ALGÆUROPE2022
13 - 15 · DECEMBER · ROME

ABSTRACT BOOK

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Benelux

EUROPEAN ALGAE BIOMASS ASSOCIATION

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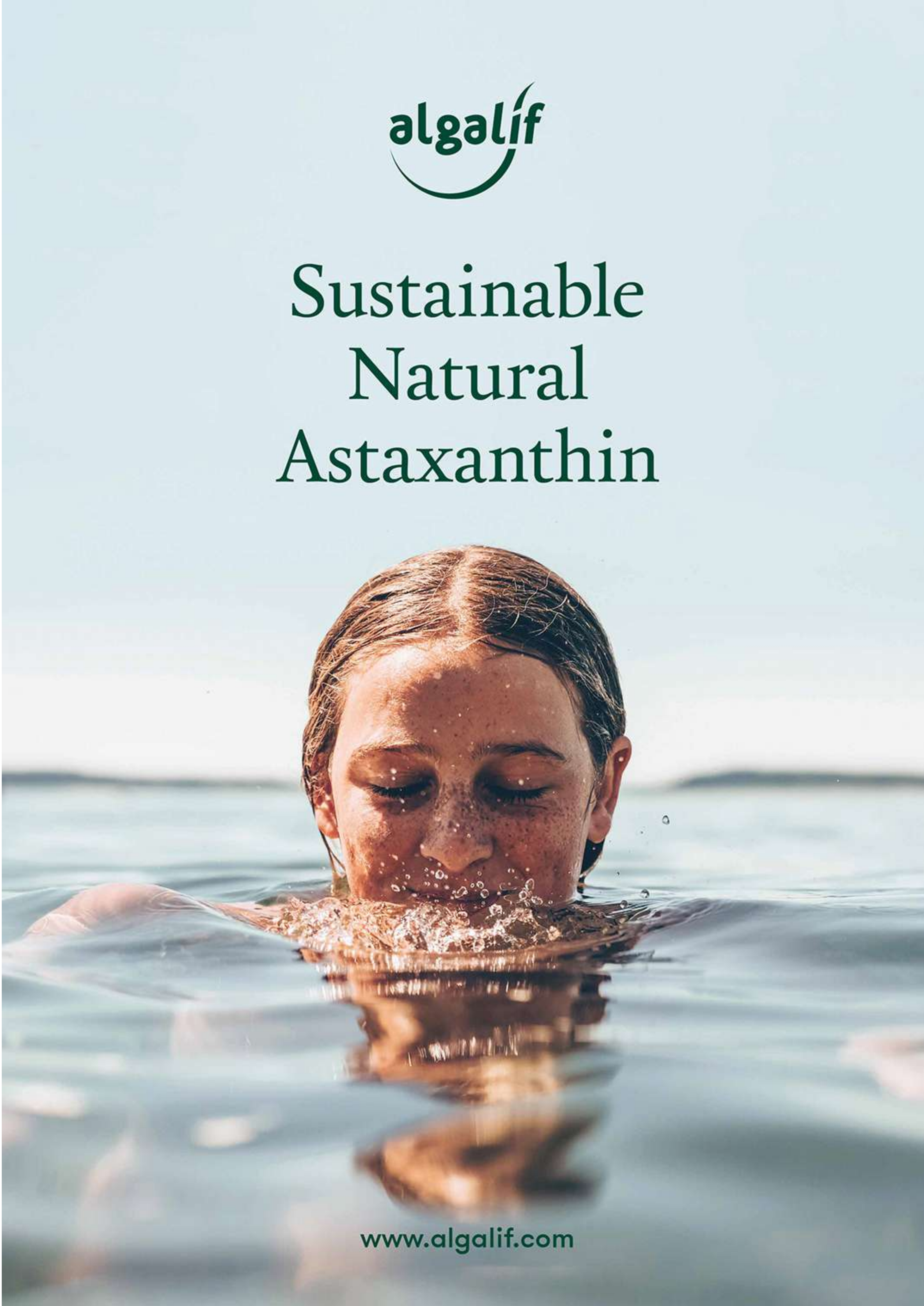
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WELCOME LETTER EABA

Dear participants

Welcome to the 2022 edition of the EABA Conference: ALGAEUROPE.

On behalf of the EABA Steering Committee, the Scientific Committee, and the Industry Committee, we are pleased to welcome you this year in a face-to-face event.

It is easy to measure how much everyone likes the return to this configuration: we have received a record number of abstract proposals: 289.

It goes without saying that this craze is accompanied by an increase in the complexity of the conference organization. Meeting all the aspirations becomes a real challenge. We need to attract universities and all academics. We need to be attractive to algae producers, equipment manufacturers and a whole private world. We must appeal to the younger generation and be aware that algae cover a disparate world where we see macroalgae on the outside and microalgae on the inside. And where finally, we must be attentive to the geographical representativeness not to say geopolitical.

Thus, it is the return of "off" moments, coffee breaks and meetings around lunches or dinners. Conviviality and live negotiations. Real meetings leading to collaborations.

The EABA and DLG teams are returning to face-to-face meetings and are organizing this event in large part thanks to the quality of the speakers and the summaries received. This edition will also be the occasion to continue to organize some original round tables. A little more business and market-oriented exchanges in addition to the usual scientific sessions.

Finally, EABA intends to continue its close collaboration with the European Commission and the European strategy in general with a session dedicated to Europe. In this respect, the Commission has chosen to back up the EU4Algae tender meetings with the EABA conference by organizing the tender feedback day on the Monday preceding AlgaEurope.

Finally, as in other years, we would like to apologize for the abstracts that were not selected for oral presentation and insist on the fact that the choices made are only the mirror of the growing success of the AlgaEurope Conference.

After Florence (2014), Lisbon (2015), Madrid (2016), Berlin (2017), Amsterdam (2018), Paris (2019), 2020 and 2021 in video conference. At the end, here is Rome 2022 where we know we can meet in real.

See you there in December!

Jean-Paul Cadoret
President EABA

Vitor Verdelho
General Manager EABA

WELCOME LETTER DLG

Dear Algae professionals,

Hereby we warmly welcome you to the AlgaEurope 2022 Conference in Rome!

After having organized the last 2 editions online, we are very excited to be back in a live format as no online format can replace the power of meeting face-to-face.

We are delighted to welcome more than 400 delegates from 43 countries, with this high interest it is once again evident that AlgaEurope is one of the most global comprehensive conferences about science, technology and business in the Algae Biomass sector organized by industry professionals.

The program of AlgaEurope 2022 is a very comprehensive one with more than 90 speakers who will share their knowledge and expertise within the field of Algae. Some 17 sessions spread out over 3 days will give the participants a full update on the Algae industry.

This years' conference is again the result of a great cooperation between EABA - European Algae Biomass Association and DLG Benelux.

I would like to take this opportunity to thank all our speakers and chairs for their cooperation and enabling us to draft such a dedicated program addressing all relevant topics in the Algae industry. Furthermore, I would like to thank our sponsors; Algalif (Platinum Sponsor), SCHOTT (Silver Sponsor), Fluid Air (Silver Sponsor), Microphyt (Silver Sponsor), A4f (Silver Sponsor), Green Aqua (Silver Sponsor), Fermentalg (Bronze Sponsor), SANI Membranes (Bronze Sponsor) and Livegreen (Bronze Sponsor).

A special thanks goes out to EABA for their commitment, dedication and support, once again it has been a pleasure to cooperate, and the conference greatly benefits from their professionalism.

I wish all our delegates, speakers, sponsors and partners a great edition of AlgaEurope 2022 and we hope to see all again next year in Prague! Thank you for your commitment and support!

Kuno Jacobs
Managing Director DLG Benelux B.V.

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A4F is a bio-industrial group focused on the development and deployment of technologies, using sustainable raw materials and renewable biological resources, to feed our future.

We are technology-driven, specialized in the production and processing of seaweeds, microalgae and other microorganisms for many applications, such as food, feed, cosmetics, pharma, fertilizers, wastewater treatment, soil remediation, bioplastics, biofuels and others.

With an eye on the future, the A4F Group seeks to develop new processes and technologies for carbon capture and utilization and for biomass valorization to built new business opportunities while fostering sustainable industrial development and reconversion.

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Green Aqua is a Portuguese investment group focused on projects in the aquatic and marine biotechnology sector. It aims at leading the algae production market for its ability to produce sustainably, for its capacity to capture and utilize CO2 emissions from industries, for the innovation of the projects it develops and for the quality of its products.

Green Aqua owns and operates at Póvoa de Santa Iria, near Lisbon, the largest microalgae production platform in Europe, having a unique concept and incorporating a biorefinery for the production of oils rich in Omega-3.

Trade Show Participants

A4F – Algafuel & Green Aqua

A4F - Algafuel is a Portuguese company deeply involved in biotechnology and sustainability. It focuses on the development and operation of industrial scale algae facilities, based on a strong track record of RD&I projects.
A4F designs, builds, operates and transfers technological solutions and systems for the industrial production and biorefining of algae, selecting the technologies that better meet customers' needs. It is part of a bioindustrial group focused on the development and deployment of technologies for bioprocesses, using marine & aquatic biomass.

Green Aqua is a Portuguese investment group focused on projects in the aquatic and marine biotechnology sector. It aims at leading the algae production market for its ability to produce sustainably, for its capacity to capture and utilize CO2 emissions from industries, for the innovation of the projects it develops and for the quality of its products.
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Algalif
Algalif® is a leading supplier of high-grade natural astaxanthin from microalgae, produced at its state-of-the-art facility in Iceland. Algalif is dedicated to solving the nutritional needs of current and future generations through the development and delivery of sustainable, high-quality microalgae ingredients.
The company is built on a foundation of quality, purity, and sustainability, and remains firmly committed to these principles. In producing its high-quality, clean, sustainable microalgae ingredients, Algalif utilizes proprietary growing and harvesting processes, and leverages renewable, sustainable resources from abundant Icelandic sources. Its Astalif™ astaxanthin is a carotenoid, shown to be one of the most potent antioxidants available. It is produced in a state-of-the-art, cGMP facility that is 100% powered by renewable geothermal energy, and incorporates stringent water conservation practices. Backed by research, Astalif is ideal for dietary supplement applications targeting: healthy aging, sports nutrition, cardiovascular health, cognitive health and eye health.

Algaverso
BAEA S.L. was born to generate the biotechnological ecosystem necessary to achieve excellence in algae cultivation: Algaverso®. Algaverso® is made up of two complementary lines:
Decarbonization of small and medium-sized companies from the production of microalgae and extraction of their products of interest (biological line, under development).
Commercialize and design lighting systems based on patented technology (line of engineering, in the industrialization phase).

Algoliner
Algoliner is a turnkey supplier for photobioreactors in any dimensions. Their lab reactors have volumes between 1 litre and 1,000 litres while their large scale have no limits.
With their unique production method, they produce the pipes for the large-scale reactors on site in any required length. Hence no packaging, transport and re-assembly with joints is necessary.
Algoliner is the only producer of photobioreactors with aseptically flanges.
The required raw material for their reactors is only 40% of those of common systems, while the pipes can be easily recycled by 100%.
Algoliner realized in the meantime many reactors in different sizes and concepts.

Bioréa
Bioréa, subsidiary of Agrial Group, relies on its patented airlift fermentation technology. Located in Bretagne, France, their new factory is mostly dedicated to microalgae.

Their operations include:
- CMO services: your partner to scale-up your technology and manufacture tailor-made biomass
- R&D services: 3 labs equipped with CiYOU benchtop bioreactors in order to elaborate and adapt the optimal fermentation parameters for your strain
- Benchtop bioreactors: design, manufacture and sell CiYOU bentchtop bioreactors. Get your own airlift fermenter for your lab.

Their facilities are food & feed grade and are certified ISO FSSC 22000 and GMP+.
Cosmetics, nutraceutics, food, feed, …, contact them and start building a collaboration.

bygora
The leading B2B marketplace to buy and sell novel proteins

Extrasynthese
Extrasynthese in an independent company, established since 1986 in Lyon area (France), specialized in natural products chemistry. They are expert in extraction, synthesis, biosynthesis, purification and analysis of small molecules from the plant kingdom. They offer a large catalog of phytochemicals analytical standards. They offer R&D technical services to their customers, and they regularly participate to collaborative research projects funded by the European Commission.

Fluid Air
Founded in 1983, Fluid Air provides custom, efficient, and effective solid dosage processing equipment and systems that can meet the most challenging powder modification and creation application. A division of Spraying Systems, Fluid Air technology is used to help the pharmaceutical, nutraceutical, food, fine chemical, agriculture, and biopharmaceutical industries daily. Learn more about Fluid Air at www.fluidairinc.com.

GICON
The GICON® Group is a group of international, independently operating engineering service providers. Sustainability in the handling of resources, in the use of energy and in the extraction of raw materials as well as a responsible approach to risks in favour of a clean environment and a society worth living in - both today and in the future - these are the guidelines that guide their daily work and this is how they understand their service to their customers.
GICON® was founded in 1994 and is an owner-managed engineering office with its headquarters in Dresden, Germany. More than 500 employees work in more than 30 departments in several branches in Germany and worldwide. Innovation through research is a GICON® trademark. Both as a service provider for their customers and for the further development of the company, they research and develop new processes, products and services. To this end, they have created an extensive network of cooperative relationships with leading research institutions.
GICON® engineers have planned a broad variety of photobioreactor plants ranging from R&D to commercial sized systems for 25 years using their detailed expertise in plant design, approval planning, process engineering, biotechnology, biology and bioenergy systems. As one of its main R&D areas, GICON® is active in several projects to further develop microalgae-based technology solutions.

Idea Bioprocess Technology
Idea Bioprocess Technology is an Italy-based company, leader in the area of smart lab equipment for applications and processes related to biotechnology (industrial biotechnology, environmental biotechnology, biogas technology, microalgal technology, etc.), that develops innovative, reliable and high-quality devices for industry and academy. All Idea devices are created with an "open technology" concept at the base. The equipment is an open system fully customizable in all its aspects (software, hardware, reaction vessels, etc.), and adaptable to future integrations/modifications. Thanks to this feature everything is easy and time-saving: all devices are sold pre-configured and ready to use.
In the field of microalgal lab technology Idea Bioprocess Technology has introduced several devices for research activities and for parallel monitoring of full-scale plants such as photo-respirometers, biomass measurement systems, microalgal light sources, modular photobioreactors and the new device MICROALGAL BOX that will be presented at the trade show.

Industrial Plankton
Founded in 2010, Industrial Plankton Inc. designs and manufactures turnkey bioreactors
Their technology is optimized for reliability, ease of use, and biosecurity.
Customers operate our photobioreactors in 24 countries around the globe.
Our equipment is highly automated, increasing reliability and saving operators countless hours. The result is trustworthy equipment capable of easily and reliably producing biosecure, algae.

Lgem
Lgem offers reliable systems for cultivating any algae at any scale. With over 16 years of experience with autotrophic algae, Lgem provides a solid basis of knowledge, skills, technology, and R&D.
Lgem's iconic two-phase PBR solutions have increased productivity and deliver stable algae production at lower operational costs. Lgem's patented PBR systems are automated, fully integrated, and industry 4.0-ready. Lgem's PBRs range from 20 litres to >1.000.000 litres.
At Lgem, investment risk reduction is a crucial success factor for its clients.
The AlgaeHUB facility (>20 FTE, 7.000 m2 with >10 production units available) allows customers to optimise and validate their business case on an industrial scale before making significant investments.
Currently, Lgem works for both established and new innovative food companies and the feed, pharma, and cosmetic industries. Lgem can successfully cultivate more than ten different microalgae strains, including some of the most fragile species/variants.

Liqoflux & Virto-Cuccolini

Liqoflux focuses on algae harvesting & water treatment for the algae industry. They offer all algae harvesting & water treatment solutions to make your algae production successful: from purifying the water for algae cultivation, harvesting your algae culture to recycling the water back to production.

Cuccolini Srl – Virto Group is an Italian Company active in the engineering, manufacturing and sale of industrial sieving equipment since 1989. Their wide experience in more than 300 different industrial separation applications and more than 20,000 sieves installed worldwide, both for powders and liquids, made it possible to create a specific vibrating sieve series for spirulina harvesting (7 to 35 µm). Their units are easy to clean and to maintain, versatile in terms of size and mesh aperture, to match with any spirulina harvesting requirements and can be used as stand-alone units or within a complete plant. Virto-Cuccolini is always open to innovating sieving needs and requirements.

Microphyt
Microphyt – Fostering a blue future
At the crossroads of life sciences and bio-processes, we develop the next generation of natural ingredients from microalgae for a better living.
Based in the South of France near Montpellier, Microphyt is a leading company in the development, production and commercialization of microalgae-based bioactives for health and beauty. With more than 10 years of expertise in the field, Microphyt leverages microalgae's previously untapped diversity to provide unique solutions for Nutrition and Wellness. Microphyt's technologies, developed in-house and patented, enable the controlled production of a wide variety of microalgae sustainably at an industrial scale. Microphyt currently employs 50 people and has filed 7 patent families.

SANI Membranes
With the unique Vibro™ Technology, SANI Membranes offers a technology where harvest, separation, and concentration can all be done in one single unit. The Vibro™ units handle harvesting, thickening, dewatering, and even separation of algae debris and protein, as well as protein concentration.
The technology makes it possible to optimize several separation processes within the algae industry, where centrifuges, hollow fibers, ceramic membranes, and other separation techniques can be replaced. Vibro™ Technology is characterized by its ability to reach very high concentrations, such as nannocloropsis up to 190 g/l and chlorella up to 290 g/l.
With Vibro™ Technology, you also get a solution that gives you a reusable permeate, scales up easily, is user-friendly, is simple to install, and has low energy consumption.

SCHOTT
SCHOTT is a manufacturer of high-tech materials based on specialty glass. Always opening up new markets and applications with a pioneering spirit and passion – this is what has driven the #glasslovers at SCHOTT for more than 130 years. SCHOTT's Business Unit Tubing is one of the world's leading manufacturers of glass tubing, rods and profiles. SCHOTT Tubing provides customized products and services for international growth markets such as pharmaceuticals and electronics as well as industrial and environmental engineering, like photobioreactors (PBR). With DURAN® borosilicate glass tubing, manifolds, bends and food-grade couplings the BU Tubing provides ideal materials for algae cultivation in PBR systems: Smooth interior surfaces are easy to clean, protect against biocontamination and ensure food grade algae quality. With a lifetime of over 50 years and a consistently high light transmission, glass offers a cost effective and productive solution.

Synoxis algae
Synoxis algae designs and markets a wide range of automated high-density algae cultivation systems: photobioreactors, barrels, raceways. From microalgae to macroalgae, it provides different solutions for algae culture experts around the world from different sectors, such as food, cosmetics, research, green chemistry, and aquaculture. As a spin-off of the plastics manufacturer Synoxis, existing since 1981, Synoxis algae has twenty years of experience in the field of aquaculture. In 2003, Synoxis participated in the thesis of Erell Olivo called "Design and study of a photobioreactor for the continuous production of microalgae in aquaculture hatcheries", in partnership with Ifremer and the University of Nantes. This led to several years of research and development on algae cultivation systems and algal biotechnology. Its range of microalgae photobioreactors is made up of three models: a small benchtop model NANO (3 L) for research laboratories, a nomadic intermediate model LUCY (16 L) for the production of inoculum and an industrial model JUMBO (285 L) for mass production. Today, Synoxis algae has 60 installations in France and abroad in all business sectors.

TMCI Padovan
A high level of specialization, a strong dedication to innovation and a natural commitment to maximizing the energy efficiency in each process, plant and machine, are the distinguishing features of TMCI Padovan, leader in the filtration technology, thanks to its spirit and mission to be always at the forefront. Grown in more than one century of history, this group invented some of the most innovative systems in the market today, winners of prestigious international awards.
Thanks to their deep market knowledge, world-wide collaborations with the finest experts in the field, partnerships with universities and research centers, experience gathered through close cooperation with top customers, today it offers the most powerful and efficient solutions to all those customers who want to be aligned with emerging needs of superior product quality, with an eye to cost saving and return on investment.

AlgaEurope 2022 Conference Program

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

All times are mentioned in Central European Time (CET)

Conference Day 1 - Tuesday 13 December 2022

Registration: 08:00 am - 09:00 am

09:00 am - 09:25 am: Conference Opening

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

09:25 am - 09:30 am: Intro Morning Chairwoman

Nyvall Collen, Pi (Olmix)

09:30 am - 10:00 am: Keynote Speaker Day 1

Prof. Falkowski, Paul G. (Rutgers The State University of New Jersey): The bottom line: The cost of light

10:00 am - 11:00 am: Session 1: Seaweed sources and applications

10:00 am - 10:15 am: Jiménez González, Camila (University of Vigo): Chondrus crispus as a potential biomass for a green biorefinery: proteins, hydrocolloids and other bio-compounds

10:15 am - 10:30 am: Camarão, Bárbara (University of Coimbra): Seaweed-based biostimulant – potential for seed germination and effect of seasonality

10:30 am - 10:45 am: Damitha Bandara, Chaturangi (Uva Wellassa University Sri Lanka): Determination of anti-microbial and anti-diabetic compunds from crude extract of red seaweed, Kappaphycus alvarezii

10:45 am - 11:00 am: Weiss, Ben (Woods Hole Oceanographic Institution): Rapid Out-Planting of Seaweed Grow Lines

11:00 am - 11:30 am: Coffee Break

11:30 am - 12:00 pm: Session 2: Process

11:30 am - 11:45 am: Di Caprio, Fabrizio (University Sapienza di Roma): Control of bacterial contamination in microalgae cultures integrated with cheese whey wastewater treatment by applying feast and famine regime

11:45 am - 12:00 pm: Enmak, Prayoon (Newcastle University): Process intensification for low-cost microalgae harvesting by using continuous foam flotation technique

12:00 pm - 12:30 pm: Special Session

Tribute to Prof. Mario Tredici (Late EABA President and Founder)

12:30 pm - 02:00 pm: Lunch Break

02:00 pm - 02:05 pm: Intro Afternoon Chairwoman

Rodolfi, Liliana (University of Florence, Department of Agriculture, Food, Environment and Forestry - DAGRI)

02:05 pm - 02:15 pm: Algalif - Sponsored Presentation

Stefánsson, Tryggvi: Algalif – How did we get here and where are we going?

02:15 pm - 03:45 pm: Session 3: Physiology

02:15 pm - 02:30 pm: Gallardo Rodríguez, Juan José (University of Almería): CFD-aided determination of shear stress thresholds in sensitive microalgae using shake flasks

02:30 pm - 02:45 pm: Bezzo, Fabrizio (University of Padova): DigitAlgaesation: A knowledge-based training network for digitalisation of photosynthetic bioprocesses

02:45 pm - 03:00 pm: Guerra, Inês (Allmicroalgae/University of Algarve): Growth, size, and biochemical evaluation of Arthrospira platensis cultivated in tubular photobioreactors driven by a centrifugal pump

03:00 pm - 03:15 pm: Van Oossanen, Sabine (Wageningen University & Research): Putting algae on the map: Genome-scale metabolic modeling of Nannochloropsis for improved lipid production

03:15 pm - 03:30 pm: Lopes, Filipa (CentraleSupelec): Microalgae-biofilm as a source of bioactive compounds

03:30 pm - 03:45 pm: Kriechbaum, Ricarda (Technical University Vienna): Potential of Lignocellulosic Waste as Substrate for Microalgal Cultivation

03:45 pm - 04:00 pm: SANI Membranes - Sponsored Presentation

Hjelmsmark, Henrik: Vibro Filtration for harvesting, concentration and refinery

04:00 pm - 04:30 pm: Coffee Break

04:30 pm - 05:00 pm: Company Presentations

04:30 pm - 04:45 pm:
Busch-Larsen, Henrik (Algiecel)
Hazewinkel, Sander (Lgem)
Lizzul, Marco (Variconaqua)

04:45 pm - 05:00 pm:
Kaplan, Claude (Kuehnle AgroSystems)
Boelens, Pieter (Liqoflux)
Goudeau, Paul (Synoxis)

05:00 pm - 06:00 pm: Session 4: Europe and the World

05:00 pm - 05:15 pm: Doumeizel, Vincent (United Nations Global Compact / Lloyd's Register Foundation): The Seaweed Manifesto and the new Safe Seaweed Coalition, working together on global regulations to enable collaboration in this emerging sector

05:15 pm - 05:30 pm: Stulgis, Maris (European Commission): EU Algae Initiative

05:30 pm - 05:45 pm: Maragna, Laura (Tender EU4Algae): EU4Algae Stakeholders Forum – Making change together

05:45 pm - 06:00 pm: Jagot, Charlotte (EMFAF): EMFAF portfolio of projects on algae and blue bioeconomy

06:00 pm - 07:00 pm: Controversis

Session Chairman: Navalho, João (Necton)
Topic: Algae and the real world

07:00 pm: Closure of the Conference Day

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

08:00 pm - 10:30 pm: Conference Dinner

Conference Day 2 - Wednesday 14 December 2022

09:00 am - 09:05 am: Conference Opening

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

09:05 am - 09:10 am: Intro Morning Chairwoman

Arvaniti, Efthalia (SUBMARINER Network)

09:10 am - 09:40 am: Keynote Speaker Day 2

Prof. Boussiba, Sammy (Microalgae Biotechnology - Ben-Gurion University of the Negev): Astaxanthin the king of Carotenoids and it's path in Nature

09:40 am - 10:55 am: Session 5: Food

09:40 am - 09:55 am: Monino Fernandez, Pedro (Wageningen University & Research): From the hot springs to the table: Scaling up mixotrophic cultivation of Galdieria sulphuraria

09:55 am - 10:10 am: Villaró, Silvia (University of Almeria): Production of Arthrospira platensis using pilot-scale raceway reactors and characterization of the nutritional, bioactive, and techno-functional properties of the biomass

10:10 am - 10:25 am: Oliveira, Sónia (School of Agriculture University of Lisbon/Ulisboa): Using 3D printing technology to develop microalgae-based snacks: Nutrition and sensory impact

10:25 am - 10:40 am: Demets, Robbe (KU Leuven KULAK): Antioxidative capacity of microalgal carotenoids for stabilizing n-3 LC-PUFA rich oil: initial quantity is key

10:40 am - 10:55 am: Bar Gil, Amikam (Yemoja Ltd): Algae based Blood substitute enriched with vitamin B-12. A complete solution for the plant-based alternatives

10:55 am - 11:25 am: Coffee Break

11:25 am - 12:25 pm: Session 5: Food

11:25 am - 11:40 am: Infante, Carlos (Fitoplancto Marino, S.L.): TetraSOD ®, a unique marine microalgae ingredient: an overview of main outputs in pre-clinical and clinical trials

11:40 am - 11:55 am: Alemán Vega, Monserrat (Instituto Tecnológico de Canarias): Long-term pilot-scale cultivation of Arthrospira platensis in seawater: Strategies for the production of alternative protein sources and high-value compounds while maximizing hydric efficiency

11:55 am - 12:10 pm: Kurpan, Daniel (University of Milan): A 2-step serial membrane filtration for the extraction and purification of phycocyanin from Arthrospira platensis

12:10 pm - 12:25 pm: Boskovic Cabrol, Marija (University of Padua): Nutritional properties of frankfurters with added chlorophyll-deficient Chlorella vulgaris mutants

12:25 pm - 12:35 pm: Algalif - Sponsored Presentation

Jessen, Jan Eric: Future potential of microalgae biotechnology

12:35 pm - 02:10 pm: Lunch Break

02:10 pm - 02:15 pm: Intro Afternoon Chairman

02:15 pm - 03:30 pm: Session 6: Biorefinery

02:15 pm - 02:30 pm: Cheel, José (Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech): Separation of eicosapentaenoic acid from the diatom Nanofrustulum shiloi via two-step high performance countercurrent chromatography

02:30 pm - 02:45 pm: Costa, Luis (A4F - Algae for Future): From wastewater to biojetfuel: a circular economy system using microalgae for wastewater bioremediation and raw material for biojetfuel through hydrothermal liquefaction

02:45 pm - 03:00 pm: Marchal, Luc (Nantes University): Eco-design of a downstream process for enriched fucoxanthin production from Tisochrysis lutea

03:00 pm - 03:15 pm: Madugu, Fatima (University of Manchester): Enhanced cultivation strategy for industrial scale algae-based biorefineries

03:15 pm - 03:30 pm: Kazbar, Antoinette (Wageningen University & Research): Multi-product biorefinery of Seaweed using green solvents

03:30 pm - 03:40 pm: Microphyt: Sponsored Presentation

Maury, Jonathan: From microalgae to the next generation of natural ingredients: focus on Microphyt expertise

03:40 pm - 04:15 pm: Coffee Break

04:15 pm - 05:00 pm: Session 7: Feed

04:15 pm - 04:30 pm: Khozin-Goldberg, Inna (Ben Gurion University of the Negev): Microalgae as sustainable and health-promoting agents in aquaculture

04:30 pm - 04:45 pm: Štěrbová (former: Ranglová), Karolína (Institute of Microbiology of the Czech Academy of Sciences, Centre Algatech): Microalgae as a feed improve the survival rate and vitality of Pikeperch (Sander Lucioperca) larvae

04:45 pm - 05:00 pm: Guiheneuf, Freddy (Inalve): Biofilm-based production of marine microalgae as live feeds for the aquaculture industry

05:00 pm - 05:45 pm: Session 8: Biostimulant

05:00 pm - 05:15 pm: Alvarez-Gil, Maria (Neoalgae Micro Seaweeds products): Life algar-bbe project: Results in microalgae application as biostimulant

05:15 pm - 05:30 pm: Schüler, Lisa M. (GreenCoLab): Novel strains of scenedesmus sp. as a potential source for agricultural applications

05:30 pm - 05:45 pm: Gitau, Margaret (Biological Research Centre): Microalgae promotes plant growth and primes plants for response to abiotic stress

05:45 pm - 05:55 pm: Fluid Air - Sponsored Presentation

Beaupeux, Elodie: Gentle drying process for thermosensitive compounds

05:55 - 06:55 pm: Session 9: Genetics

05:55 pm - 06:10 pm: D'Adamo, Sarah (Wageningen University & Research): Genetic engineering tools and approaches for understanding and revising lipid metabolism in the microalga N. oceanica

06:10 pm - 06:25 pm: Mokakabye, Ngokoana (University of Cape Town): Recombinant expression of Thermostable Phycocyanin in Spirulina

06:25 pm - 06:40 pm: Betterle, Nico (University of Verona): Engineering of the recently-discovered Synechococcus PCC 11901, a fast-growing cyanobacteria, for the synthesis of high added-value carotenoids

06:40 pm - 06:55 pm: Kichouh Aiadi, Salim (University of Almeria): Exposure of a marine dinoflagellate microalgae to epigenetic

06:55 pm: Closure of the Conference Day

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

08:30 pm - 10:30 pm: The Mentor's Evening

Moderator: Cadoret, Jean-Paul (EABA President)

Sommer Ferreira, Bruno (Biotrend SA)
Sassi, Jean Francois (CEA - Commissariat a l'Energie Atomique et aux Energies Alternatives)
Reinhardt, Robert (Algen, Algal Technology Centre, LLC)
Raymundo, Anabela (University of Lisbon)
Pruvost, Jeremy (GEPEA - Nantes University)
Nyvall Collen, Pi (Olmix)
Hennequart, Franck (ALGAIA)
Griffths, Hywel (Fermentalg)
Fuentes Grünewald, Claudio (King Abdullah University of Science and Technology)
Benemann, John (MicroBio Engineering Inc)
Barbosa, Maria (Wageningen University & Research)
Acien, Gabriel (University of Almeria)

Conference Day 3 - Thursday 15 December 2022

09:00 am - 09:05 am: Opening Day 3

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

09:05 am - 09:10 am: Intro Morning Chairman

Verdelho, Vitor (EABA General Manager)

09:10 am - 09:40 am: Keynote Speaker Day 3

Prof. Smith, Alison (University of Cambridge): How can we exploit the biodiversity of algae for biotechnology?

09:40 am - 10:55 am: Session 10: Original

09:40 am - 09:55 am: Ende, Stephan (Alfred-Wegener-Institute): New Microalgae media formulated with completely recycled phosphous originating from agricultural sidestream

09:55 am - 10:10 am: Pozo-Dengra, Joaquin (Biorizon Biotech): EMFF- ALGAENAUTS Project: Eco-friendly and sustainable new family of biopesticides based on microalgae

10:10 am - 10:25 am: Berden Zrimec, Maja (Algen, Algal Technology Centre, LLC): Relevance of zoosporic parasites in aquatic systems (Cost ParAqua)

10:25 am - 10:40 am: Nascimento, Francisco (iBET - Institute of Experimental Biology and Technology): Insights into the role of the microbiome in microalgae growth and development: Microbiome recruitment and assembly dynamics in Nannochloropsis oceanica and Phaeodactylum tricornutum cultivations

10:40 am - 10:55 am: Parreira, Celina (A4F - Algae for Future): Extratoteca – Microalgae Extracts for High Value Products

10:55 am - 11:25 am: Coffee Break

11:25 am - 12:25 pm: Session 11: Process B

11:25 am - 11:40 am: Davila, Javier (Universidad de Sevilla): Time scales of the use of light in photobioreactors

11:40 am - 11:55 am: Castejón, Natalia (University of Vienna): Unlocking the lipidome of Nannochloropsis gaditana: a source of high-value lipids with potential biological activities

11:55 am - 12:10 pm: Silva, Samara (University of Porto & Polytechnic Institute of Bragança): C-phycocyanin extraction using spirulina (Arthrospira platensis) biomass immobilization in calcium-alginate beads

12:10 pm - 12:25 pm: Manoel, João (Institute of Microbiology, Centrum Algatech) - Deciphering the communication between Algae and Bacteria

12:25 pm - 12:35 pm: SCHOTT - Sponsored Presentation

Wintersteller, Fritz: Why Tubular Glass Photobioreactors?

12:35 pm - 02:00 pm: Lunch Break

02:00 pm - 02:05 pm: Intro Afternoon Chairman

Unamunzaga, Carlos (Fitoplancton Marino)

02:05 pm - 02:15 pm: A4F- Sponsored Presentation

Costa, Luís: Replicable large scale exploitation of algae in a circular economy symbiosis with other industries

02:15 pm - 03:00 pm: Session 12: Transversal

02:15 pm - 02:30 pm: Leão, Susana (LEITAT): REDWine LCA: CO2 from wine fermentation to produce Chlorella as feedstock for the industry

02:30 pm - 02:45 pm: Braud, Léa (University College Dublin): Improving transparency in life cycle assessment of algae systems: A case study on the spiralg biorefinery

02:45 pm - 03:00 pm: Speranza, Lais (GreenCoLab): LCA of commerical scale Tetraselmis sp. production

03:00 pm - 04:15 pm: Session 13: Bioremediation

03:00 pm - 03:15 pm: Beigbeder, Jean-Baptiste (APESA - Association for the Environment and Safety in Aquitaine): Coupling anaerobic digestion and microalgal cultivation for efficient nutrients and inorganic carbon uptake

03:15 pm - 03:30 pm: Costa, Margarida (NIVA - Norwegian Institute for Water Research): Filamentous microalgae as tertiary wastewater treatment

03:30 pm - 03:45 pm: Futó, Peter (Albitech Biotechnology Ltd.): Comparative analysis of soil degradation and soil structure in croplands affected by erosion and soil dehydration treated with a biological soil crust forming algal culture

03:45 pm - 04:00 pm: Carneiro, Mariana (Necton): Reusing Effluents from Agriculture to unlock the potential of Microalgae (REALM)

04:00 pm - 04:15 pm: Casagli, Francesca (INRIA): Outdoor N2O emissions measurements in algae-bacteria systems guided by modelling

04:15 pm - 04:45 pm: Coffee Break

04:45 pm - 05:45 pm: Session 14: Biomaterial

04:45 pm - 05:00 pm: Rizzo, Arianna (YAS - Young Algaeneers Symposium): YAS, the next Algaeneers generation

05:00 pm - 05:15 pm: Carletti, Marta (Ben-Gurion University of the Negev): Effective conversion of atmospheric nitrogen into cyanophycin: mutagenesis and optimization of cultivation of Nostoc sp. PCC 7120

05:15 pm - 05:30 pm: Gao, Fengzheng (Wageningen University & Research): Oxygen production from 3D bioprinted living microalgae for tissue engineering

05:30 pm - 05:45 pm: Van Miert, Sabine (Thomas More University of Applied Sciences) - Pilot-scale Cultivation of Chloromonas typhlos in a photobioreactor

05:45 pm - 06:00 pm: Closure of the Conference

Cadoret, Jean-Paul (EABA President)
Verdelho, Vitor (EABA General Manager)

CONFERENCE DAY 1

ALGÆUROPE2022

13 - 15·DECEMBER·ROME

Session 1: Seaweed sources and applications

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

CHONDURUS CRISPUS AS A POTENTIAL BIOMASS FOR A GREEN BIOREFINERY: PROTEINS, HYDROCOLLOIDS AND OTHER BIO-CPMPOUNDS

Camila J.G[1,2]

Andrea L[1], Tania B.O[3], Martiña F.N[3], Clara F.G[2]

[1]Biochemistry Laboratory, Universidade de Vigo, Spain
[2]Endocrinology Laboratory, Center for Biomedical Research (CINBIO), Spain
[3]Division of Marine resources and Aquaculture, ANFACO-CECOPESCA, Spain

ABSTRACT

Food production is accelerating, and therefore, its environmental impacts. Regarding sustainability, there is a growing consensus that animal protein has a disproportionate impact on the environment, especially when it is produced in intensive systems using large amounts of feed crops [1]. As such, the use of alternative feedstocks will enable society to transition to a bio-based bioeconomy. The EU amend their bio-economy strategy in 2018 with the aim of giving more importance to economy, society and environment to line up with the 17 Sustainable Development Goal's (SDG) regulated by the United Nations [2–4]. Besides, undersupply of land cultivation, limitation of crops, more food requirement, demand of new compounds for pharmacology, and so many other requests contrived researchers to look deep into the ocean to find new natural resources [5].

The oceans cover more than 70% of the Earth's surface with marine species compromising approximately half of the total global biodiversity which plays a key ecological role in coastal ecosystems [6]. Macroalgae have been perceived as one of the most reasonable and seemly feedstocks for a change into a blue bio-economy. Despite the clear advantages that macroalgal species offer for a biorefinery development, both the research and commercialization aspects of macroalgal biorefineries are still in their infancy and few have progressed beyond laboratory scale [2]. Currently the seaweed market has two major business segments: it is targeted either directly for consumption or is used as a basis for extraction of seaweed-based hydrocolloids.

Chondrus crispus is one of the most harvested red seaweed species in Europe. *C. crispus* is primarily used for the extraction of carrageenans, which represents an economic interest for European industries [7,8]. Carrageenan's global market size represented USD 780.5 million by 2020 and is expected to increase 6.0% by 2028 [9]. Besides the high hydrocolloid content, *C. crispus* represents a source of valuable proteins, and other compounds.

Therefore, this investigation aims to probe the feasibility of integrate the recovery of proteins, hydrocolloids and other interesting compounds on the framework of a green full biorefinery process from wild-collected, and lab-cultivated red macroalgae *Chondrus crispus*.

Keywords
Chondrus crispus; biorefinery; proteins; hydrocolloids; applications

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SPEAKER INFORMATION

Camila Jiménez González
PhD student / Universidade de Vigo
Spain

BIOGRAPHY

Camila Jiménez González is a Dietitian with a background in both clinical nutrition and research. Camila began her career at the Instituto Universitario CEMIC, Argentina where she received her Bachelor's Degree in Nutrition. Afterwards, Camila obtained a Master Degree in Nutrition at Universidade de Vigo, Spain. She is currently a PhD student in the Doctorate program of Endocrinology imparted by Universidade de Vigo. Her current field of research is focused on vegetable proteins from legumes and macroalgae. Camila has experience in extraction and purification of proteins and is now working with bioavailability *in vivo* studies with animal models. Camila hopes to investigate different biomolecules from macroalgae and their possible positive effect on metabolism.

SEAWEED-BASED BIOSTIMULANT – POTENTIAL FOR SEED GERMINATION AND EFFECT OF SEASONALITY

Abusaid,A.[1], **Camarão,B.**[2], Resende, L.[2], Cotas, J.[2], Pereira,L.[2], Bahcevandziev,K.[3], Rocha, A. Cristina[2]

[1]Department Environment Engineer and Energy-INSA, Lyon, France
[2]Universidade de Coimbra, MARE-UC(MAREFOZ)/ARNET - Aquatic Research Network, Departamento de Ciências da Vida, Coimbra, Portugal
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ABSTRACT

"Sargaço" is a mixture of seaweeds that grow on the rocks of northern Portuguese coast and that are washed ashore on the beach. Traditionally, "sargaço" was collected by local farmers and fishermen and applied in agriculture. This activity has fallen into disuse. So, this natural biomass is currently under-exploited.

Macroalgae are known to be rich in minerals and bioactive compounds that are prone to stimulate seed germination and plant development and has been successfully used in agriculture. Nevertheless, research has been focused on specific macroalgae species or a mixture of two or three macroalgae. Little is known about the biostimulant potential of a mixture of algae as "sargaço".

Therefore, this work aims to assess the potential of extracts from "sargaço" as biostimulant and evaluate the effect of seasonality on its performance. For that, "sargaço" was collected at A-ver-o-Mar beach in autumn, winter, spring and summer. The collect seaweeds were dried and milled and aqueous extracts were prepared.

Four germination assays were performed with tomato seeds, which were exposed to the following treatments (n=3): water(Control); diluted extract 10%(T10), diluted extract 25%(T25), diluted extract 50%(T50) and extract(T100). During the experiment, germination rate and total weight difference were recorded. At the end, root and shoot length were measured as well as fresh plantlet weight.

This work is still being conducted.

Preliminary data indicate that "sargaço" collected in different seasons, slightly change in terms of algae composition. Germination percentages above 80% were obtained especially for T10, T25 and T50. The seaweed-based biostimulant seem to stimulate seed germination and the growth of root and plantlet. Results will be presented further with more detail.

"ValSar: Valorização do Sargaço da Costa Litoral Norte" (MAR-04.03.01-FEAMP-0502) is financed by MAR2020, PT2020 through European funds (FEAMP) and supported by GAL Costeiro Litoral Norte.

Keywords
Macroalgae; Valorization; Blue economy; Agriculture

SPEAKER INFORMATION

Bárbara Costa Camarão
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Portugal
LinkedIn: linkedin.com/in/bárbara-camarão-4047a282

BIOGRAPHY

Bárbara Camarão is a researcher from MARE-University of Coimbra, with a MSc in Marine Biology (2017). Her research has been focused on marine life conservation and protection and assessment of water quality and environmental risk. She has worked in the rehabilitation of marine animals and in several research projects, related to marine/estuarine fauna, aquaculture, blue economy and resources valorisation. In MAREFOZ Laboratory, she has gained expertise in field work, sampling, chemical compounds extraction and measurement in several environmental matrices, and analytical techniques. Recently she integrated the project VALSAR that aims to valorise the “Sargaço” of the North Coast of Portugal.

DETERMINATION OF ANTI-MICROBIAL AND ANTI-DIABETIC COMPOUNDS FROM CRUDE EXTRACT OF RED SEAWEED, *KAPPAPHYCUS ALVAREZII*

Bandara,I.M.C.D.

Abeyrathne,E.D.N.S. / Alakolanga,A.G.A.W.
Uva Wellassa University, Sri Lanka

ABSTRACT

Seaweeds are common botanical species naturally found along all coastlines in Sri Lanka, recognized as one of nature's greatest biologically active resources and possess large amount of bioactive compounds. Red seaweed *Kappaphycus alvarezii* is one of the red seaweed which has high demand and contains bioactive compounds for different industries. Therefore, the objective of this study is to determine bioactive compounds from red seaweed, *Kappaphycus alvarezii* under different treatments and check the antidiabetic and antimicrobial properties of the crude extract isolated. Extraction of secondary metabolites was carried out using distilled water (DW) under 4^{o}C and room temperature with washed and unwashed conditions and determined antimicrobial and antidiabetic properties of the extract. Isolated compounds were separated at 24 and 48 hrs intervals and lyophilized. All samples were replicated (n=3). Isolated from different treatments showed a colour variation from purple to pink resulting a structure change in the anthocyanin. Antimicrobial activity was determined using well diffusion method with locally isolated *E coli* strain and Augmentin^{XR} (0.001 ppm) as the positive and distilled water as negative control. All four extracts inhibited the gram-negative *Escherichia coli* after 48 hours of incubation ($p<0.05$) resulting storage temperature does not have an effect on the antimicrobial properties. More than 50% amylase inhibition activity was recorded in the samples which were not washed from DW and stored in room temperature (54.54) and in ice (59.58%) and washed samples which were stored in room temperature (50.32%) at 400 ppm concentration. As conclusion, crude extract without washing which are stored under room temperature can be used as a potential antimicrobial and antidiabetic agent. However further studies required for the analysis of the structure and other functional properties of the crude extract of *Kappaphycus alvarezii* with different extraction methods and isolates.

Keywords
Seaweeds, functional properties, antimicrobial, anti-diabetic, secondary metabolites

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SPEAKER INFORMATION

Imihami Mudiyanselage Chaturangi Damitha Bandara
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BIOGRAPHY

Graduate student of Uva Wellassa University with the BSc (Special) in Aquatic Resources Technology degree willing to gather new knowledge in seaweed industry. Being a trainee at National Aquatic Resources Research and Development Agency (NARA) in Sri Lanka I realized that algae and their natural products have a huge range of applications for humans as well as the environment. So started my master’s degree to gain knowledge in food science at PGIA, University of Peradeniya to improve the techniques which related to different applications of seaweeds in food science.

I would like to extend my further studies in the same field in relation to the characterization of ecologically significant secondary metabolites from marine algae (Determination of seasonal /geographical variations in the chemistry of different varieties of algae). As well, I am keen to explore my knowledge in closely related fields like Blue economy and algal biotechnology.

My career plan is to pursue a Master’s and then a doctoral degree in the Algal Biotechnology field to become a female researcher in the future to contribute my time and effort for the better future for seaweed industry.

RAPID OUT-PLANTING OF SEAWEED GROW LINES

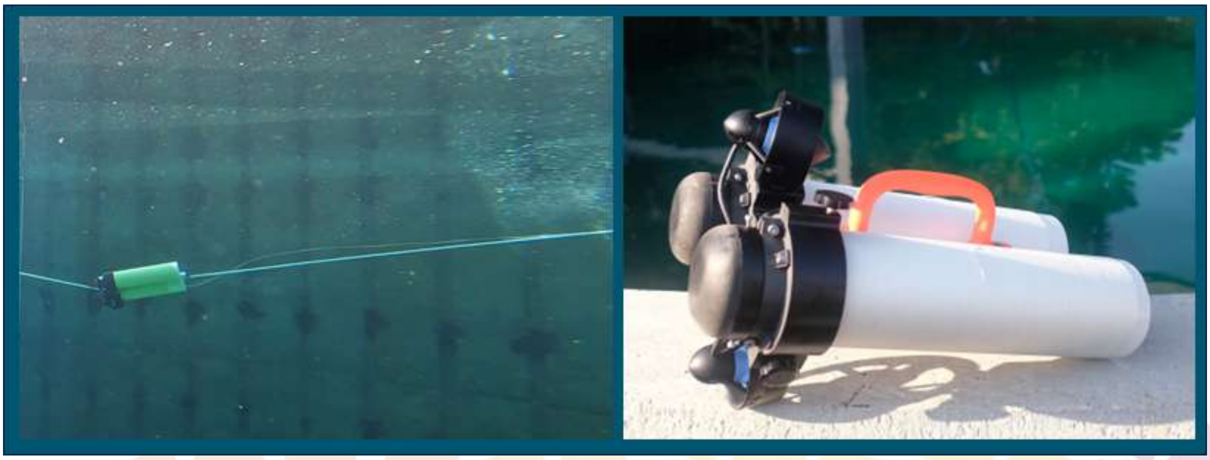
Littlefield, R.

Weiss, B. Bailey, D.

Woods Hole Oceanographic Institution, United States

ABSTRACT

Researchers at the Woods Hole Oceanographic Institution (WHOI) are developing equipment for rapidly seeding kelp farms with juvenile seaweed. The farming techniques enabled by the described equipment will allow farmers to out-plant juvenile seaweed to farm structures without the existing need to manipulate the farm or pull a boat up and down the grow-lines to apply seed string. The associated reduction in labor is expected to result in significant time and costs saving across the seeding process. This method also reduces juvenile seaweed exposure to air, as the seeding process occurs under water, improving successful seeding rates and increasing the weather windows for seeding operations. This reduced stress on the juvenile seaweeds and access to optimum out-planning times is expected to increase total biomass yield of a farm. A farmer using the seeding device clips it onto an installed grow line, attaches the free end of the seed string and activates the thrusters with an oversized power switch. The seeder then uses two thrusters to propel itself down the grow line while rotating to evenly apply seed string at a specified number of wraps per distance traveled. Upon reaching the transverse headrope at the end of the grow-line, the power switch is triggered, and the thrusters automatically stop. The second of two seed spools can be attached to the next grow-line, and the seeder can be sent back, seeding along the way. WHOI is conducting tests with partner seaweed farms in Alaska and Connecticut to evaluate the usability of equipment and quantify out-planting labor and resulting biomass yield as compared to existing farming practices.



Keywords
Mariculture, aquaculture, seaweed, kelp, farm

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Teis Boderskov, Mette Møller Nielsen, Michael Bo Rasmussen, Thorsten Johannes Skovbjerg Balsby, Adrian Macleod, Susan Løvstad Holdt, Jens Jørgen Sloth, Annette Bruhn,
Effects of seeding method, timing and site selection on the production and quality of sugar kelp, Saccharina latissima: A Danish case study,
Algal Research, Volume 53, 2021, 102160, ISSN 2211-9264,
https://doi.org/10.1016/j.algal.2020.102160.

SPEAKER INFORMATION

Robin H. Littlefield
Research Engineer / Applied Ocean Physics and Engineering / Woods Hole Oceanographic Institution
United States

BIOGRAPHY

Robin Littlefield is a research engineer at the Woods Hole Oceanograohic Institution investigating novel approaches to extend the reach of scientific understanding through the development of new systems and methods for use in undersea applications. With a background is in mechanical engineering and autonomous underwater vehicle design, Robin's research is focused on ocean exploration, ocean-based renewable energy, and aquacuture supporting carbon capture and the green economy. Mariculture, aquaculture, seaweed, kelp, farm

COMPANY PROFILE

The Woods Hole Oceanographic Institution is dedicated to advancing knowledge of the ocean and its connection with the Earth system through a sustained commitment to excellence in science, engineering, and education, and to the application of this knowledge to problems facing society. Since 1930, WHOI scientists and engineers have laid the groundwork for many of the discoveries that have revolutionized our knowledge about the ocean and its impacts on our planet and our lives. From the discovery of hydrothermal vents to the development of revolutionary ocean technology to mapping global ocean circulation, our research matters now more than ever before.

Session 2: Process

ALGÆUROPE2022
13 - 15 · DECEMBER · ROME

CONTROL OF BACTERIAL CONTAMINATION IN MICROALGAE CULTURES INTEGRATED WITH CHEESE WHEY WASTEWATER TREATMENT BY APPLYING FEAST AND FAMINE REGIME

Di Caprio F.[1]

Proietti Tocca G.[2,3], Pagnanelli F.[1], Altimari P.[1]

[1]*Università Sapienza di Roma, Italy.*
[2]*Politecnico di Torino, Italy,* [3]*Istituto Italiano di Tecnologia, Italy.*

ABSTRACT

Microalgae productivity in photobioreactors is usually highly limited by the reduced cellular light supply rate resulting from the self-shading effect. Microalgal heterotrophic metabolism can be exploited to overcome this problem by sustaining microalgae growth through pathways independent of the light supply. Such strategy is particularly attractive when wastewaters are used as source of organic substrate, integrating microalgae growth with pollutant removal. However, as wastewater sterilization is unfeasible at large scale, contamination by heterotrophic bacteria is a main bottleneck currently limiting the application of mixotrophic and heterotrophic processes integrated with wastewater treatment.

When wastewaters are supplied without an appropriate control, bacteria contamination can increase exponentially because of their higher specific growth rate in nutrient replete conditions.

In our research group we study the development of process configurations to control bacteria contamination in unsterilized reactors by exploiting tailored feeding strategies [1–3]. Nutrient deplete conditions can be exploited to make microalgae more competitive [4].Our most relevant findings have been obtained in fed batch [1] and sequencing batch reactors [2], by uncoupling organic substrate and nitrogen supply, which uncouples cell duplication and biomass production. However, this uncoupling is difficult to be achieved by using real wastewaters as nutrient source, because wastewaters typically contain both organic substrate and nitrogen.

Here our latest findings obtained by using real wastewater (cheese whey) containing both organic carbon and nitrogen are reported.

To control bacteria contamination, we exploited the implementation of feast (organic substrate + nitrogen available) and famine (no organic substrate available) conditions [5], in heterotrophic and mixotrophic conditions. Grow and decay rates were measured in feast and famine condition, respectively, for bacteria and microalgae, by using flow cytometry. The findings allowed us to provide preliminary general guidelines on how to supply wastewaters to maximize biomass productivity and pollutant removal, by maintaining contamination under a certain threshold level. Our findings also indicate the importance of taking into account for the cell-to-cell heterogeneity to fully understand the mechanisms determining the different dynamics under famine condition, and, in turn, the effectiveness and predictability of the control strategy.

Keywords
Mixotrophy; Heterotrophy, Wastewater treatment; Bacteria contamination; Process control

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SPEAKER INFORMATION

Fabrizio Di Caprio
Assistant Professor / Department of Chemistry / Università Sapienza
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BIOGRAPHY

He obtained a bachelor's degree in agro-industrial biotechnology and a master's degree in industrial chemistry at the Università Sapienza of Rome. At the same University he got his PhD in chemical engineering (2017). He spent 6 months of his PhD at the University of Wageningen (Bioprocess Engineering group). Since 2017 he works as postdoctoral researcher at the Department of Chemistry of the Università Sapienza of Rome, where he is currently an assistant professor.
He works on the development of innovative industrial processes based on microalgae cultivation. The main research field is about development of processes to integrate microalgae cultivation with wastewater treatment, to couple high-value microalgae biomass production with pollutant removal. More in detail, a relevant part of this research is focused on the development of innovative feeding strategies to control bacteria contaminations in mixotrophic and heterotrophic cultures, with an ecological engineering approach to control the competition inside the bioreactor, and in turn the cell-to-cell heterogeneity. Another part of the research activity is towards the development of biorefinery processes to obtain starch and pigments from microalgae and utilization of biomass to synthetize adsorbents for heavy metal removal from contaminated water.

COMPANY PROFILE

Università Sapienza is the largest Italian public university. It is located in the center of Rome, at Piazzale Aldo Moro 5. It is among the oldest universities, since it was funded in 1303. Università Sapienza is divided into 11 Faculties and 58 Departments. The Department of Chemistry is part of Mathematics, Physics and Natural Sciences Faculty and includes more than 90 people (professors and researchers) working in different scientific fields: industrial chemistry, inorganic chemistry, analytical chemistry, organic chemistry, physic chemistry. The research activities carried out in this Department have applications on the development of scientific knowledge and technology in biotechnology, materials science, industrial processes, biology, applied physics, cultural heritage preservation, microscopy, medicine, human health, geology and environmental science, among other fields.

PROCESS INTENSIFICATION FOR LOW-COST MICROALGAE HARVESTING BY USING CONTINUOS FOAM FLOTATION TECHNIQUE

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ABSTRACT

Microalgae are photosynthetic microorganisms, mainly composed of proteins, carbohydrates, and lipids. They can be used as raw materials for bioactive compounds, food, and biofuel production. However, downstream processing of microalgae biomass production, especially the harvesting processes is expensive and has so far limited the commercialization of algae production.

Foam flotation is a form of cell separation by inverted sedimentation. Bubbles pass through the culture media and capture algal cells and transport them to the upper surface, where they are transported and concentrated by a rising foam. The benefits of this form of microalgae harvesting are high recovery efficiency, lower equipment costs, and low energy requirement. The main objective of the research was to maximize algae recovery via design of “foam risers”, which accelerate the foam by constricting the flow. Parameters included the angle, constriction ratio and riser length. *Chlorella vulgaris* was used as biomass for the continuous foam-flotation. It was cultivated under autotrophic conditions, using BG-11 medium for 15 days period. Steady state foam production was found to occur after 15 minutes. The highest concentration factors were achieved at the greatest degrees of constriction and the greatest lengths of riser (constriction ratio of 0.25 and 15 cm length, respectively). The recovery efficiency was increased by 57% at an air flow rate 2 L/min. However, riser angle had little effect. This was because the foam tended to form its own angle, between moving and stationary foam phases at the inlet to the riser.

Keywords
Continuous foam flotation, microalgae, foam riser, concentration factor, recovery efficiency

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BIOGRAPHY

Research Experiences

- Research & development on sugar technology to improve the efficiency of sugar production and value-added by-products and zero waste.
- Research & development on mass cultivation and harvesting of local microalgae strain for bioactive compounds and biopolymer production.

COMPANY PROFILE

The mandates of the Department of Agriculture, Ministry of Agriculture and Cooperatives are including with

- Conduct research and development studies on various agricultural disciplines concerning crops, soil, fertilizer, biodiversity resources, and farm mechanization.
- Transfer of agricultural technology to concerned government officials, farmers, and the private sector.

The goal of microalgae projects

- To improve the processing of microalgal cultivation, bioreactor, harvesting design, and extraction technology
- Contribute the knowledge of microalgae technology to farmers and local cooperatives, especially, the salted area in the North-East of Thailand which is unsuitable for food crop production.

Session 3: Physiology

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CFD-AIDED DETERMINATION OF SHEAR STRESS THRESHOLDS IN SENSITIVE MICROALGAE USING SHAKE FLASKS

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ABSTRACT

The culture of dinoflagellate microalgae has a huge biotechnological interest due to the interesting bioactives produced by certain strains (Gallardo-Rodríguez et al., 2012). Although significant progress has been made with a few species in recent years, the main bottleneck that hinders their commercial development continues to be the difficulty in mass culturing them. In general, this group of microorganisms shows a high hydrodynamic sensibility to shear stress. Turbulent environments are associated with several adverse effects that may finally result in cellular death (Gallardo-Rodríguez et al., 2016). In suspension cultures, turbulence is essential to provide a homogenous ambient where all cells are periodically exposed to light, oxygen is degassed, and CO_2 homogenously distributed. Thus, to successfully scale-up the culture system with dinoflagellate species, it is necessary to know in detail its hydrodynamics and the shear threshold that a selected strain can withstand. Shake flasks can be used to expose cells to quantified hydrodynamic stresses. In the present wok, shake flasks' hydrodynamics were studied using the computational fluid dynamic technique (CFD). The commercial software ANSYS Fluent was used. Three different mesh resolution have been evaluated, as well as the three k-ε turbulence models available. The volume of fluid (VOF) two-phase model was used. The gas-liquid volumetric mass transfer gas-liquid (k_{La}) coefficient was used as validation parameter. The average energy dissipation rate (EDR) was calculated on the liquid phase for different cases, comparing the values with an empirical correlation (Büchs et al., 2000). In addition, 50 particles were tracked using a Lagrangian model to obtain exposition stories. The simulations predicted adequately the average EDR, in good agreement with empirical correlations (Büchs et al., 2000). In addition, particle EDR stories allowed observing that the maximum values (EDR_{max}) that a cell must withstand are orders of magnitude higher than the average values. This must be considered for sensitive species whose response to hydrodynamics can be triggered after a single exposition event (Gallardo-Rodriguez et al., 2016).

Acknowledgements
This research was funded by the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund Program (Project PID2019-109476RB-C22), and the Operative Program FEDER Andalucía 2014–2020 Framework (grant UAL2020-BIO-A2078).

Keywords
Sensitive cell; microalgae; bioprocess; hydrodynamics; mixing

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SPEAKER INFORMATION

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BIOGRAPHY

My research trajectory has been focused on microalgal bioprocessing for high value bioactives production. I've specifically worked on the design, characterization, optimization and scaling of culture systems for photosynthetic microorganisms. My main research interest is the bioreactors' fluid dynamics and its effect on sensitive cells. Additionally, I have some experience in other biotechnological fields such as water treatment, bioremediation and animal cell culture. I have participated in a number of projects of competitive public financing (three as IP, and two as Co-IP) and private financing projects. Since 2018, I am affiliated as assistant professor at the Department of Chemical Engineering of the University of Almería.

DIGITALGAESATION: A KNOWLEDGE-BASED TRAINING NETWORK FOR DIGITALISATION OF PHOTOSYNTHETIC BIOPROCESSES

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ABSTRACT

Microalgae and other photosynthetic microorganisms represent a highly promising source for food, feed, chemicals, and fuels. Europe has been leading world research and industrial deployment of microalgae-based technologies. However, despite the enormous potential and the impressive R&D effort, industrial use of microalgae is still at its first developmental stage. A major step forward can derive by the development and implementation of digital technologies, capable of automatizing and optimising culture conditions at industrial scale. The H2020 ITN Project DigitAlgaesation will aim at:

1. Developing a sound modelling approach at the process scale to describe the dynamics of the photosynthetic phenomena (response to light and temperature fluctuations) and metabolic fluxes that are relevant to an effective automatic control strategy in an industrial environment; and be representative of different microalgae of industrial interest;

2. Developing a reliable smart monitoring approach for measuring and/or estimating the biological key performance indicators required for model identification and to support an effective control

System: new optical online in-situ sensors will be developed as well as AI and machine learning technology for soft sensors development

3. Developing and implement automatic control strategies in real industrial systems characterised by non-ideal behaviour in the presence of uncertainty, to achieve high level of productivity, and reduce manpower and energy costs while maintaining a constant product quality.

The goal is to provide tools for designing the next generation of sustainable microalgae-based production process with enhanced productivity, reduced environmental impact and costs. DigitAlgaesation will train 15 early-stage researchers in all aspects of microalgae technological innovation to pave the way towards a knowledge-based breakthrough in monitoring methods and instrumentation, biological modelling and simulation, and automatic control. In this contribution, we will present and critically discuss the project research goals, and the consortium state of advancement after one year of activities.

Keywords

Microalgae, Digitalisation, Modelling, Automatic control, Monitoring

References

https://digitalgaesation.eu

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BIOGRAPHY

Dr. Fabrizio Bezzo is Professor of Chemical Engineering at the University of Padova. He received his degree in Chemical Engineering at the University of Padova and his PhD at Imperial College London (UK). His research interests comprise process and supply chain design and optimization of production systems for renewable energy, biofuels and biochemicals (including microalgae-based processes), and the modeling and scaling-up of chemical and pharmaceutical processes. He has published 200 scientific papers in international research journals and conference proceedings. He co-chairs the Energy Section of the European Federation of Chemical Engineering. He currently coordinates the H2020-MSCA-ITN-2020 project “DigitAlgaesation”.

COMPANY PROFILE

Dating back to 1222, the University of Padova (UniPD) is one of the oldest and most prestigious seats of learning in Europe. Its 32 departments, 40 doctoral degree courses and 44 interdisciplinary research centers with about 2500 professors and researchers employed, cover an exceptionally broad research scope. UniPD is active in algae research thanks to the activity of an interdisciplinary disciplinary group between the Departments of Industrial Engineering and Biology, involving about 25 people working on various aspects of algae biotechnology and process design.

GROWTH, SIZE, AND BIOCHEMICAL EVALUATION OF *ARTHROSPIRA PLATENSIS* CULTIVATED IN TUBULAR PHOTOBIOREACTORS DRIVEN BY A CENTRIFUGAL PUMP

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ABSTRACT

Arthrospira platensis, commonly known as Spirulina, is a protein-rich cyanobacterium approved for human consumption [1], [2]. Industrial production of Spirulina occurs mainly in open ponds, since they are cost-effective due to low construction costs and low-energy requirements for mixing [3], [4]. Nevertheless, closed systems present advantages such as better control of abiotic factors, decreased contamination risk and minimization of water loss due to evaporation [5], [6]. Tubular photobioreactors (PBRs) are considered to be the most suitable closed reactors for outdoor cultivation, since they present good light distribution, leading to higher productivities [6]–[8]. However, these reactors usually operate with energy demanding pumps, to assure proper mixture and aeration. Besides the increase in energy costs, these pumps can induce high levels of stress on the cultures, which can be problematic for shear-stress sensitive species like Spirulina [9], [10].

In this work, a culture of *A. platensis* obtained from a flat panel (FP) was cultivated for 14 days in 2.6-m^3 tubular PBRs, driven by centrifugal pumps. After 8 days, this culture was used to inoculate a 3.6-m^3 raceway pond (RW). This assay was performed 3 times, during Summer, in Pataias, Portugal. The culture grown in the tubular PBR presented 3-fold higher volumetric and areal productivities, compared to the RW (0.176±0.057 and 0.053±0.007 $g.L^{-1}$day-1 and 17.4±5.6 and 6.9±1.0 $g.m^{-2}day^{-1}$, for the PBR and RW, respectively). The cells cultivated in the tubular PBR presented a significant decrease in filament size, when compared to the size presented in the FP, but they could recover the regular size distribution after only 6 days of cultivation in a RW (Figure 1). Phycocyanin content was also evaluated throughout the cultivation period, being 9.2±1.4 % after 14 days of cultivation in the PBR and 6.8±1.8 % after 6 cultivation days in the RW. In conclusion, even though Spirulina cultivated in tubular PBRs showed signs of shear stress, it still presented higher productivities than in raceways and displayed stable levels of phycocyanin during cultivation.

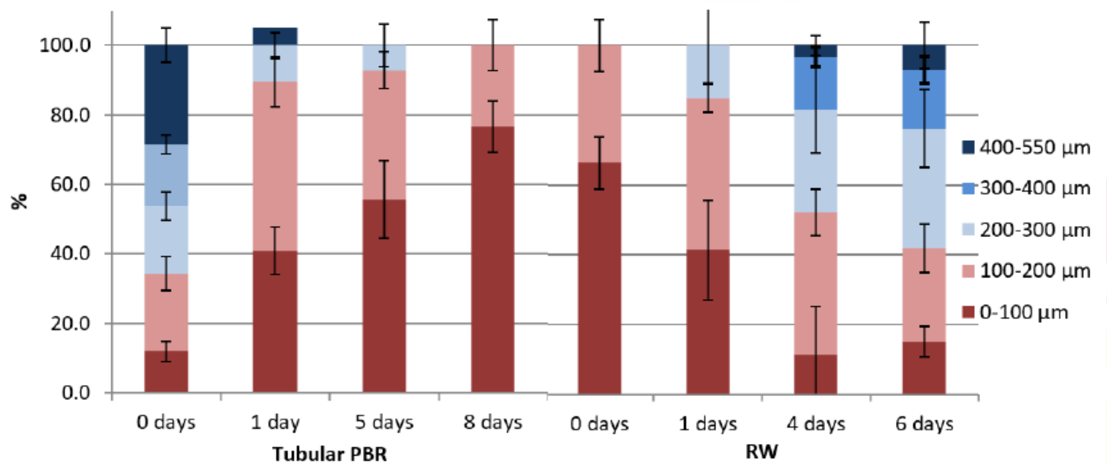

Figure 1 – Filament length distribution of an *Arthrospira platensis* grown in a tubular PBR moved by a centrifugal pump and subsequently grown in a raceway pond.

Keywords
Arthrospira platensis; industrial production; outdoor cultivation; tubular photobioreactors; shear stress

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BIOGRAPHY

Inês Guerra has a master in Biological Engineering from Instituto Superior Técnico. She worked as a Research Fellow for ALGAVALOR project in the R&D department of Allmicroalgae-Natural Products, holding experience in microalgae cultivation, from laboratorial to pilot-scale. Currently, she is doing a PhD in Universidade do Algarve to optimize the growth of organic *Arthrospira platensis* and formulate an *A. platensis* protein-based meat analogue. This first year Inês has been working in Allmicroalgae's facilities on medium optimization in terms of biomass and protein production of *A. platensis* and in the comparison of the growth and biochemical profile of *A. platensis* in different pilot-scale reactors. Inês has 3 publication, 1 book chapter and performed 3 oral presentations in international conferences.

PUTTING ALGAE ON THE MAP: GENOME-SCALE METABOLIC MODELING OF *NANNOCHLOROPSIS* FOR IMPROVED LIPID PRODUCTION

Sabine Van Oossanen

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ABSTRACT

Microalgae show great potential for replacing fossil fuels in the near future. However, strains and technologies must be improved in order to reach an economically feasible bulk production. Our research focuses on developing and testing a genome-scale constraint-based metabolic model (GEM) for the oleaginous microalga *Nannochloropsis oceanica*. Metabolic modeling is key to understanding carbon partitioning mechanisms, and it provides insights for strain engineering to further advance *Nannochloropsis* as a photosynthetic cell factory.
We constructed a first GEM of *N. oceanica* in an orthology-based approach, using curated scaffold GEMs (*N. salina*, *N. gaditana, P. tricornutum, E. silicolosus, C. reinhardtii* and *C. oleaginosus*), available genomics data (Gong et al. 2020) and physiological data such as biochemical compositions, growth rates and gas exchange rates. Through flux sampling of the GEM in high lipid, low growth and high growth phenotypes, we predicted enzymatic targets for overexpression that result in lipid accumulation while ensuring growth.

From the predicted targets, we have selected two enzymes involved in the lipid metabolism. Of these, overexpression mutants have been generated using a novel gene expression system (Südfeld et al. 2022), and phenotypically characterized to validate the GEM predictions. Current targets show increased lipid production rates (g/gDW/d) of 149% and 123% compared to the wildtype, in nutrient replete conditions.

This study allowed the selection of novel targets involved in lipid accumulation and increased the understanding on lipid metabolism, in *N. oceanica*. Future investigations are focusing on GEM validation and curation.

Keywords
Metabolic engineering, Nannochloropsis, modelling, lipids

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BIOGRAPHY

Sabine likes numbers, bioreactors, and cats. In her PhD she combines the first two, merging the fields of systems biology and bioprocess engineering to uncover hidden potential of algae metabolisms. After an early start with microalgae at a high-school graduation project, Sabine decided to study both the BSc and MSc Biotechnology at Wageningen University. Here she learned how to see biology as a highly intricate and integrated system, one that allows for steering and tweaking when using the right tools. Following research projects at Systems and synthetic biology and Environmental Technology including an iGEM project, Sabine decided in 2019 to move on to the microalgae field, where the first steps were being taken in creating photosynthetic cell factories. In her project, taking place in the Laboratory of Systems and Synthetic biology and the Department of Bioprocess Engineering, Sabine aims to build and use genome-based metabolic models to lift metabolic engineering of microalgae to a next level.

COMPANY PROFILE

At Wageningen University, the department of Bioprocess Engineering teaches and develops innovative bio-based processes. We work on a sustainable and healthy future by engineering efficient bioprocesses for high quality products. We study and develop photoautotrophic and heterotrophic production systems for biobased products, as well as high-quality processes for the production of biopharmaceuticals.
Photosynthetic microorganisms use a direct route to convert inorganic carbon into functional molecules while employing sunlight. Our mission is to develop a commercial and sustainable production chain for food, feed, chemistry and energy from microalgae and cyanobacteria. We integrate biological and engineering studies of cellular processes, work on strain improvement, cultivation optimization and scale-up. We bridge fundamental research to applications in outdoor pilot facilities (www.AlgaePARC.com) and use techno-economic models to assess and guide our research program.

MICROALGAE-BIOFILM AS A SOURCE OF BIOACTIVE COMPOUNDS

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ABSTRACT

Microalgae are now clearly recognized as a valuable source of bioactive compounds (pigments, fatty acids, …) for the health market. In addition, in the last decades, an increased resistance to antibiotics has been largely reported, especially for bacteria-forming biofilms with serious infections consequences. Therefore, there is an urgent need to identify and produce alternative agents, especially of natural origin, to face this huge health challenge. In particular, promising anti-biofilm activity of compounds from microalgae cultivated in conventional suspended systems has been reported (Nag et al., 2021). However, microalgae are also known to grow in assembled structures irreversibly associated to surfaces, embedded in an EPS (Extracellular polymeric substances) matrix, forming photosynthetic biofilms. Such a self-organized community can be the source of new and more effective antimicrobial agents for struggling against biofouling and, in particular bacterial biofilms of health concern. However, no information is available regarding the effect of bioactive molecules extracted from microalgae biofilms to combat human pathogens. On the other hand, a strong interest for biofilm-based systems for microalgae cultivation and associated compounds production has raised lately (Benret et al., 2015). In this study, microalgae biofilms (i.e. *Tetraselmis suecica* and *Cylindrotheca closterium*) were produced in an industrial rotating system placed in a greenhouse and the anti-adhesion potential of the associated extracts were tested on eight human pathogenic bacteria including several strains of *Escherichia coli*, *Staphylococcus aureus*, *Listeria* sp. and *Salmonella* sp. EPS from both microalgae biofilms were extracted and first characterized showing fractions rich in sulfated polysaccharides (up to 40% in dry weight). They were mainly composed of elongated linear or branched chains of monosaccharides (galactose, glucose, fucose, rhamnose, xylose, uronic acids, etc.). Some of those, such as glucuronic and galacturonic acids, are commonly associated with biological activities (Minzanova et al., 2018). Our results also show an adhesion inhibition ranging between 35 to 60% for *S. aureus, L. monocytogenes* and *E.coli strains*. On the whole, an inhibiting effect of *T. suecica* extracts on the adhesion of a broad range of bacteria was therefore demonstrated. The anti-biofilm activity seems to be bacteria-dependent, as expected. Finally, our work suggests that promising anti-biofilm drugs can be produced through microalgae biofilm-based systems.

Keywords
Microalgae, biofilm, bacterial adhesion

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BIOGRAPHY

Anne-Sophie is a postdoctoral researcher in microalgal biotechnology at CentraleSupelec, University of Paris Saclay. She obtained her PhD in marine chemical ecology in 2019 at Sorbonne University and a master's degree in Marine sciences from the Université du Littoral Côte d'Opale. Her current work is about microalgae biofilms and the production of antimicrobial compounds form microalgae cultivated in biofilms.

POTENTIAL OF LIGNOCELLULOSIC WASTE AS SUBSTRATE FOR MICROALGAL CULTIVATION

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ABSTRACT

As the human population is rising, the need for novel protein sources and a reduction of greenhouse gas emission is of utmost importance. An approach tackling both concerns at once, is given via microalgal cultivation. Microalgae can be cultivated on non-arable land and require less space than the production of currently established protein sources, such as meat and soy.

Trying to implement circular economy, hydrolysates from abundantly available lignocellulosic waste streams could be used as substrates for heterotrophic and mixotrophic cultivations of *Chlorella vulgaris*. Even though hydrolysis is a powerful method to degrade oligomeric C5 and C6 sugars of these waste-streams, inhibitors will be formed as a by-product. Applying liquid hot water hydrolyzation to lignocellulosic biomasses, furfural and 5-Hydroxymethylfurfural (HMF) are formed in the process. Furfural and HMF are known fermentation inhibitors to a diverse set of microorganisms.

In this presentation, I discuss the impact of furfural and HMF on the mixotrophic and heterotrophic cultivation of *Chlorella vulgaris*. Results demonstrate that substrates containing furfural and HMF are suitable for microalgal cultivation. Using mass spectrometry analysis, we identified two metabolites stemming from *Chlorella vulgaris* cultivated in media containing those inhibitors. Even though, metabolic pathways of these reactions are still unknown as of now, the two metabolites have not yet been described in any microalgal species. The conversions of these inhibitors might open up new future applications in microalgal cultivation.

Keywords
Chlorella vulgaris, Lignocellulosic waste, Substrate, Sustainability, Metabolism

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BIOGRAPHY

After concluding my school education in Linz (Austria), I moved to Vienna to study biotechnology at the University of Life Sciences - BOKU. There I finished my Bachelor's and Master's programme, focusing on the identification and characterization of β-Galactosidases acting on N-glycans in *Nicothiana bentamiana*. After finishing my Master thesis, I worked for another 6 months at BOKU as a scientific assistant, before starting my PhD Thesis at the Technical University of Vienna. I am currently employed at the Integrated Bioprocess Development Group focusing on the implementation of waste streams as substrates for microalgal cultivation. There I have been working mainly with *Chlorella vulgaris*, *Haematococcus pluvialis* for food and feed production and *Synechocystis* sp. for the production of biodegradable bioplastic PHB (polyhydroxybutyrate) and the sustainable extraction thereof.

COMPANY PROFILE

Integrated Bioprocess Development: from genome to product – We cover all process steps from identification of interesting gene/product, strain generation, production in the bioreactor, physiological strain characterization, product purification to product characterization and analyze interdependencies of these steps → create process understanding!

Session 4: Europe and the World

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

THE SEAWEED MANIFESTO AND THE NEW SAFE SEAWEED COALITION, WORKING ON GLOBAL REGULATIONS TO ENABLE COLLABORATION IN THIS EMERGING SECTOR

Vincent Doumeizel

ABSTRACT

Businesses, governments, academia, non-governmental organisations and the UN must act together and deliver solutions addressing key issues such as hunger, poverty and climate change, to name a few. Ocean and seaweed specifically hold a huge potential to deliver on UN SDG's and mitigate food security issues all over the world.

Seaweed is today only cultivated in Asia with 99,5% of the global farming capacities. Seaweed is currently not high in non-Asian consumer preferences. A shift in western diets would be needed for an increase in direct consumption of seaweed as food, and there is an opportunity to increase consumer awareness regarding the benefits. An increase in animal based diets would drive the demand for seaweed for feed.

Lloyd's Register & UN Global Compact led the redaction of a a manifesto (www.seaweedmanifesto.com) gathering input from World leading organizations (FAO, World Bank, Cargill, WWF, Riken Food, Ningbo University, Metro, BNP Paris, SAMS, etc…). Released last June the document outlines the opportunities and barriers ahead of us and the need for harmonizing rules and regulations, sharing science and safety best practices, enabling innovations; good marine spatial planning and new investment efforts can ensure this industry accelerates to the next level.

Amongst the needed actions highlighted by this Manifesto is the need for more collaboration within the industry and more global safety standards/regulations for final products, environment and workers in the Seaweed Industry.

As a direct consequence, Lloyd's Register Foundation agreed to grant a multi-million $ grant in order to build a global coalition for Safe Seaweed Production (Safe Seaweed Coalition) to enable all Seaweed stakeholders to work on common principles for safety in this emerging industry.

Objective of the coalition is to improve global standards and regulations for safe operation and production, improve public education/consumer awareness, generate more investment in scalable production initiatives, particularly in developing economies, greater collaboration between manufacturers, regulators, academia. IGOs, NGOs and industry, leading to an improved, safe and better developed global seaweed market.

Safety as a pure non competitive topic hold the potential to enable collaboration and actions to meet the Manifesto ambition beyond safety.

The Coalition already delivered 24 grants in 20 different countries and now gathers over 800 seaweed stakeholders worldwide. Altogether, they voiced actively the potential for seaweed in high level meeting (COP26 & COP 27, UN Food System Summit, UN Ocean Conference, UN General Assembly,…) and are working with large institutions at national or global level (EU, World Bank, GEF, Blue Food, EDF, WWF, etc…)

Objective of the initiative is to scale up Seaweed production all around the world, and notably in Europe, and enable a real new global supply chain to address some of the world global challenges

CONFERENCE DAY 2

ALGÆUROPE2022
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Session 5: Food

ALGÆUROPE2022

13 - 15·DECEMBER·ROME

FROM THE HOT SPRINGS TO THE TABLE:
SCALING UP MIXOTROPHIC CULTIVATION OF *GALDIERIA SULPHURARIA*

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ABSTRACT

Galdieria sulphuraria is a species of red microalgae that colonizes acidic hot springs and sulfur fumes worldwide. Because of this unique lifestyle, these photosynthetic microorganisms have adapted to high temperatures, low pH and high concentrations of salts and metals [1]. In addition, they possess a versatile metabolism, being able to grow with more than 25 organic substrates [2]. We have exploited these characteristics to develop a new type of mixotrophic cultivation: oxygen-balanced mixotrophy (OBM). By adjusting organic substrate supply to photosynthetic oxygen production, the need for aeration in the culture is removed while boosting productivity. Because of the stringent cultivation conditions, bacterial and fungal contamination are avoided even in the presence of organic substrates. At lab scale, OBM doubled autotrophic productivity and achieved a high yield on substrate (0.9 C-g·C-g^{-1}). The biomass produced in this manner contained a high fraction of proteins (>63% w/w) and was rich in essential amino acids, especially cysteine and methionine. Moreover, we obtained the highest productivity of the blue pigment C-phycocyanin ever reported in illuminated cultures [3].

After these promising results in the lab, the next step was adapting OBM to large scale systems, in particular tubular photobioreactors. This is not a straightforward procedure, as fluid behavior in these reactors resembles plug flow, affecting the dynamics of the process. In order to address this issue, we designed a scale-up approach consisting of 3 blocks: (1) scale-down of glucose and oxygen fluctuations in tubular photobioreactors, (2) model-based validation of substrate feeding control strategy and (3) experimental validation in our pilot facility. The results from (1) showed that oxygen limitation was a critical factor in tubular reactors, but based on (2) it could be avoided with a proper substrate feeding strategy. Finally, using these inputs OBM was implemented in a pilot scale tubular photobioreactor (3), obtaining equivalent results to the lab experiments. Overall, the results open the door for cost-effective production of high-quality protein with *G. sulphuraria* and potentially of other products at large scale via OBM. Additionally, our research is a proof-of-concept of a systematic procedure for the scale-up of mixotrophic cultivation with microalgae.

Keywords
Galdieria sulphuraria, mixotrophy, protein, scale-up, tubular photobioreactors

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SPEAKER INFORMATION

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BIOGRAPHY

Pedro Moñino Fernández was born in Spain in 1995. In 2013, he started his scientific path with a BSc degree in Biotechnology in the Technical University of Madrid. Later he moved to The Netherlands to continue his studies with a MSc in Biotechnology at Wageningen University. During this period, he worked in metabolic engineering and metabolic modelling of bacteria. Since 2020, he is pursuing a PhD in the chair group of Bioprocess Engineering in the same university. In his PhD he is working in the scale-up of mixotrophic cultivation with extremophilic microalgae.

PRODUCTION OF *ARTHROSPIRA PLATENSIS* USING PILOT-SCALE RACEWAY REACTORS AND CHARACTERIZATION OF THE NUTRITIONAL, BIOACTIVE, AND TECHNO-FUNCTIONAL PROPERTIES OF THE BIOMASS

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ABSTRACT

The present work aimed at maximizing the productivity of the strain *Arthrospira platensis* BEA005B in 80 m^2 raceway reactors and to assess the potential of the biomass for being used as a source of bioactive food ingredients. The main operational parameters were the dilution rate and the depth of the culture. The optimum combination, optimized using a surface response methodology, was a depth of 0.10 m and a dilution rate of 0.33 day^{-1}. These conditions led to a biomass productivity of 30.2 $g\cdot m^{-2}\cdot day^{-1}$ when operating the reactors in semi-continuous mode.

Despite being produced in an open system, *A. platensis* managed to be the most abundant strain in the culture after 30 days of semi-continuous production. Illumina sequencing analyses revealed that the relative abundance of *Arthrospira* strains was higher than 85%. Other microalgae including *Tetradesmus obliquus*, which was being produced in a photobioreactor nearby, were also present but with a relative abundance lower than 5%. The analyses did not detect any pathogenic microorganism. The composition of the produced biomass was 62.2% proteins, 22.5% carbohydrates, 11.6% ashes, and 8.1% lipids. The content of phycocyanins and allophycocyanins was estimated as 115.4 and 36.9 $mg\cdot g^{-1}$, respectively.

An ultrasound-assisted methodology was used to recover the proteins from the biomass. Ultrasounds allowed recovering approximately 90% of the initial protein content of the biomass. The recovered proteins contained all the essential amino acids (except for tryptophan, which was not determined); highlighting the content of valine (6.8%), histidine (8.3%), and lysine (7.5%). An *in silico* analysis was carried out to assess the potential utilization of the proteins as a source of bioactive peptides. Four enzymes were then utilized *in vitro* and different enzymatic hydrolysates were generated including one that is able to inhibit the enzyme renin with an IC_{50} value of 0.85 ± 0.09 $mg\cdot mL^{-1}$ and another one that inhibited the enzyme ACE-I with an IC50 value of 0.49 ± 0.05 $mg\cdot mL^{-1}$. Both enzymes are involved in hypertension. The proteins were also characterized in terms of their techno-functional properties (e.g. foaming capacity) demonstrating a huge potential for being used in the manufacture of safe, healthy, and innovative foods.

Keywords
Funtional foods, bioactive peptides, Spirulina, techno-functional properties, novel food ingredients.

SPEAKER INFORMATION

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BIOGRAPHY

BSc in Food Science and Technology (University of Lleida, Spain) and MSc in Industrial and Agri-Food Biotechnology (University of Almeria, Spain). Silvia is currently a PhD candidate at the Department of Chemical Engineering of the University of Almeria. Ms Villaró is also a member of the Desalination and Photosynthesis Functional Unit of CIESOL Solar Energy Research Centre (Almeria, Spain). Her research is related with the optimization of the production of microalgae for food applications and in the development of novel functional foods with improved quality.

COMPANY PROFILE

www.ual.es

USING 3D PRINTING TECHNOLOGY TO DEVELOP MICROALGAE-BASED SNACKS: NUTRITION AND SENSORY IMPACT

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ABSTRACT

Health impact of food has seen growing attention from consumers who have expressed an increased demand for foods with positive health effects. Among these food products, algae have been shown to be a potential ingredient for functional foods, rich in valuable nutrients and bioactive molecules, such as proteins, minerals, dietary fiber and antioxidants[1]. Additionally, developing microalgae-based food solutions for the global market could have significant impacts on securing future food demands. However, efforts are often constrained by the palate of consumers[2]. Strategies to overcome unfamiliarity and aversion to microalgae-based foods have been explored. 3D Food Printing (3DFP) is a potential emerging technology for meeting current growing market demands for tailored nutrition, while, simultaneously, creating a positive sensory impact on consumers by providing attractive multi-flavoured, and layered textured structures, altering consumers' perception of algae food[3].

In the present work, cereal-based snacks were enriched with *Chlorella vulgaris* (Cv) biomass. Levels of Cv incorporation were set according to raw dough's printability (2-18%). Snacks' formulation was previously optimized to acquire the optimal extrudability properties before printing (Foodini, Natural machines, Spain). The health impact of baked snacks (170°C, 15 min) enriched with the Cv was analyzed. Parameters such as nutritional composition, including mineral and aminoacids profiles*, in vitro* antioxidant activity (FRAP, ABTS, DPPH assays), total phenolics (Folin-Ciocalteu) and *in vitro* digestibility and bioaccessibility (Infogest model), were analyzed. Additionally, sensory analysis was also conducted to investigate the sensory acceptance of snacks.

Increasing incorporations of Cv in snacks positively impacted protein and lipid contents, as well as promoting an increase of the bioactive compounds such as total phenolic compounds and minerals. Snacks enriched with the highest levels of Cv incorporation presented higher antioxidant activities and increased mineral bioaccessibility. However, higher microalga inclusions also led to lower insoluble protein recovery and thus, lower protein digestibility. Additionally, sensory results revealed the inclusion of this microalga in snacks contributed to improve snacks' sensory acceptance.

Keywords

3D food printing, microalgae, antioxidants, bioaccessibility, sensory analysis

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SPEAKER INFORMATION

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BIOGRAPHY

PhD student in Food Engineering at the LEAF Food and Feed Group LEAF research unit (https://www.isa.ulisboa.pt/en/leaf/research-groups/group-3-food-feed). Research work has been carried out developing healthy snacks through 3D printing technology with the incorporation of microalgae biomass. Major focus in boosting consumers' acceptance of algae-based foods through the development of 3D intricate, visually stimulating structures. Participated in two food product development research projects in collaboration with industry. Participation in national and international congresses: six oral and six poster presentations. Further participations in organizing molecular gastronomy workshops and other gastronomic events based on sensory perception of English literature (https://orcid.org/0000-0002-6498-7858).

ANTIOXIDATIVE CAPACITY OF MICROALGAL CAROTENOIDS FOR STABILIZING N-3 LC-PUFA RICH OIL: INITIAL QUANTITY IS KEY

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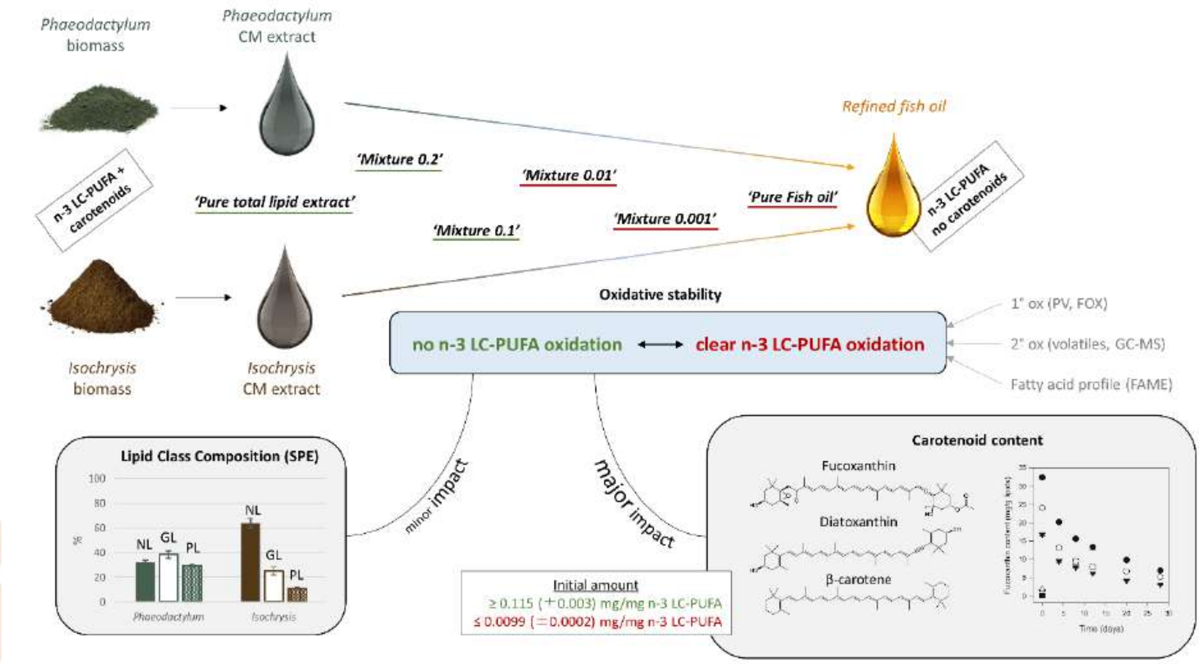
ABSTRACT

The health-beneficial long chain omega-3 polyunsaturated fatty acids (n-3 LC-PUFA) are easily affected by the undesired process of lipid oxidation in fish oil (the most important bulk oil source), while being stable in the lipid extracts of photoautotrophic microalgae. The current research investigates the role of carotenoids in this by evaluating the oxidative stability of mixtures of fish oil with total lipid extracts of two different microalgae (*Phaeodactylum sp.* and *Tisochrysis lutea*) throughout an accelerated storage experiment of 4 weeks at 37 °C. Analysis of both primary (PV) and secondary (GC-MS) lipid oxidation compounds as well as fatty acid profile and carotenoids throughout storage, gave an indication on the carotenoids' capacity to prevent bulk oil n-3 LC-PUFA oxidation. A clear separation between oxidatively stable and oxidatively unstable mixtures was observed for which the amount of carotenoids relative to the amount of n-3 LC-PUFA was a good indicator. The lipid class composition, clearly differing between the two microalgae, was of no influence. The antioxidative role of fucoxanthin, and diatoxanthin and β-carotene as minor carotenoids, was illustrated by their gradual degradation throughout storage. However, when their initial contents were too low, this role could not be exerted leading to thorough lipid oxidation. The results therefore illustrated the antioxidative potential of microalgal carotenoids, offering possibilities regarding the use of natural antioxidants for obtaining oxidatively stable food products, while pointing out the importance of quantity in doing so.



Keywords
n-3 LC-PUFA, carotenoids, *Phaeodactylum*, *Isochrysis*, lipid oxidation

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BIOGRAPHY

Robbe Demets graduated as Master of Bioscience Engineering at the university of KU Leuven (Belgium) in 2018 and is currently doing research as a PhD student in the laboratory of Food & Lipids under the supervision of Prof. Imogen Foubert. He investigates the role of microalgal carotenoids in preventing lipid oxidation in view of obtaining oxidatively stable food products enriched with n-3 LC-PUFA rich microalgae. The research is being funded by Fonds Wetenschappelijk Onderzoek Vlaanderen (FWO).

COMPANY PROFILE

The laboratory Food & Lipids concentrates her research on the lipid (fat) fraction of food stuffs. On the one hand, lipids are often under pressure from a health conscious point of view but on the other hand they are essential for the sensory (e.g. taste), nutritional (e.g. essential fatty acids, fat soluble vitamins) and technological (e.g. spreadability) properties of fat rich food products. The mission of the laboratory is thus to conduct research facilitating the production of healthy/ier fat rich food products without compromising the taste or the technological functionality.

ALGAE BASED BLOOD SUBSTITUTE ENRICHED WITH VITAMIN B-12. A COMPLETE SOLUTION FOR THE PLANT-BASED ALTERNATIVES

Amikam Bar Gil Ph.D

Yemoja ltd.

ABSTRACT

The plant-based meat alternatives are in constant increase for the last decade now. Numerous companies' worldwide is trying to deliver the ultimate "Bite" that doesn't contain ingredients from any animal source. The most abundant source of proteins in these meat alternatives is soy and pea. These almost tasteless ingredients need to be added with flavors, fat odor and other supplements that will deliver the complete sensation of a "real" meat product. The first sense that we judge our food with, is sight. "We eat with our eyes first". Therefore, one of the most crucial ingredients that is added to the product is color. When we think of meaty color we think: Red, Pink, brown and grey. Each color belongs to a different stage or product of bovine meat. Red represents whole cuts. Pink represents grinded meat and brown/ represents both products after exposure to temperatures above 53^0C for more than 10 seconds. Most colorants that are being used nowadays are mainly focusing on either delivering the end color (brown/greyish) or the raw and uncooked red. These colorants are mainly beetroot, lycopene, astaxanthin, berries extract, black carrot, scorched sugar, red cabbage extract and few others. All of these colorants fall under the category of stable colorants. Meaning, they do not change their properties when subjected to changing environment. Yemoja has launched a colorant that can deliver the ultimate appearance both in raw and cooked products. The porphyridium based "Blood Alternative", AKA, Ounje, will change its color just like blood. Furthermore, it contains a huge amount of vitamin B12 thanks to the ability to cultivate both, the algae and the synergistic bacteria that is responsible for Vitamin B-12 synthesis. Using a unique cultivation protocol Yemoja is able to deliver a colorant that is closest to the real thing in terms of taste appearance odor and enriched with high nutritional values. The concentration of vitamin B-12 in Ounje is more than 10 times the one found in bovine (more than 14 ug/100gr).

Keywords
Plant-based, microalgae, Blood Substitute, colorant, Ounje, Yemoja

SPEAKER INFORMATION

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BIOGRAPHY

With almost 15 years of experience in the micro-algae world, I am now the co-founder and CTO of Yemoja ltd. My work experience includes: Lead researcher in TransAlgae, Israel, VP. R&D at Asta Technologies, Israel, researcher in Univerve Biofuels, Israel. All focuses on the microalgae field. My Ph.D focused on the influence of environmental conditions on the physiology and biochemistry of micro and macro algae. I had the honor of receiving Marie Curie scholarship as an Early-Stage Researcher (ESR) under the program of: “From light to harvest”.

TETRASOD®, A UNIQUES MARINE MICROALGAE INGREDIENT: AN OVERVIEW OF MAIN OUTPUTS IN PRE-CLINICAL AND CLINICAL TRIALS

Infante C

Mantecón M, Unamunzaga C

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ABSTRACT

TetraSOD® is a novel natural marine ingredient produced by the company Fitoplancton Marino, S.L. (El Puerto de Santa María, Cádiz, Spain). It is derived from the green microalgae *Tetraselmis chuii*, which is grown in outdoor closed photobioreactors under patent-protected technology ensuring high SOD activity. Outputs obtained in a range of different trials conducted both in human and in animal (rat) models have begun to reveal some of its bioactivities after dietary supplementation. In this regard, TetraSOD® elicited significantly greater and favourable changes in maximal endurance capacity and ergoespirometric values, body composition, blood markers of oxidative stress, and blood hemoglobin in young football players. As an extended follow-up of this first study, in a new trial conducted with good fitness university students, TetraSOD® supplementation induced favourable changes not only on anthropometric and hematological but also on hormonal levels. By the other side, additional clinical trials in trained subjects showed the capacity of TetraSOD®: i) to improve recovery, to sustain power, and to prevent declines in strength across repeated endurance and cross-training bouts, and to reduce muscle damage, ii) to improve perceptual and functional indices of exercise recovery in individuals following a non-functional overreaching resistance-training programme, and iii) to prevent decrements in indices of functional exercise recovery and immune function in subjects following a high-intensity resistance-training programme. According to results in a mechanistic rat model, these outcomes in humans could be prompted by modulating the oxidative stress and pro-inflammatory cytokine response, as well as through up-regulation of positive and down-regulation of negative myogenic factors. In an animal model of induced metabolic syndrome, dietary TetraSOD® supplementation promoted endogenous antioxidant defence mechanisms in liver, modulated oxidative stress and inflammatory markers in plasma, and regulated genes involved in antioxidant, anti-inflammatory and immunomodulatory pathways in liver, adipose tissue, thymus, and spleen. Although the precise mechanism of action of TetraSOD® is currently not well understood, *in vitro* testing in human muscle cells have revealed a unique signalling effect of TetraSOD®, up-regulating gene expression of the key transcription factor NRF2. On-going clinical trials will probably help to shed light on the molecular pathways that mediate TetraSOD® induced response.

Keywords
TetraSOD®, microalgae, recovery, antioxidant, anti-inflammatory

SPEAKER INFORMATION

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BIOGRAPHY

Ph D in Biology, with more than 20 years of experience as a researcher, and close to 70 papers published in scientific journals included in the SCI. Started at Fitoplancton Marino, S.L. in 2012 to be in charge of all experimental activities developed in the framework of European Union and national projects in which the company takes part. Also acting as a coordinator with different institutions and research centers which collaborates with the company in clinical trials to test the effects of the microalgae derived products, mainly TetraSOD®.

COMPANY PROFILE

Fitoplancton Marino, S.L. is a company with professionals who are highly qualified in microalgae production systems, conservation systems and nutrition.The company was established in 2002 focused on satisfying the market needs of various sectors including aquaculture, aquarium keeping, cosmetics, health and nutrition, guaranteeing the best standards in quality and experience. The company develops and supplies microalgae based products and functional ingredients that contribute to improve the good health and quality of life to our consumers. The objective for the next decade is to consolidate Fitoplancton Marino as a leader in the production and commercialisation of products with high added value derived from microalgae.

LONG-TERM PILOT-SCALE CULTIVATION OF *ARTHROSPIRA PLATENSIS* IN SEAWATER: STRATEGIES FOR THE PRODUCTION OF ALTERNATIVE PROTEIN SOURCES AND HIGH-VALUE COMPOUNDS WHILE MAXIMIZING HYDRIC EFFICIENCY

Alemán, M.*[1,2]

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ABSTRACT

The edible cyanobacterium *Arthrospira platensis* (*Spirulina*) produces a valuable biomass which has multiple market applications with high demand, such as the incorporation in conventional food and feed, as well as natural colorants (i.e., blue pigment phycocyanin). Gran Canaria (Spain) is a strategic site for microalgae production due to ideal climatological conditions and large seawater availability, factors that reduce production costs. For this reason, the objective of this study was to culture the native canarian strain *A. platensis* BEA 1257B in different seawater concentrations while maintaining high-valuable compound contents in the biomass. In addition, production was previously optimized by reusing culture medium, reduction in nitrogen addition and improvement in biomass harvesting process. A long-term culture in full seawater medium was grown semi-continuously for a year in 8000-L raceway (RW) under a greenhouse, while recycling the culture medium. The culture showed an average productivity of 0.041 g L^{-1} day^{-1} of dry biomass (15 tons ha^{-1} $year^{-1}$) for cycles in the concentration range of 0.6-0.9 g L^{-1}, which is only 30% lower than that previously obtained in a freshwater medium (control; 21.9 tons ha^{-1} $year^{-1}$). Biochemical compositions of the biomass cultivated at higher salinities (10%, 50% and 100% seawater) were similar to those obtained in the freshwater medium; C-phycocyanin contents at 100% seawater were within the high range of commercial Spirulina (8-9% DW). The fatty acid profile remained generally constant throughout the trial, and the observed differences between treatments were not related to the seawater percentage. The dried biomass complies with the international standards for human consumption, due to low heavy metal content and general absence of pathogens. Altogether, this data makes *A. platensis* BEA 1257B cultivated in seawater promising for food and extraction of natural pigments and antioxidants. As far as we know, this is the first study reporting on *A. platensis* culture in full seawater at semi-industrial scale while recycling the culture medium. Our results suggest the possibility for year-round stable production of *A. platensis* in open ponds with full seawater by recycling the culture medium. Our findings pave the way for a sustainable microalgae-based blue bioeconomy in the Canary Islands.

Keywords
Arthrospira (Spirulina), cultivation process optimization, human consumption, phycocyanin, seawater

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BIOGRAPHY

Monserrat Alemán-Vega, M.Sc. in Biotechnology at Universidad de Vigo, specialized in environmental biotechnology (Thesis research theme: Evaluation of antifouling activity of natural and synthetic compounds on lignocellulosic surfaces), B.Sc. in Marine Science at Universidad de Las Palmas de Gran Canaria (Thesis research theme: Evaluation of antifouling activity of marine bacteria). Currently, Research Scientist at the Biotechnology Department of Instituto Tecnológico de Canarias (https://www.itccanarias.org/web/es/areas/biotecnologia) and PhD student in Oceanography and Global Change (DOYCAG) in the University of Las Palmas de Gran Canaria, in the research area of Biological Oceanography, Biotechnology and Environment (Thesis research theme: Production optimization of canarian native microalgae strains and preliminary development of post-processing stages for the extraction of metabolites of commercial interest). Main field of research: Optimization of the cultivation processes of microalgal native strains for food and feed purposes, through valorization of the locally available energetic and hydric resources.

COMPANY PROFILE

Instituto Tecnológico de Canarias is a public company of the Canary Islands Government (Spain). Its main objective is to foster the industrial development of the region through R&D activities and technical services to SMEs in the field of Renewable Energies, Water Treatment & Management, Environmental Sciences, Medical Engineering and Biotechnology.

A 2-STEP SERIAL MEMBRANE FILTRATION FOR THE EXTRACTION AND PURIFICATION OF PHYCOCYANIN FROM *ARTHROSPIRA PLATENSIS*

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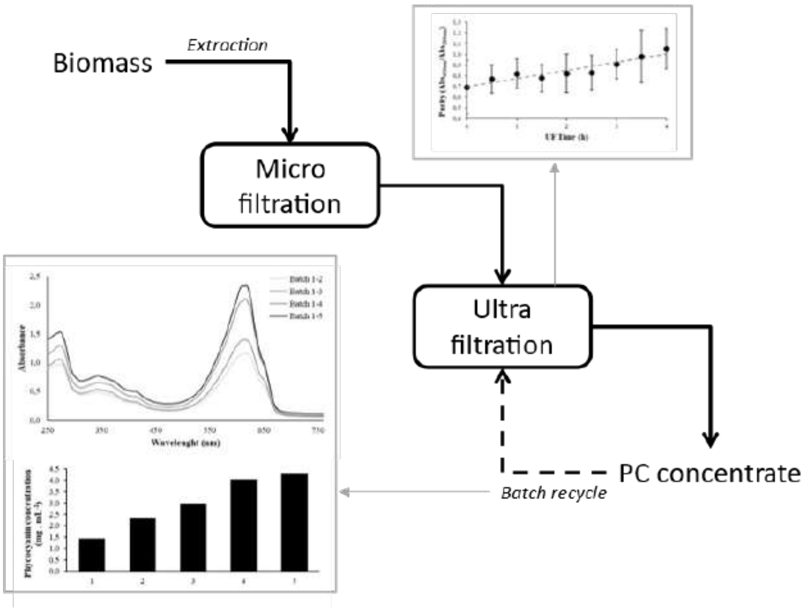
ABSTRACT

Phycocyanin (PC) is a natural, non-toxic dye, with an intense blue color and high fluorescence, applied in cosmetic industries; preventive medicine, as a fluorescent reagent; and over the last years it has been widely disseminated in the food sector [1]. PC is the main accessory photosynthetic pigment of *Arthrospira* spp. (commonly known as Spirulina), composing up to 20% of the microalgae dry biomass [2]. Commercial production of Spirulina is, therefore, frequently used as a source of phycocyanin, to supply the increasing demand for natural food colorings. Although it is a relatively novel industry, various methods of extracting and purifying phycocyanin from different microalgae have been studied [3,4]. Among those, membrane filtration stands out as the technique present in more than half of the patents issued from 2015 to 2021 and a substantial number of research articles published in the same period [5]. Considering the state of the art and future trends observed in literature, this study assesses a 2-step serial tangential membrane filtration prototype with 3 kg-DW/day treatment capacity (see Graphical abstract). Tests were carried out using a commercial Spirulina biomass (*A. platensis* – Algaria Srl), having a total phycocyanin and allophycocyanin (another Spirulina bluish accessory pigment) concentration of about 135 g / kg DW. The system reached extraction yields of around 100 g PC / kg DW (about 75% extraction yield) and purity (absorbance ratios A_{620}/A_{280}) typically in the range of 1.5 - 2.5, comparable to laboratory assays reported on literature (>1.5) and well above that required for use in the food sector (>0.7). However, the phycocyanin concentration in the liquid extract was considerably low (1 - 2 g PC /L), compared to the requirements of downstream concentration/drying processes. Assembling extracts of subsequent batch cycles through the ultrafiltration step resulted in a promising way to increase phycocyanin concentration up to 5-10 gPC /L in the final concentrate. Such method is only limited by risks of partial oxidation of the PC solution, due to longer processing times. The tested prototype, when improved by the correct dimensioning and process automation, can be scalable up to industrial purposes for the concentration and purification of phycocyanin.



Graphic abstract: General scheme of the 2-step membrane filtration procedure for extraction and purification of phycocyanin (PC); and partial results of purification during ultra-filtration and phycocyanin concentration during batch recycling.

Keywords
Microalgae; Spirulina; Phycocyanin; Membrane filtration; Tangential filtration

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NUTRITIONAL PROPERTIES OF FRANKFURTERS WITH ADDED CHLOROPHYLL-DEFICIENT *CHLORELLA VULGARIS* MUTANTS

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ABSTRACT

Microalgae have been proven to be a promising functional and sustainable ingredient in different food matrices, including some meat products [1,2,3,4,5,6]. However, the coloring of meat products containing microalgae *Spirulina maxima* and *Chlorella vulgaris* in a dark green could lead to consumers` rejection of these products. To overcome the color barrier, we used white and yellow chlorophyll-deficient *C. vulgaris* mutants, commercially available (Honey Chlorella), in designing acceptable products. The study aimed to analyze the nutritional composition (chemical, fatty, amino acid, and mineral composition) of pork frankfurters enriched with 3% of yellow and white *C. vulgaris*. The inclusion of these microalgae resulted in a significant ($P < 0.05$) increase in protein (0.8- 0.85%) and ash, while moisture and fat content decreased. Following current Regulation (EC) No 1924/2006 and the amended Commission Regulation (EU) No 1047/2012, the ˝high protein˝ content claims can be assumed for microalgae enriched frankfurters. Carbohydrates increased with microalgae addition due to the increase in fibers. The sum of total essential amino acids and essential/nonessential amino acid ratio was higher ($P < 0.05$) in groups containing microalgae. In addition, white *C. vulgaris* samples had better Lys:Arg ratio compared to the control and honey *C. vulgaris* groups. Frankfurters with microalgae had significantly more ($P < 0.05$) n-3 PUFAs, mainly alfa-linolenic, gamma-linolenic, and eicosatrienoic acid, along with significantly lower amounts ($P < 0.05$) of SFAs, mainly palmitic and stearic acid. *C. vulgaris* mutants resulted in increased total PUFA, lower PUFA/SFA ratio, n−6/n−3 ratios, lower atherogenic index (AI), and thrombogenicity index (IT) compared to reference frankfurters. *C. vulgaris* inclusion in formulations increased ($P < 0.05$) Na, K, Ca, P and Zn, but lowered Mn compared to control frankfurters. In addition, frankfurters fortified with white *C. vulgaris* resulted in higher iron content and lower copper content compared to the control. Moreover, microalgae enrichment lowered the Na/K ratio by 34.74% and 16.42%, respectively, resulting in a significantly better ratio closer to the recommended one. Using microalgae to create innovative meat products can bring potential health benefits to consumers due to the enhanced nutritional profile of these products.

Keywords
Frankfurters, microalgae, PUFA, minerals, amino acids

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Over the following ten years she published more than 100 journals and conference papers and three book chapters about animal science, meat quality, safety, innovation and functional food.

Session 6: Biorefinery

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SEPARATION OF EICOSAPENTAENOIC ACID FROM THE DIATOM *NANOFRUSTULUM SHILOI* VIA TWO-STEP HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY

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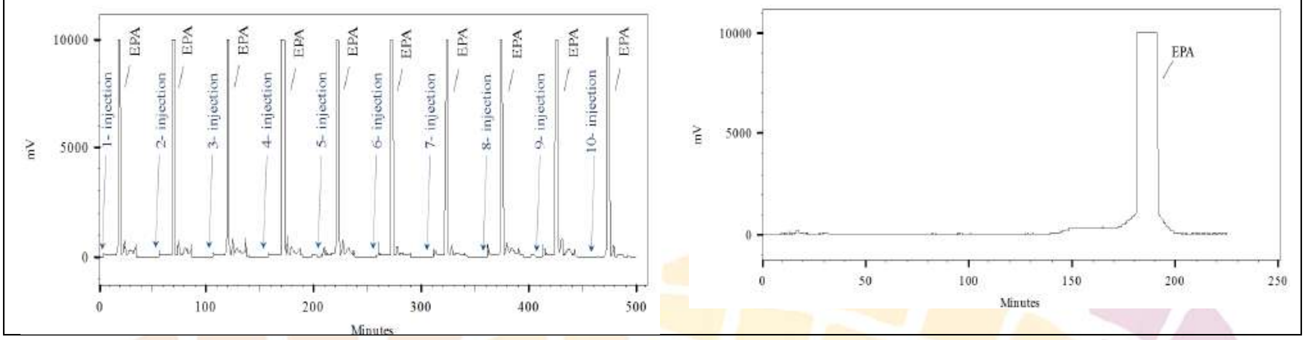
ABSTRACT

Eicosapentaenoic acid (EPA) is an essential polyunsaturated fatty acid of nutritional and physiological relevance to humans [1]. It is commercially produced mainly from fish oil, but new sustainable sources are needed. In this study, pure EPA ethyl ester was produced from the biomass of the diatom *Nanofrustulum shiloi* by integrating two separation steps using high performance countercurrent chromatography (HPCCC). The first HPCCC step consisted of 10 separation cycles (1200 mg sample per cycle), with each cycle consisting of three stages: EPA fraction separation in reverse phase elution mode (lower phase used as mobile phase); with extrusion of stationary phase (by switching mobile phase pumping to stationary phase, while maintaining column rotation); and reaching hydrodynamic equilibrium in the column. The procedure yielded 982.1 mg of a fraction rich in EPA with a purity of 71% (Fig. 1A). To improve the purity of the EPA fraction, a second HPCCC step was performed resulting in 698.97 mg of EPA ethyl ester with a purity of 99%, and a recovery of 98% (Fig. 1B). To avoid unnecessary waste of solvents, the liquid phases were formulated based on a priori NMR measurements. The HPCCC process throughput was 0.936 g/h, while the efficiency per gram of EPA was 0.054 g/h. Environmental risk and process evaluation factors were used to evaluate the separation process. Overall, this isolation approach may provide a useful model for the efficient production of pure EPA from microalgae.

Figure 1.HPCCC separation of EPA ethyl ester from the diatom *Nanofrustulum shiloi*.

A) First HPCCC step B) Second HPCCC step



Keywords
Eicosapentaenoic acid; *Nanofrustulum shiloi*; diatoms; high performance countercurrent chromatography; HPCCC.

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PhD in Pharmacognosy with experience in research & development of natural cosmetic ingredients and pharmaceutical manufacturing. As a scientist of Centre Algatech - CAS, he is currently engaged in research and development of algal biorefinery processes using high performance countercurrent chromatography (HPCCC). He has developed and applied new concepts that support automation and profitability of developed separation processes for the efficient isolation of valuable compounds from algae. He has been involved in national academy-industry projects (TAČR, MPO-TRIO and NCK) and currently in a European project (Horizon 2020) in the field of microalgal biorefinery using HPCCC technology to recover valuable algal compounds. He has made contributions to the development of multiple sequential injections-HPCCC methods for the isolation of algae-derived compounds such as astaxanthin, lutein, fucoxanthin, polyphenols, peptides, mycosporine-like amino acids (MAAs), and polyunsaturated fatty acids. Currently, his efforts are focused on creating new value chains through the development of comprehensive multi-target biorefinery processes using a combined approach of novel biotargets and in-demand active ingredients. Complementary to this, the development of specific biphasic solvent systems is one of his goals to meet food, cosmetic and pharmaceutical regulations.

FROM WASTEWATER TO BIOJETFUEL: A CIRCULAR ECONOMY SYSTEM USING MICROALGAE FOR WASTEWATER BIOREMEDIATION AND RAW MATERIAL FOR BIOJETFUEL THROUGH HYDROTHERMAL LUQUEFACTION

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ABSTRACT

The decarbonization of the air transport is key to mitigate climate change. In contrasts with the road transport sector electrical mobility is not a suitable viable solution at the moment for air transport. Therefore, biofuels appear as the only technical viable option to achieve zero emissions from aviation fuel combustion. The main goal of this project is to produce biojetfuel from microalgae biomass in a circular economy concept, as microalgae are grown in wastewater form the fertilizers industry. In this way microalgae are used as biocatalysts in wastewater treatment and as biomass in the production of biojetfuel. This strategy will allow to decrease the costs associated with the production of microalgae cultivation medium and at the same time mitigate the environmental impacts (such as eutrophication) associated with wastewater disposal and treatment.
In order to achieve the mentioned objectives an operational strategy was developed in two stages: 1) a pilot stage where a 5 m^2 conventional raceway was used to optimise all the operational parameters and establish a microalgae consortium able to remove nitrogen from the effluent, a wastewater culture medium formulation was developed and the seasonality of the system was study; 2) all the optimised conditions were used in a demonstration stage where a 2200 m^2 cascade raceway was used for the scale up of the system. The system achieved a maximum annual N removal of 0.4 kg N/m^2/year and a biomass production of 3.2 kg/m^2/year.

The microalgae biomass produced was then used in hydrothermal liquefaction (HTL). HTL is a thermochemical process used to convert biomass with high moisture content into biocrude and value-added chemicals. The results showed that the obtained biocrude had as main components long chain hydrocarbons and aromatic ring type structures like phenols or nitrogen heterocyclics such as indole or pyrrole. The increases in the reaction temperature decreased the water-soluble products, increasing biocrude production with a maximum at 325 ºC.

The results achieved demonstrate the feasibility of the system, which allows for N removal, biomass and biocrude production, next steps will be focused on biocrude catalytic upgrading towards final biojetfuel production.

Keywords
Biojefuel; bioremediation of wastewaters; cascade raceway; hydrothermal liquefaction (HTL), circular economy

Acknowledgements
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COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions, circular economy opportunities and Customer goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

ECO-DESIGN OF A DOWNSTREAM PROCESS FOR ENRICHED FUCOXANTHIN PRODUCTION FROM *TISOCHRYSIS LUTEA*

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ABSTRACT

Fucoxanthin is the most abundant carotenoid in oceans. It is produced by brown algae, the best producers being microalgae. Among them, the haptophyte *Tisochrysis lutea* is well studied for its high fucoxanthin content (17.8 $mg.g^{-1}$ dry weight). This species is commonly used as food in aquaculture for its lipid content and anti-oxidant properties mainly due to fucoxanthin. In mammal cells, this pigment has even more properties such as anti-cancer, anti-diabetes, anti-inflammatory, etc. Therefore, fucoxanthin potential as a nutraceutical and as a bioactive molecule in pharmacology is important; DownStream Processes development is then a key point of its valorization.
In this project we proposed in to integrate green chemistry and eco-design principles to propose a more sustainable way of extraction and purification for biomolecules recovery. This work presents the development of pigment extraction method from *Tisochrysis lutea* with green solvent, followed by the fractionation and the purification of fucoxanthin from the extract using centrifugal partition chromatography, and by treatment of the residue by enzymatic hydrolysis (alcalase) and multi-stages membrane fractionation (ultra then nanofiltration) in order to recover some peptides. We obtained both high recovery and purity of fucoxanthin up to 18.0 $mg.g^{-1}$ dry weight and >99% HPLC purity. CPC development was achieved using alternative solvents, green or with low energetical demand, high recyclability. Overall impact of the process and scale-up constrains and performances will be discussed. The tested process scheme leaded to multiple organic and aqueous fractions for a high yield of biomass valorization.

CPC fractions of alcoholic extract on the left, alcalase hydrolysis of the residue in the middle and 10kDa filtration on the right

Keywords
Fucoxanthin, extraction, purification, eco-design, biorefinery

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Pr Luc Marchal, head of Process and Bioprocess department of the Graduate School of Engineering of Nantes University, is a specialist of mass transfer phenomenon at solid-liquid and liquid-liquid interfaces. His work is applied to extraction/purification process development (see https://capacites.fr/en/expertises/centrifugal-partition-chromatography/) and to microalgae biomass refinery.

COMPANY PROFILE

GEPEA laboratory, especially “Bioprocess Applied to Microalgae” team, is a research group dedicated to process studies and development (from photobioreactor to purification steps) (https://www.gepea.fr/)

ENHANCED CULTIVATION STRATEGY FOR INDUSTRIAL SCALE ALGAE-BASED BIOREFINERIES

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ABSTRACT

Microalgae-based biorefineries are not yet economically viable compared to their fossil fuel counterparts[1]–[3]. Production of algal biofuels together with multiple valuable chemicals provides microalgae-based biorefineries with a promising cost-effective future[10]. There are numerous cultivation strategies for increasing biomass and other valuable chemicals at lab scale [4]–[6]. Nevertheless, industrial-scale cultivation, is a complex system dynamics, and therefore the system performance heavily relies on the culture mode, making it challenging to identify optimal cultivation strategies. Promising autotrophic large-scale cultivation technologies are bubble column photobioreactors and open pond systems [7], [8]. However, optimization of these systems requires understanding the intricate interactions between nutrient supply, CO_2 fixation, and light intensity [9], [10]. Although nitrogen and carbon limitation have been reported as promising strategies for starch and lipid accumulation [11]–[13], few studies have evaluated strategies for both open and closed large-scale systems under different nutrient supply rates.

This work develops a kinetic model of microalgae growth with predictive capabilities by exploring the effect of nutrient supply, carbon dioxide fixation and light intensity, to determine optimal nutrient(s) composition for enhancing microalgae biomass, starch, and lipid accumulation in large-scale systems, in conjunction with experiments in photobioreactors and open ponds. In particular, experiments are carried in a 12L bubble column photobioreactor and 500L (300L workin volume) open raceway ponds. The microalgae strain used is *porphyridium purpureum*, which is subjected to different nutrient, light and CO2 supply conditions.

The proposed macroscopic model describing cultivation dynamics is constructed around the concept of the specific growth rate (μ), which defines the rate at which cells grow over time in response to any growth-limiting factor [11] and consists of a set of ordinary differential equations. It includes a number of state variables: total biomass X gC L^{-1} (i.e sum of biomass X + Starch S + Lipids L, nitrogen uptake gN L^{-1}, nitrogen quota *Nq*.gN gCx^{-1}, carbon dioxide CO_2 gC L^{-1}, starch concentration gC gC^{-1} and lipids concentration L gC^{-1}. The model parameters are fitted against experimental data at different consiftions and cultivation modes and its predictions are validated extensively with different sets of experiments. The validated model is subsequently used to compute optimal operating cultivation policies and can be employed for scale-up purposes.

Keywords
Biorefinery, Modelling, Microalgae, Photobioreactor, Open Ponds

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I am a Daphne Jackson fellow working as a Post-doctoral Research Associate based in the Department of Chemical Engineering at the University of Manchester (UofM) under Professor Costantinos Theodoropoulos. My fellowship is sponsored by the Biotechnology and Biological Science Research Council (BBSRC). I use a combination of experimental and computation modelling to enhance microalgae cultivation and evaluate the biochemical production of various high-value products used as raw materials for fuel, food, drug and beauty industries. In my project, I use ingenious techniques to cultivate different microalgae strains, I currently work with *Chlorella sorokiniana*, *Porphyridium purpureum, Nannochloropsis gaditiana, and chlamydomonas reinhardtii*. I grow cultures in vessels of different sizes depending on the target of experimentation, which includes duran 500 mL bottles, Bubble column 12 L Photobioreactor (PBR) or pond 500 L volume of each pond in nitrogen content media in photoautotrophic or mixotrophic mode.I build predictive models for microalgae growth kinetics, including models related to algae growth rate and substrate concentration in culture media. I am a member of the BBSRC People and Talent Strategy Advisory Panel (PAT SAP) group. I supervise foundation stage projects at the University of Manchester. I also mentor post-doctoral researchers and PhD students in my group.

COMPANY PROFILE

The University of Manchester is renowned as a major research and development centre and a Russell Group member. In the 2014 Research Excellence Framework (REF), the university of Manchester was ranked 5th in positions of research power and 17th for grade point quality when including specialist universities. In 2018 Manchester was awarded the Athena AWAN Bronze 2018 and had been a member of the Athena SWAN Charter since 2008. The university has a reputation for its commitment to cultural change, diversity, and inclusion. It demonstrates good practice and aligns itself with the SWAN criteria of excellence with one of the best in the world, especially the Chemical Engineering Department. The Department promotes Biochemical and Bioprocessing Engineering as a discipline that creates practical and sustainable advances for the overall benefit of society.

MULTI-PRODUCT BIOREFINERY OF SEAWEED USING GREEN SOLVENTS

Antoinette Kazbar (AK)

Iulian Boboescu (IB), Michel Eppink (ME), Isa Hiemstra (IH), Wimar Reynaga Navarro (WRN), Rene Wijffels (RW)

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ABSTRACT

Macroalgae (seaweed) contain phycocolloids, essential amino acids, proteins, pigments and polyphenols, useful for the production of high-value products for food, nutraceuticals or pharmaceuticals. Their current extraction processes employ acid or alkaline conditions under high water and energy consumption, followed by costly product recovery. With this process phycocolloids are extracted and multiple products cannot simultaneously be extracted as these components are destroyed. The new developed techniques such as Ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), enzyme assisted extraction (EAE), homogenization and supercritical fluid extraction (SFE) were proposed as a solution but they still present many disadvantages: low yield, high costs, product degradation and the difficulties to scaleup. In this proposal, we are developing an efficient multiproduct-biorefinery for the extraction of high-value compounds from seaweeds using Deep Eutectic solvents (DES). This new class of sustainable solvents share the characteristics of ionic liquids (ILs), such as thermal and chemical stability, low vapor pressure and design ability. They offer many advantages such as low price, chemical inertness with water, and ease of preparation, and most of them are biodegradable with very low toxicity. DESs have been considered to be a potential alternative to replace conventional organic solvents for the extraction of interesting metabolites (flavonoids, proteins, lipids and polysaccharides) from different biomass such as plants, microalgae, and lignocellulosic biomass. Besides, they bring outstanding features considering that DESs are “tailor” made and switchable solvents that could be created during a desired process. The physicochemical interactions between DES molecules and the desired products (proteins, polysaccharides and lipids) need further investigations, same as their efficiency and recyclability. All these aspects will be tackled for the first time in the concept of a seaweed multi-product biorefinery with the aim for effective and gentle fractionation methods able to extract substances (biochemicals, polymers and food/feed additives) in the right order protecting the fragile components (e.g. proteins).

Keywords
Seaweed, biorefinery, zero waste, Deep eutectic solvents

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SPEAKER INFORMATION

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BIOGRAPHY

7 years ago I moved from Lebanon to do my Ph.D. in Bioprocess engineering at Nantes University. The topic was on modeling the impact of design dark fraction and high dissolved oxygen concentration on microalgal productivities in photobioreactors. After 2 years working with AlgoSource, France, as a microalgal biorefinery project manager, I joined WUR in 2020 to be a lecturer and researcher in biorefinery and process design for a plethora of biomass with specifically microalgae and seaweed.

COMPANY PROFILE

At Bioprocess engineering, biorefinery theme, we develop mild separation processes that enable us to obtain a plethora of valuable biomolecules from biomass (e.g. algae, seaweed and (cyano) bacteria). By taking cell disentanglement, mild extraction/separations and product functionality into account we are developing processes with a positive economic value and ecological impact.

Session 7: Feed

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

MICROALGAE AS SUSTAINABLE AND HEALTH-PROMOTING AGENTS IN AQUACULTURE

Dina Zilberg, Sagar Nayak, Ekaterina Novichkova, Arunjyothi Jawaji, Sammy Boussiba, Inna Khozin-Goldberg*

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ABSTRACT

Aquaculture is currently the fastest growing animal food production sector, driven by the high nutritional value, increased human demand and market acceptance. Microalgae are primary producers of high-value biomolecules with health-promoting and immunomodulatory properties, such as LC-PUFA, lipids, proteins, pigments, and polysaccharides.

We will present the results of our recent studies, demonstrating the multifunctional potential of LC-PUFA-producing microalgae as therapeutants for important parasitic diseases, for reducing gut inflammation and as a sustainable alternative to key ingredients of fish feeds, fish meal and fish oil (FMFO). The latter is being one of the main constrains to sustainable fish production in aquaculture.

Substantial loses in commercial fish farming are caused by microscopic ectoparasitic flatworms of the class Monogenea, which attach and wound the skin and gills, leading to fish mortality. Our research has demonstrated that extracts produced from the diatom *Phaeodactylum tricornutum* are effective against monogenean parasites and have antibacterial properties, mainly associated with fatty acids liberated from glycerolipids during cell breakage and extraction. To further develop a sustainable and cost-effective anti-parasitic treatment, the use of residue material from commercial *P. tricornutum* production was demonstrated.

Dietary application of the green microalga *Lobosphaera incisa* biomass at two physiological states, nutrient replete and nitrogen starved, was shown to significantly ameliorate TNBS-induced gut inflammation in the zebrafish model of IBD, signifying the potential of microalgae in treating inflammatory gut conditions and diseases.

A feeding trial was performed with Baramundi (*Lates calcarifer*), a carnivorous high-valued food fish, fed FMFO-free diets supplemented with microalgae (*Nannochloropsis oceanica*, *Isochrhysis galbana*, *Porphyridium purpureum*), producing omega-3 and omega-6 LC-PUFA. Compared to the commercial FMFO-containing feed, results demonstrated significantly better growth performance, lower mortality following bacterial challenge, enhanced lysozyme and antiprotease activities in the plasma, and a modulated expression of immune-related genes. Lipidome analysis of fish tissues is underway to elucidate the modifications in lipid metabolism resulted from microalgae-feeding.

These results will be discussed in view of the extended use of microalgae in aquaculture.

Keywords
LC-PUFA, fish-free diets, gut inflammation, parasitic diseases.

SPEAKER INFORMATION

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BIOGRAPHY

Inna Khozin-Goldberg specializes in physiology, biochemistry, and biotechnology of microalgae, particularly in lipid metabolism in microalgae. She is the author of over 120 publications, reviews, book chapters, and patents in the field of microalgal biotechnology and lipid biochemistry https://cris.bgu.ac.il/en/persons/inna-khozin-goldberg. She serves on the editorial boards of Journal of Applied Phycology, Algal research, and Frontiers in Plant Science. Her research includes genetic engineering and genome editing of microalgae; omics approaches, application of microalgae as anti-inflammatory agents in fish nutrition to modulate stress and disease resistance; application of microalgae in agriculture and wastewater treatment for nutrient recovery.

MICROALGAE AS A FEED IMPROVE THE SURVIVAL RATE AND VITALITY OF *PIKEPERCH (SANDER LUCIOPERCA) LARVAE*

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ABSTRACT

Microalgae cultures can synthesize high-value compounds, such as pigments and polyunsaturated fatty acids (PUFA) that are crucial for nutrition in aquaculture applications. For aquafeed preparations, the most important fatty acids are linoleic acid (LA; C18:2, n6), α-linoleic acid (ALA; C18:3, n3), eicosapentaenoic acid (EPA; C20:5, n3) and docosahexaenoic acid (DHA; C22:6, n3) which are essential for fish larvae development. Pikeperch (*Sander lucioperca*) is a highly demanded fresh and brackish water fish. The survival rate of pikeperch larvae is usually low, below 20%, due to unsuitable nutrition. The feeding of some microalgae strains to rotifers (*Brachionus plicatilis*) and subsequently to fish larvae confirmed increase of the survival rate of pikeperch [1]. In general, rotifers retain the nutritional composition of microalgae and represent live-feed capsules for the transfer of crucial nutrients, mainly polyunsaturated fatty acids (20:5, n-3 and 22:6, n-3), to fish larvae [2].

In this work, microalgae with a suitable fatty acid profile were used as a feed for rotifers, thus enhancing the rearing success of pikeperch in a recirculating aquaculture system (RAS). In a short light-path, annular-column photobioreactor (AC-PBR) with internal LED illumination developed for obtaining microalgae biomass of required quality, five microalgae strains, either Chlorophyceae or Eustigmatophyceae (*Chlorella vulgaris, Monoraphidium* sp., *Trachydiscus minutus, Vischeria helvetica* and *Monodopsis* sp.) were cultivated. Under continuous illumination, the highest biomass density over 5 g DW L^{-1} was achieved in *Chlorella* cultures. The volumetric biomass productivities between 0.33 and 0.52 g DW L^{-1} d^{-1} were found, corresponding to the areal productivities between 15 and 22 g DW m^{-2} d^{-1}. All microalgae strains used in this work represent suitable replacements for expensive commercially available aquaculture feed. The microalgae cultures were used for feeding rotifers that were further supplied to pikeperch larviculture in RAS as a live feed and the effect on the survival rate and overall vitality of larvae was studied. By 50% higher survival rate was found when *Chlorella* biomass was used for feeding fish larvae as compared to the control (commercially used *Nannochloropsis* paste in hatcheries).

Keywords
Microalgae feed, aquaculture, pikeperch, *Brachionus plicatilis*, survival

Funding
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SPEAKER INFORMATION

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BIOGRAPHY

Karolína Štěrbová (former: Ranglová) is a Postdoc at the Institute of Microbiology of the CAS, Centre Algatech in Třeboň. In 2014 she obtained MSc. in Chemistry of Natural Compounds at University of Chemistry and Technology in Prague. She started to work in Centre Algatech, Laboratory of Algal Biotechnology as a research assistant. In 2020 she graduated Ph.D. in Biotechnology at University of South Bohemia in České Budějovice and started the position of Postdoc in the same laboratory. Her main research interests are microalgae cultivation for application, studying of bioactivity and photosynthesis. She was/is member of 9 international projects and from 2020 she is a principal investigator of Interreg Project Alage4Fish dealing with a selection of microalgae according to fatty acid composition and their use as a feed to rotifers and pikeperch larvae to improve survival rate and quality of larvae. She published 24 papers in impacted journal so far, 1 book chapter, h-index 9.

COMPANY PROFILE

ALGATECH – The Centre of Algal Biotechnology evolved from the Laboratory of Algal Research, founded in 1960 in Třeboň. Throughout its history, the Třeboň's site of the Institute of Microbiology of the CAS has focused on microscopic algae and their use in food and feed industries and in human and veterinary medicine. At present, the ALGATECH Centre is an internationally recognised centre for basic and applied research of microalgae, cyanobacteria and photosynthetic bacteria, including the development of algal biotechnology. It is the largest centre of microalgae research in the Czech Republic.The ALGATECH Centre is housed in the historic building of Opatovický Mill from the 18th century, which has been recently renovated. The Centre also operates unique thin - layer cultivation units for autotrophic cultivation and a biotechnological hall for heterotrophic cultivation of microalgae, including the equipment for processing algal biomass. The Centre is known for its excellent research in photosynthesis and algal cell cycles.

BIOFILM-BASED PRODUCTION OF MARINE MICROALGAE AS LIVE FEEDS FOR THE AQUACULTURE INDUSTRY

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ABSTRACT

Current microalgae production systems are mainly based on suspended cultures using large volumes of water such as in raceway ponds and photobioreactors. The relatively low cell density or biomass concentration observed within these planktonic-based systems requires energetic expenses for culture agitation, and for biomass harvesting and dewatering. Therefore, an innovative technology has been developed and patented by the inalve company to further enhance productivity at reduced costs, overcoming the drawbacks of conventional systems. Created in 2016, inalve produces microalgae and formulates enriched-products used as live feeds for the aquaculture industry. Indeed, microalgae are required during the first-feeding of fish larvae, either for direct consumption or indirectly as food for living preys. Based on Rotating Algal Biofilm (RAB), the cultivation process does not spend energy to mix the culture, and harvesting is simply carried out by scraping the attached biomass with minimal energy demand. This gentle harvesting procedure allows to produce a concentrated algae paste (15-20% DW) with high cell viability and long shelf-life conservation (>50% alive cells after one month stored at 4 °C). Therefore, the presentation will describe the recent advances on microalgal biofilm production systems done by inalve from the optimization of biofilm cultivation to the use of the produced microalgae and formulated products for marine hatcheries as feedstock for rotifers, copepods and shrimp larvae. The specificity of each alive microalgae formulation will detailed.

Keywords
Microalgae, rotating biofilm, culture optimization, live feeds, hatcheries.

SPEAKER INFORMATION

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BIOGRAPHY

After a MSc degree in Marine Bioproduction from University of Nantes (France). F. Guihéneuf received a Ph.D. from Le Mans University (France) on the regulation by environmental factors of the long chain fatty acids synthesis in marine microalgae. F. Guihéneuf has more than 10 years of international experience (France, Israel, Ireland) on research and development projects focused on algal biotechnology, strain improvement, genetic engineering, and extraction technologies of bioactive compounds. As CSO, he is in charge of the scientific projects and R&D management at inalve company. He is the co-author of 40 peer reviewed international journals and 2 patents.

COMPANY PROFILE

Inalve is a french green-biotech company producing microalgae for the aquaculture industry. Our patented technology based on algal biofilm cultivation can produce concentrated microalgae on an industrial scale. Our recent life cycle analysis confirmed that we consume 20-30% less energy, and 70% less water than current technologies for microalgae production. Created in April 2016, inalve launched its pilot plant in October 2020 aiming to develop a demonstrator in effective operation from mid-2024.

Session 8: Biostimulant

ALGÆUROPE2022

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LIFE ALGAR-BBE PROJECT: RESULTS IN MICROALGAE APPLICATION AS BIOSTIMULANT

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ABSTRACT

ALGAR-BBE is a LIFE project focused on the development and study of the production and application of biostimulants with biocide effect, which were made of microalgae and aromatic plants, which was funded by LIFE financial instrument of the European Union under grant Agreement LIFE18 ENV/ES/000518.

Pesticide residues are a widespread risk which are found on more than the 40% of the food marketed on UE, which presence on food chains may cause different diseases or disorders. Moreover, the combined effect with other pollutants of concern is still a field under study that ignores the consequences of the exposure to these compounds. Perhaps their effects on human and environmental health, pesticides are still necessary for industrialized agronomy, in order to achieve a desirable yield of production.

On this basis, the biocide effectiveness of different biostimulant formulates from microalgal biomass and aromatic plants extracts was tested. Several species were selected for this project, such as *Origanum vulgare*, according to their proved antioxidant and antimicrobial properties. Furthermore, microalgae are great source of biostimulant compounds such as phytohormones, among others, while being the perfect atmospheric carbon sinks. Therefore, their inclusion into ALGAR-BBE aimed recover biostimulant compounds while using these organisms to remediate power plant flue gases. Accordingly, the project main objective was to produce a novel formulation with a mixed biostimulant and biocide action. On this basis, culturing optimization of 2 microalgal strains (*Nannochloropsis gaditana* and *Tetradesmus obliquus*) was done while enhancing the extraction yield of aromatic plants essential oils.

Different organic wastes were also harnessed as source of innovative compounds that could optimise the biostimulant development, while increasing the sustainability of the final formulas.

Once formulated, the developed compound was tested on tomato and corn crops with the objective to reduce, at least, the needing of56% of pesticides. In order to prove the effectiveness of the developed formulates, intensive trials were done in different crop fields in Spain and Portugal (mainly commercial crops as tomato and corn, but its effectiveness was also demonstrated in pepper and potato), reaching up to 20 ha treated.

LIFE ALGAR-BBE partners are NEOALGAE, CTAEX and ENDESA

Keywords
Bioestimulant, biofertilizer, biocide, *Nannochloropsis gaditana, Tetradesmus obliquus,* bioactivity

References

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SPEAKER INFORMATION

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BIOGRAPHY

As Head of the Technology Department in NEOALGAE Micro Seaweeds Products, Maria Alvarez is boosting this Spanish SME company through the pathway of microalgae biotechnology. She graduated in Biology from University of Oviedo in 2003 and as a Master of Environmental Engineering from University of Santiago de Compostela in 2006 (both in Spain). Currently she is focused on the development of biostimulants from microalgae biomass as part of a PhD program that will drive her to obtain the PhD diploma. She started her professional life linked to environmental sustainability and management in different companies. Prior to start working in NEOLGAE, Maria has worked in environmental and energy management areas in a leading electric utility in Spain, where she stumbled into microalgae technology in 2010. She began to be involved in the management of the microalgae pilot plant constructed in a coal power, carrying out national R&D projects mainly related to CO2 capture and energy uses. Nowadays, at NEOALGAE, she is focused on different microalgae technologies and applications, with special emphasis on the development of new microalgae- based products in food, feed, energy and agronomic sectors.

COMPANY PROFILE

NEOALGAE is a highly qualified microalgae biotechnology SME created to fill the current gap between laboratory scale and industrial processes in the field of microalgae applications. This objective is achieved by addressing R&D projects resulting in the commercialization of microalgal biomass and extracts for different uses and applications.
NEOALGAE´s mission is to contribute to the nutrition and health of people, as well as the care of the environment, through the development of different products based on microalgae that guarantee personal wellbeing in different areas (food, cosmetics, health) and sustainability environmental (through agricultural products and animals).
The company has three main interconnected divisions (technological, production and R&D), in order to be more effective in the development of the company skills and addressing the main strategic areas: biotech, aggrotech, cosmetic, foodtech, petfood an bioenergy.
The main facility is a microalgae plant in north Spain with funds by Phase II of H2020 SME Instrument, and equipped with offices, two greenhouses (with the required equipment for microalgae cultivation and downstreaming) and laboratories for different approches (biotech, extraction and purification, cosmetic, etc.). In addition, there are other areas such as biofertilizer production area. NEOALGAE has also another facility for foodtech product elaboration in Asturias.

NOVEL STRAINS OF *SCENEDESMUS SP.* AS A POTENTIAL SOURCE FOR AGRICULTURAL APPLICATIONS

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ABSTRACT

Microalgae have emerged as an alternative source of bioproducts due to their fast growth rates and relevant biochemical composition, including functional peptides, key free amino acids and fatty acids, antioxidants, terpenoids, vitamins and phytohormones. Besides the development of microalgae-based food, feed and nutraceutical products, microalgae have recently been marketed as sources of biostimulants to improve crop productivity with the aim of securing food supply in the form of sustainable, organic products [1]. However, high production costs and lack of research still hamper the broad application of microalgae-based biostimulants. In this study, random mutagenesis was used to improve biomass/target compound productivities of *Scenedesmus* sp. under heterotrophic conditions. Upon random mutagenesis using an already successfully developed pipeline [2], strains were selected based on pathway inhibitor screening using paclobutrazol, diphenylamine (DPA) and nicotine to target gibberellins and carotenoid biosynthetic pathways. After assessment of phenotypic stability by cultivation over 10 generations, biomass productivity of the novel strains was compared against the wildtype since higher growth usually leads to higher productivity of target compounds. The six best-performing strains had similar or higher (up to 50%) growth rates than the wildtype and different hues, ranging from orange, yellow and light green to dark green. When these strains were scaled up in 1-L fermenters, their productivity and macroscopic appearance remained similar to that of the original inocula. Preliminary test trials on the two DPA-resistant strains indicated a significant (up to 36%) increase in *Lepidium sativum* (garden cress) seed germination after 3 days of treatment using a biomass concentration of 0.1 g/L when compared to water. To better understand which compounds are responsible for this effect, the biomass will be characterized by their proximate composition, amino acid, carotenoid profiles, and phytohormone content. In conclusion, strain improvement is an interesting technology to enhance the application of *Scenedesmus* biomass as a plant biostimulants source.

Keywords
Microalgae, strain improvement, random mutagenesis, pathway inhibitors, biostimulants

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SPEAKER INFORMATION

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BIOGRAPHY

Lisa M. Schüler is part of the GreenCoLab team as a biotechnologist researcher leading the algal cultivation action line. Holding a BSc and MSc in Life Sciences, University of Hannover, Germany and a PhD in Biotechnology, University of Algarve, Portugal, Lisa has profound knowledge in biotechnology of microalgae, analytical chemistry, and molecular biology. Currently, her research is focused on strain development and improvement to enhance the contents of high-value compounds and the overall productivity of the strains using physiological and genetic approaches. Moreover, Lisa is developing detection methods of biological contamination in algal cultures using molecular techniques and mitigation strategies of these harmful contaminants. Her overall aim is to enhance the production of high-quality algal biomass as well as to develop new products and pipelines to the algae industries. Lisa has published 18 articles and one book chapter in international peer-reviewed journals and participated in several international conferences.

COMPANY PROFILE

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law.

GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology. The GreenCoLab's multidisciplinary team covers expertise across the whole algae value chain; from the lab to large scale production, strain optimization, scale-up, harvesting, biorefining to commercialization.

MICROALGAE PROMOTES PLANT GROWTH AND PRIMES PLANTS FOR RESPONSE TO ABIOTIC STRESS

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ABSTRACT

Microalgae have been found to positively influence plant growth. The objective of this study was to investigate the growth-promoting effects of a *Chlorella* green algae strain (MACC-360) on *Solanum lycopersicum* (tomato) model plant grown under controlled greenhouse conditions. The plants were cultivated in pots containing a mixture of vermiculite and soil layered with clay at the bottom. Algal whole-cell suspensions were applied to plants via soil drench method from the first week of germination until the 12th week of growth. Foliar application of algae extract prepared by homogenization of microalgae cells in liquid nitrogen was initiated on the fifth week of plant growth. We analyzed the physiological responses of *S. lycopersicum* to algal biomass addition and performed whole transcriptome analysis of unopened flower buds. Microalgae application significantly affected flowering, pigment content and fruit parameters. Transcriptomic studies revealed differential expression of several genes involved in defense and response to abiotic stresses. These results show the potential of microalgae to stimulate growth and enhance plants' stress tolerance and pathogen resistance.

Keywords
Eukaryotic microalgae, *Chlorella*, Biostimulant, Tomato, Transcriptome

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SPEAKER INFORMATION

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BIOGRAPHY

Gitau Margaret Mukami received Msc. Natural Science in Botany degree in July 2017 from the University of Chinese Academy of Science, China. She joined the Institute of Genetics in Biological Research Centre (BRC), Szeged Hungary for a 1-year International Training Course. In September 2018, she joined the research group of Dr. Gergely Maróti at the Institute of Plant Biology at BRC and enrolled for PhD in Plant Molecular Biology at the University of Szeged. Her current work involves discovering the plant biostimulant potential of microalgae. She has published 13 articles in SCI(E) journals, 2 of which report about plant biostimulants.

Session 9: Genetics

ALGÆUROPE2022
13 - 15 • DECEMBER • ROME

GENETIC ENGINEERING TOOLS AND APPROACHES FOR UNDERSTANDING AND REVISING LIPID METABOLISM IN THE MICROALGA *N. OCEANICA*

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ABSTRACT

Microalgae have caught industrial interest as promising sustainable photosynthetic production platforms for several compounds (i.e. proteins, hydrocarbons, fatty acids) exploitable in food and feed, aquaculture, and fuel sectors. However, in order to become an economically viable platform, these microorganisms must be optimized in solar energy conversion, carbon capture and utilization, and the partitioning of metabolic fluxes (1). Being light to lipid conversion one of the major causes of the high costs of bulk lipid production (2), how can we optimize this process? To fulfill the necessity for improved yields in microalgal lipid production, our research moves towards targeted strain selection and engineering, focusing on the oleaginous microalgae *Nannochloropsis oceanica*. In this talk, we present our recent developments on cutting-edge gene editing tools by using CRISPR-Cas systems (3,4), a new developed expression system based on RNA polymerase I activity (5), and high-throughput screening techniques, which have been successfully used for the identification and selection of new mutant lines. In this regard, we are expressing foreign enzymes to tailor novel lipid classes (6,7). Through insertional mutagenesis, we have reported the identification of novel genes associated with lipid metabolism, selected for their capability of accumulating lipid in standard growing conditions (8). Among those, we isolated and performed transcriptomic analysis for a knock-out mutant of a transcription factor, which shares DNA-binding domains similar to the ones of MYB and AP2/EREBP plant transcription factors. Mutant showed increased photosynthetic efficiency, upregulation of genes involved in the central carbon metabolism, sugar and fatty acid biosynthesis (8).

Keywords
Genetic engineering, lipid metabolism, *Nannochloropsis*

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Sarah D’Adamo holds a MSc. In Industrial biotechnology and PhD degree in biotechnology, curriculum: biochemistry and biophysics, both obtained at the University of Study of Padova, Italy. From January 2018, she is a tenure-track Assistant Professor in microalgal cell factory design at the Bioprocess Engineering division of Wageningen University (WUR), The Netherlands. She worked as post-doctoral fellow and research associate at the Chemistry Dept.ment of Colorado School of Mines, United States of America, following projects on redox and hydrogen metabolism of green microalgae. She has been senior scientist and lead scientist at Algenuity, a microalgal biotech start-up company based in England. Overall, she has >12 years’ of both academic and industrial experience in the field of microalgae physiology and biotechnology, conducted in Italy, USA, UK and, NL. Expertise includes analytical chemistry, biochemistry, molecular biology, biophysics, redox reactions, photosynthesis, metabolic engineering, strain engineering, bioprocess design, nutraceutical production.

COMPANY PROFILE

Wageningen University and Research (WUR) is a collaboration of Wageningen University and specialized research institutes of the Wageningen Research foundation, which have joined forces `to explore the potential of nature to improve the quality of life´. The Bioprocess Engineering division (BPE) of WUR is performing research on the development of novel biotechnological processes for production of bio-based chemicals, biofuels, food additives, feed and pharmaceuticals. WUR-BPE’s challenge is to produce high quality biobased products in a sustainable and economical way. WUR-BPE has over 15 years’ experience with the cultivation of algae and has a solid scientific basis in the expertise of bioreactor design, medium optimization, strain improvement, scale-up, biorefinery and LCA’s in this area of application. 25 PhD students, 2 professors, 2 assistant professors and 2 PostDocs from our staff are working in microalgae field. We collaborate already with over 40 companies and we coordinate large public private partnership programs (AlgaePARC pilot facilities, AlgaePARC Biorefinery and 3 EU research programs).

RECOMBINANT EXPRESSION OF THERMOSTABLE PHYCOCYANIN IN *SPIRULINA*

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ABSTRACT

Spirulina is an important cyanobacterial strain that is widely used as a protein supplement and as a rich source of the blue pigment phycocyanin. Phycocyanin is currently in high demand due to an increasing trend towards the use of natural dyes over synthetic dyes. Commercially, phycocyanin is currently produced from Spirulina. However, phycocyanin from this cyanobacteria is limited by its vulnerability to high temperatures, it denatures at temperatures above 50°C. Phycocyanin's instability at higher temperatures reduces its use in any industrial application requiring temperatures above 50°C. Various methods have been used to improve the thermal stability of phycocyanin, these include the addition of sugars, consumable acids and salts. However, these methods only enhance thermal stability for short periods, therefore, a more feasible approach to the production of thermostable phycocyanin is sought. Extremophilic species such as *Galdieria sulphuraria* and *Synechococcus elongatus* are potential sources of natural thermostable phycocyanin, they produce phycocyanin that is stable at temperatures above 60°C. However, the commercial production of phycocyanin from these strains is hampered by their slow growth rate and recalcitrant cell walls. In this project, we aim to improve the yield and quality of phycocyanin produced by Spirulina. This will be achieved by genetically modifying Spirulina to express thermostable phycocyanin genes from *G. sulphuraria.* The genetic modification of Spirulina was carried out through an Agrobacterium mediated transformation. The progress made thus far in the project includes successful transformation of Spirulina and *Chlorella vulgaris* (a freshwater microalgal strain that is used as a positive control) with thermostable phycocyanin genes from *G. sulphuraria*. The preliminary results obtained have confirmed the presence of the thermostable genes in the genomic DNA of Spirulina and *C. vulgaris*. Work is currently underway to characterize the transformants that were obtained, with regards to phycocyanin yields and thermostability.

Keywords
Biorefinery; Genetics; Synthetic biology; Bioprocessing; Algal biotechnology

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Ngokoana Mokakabye is currently a Master of Engineering (Chemical) candidate at the Centre for Bioprocess Engineering Research (CeBER), Department of Chemical Engineering, University of Cape Town, South Africa. Ngokoana started off as a research intern at CeBER, where she worked on a phycocyanin related project. Following the internship Ngokoana was recruited as an MSc students and her project looks at improving the quality and yield of phycocyanin from Spirulina. This project is situated in CeBER's algal biotechnology platform, which conducts research across these themes: algal strain selection and development, product based research, waste water treatment and reactor design modifications. Ngokoana carried out her undergraduate studies at the University of Pretoria, South Africa, where she obtained the following degrees: BSc in Biotechnology majoring in Microbiology & Genetics (2012-2016) and BSc Honours in Microbiology (2018). Apart from her MSc project, Ngokoana also has a Teaching assistant role for the Chemical Engineering I and II courses offered to the Chemical Engineering first and second year students respectively.

ENGINEERING OF THE RECENTLY-DISCOVERED *SYNECHOCOCCUS* PCC 11901, A FAST-GROWING CYANOBACTERIA, FOR THE SYNTHESIS OF HIGH ADDED-VALUE CAROTENOIDS

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ABSTRACT

The industrial-scale production of high added value molecules in microalgae is limited by the growth performance of the latter in industrial systems. *Synechococcus* PCC 11901 (hereafter *Synechococcus*), a prokaryotic microalgal strain, has been recently isolated [Włodarczyk et al. (2020)]. This microalga showed exceptional properties suitable for large-scale cultivation. In fact, this microalga, using light and CO2, is a) capable of accumulating biomass in large quantities, b) has a rapid duplication rate, c) grows in soils with a high salt concentration and d) is genetically manipulable. This work focused on the engineering of *Synechococcus* to produce astaxanthin, a high added value ketocarotenoid not produced in this microalgal strain. Specifically, heterologous β-ketolase from *Chlamydomonas reinhardtii* [Perozeni et al. (2020)] and β-hydroxylase from *Brevundimonas* sp. SD-212 [Menin et al. (2020)] genes were constitutively expressed. Transformed cells (hereafter called BC) efficiently accumulated astaxanthin (~90% of total carotenoids) during photo-autotrophic growth. Moreover, preliminary experiments showed that BC cells grew faster than WT cells in the presence of high-light and continuous bubbling with CO2, possibly because of the photoprotective activity exerted by astaxanthin. In the stationary phase, WT and BC cells showed a comparable biomass accumulation, reaching ~4g/L dcw upon only 4 days of growth in a photobioreactor. BC cells accumulated ~0.8g of astaxanthin per Kg of biomass produced. Of interest was the evidence that the synthesis of astaxanthin caused a rearrangement in the composition of thylakoidal complexes. In fact, sucrose gradient fractionation showed that accumulation of both trimeric Photosystem I and dimeric Photosystem II was impacted in BC cells compared with WT cells. It is noteworthy that this phenomenon did not impair photoautotrophic growth of the BC transformants. In conclusion, the engineered *Synechococcus* succeeded in accumulating astaxanthin, without impairing cell growth rate, making this fast-growing cyanobacterium an ideal platform for the industrial photoautotrophic synthesis of astaxanthin.

Keywords
Fast-growing, *Synechococcus*, engineering, carotenoids, astaxanthin

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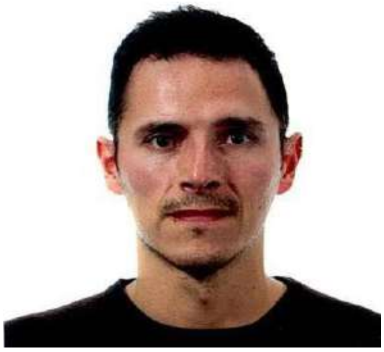
SPEAKER INFORMATION



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BIOGRAPHY

Nico Betterle is a researcher at the SOLE Lab, guided by prof. Matteo Ballottari, at the University of Verona (UNI-VR). His studies are focused on the production of high added-value molecules in microalgae and plants grown in indoor controlled systems. Nico was a Project Scientist at University of California Berkeley (> 4 years), having prof. Anastasios Melis as PI. Previously he was a Post-Doc researcher in the laboratory of prof. Roberto Bassi (UNI-VR). He holds a degree (2007) and a PhD in Industrial and Environmental Molecular Biotechnology (2011) at UNI-VR.

COMPANY PROFILE

Department of Biotechnology – University of Verona. This department is an institution leading Italian research on the field of biotechnologies applied to agricultural and industrial fields. In particular, the Sole-Lab guided by prof. Matteo Ballottari is solidly focused on the exploitation of photosynthetic (micro)organisms for the synthesis of high-added value products

EXPOSURE OF A MARINE DINOFLAGELLATE MICROALGAE TO EPIGENETIC MODIFIERS

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ABSTRACT

Marine dinoflagellate microalgae are source of a wide variety of molecules with pharmaceutical, nutraceutical and metabolomic applications. The search for mechanisms and techniques to improve the production of molecules of interest, which in many cases are obtained at low yields, is crucial to their commercial exploitation. *Amphidinium carterae* is known to produce amphidinols, molecules of the polihydroxylated polycetide group with antifungal, haemolytic, antibiotic, and anticancer properties. In addition, its biomass contains valuable compounds such as the pigment peridinin[1] and omega-3 fatty acids[2]. In this work, we cultured *A. carterae* in presence of different epigenetic modifiers for the purpose to study their effect on the cellular quota of interesting metabolites and their production in batch cultures. Six epigenetic modifiers were used at three different concentrations: suberoylanilide hydroxamic acid (SAHA), sodium butyrate (SOBUT), 5-azacitidine (5-AZA), tricyclazole (TCZ), metyrapone (MTY) and jasplakinolide (JASP). The results obtained, showed that the yield of total saponifiable fatty acids on biomass dry weight did not vary significantly with respect to the control culture ($p<0.01$), although the profile of free fatty acids varied greatly depending on the epigenetic chemical. For example, metyrapone, at its maximum non-lethal concentration (0.15 µM), the percentage of polyunsaturated fatty acids (PUFAs) decreased whereas monounsaturated fatty acids (MUFAs) increased. Similarly, sodium butyrate, at the highest concentration tested (0.6 mM), decreased PUFAs and increased Saturated Fatty Acids (SFAs). Interestingly, *A. carterae* grown with SAHA or tricyclazole produced the pigment peridinin three times more than the control at the highest non-lethal concentration tested for both chemicals (0.15 mM and 31.7 µM, respectively) ($p<0.01$). Thus, SAHA and tricyclazole seem to be involved in the pigment biosynthesis routes, promising elicitors being for the overproduction of peridinin. In contrast, the yield of bioactive molecules related to amphidinols -evaluated with a haemolytic assay[3]- showed no statistically significant difference among experimental and control groups. To our best knowledge, the treatment based on epigenetic modifiers is tested for the first time in marine dinoflagellate microalgae. Our positive results in altering the biomass composition of *A. carterae* allow considering them as a promising new approach for the microalgal industry.

Keywords
OSMAC, marine dinoflagellate, epigenetics, peridinin, fatty acids, amphidinol

Acknowledments
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Graduated in Chemical Engineering and master's degree in Chemical Engineering from the University of Almeria, Spain. Currently, PhD student at the Doctorate in Biotechnology and Industrial Bioprocesses Applied to Agri-Food and the Environment Program by the University of Almeria, thanks to a predoctoral contract at the University of Almería associated to the project PID2019-109476RB-C22 in Biotechnology of Marine Microalgae Group (BIO-173). PhD thesis focused on the bio-guided microalgae-based bioprocess research and the optimization for agrifood industry applications.

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NEW MICROALGAE MEDIA FORMULATED WITH COMPLETELY RECYLCED PHOSPHOUS ORIGINATING FROM AGRICULTURAL SIDESTREAMS

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ABSTRACT

Economically viable microalgae companies in Northern Europe mainly produce food supplements or extracts for cosmetic or pharmaceutical applications. A considerably reduction in production cost would potentially allow entering the feed market expanding the number of producers and volumes of microalgae production in Europe. The dependency of importing bulk microalgae currently included in feeds would be reduced. One possible way of reducing production costs and simultaneously increasing sustainability is using sidestreams from agricultural production processes.

The aim of the project ‘PhycoKult’ is 1) to develop practical and cost-effective methods to recover especially phosphorus from agricultural sidestreams to replace none sustainable and expensive basic components such as K2HPO4, KH2PO4, MgSO4-7H2O and NaNO3 in microalgae media and 2) to demonstrate that performance of the model species *Arthrospira platensis* cultivated on phosphorus recycled originating from agricultural residue is not impaired. Pre-trials conducted so far confirm high phosphorus and ammonia recovery efficiencies (0.16g P2O5 per L cow urine, Figure) as well as similar growth performance of Arthrospira grown on media in which phosphorus originated from agricultural residues vs conventional growth media.

Fig: Produced struvite (left from pig urine and right from cattle urine) containing P205, NH4 and Mg (left) (© A. Beyer, AWI).

Presentation content at Algae Europe

At Algae Europe we present comprehensive phosphorus and ammonia recovery efficiencies when recovered from cow and pig urine as well as results from growth and C-phycocyanin contents in Arthrospira grown on four different growth, i.e. standard Spirul Medium (SAG Göttingen), commercial media supplemented with commercial struvit, pre-treated cow struvite media and cow struvite media without a pre-treatment. Growth trial started on 13.09.2022 and will run for a period of 40-d at AWI microalgae facilitates applying standardized culture conditions.

Keywords

Sidestreams, struvite, phosphorus, *Arthrospira platensis*, C-phycocyanin

EMFF- ALGAENAUTS PROJECT: ECO-FRIENDLY AND SUSTAINABLE NEW FAMILY OF BIOPESTICIDES BASED ON MICROALGAE

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ABSTRACT

Microalgae, especially cyanobacteria, are promising as biological agents for the control of pathogenic fungi and soil-borne diseases in plants because they produce biologically active compounds with antifungal, anti-biotic and against nematodes[1]. In this sense, ALGAENAUTS EMFF-BEW-2020 innovation project is focused on the development of a sustainable and eco-friendly biopesticides line of products for agriculture based on microalgae, produced sustainably. Technologies to be developed as part of the ALGAENAUTS agree with the principles of the circular economy approach thus aiming to achieve the integral utilization of biomass. Thus, after the extraction of biopesticides the biomass will be used for the production of biofertilizers, the quality of end-products being validated in field trials with farmers.

Production conditions for five selected microalgae and cyanobacteria strains (fresh and seawater), with antifungal and bactericidal activity, have been optimised using residual streams (wastewater and pig manure) as the source of nutrients. Processing of wet biomass allows for reducing the production cost and simply the overall production process, the final end-products being formulated based on extracts from wet biomass.

The end-products are being evaluated as biopesticides for agricultural uses. Bioassays were performed to detect the antagonistic effect against phytopathogenic bacteria and fungi. Bactericidal activity of up to 14% and antifungal activity of up to 55% have been detected during the *in vitro* trials. BZN-4 strain showed a broad antifungal activity to inhibit the growth of *Fusarium* spp., *Phytophthora capsici*, *Pythium ultimun*, *Alternaria alternata* and *Botrytis cinerea* and no antibacterial activity against the model bacteria. Similarly, the selected marine microalgae, BZN-6 and BZN-7 showed an identical spectrum of action. Meanwhile, BZN-8 showed only antifungal activity against, *Fusarium oxysporum*, Phytophthora *capsici* and *Pythium ultimum* and no antibacterial activity. BZN-5 was the only selected strain with antibacterial activity against *Xantomonas campestris* and antifungal activity against *Rhizoctonia solani*, *Fusarium oxysporum*, *Phytophthora capsica* and *Pythium ultimun*.

ALGAENAUTS project considers the validation of products, in collaboration with farmers, and to acquire of data for robust techno-economic and sustainability/LCA assessment, in addition to marketability and legal framework evaluation. The successful development of the project allows for the expansion of new sustainable biotools for agriculture.

Keywords
Microalgae, Circular Economy, Biopesticides, Biostimulants, Large Scale

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COMPANY PROFILE

Biorizon Biotech SL is a biotechnology company located in Almería (Spain). Biorizon Biotech is the world pioneering developing and commercialising agricultural products based on microalgae (biostimulants and biofertilisers). Biorizon Biotech has successfully marketed microalgae-based products for agriculture in worldwide.

RELEVANCE OF ZOOSPORIC PARASITES IN AQUATIC SYSTEMS (COST PARAQUA)

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ABSTRACT

Parasites, pathogens, and grazers are some of the most important biological constraints of large-scale algal biomass production. For example, fungal parasite infection significantly affects the astaxanthin production of *Haematococcus pluvialis*, reducing biomass production by up to 50% and astaxanthin yield by as much as 80%. Current solutions to tackle infections are not satisfactory. As the whole biomass is usually harvested before the contamination spreads, the culture must be subsequently initiated from scratch, resulting in serious yield loss. Various patents and protocols include mostly chemical treatments (fungicides, hydrogen peroxide). Regulation of physical parameters has been studied as well, but it mostly results in lower production.

While we still don't know enough about the parasite infections' drivers in algal biomass production, parasites have been intensely researched in natural systems where they have already been recognized as significant regulators of algae population growth. Like high-density algae blooms in nature, intensive algal production is prone to disease outbreaks caused by pathogens and parasites. With mass algal production on the rise, together with an increasing number of cultivated species, a wide array of known and still unrecognized parasites will pose significant biological, and consequently economic challenges to commercial cultivation. Early detection methods based on an improved understanding of key abiotic and biotic drivers would thus greatly contribute to the control of parasites in the algal production industry, increasing safety and reducing chemical treatment of infections.

ParAqua reaches out to environmental science knowledge to compile and test standardized procedures and rapid detection methods (molecular, real-time automated monitoring) for monitoring parasites in algal systems. ParAqua database will include observations of zoosporic parasites in biotech and nature, including their phenology, prevalence of infection, and diversity, abiotic and biotic conditions under which infections occur, inventory of methods for monitoring and early detection of infection, a compilation of infection control methods used in algal biotech weighing their feasibility and (cost) effectiveness. Possibilities of utilizing host-parasite relationships in new products will be studied as well. ParAqua thus brings together researchers, industry, and stakeholders from environmental studies and biomass production.

Keywords
Parasites, pathogens, grazers, microalgae, fungi, early-detection methods

References
COST ParAqua - Applications for Zoosporic Parasites in Aquatic Systems, https://paraqua-cost.eu

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Maja Berden Zrimec has a PhD in Biology from Biotechnical faculty at University of Ljubljana (Slovenia). She works in the fields of biology and circular economy in Algen, algal technology centre, llc, and Anteja ECG, where she is the Head of research group. She is a senior expert, project manager and content provider in the EU and national projects in the field of algal technologies, circular economy and bioresources: Interreg - GoDanuBio, Ardia-Net and AlpLinkBioEco, Horizon 2020 - Water2Return & Saltgae, Horizon Europe – BioRural & Cronus, COST ParAqua (WG3 co-leader), and LIFE AlgaeCan. She is the editor of Frontiers scientific journals topic “Boosting the Potential of Algae for Biomass Production, Valorisation, and Bioremediation”. She has a certificate in Ecosystem Services: A Method for Sustainable Development from the University of Geneva through Coursera.

In her early career, she co-founded the Institute of Physical Biology, where she was the Head of scientific research and development, working on ecotoxicology, algae (eco)physiology, bioremediations, and environmental monitoring. She cooperated in building an international educational platform on Harnessing the Power of Biopolymers for Human Well-Being (including Biopolymer Workshops, Talent Camps, Training Courses, Global Biopolymer Network) in the Centre of Excellence for Polymer Materials and Technologies. She closely cooperated as a Subject Matter Expert and content provider in the American Chemical Society educational programme Sci-Mind^{TM}. She was the Head of biology department working on seabed habitats research with remote sensing and photogrammetry at the company Harpha Sea.

COMPANY PROFILE

Algen is a micro-SME established in 2010, specialized in the development & system integration of algae technology systems, providing consulting, algal cultivation, wastewater treatment and engineering services. R&D is currently focused on the cultivation of algae in waste streams (wastewater from different industries, digestate, side-streams from food and drink production), simultaneously treating the water by the algae-bacterial process for recycling of nutrients and CO_2 and producing the algal biomass for various valorisations, from valuable extracts to fertilisers.
Algen has been responsible for the R&D of algal systems in the scope of several European projects, including anaerobic digestate from biogas plants (AlgaeBioGas, Saltgae), wastewater from the slaughterhouse (Waster2Return) and fruit & vegetable processing industries (LIFE AlgaeCan), capture and reuse of biogenic gas (Cronus), and providing circular biobased solutions for rural areas (BioRural). Algen is part of the COST action “Applications for zoosporic parasites in aquatic systems”. On the national level, Algen is involved in research of lactic acid fermentation for enrichment of the microalgae biomass with new nutrients, valorisation of algae for cosmetic products and bioremediation. Algen has designed, engineered, and built several high-grade Spirulina production facilities and algal systems in demo centres (Slovenia, Italy, Spain).

INSIGHTS INTO THE ROLE OF THE MICROBIOME IN MICROALGAE GROWTH AND DEVELOPMENT: MICROBIOME RECRUITMENT AND ASSEMBLY DYNAMICS IN *NANNOCHLOROPSIS OCEANICA* AND *PHAEODACTYLUM TRICORNUTUM* CULTIVATIONS

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ABSTRACT

Recent studies have unveiled the vital role of the microbiome in regulating and potentiating the development of several eukaryotic hosts, including microalgae. In this sense, beneficial symbiotic and mutualistic members of the microbiome can promote microalgae growth and production of bioactive compounds, through a variety of beneficial syntrophic relationships. On the other hand, opportunistic pathogens can greatly impact microalgae leading to cell lysis and disruption, and ultimately cultivation collapses [1,2]. Despite the known beneficial/detrimental properties of some bacterial members of the microalgae microbiome, not much is understood about the microbiome assembly of relevant microalgae such as *Nannochloropsis oceanica* or *Phaeodactylum tricornutum* nor the mechanisms involved in microalgae-bacteria interactions.

In our work, a detailed characterization of the *N. oceanica* and *P. tricornutum* microbiomes was performed. The two microalgae were cultivated in natural waters obtained from two distinct locations in Portugal and microbiome assembly studied throughout cultivation times. Members of the microalgae microbiomes were isolated and characterized.

The obtained results demonstrated different microbiome recruitment and assembly abilities, as well as different growth performance by the two different microalgae despite being cultivated in identical waters. Moreover, different water sources induced similar microbiome compositions in both microalgae, suggesting a strong selection of the microbiome by the microalgae host. The microalgae microbiome assembly occurred differently throughout the cultivation time, indicating that this is a highly dynamic process. The characterization of the bacterial members of the microbiome revealed different activities (e.g., production of phytohormones, lytic enzymes) between the different members. Lytic enzymatic activities were found in specific bacterial groups.

Ultimately, the obtained data brings new relevant insights into the importance of microbiome assembly and function in *N. oceanica* or *P. tricornutum* cultivations which may be key to the development of a wide range of industrial applications.

Keywords

Microbiome, *Nannochloropsis oceanica*, *Phaeodactylum tricornutum,* Symbiosis, Pathogens

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SPEAKER INFORMATION

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BIOGRAPHY

Francisco X. Nascimento, Ph.D. is an applied microbiology enthusiast and a versatile and motivated researcher that tackles several aspects of microbial biotechnology. During his research career published 38 articles in international peer-reviewed journals (h index=19) and three book chapters. These publications result from multidisciplinary works and focus on several scientific areas, such as applied microbiology, bioprocesses, microbial ecology, genomics, eukaryote-microbe interactions, evolutionary biology, and bioinformatics. Francisco participated in several national and international projects and worked in several labs (Portugal, Canada, Brazil, Netherlands). In his professional activities, he interacted with several collaborators in co-authorship of scientific papers, supervises 4 PhD students and 1 master student, participated in teaching activities, as a jury of dissertations, presented his work as an invited speaker, participated as Editor (microorganisms, MDPI) and reviewer for several international peer-reviewed journals. Currently, he is affiliated with Instituto de Biologia Experimental e Biotecnológica (iBET, Portugal), acting as Principal Investigator of a national-funded project, "PhycoµBiome: Understanding and harnessing the power of the microalgae microbiome aiming the maximization of marine microalgae productivity" and participating as an active researcher in the project "MULTI-STR3AM: A sustainable multi-biorefinery for microalgae integrating industrial side streams to create high-value products".

COMPANY PROFILE

iBET (www.ibet.pt) is a private non-profit institution, a Biotechnology Research Organization acting as an interface between academic and private institutions while also creating and organizing autonomous knowledge and expertise. Target areas are biopharmaceuticals and novel therapies (ii) food and health with the development of new bioactive food supplement, new solutions for the detection of food frauds, food, and water-borne pathogens (iii) environment, with the development of new sustainable, energy saving, processes for both food and water industries.
iBET's infrastructure comprises 16 cutting edge laboratories, a GMP certified Analytical Services Unit and a Mass Spectrometry Unit, a Late-Stage R&D and Bioproduction Unit (2000 m2) and access to the GMP manufacturers which, together, allows iBET to develop integrated solutions from R&D to phase I/II clinical trials.
As an R&D institution iBET has coordinated over 30 international projects and participated as work-package leader in more than 100 projects supported by the European Commission. iBET has several well-established collaborations in Networks of Excellence and long-term partnerships with companies from the Agro-food industry.

EXTRATOTECA – MICROALGAE EXTRACTS FOR HIGH VALUE PRODUCTS

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ABSTRACT

The growing demand for natural products, sustainable food and renewable energy sources led to a rapid growth in the microalgae research field. Microalgae were found to be rich in vitamins, minerals, proteins, lipids and carbohydrates, extremely useful active ingredients, drawing the attention from the food, cosmetic and nutraceutical industries. Advances in technology enhanced the production and recovery of microalgal compounds. Now, the challenge is to find an efficient, high quality, sustainable and scalable system, to fully explore the potential of microalgae as a rich and natural source of such high-value compounds.
EXTRATOTECA is a 4-year project funded by the Portugal 2020 program aiming to develop a microalgae "extract bank" with worldwide relevance. A catalogue of selected extracts is being developed from isolated strains available in the consortium culture collection and is expected to be validated by relevant stakeholders.
Throughout the project, several production technologies and strategies were evaluated for each strain in order to promote the synthesis of high value compounds. At A4F, at pilot scale, a total of 12 strains (*Arthrospira* sp., *Chlorella* sp., *Dunaliella* sp., *Galdieria* sp., *Haematococcus* sp., *Lobosphaera* sp., *Nannochloropsis* sp., *Odontella* sp., *Phaeodactylum* sp., *Porphyridium* sp., *Tetraselmis* sp. and *Tisochrysis* sp.) were cultivated using several production technologies, such as raceways, flat panel and tubular photobioreactors. Also, different extraction processes (solvents, ionic liquids, super-critical liquids, ultrasounds, high pressure homogenization and electric fields) and methodologies for production and stabilization of extracts were developed by project partners. As this project's targets were the health, cosmetics, food, feed and fertilizers markets, the most sustainable technologies (high pressure processing and pulsed electric field) were selected. These technologies do not require the use of chemicals or preservatives, and also reduce waste production, cause lower emissions and improve shelf-life capabilities.
In the final stages of the project, the potential applications for the microalgae extracts will be determined by a full chemical, biochemical and functional characterization of both biomass and integral extracts.
In this communication, an insight into the microalgae extracts production and subsequent bioactivity determination and application potential will be given.

Keywords
EXTRATOTECA, microalgae extract, high value product, biological activity

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BIOGRAPHY

Biologist with a Master's degree in Cellular and Molecular Biology (2013, University of Coimbra). At A4F, she has accumulating over 8 years' experience in microalgae production from laboratory scale to industrial scale, with participation in several European bioengineering R&D and client projects. She also participates in consultancy activities and gives support to the prospecting team.

COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions, circular economy opportunities and Customer goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices

Session 11: Process B

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TIME SCALES OF THE USE OF LIGHT IN PHOTOBIOREACTORS

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ABSTRACT

To increase the global economic impact of microalgae photobioreactors the use ofiIn these situations, light absorption by microalgae is usually the bottleneck for culture growth (, y más referencias). Because of microalgae's own shadow a photobioreactor necessarily contains illuminated and dark zones. The movement of fluid particles between these zones unavoidably subjects the cells to fluctuating illumination and thus defines different time scales of irradiance.

During photosynthesis, a microalgae photosynthetic unit that is in a non-activated state is first activated by absorbing photons. In subsequent steps, the activated photosynthetic unit is consumed in enzyme-mediated reactions to return to its non-activated state, providing energy for maintenance and producing biomass (). The photosynthetic activation-deactivation has a characteristic time scale, *t_ad* < 1 s and is related with the well-known “flashing light effect”.

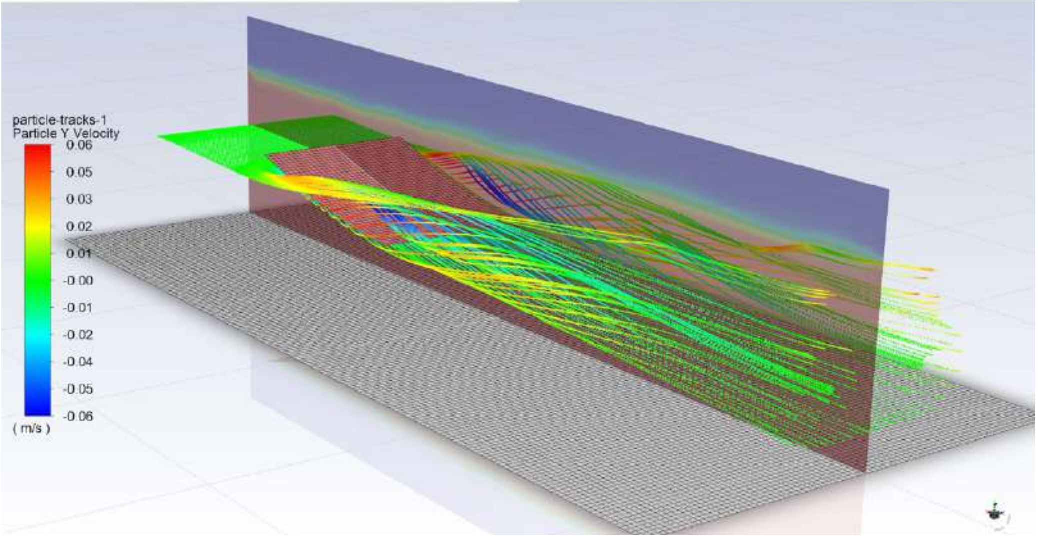
A larger characteristic time (*t_cr*) can be defined as the time required for a cell to go from having photosynthetic activity to carrying out cellular respiration and vice versa. A third characteristic time can be defined in relation to the photoinhibition effect, a much slower phenomenon than the previous ones, with a characteristic time *t_f* ~ 1 h. Also, phytoplankton slowly respond to the amount of light available by varying the size and concentration of the photosynthetic units by photo-acclimation (*t_ac*).

The objective of this work is to improve the efficiency of light use by optimizing the trajectories of fluid particles, to adapt their irradiance time scales and thus allow microalgae cells to make the most of the available light. To achieve this, we use:

- CFD (Computation Fluid Dynamics) for the design of the photobioreactors dynamic models of light integration

to determine trajectories of fluid particles, theand the flow power consumption.
increase the oxygen production rate usingphotobio maintaining low power consumption and CAPEX

Keywords
Light integration, photobioreactor mixing, dynamic models, time scales, CFD



Simulation of cell trajectories around an airfoil using Computational Fluid Dynamics.

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BIOGRAPHY

He has been doing R&D on design of microalgae photobioreactors and on microalgae based WWT since 2014.

COMPANY PROFILE

The company's D&B Tech design raceway reactors to grow microalgae and for microalgae based WWT.
D&BTech exploits several international patents related with flow dynamics, mixing and bioflocculation applied to chemical and biological reactors.

UNLOCKING THE LIPIDOME OF *NANNOCHLOROPSIS GADITANA*: A SOURCE OF HIGH-VALUE LIPIDS WITH POTENTIAL BIOLOGICAL ACTIVITIES

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ABSTRACT

Microalgae are crucial sources for meeting the global population's needs in terms of more sustainable food supplies, specifically concerning lipid demand. Microalgae produce a wide range of high-value lipids, including omega-3 and omega-6 long chain-polyunsaturated fatty acids (ω-3 and ω-6 LC-PUFAs), neutral lipids, and more complex lipids, which have important roles in cell structure, such as glycolipids and phospholipids [1]. *Nannochloropsis gaditana* is one of the most promising microalgae specie for producing valuable lipids due to the relatively high lipid content and excellent lipid composition containing ω-3 LC-PUFAs, mainly eicosapentaenoic acid (EPA, 20:5) [2]. However, the complexity of *Nannochloropsis gaditana* lipids and their potential biological activities remains largely unexplored. In this work, the use of enzyme-based methods in combination with ultrasounds was investigated as eco-friendly approaches to produce high-quality lipid extracts from this oleaginous microalga. Moreover, the effect of the green approaches on the lipidome profiles and the bioactivity of microalgal lipids were also investigated to evaluate the potential of these valued lipids in health and food applications. A novel lipidomics workflow was applied for the analysis of the lipidome of *Nannochloropsis gaditana* based on liquid chromatography high-resolution mass spectrometry (LC-HRMS). Different lipids containing ω-3 and ω-6 LC-PUFAs of nutritional importance were identified, including neutral and polar lipids, such as triglycerides (TG), diglycerides (DG), phosphatidylcholines (PC), as well as galactolipids, including monogalactosyldiacylglycerols (MGDG), and digalactosyldiacylglycerols (DGDG). The lipid composition differed from the extraction methods applied depending on the enzymatic solution used, leading to enriching the extracts with different lipid classes. In terms of bioactivity, the cytotoxicity of the produced lipids was assessed by comparing human colon cancer cells (HCT 116) and epithelial nontumorigenic immortalized cells (HCEC-1CT). Results suggest that the lipid extracts have a selective cytotoxic effect, reducing the viability of the colon carcinoma cells but not the nontumorigenic cells. Thus, this multidisciplinary approach clears up the lipidome of *Nannochloropsis gaditana*, and at the same time, provides new eco-innovative methods for extracting valuable lipids enriched in ω-3 and ω-6 LC-PUFAs from microalgae with promising biological properties.

Keywords
Omega-3 fatty acids, bioactive lipids, eco-friendly approaches, lipidomics, cytotoxicity.

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SPEAKER INFORMATION

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BIOGRAPHY

Natalia is a food scientist with extensive experience in novel and eco-friendly techniques to extract a broad spectrum of bioactive compounds (omega-3 lipids and other minor compounds) from microalgae and seaweeds. She holds a Ph.D. in Food Chemistry from the Autonomous University of Madrid (2018). After a one-year postdoc at the University of Iceland (2019), she moved to the University of Pau and the Adour Region (Anglet, France), where she was working as an Assistant professor. In 2020, she was awarded the H.P. Kaufmann Award granted by the German Society for Fat Science (DGF). Moreover, in 2020, the AOCS acknowledged her carrier giving her the recognition "Young Scientists to Watch." Recently, she received a 3-year postdoctoral fellowship (2021-2024) from the REinforcing Women In Research (REWIRE) Programme, a Marie Sklodowska Curie Actions co-funded by the University of Vienna and the European Commission. Her project, BioactALGAE, explores for the first time the valorization of microalgal biomass by exhibiting biological activities of produced extracts using a multi-step extraction method implying eco-friendly techniques.

C-PHYCOCYANIN EXTRACTION USING *SPIRULINA* (*ARTHROSPIRA PLATENSIS*) BIOMASS IMMOBILIZATION IN CALCIUM-ALGINATE BEADS

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ABSTRACT

Spirulina (*Arthrospira platensis*) is a photosynthetic cyanobacterium rich in pigment-protein complexes, the phycobiliproteins. C-phycocyanin (C-PC) is the major phycobiliprotein found in Spirulina, achieving up to 20% of its dry weight. It is a blue and water-soluble pigment, commonly extracted with saline and buffer solutions. Freezing/thawing, bead milling, mixing, and homogenization are the most used techniques for C-PC extraction. However, they are time- and energy-consuming and often lead to low C-PC purity extracts. Moreover, C-PC has low stability requiring the addition of stabilizers (e.g., sugars, $CaCl_2$, citric acid) to delay its degradation and enable its industrial application [1]. Calcium-alginate matrices have been investigated for protein delivery systems showing relevant outcomes for retaining and releasing proteins from food products along the gastrointestinal tract [2]. Thus, in this work, Spirulina biomass was immobilized in calcium-alginate beads (SAC), promoting C-PC recovery in the aqueous crosslinking solution while the biomass keeps retained, facilitating separation steps. SAC beads were prepared from a mixture containing the Spirulina biomass powder and alginate at 2% (PC2%) and 4% (PC4%) and formed by ionic gelation upon being added dropwise to the $CaCl_2$ crosslinking solution. Four Spirulina:$CaCl_2$Solution (S:S) ratios were studied (1:33, 1:42, 1:83, 1:125 (w:v)). The C-PC was quantified according to [3], and the C-PC purity was determined by [4] after 24 h of extraction at room temperature. From the C-PC concentration (mg mL^{-1}), the C-PC extraction yield (mg $g^{-1}_{biomass}$) was found. Higher S:S ratios result in higher C-PC extraction yields for both used calcium-alginate concentrations (2 and 4%) (Figure 1). For all studied S:S ratios, the PC4% extracts presented purities higher than those established for food grade (≥ 0.7). For PC4%, the higher obtained purity (0.83) corresponds to the 1:42 S:S ratio. For PC2%, the higher purity (0.68) was found for the 1:83 S:S ratio. Overall, the proposed technique enables the C-PC release to the $CaCl_2$ solution, an investigated C-PC stabilizing agent, achieving food-grade purity at relevant extraction yields. Besides, high levels of protein (≥ 30 % DMB) still remain in the SAC beads, which can be further valorized within a biorefinery concept [5].

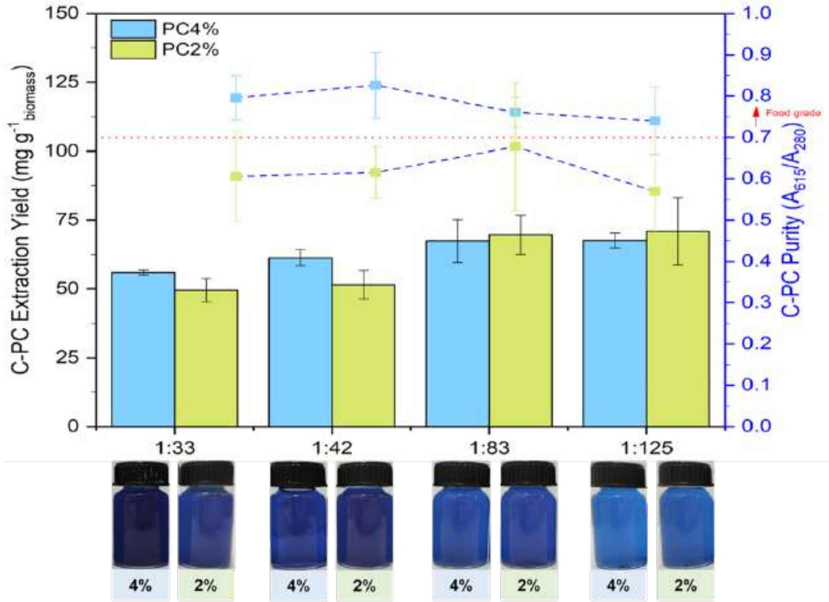

Figure 1: C-PC extraction yield (mg $g^{-1}_{biomass}$), purity (absorbance ratio A_{615}/A_{280}), and visual appearance for different calcium-alginate contents (2% (PC2%) and 4% (PC4%)) and Spirulina:$CaCl_2$Solution ratios (w:v) (1:33, 1:42, 1:83 and 1:125).

Keywords
Phycocyanin extraction; Calcium alginate beads; Protein release; Food grade phycocyanin, Microalgae biorefinery.

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CIMO (Centro de Investigação de Montanha) (UIDB/00690/2020 and UIDP/00690/2020), LSRE-LCM (Laboratory of Separation and Reaction Engineering – Laboratory of Catalysis and Materials) (UIDB/50020/2020 and UIDP/50020/2020), ALiCE (Associate Laboratory in Chemical Engineering) (LA/P/0045/2020), and SusTEC (Associate Laboratory for Sustainability and Technology in Mountain Regions) (LA/P/0007/2020) funded by national funds through Fundação para Ciência e a Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES) (PIDDAC). FCT for the PhD research grant of Samara Cristina da Silva (SFRH/BD/148281/2019). Algikey – Algae-based Solutions S.A for the kindly donation of the Spirulina biomass.

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BIOGRAPHY

Samara Silva is currently a PhD Candidate in Chemical and Biological Engineering at the Faculty of Engineering (FEUP), University of Porto, Portugal. Her PhD work is focused on developing an integrative and sustainable approach to valorise protein fractions of Spirulina biomass in natural emulsifiers and colourants. She is a Food Engineer and holds a Master's Degree in Food Quality and Safety from the Federal University of Technology (UTFPR), Brazil, and the Polytechnic Institute of Bragança (IPB), Portugal. In 2013, she received an award for best student in the Chemistry technician program at the Centro Paula Souza, Brazil, which enabled her to do a cultural exchange program in San Francisco, USA. She also participated in the double diploma degree program between UTFPR and IPB from 2017 to 2018. She was a very proactive undergraduate student and participated in scientific initiation (undergraduate research) and mentoring programmes, as well as a quality assistant at the UP Foods Junior Enterprise. Besides, Samara also participated as a volunteer in social projects aiming at digital inclusion, education and craft activities for the elderly. She was involved in the organising committee of the Innoevent Bragança (2018 ed.) and the Double Diploma Summer School & Symposium (2018 and 2019 ed.). She appreciates solving problems and working in teams. She is passionate about food and how it can be transformed by the science of cooking. Her main interests include microalgal proteins, protein-stabilised emulsions, protein extraction, microalgae biorefinery, microencapsulation, phycocyanin extraction, natural colourants and biocompounds.

DECIPHERING THE COMMUNICATION BETWEEN ALGAE AND BACTERIA

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ABSTRACT

In plankton communities, microalgae and heterotrophic bacteria can establish relationships that benefit both partners[1]. These relationships have been gaining more attentions these days due to their potential applications in large scale microalgae culturing[2], opening the doors to the discovery of new products of interest. It has been shown that bacteria cannot just have a protective effect against predators, but also to promote their growth parameters[3]. Some work has been done to better understand these interactions between cyanobacteria and bacteria, with particular emphasis on quorum sensing[4], whilst the relations between eukaryotic microalgae and bacteria are not well understood, with most of the works focusing more into understanding the effect on bacterial growth. This gathers more importance when considering the difficulty of axenification of certain biotechnologically relevant species. In the current work, we try to emphasise the effect of bacterial metabolites on the physiology and the growth of the green algae *Monoraphidium* sp., isolated in Antarctica, which has shown potential to produce biotechnological important compounds[5]. Using classical culturing methods, we have managed to isolate some of the bacteria closely associated with this *Monoraphidium* sp. We have tried to isolate some of the closely associated bacteria and identify them. One of the bacteria isolated showed enhancements in the production of a biotechnologically important pigment, Lutein. Our results have demonstrated the potential role of bacterial metabolites, possibly via quorum sensing in the regulation of Lutein production and show that these processes might play a key role in epibiont-algae interactions.

Keywords
Microalgae-bacteria interactions; Co-culturing; Quorum sensing; Pigments

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Session 12: Transversal

ALGÆUROPE2022

13 - 15·DECEMBER·ROME

REDWINE LCA: CO_2 FROM WINE FERMENTATION TO PRODUCE *CHLORELLA* AS FEEDSTOCK FOR THE INDUSTRY

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ABSTRACT

Climate Change is a serious worldwide concern. Among all the production sectors, the food industry is a major contributor to greenhouse gas (GHG) emissions, accounting for an estimated 29% of all anthropogenic emissions. These emissions have an impact on the global climate, affecting resource availability and food production. The wine sector is under pressure, with studies showing the possibility of vast portions of Europe on the Mediterranean coast will become completely inhospitable to grape production by 2050 (Mozell and Thachn, 2014).

In this context, REDWine project aims to contribute to climate change mitigation by using CO_2 from red wine fermentation off-gas and liquid effluents to produce Chlorella and valorize it as feedstock for the industry (food, cosmetics, and agricultural products). This objective will be achieved through the efficient capture, storage, and supply of wine fermentation off-gas (mainly composed of CO_2 (>90%)), to be used to feed microalgae production systems, along with the liquid effluent resulting from washing wine fermentation tanks.

To ensure the best environmental performance of REDWine processes and configurations, a life cycle assessment (LCA) study (ISO, 2006a, 2006b) is being carried out from an early stage of the project and the preliminary results obtained so far will be presented. The selected REDWine process configuration will be fully evaluated, and a final comparison will be made with the competing technologies currently used. In addition to the reduction of climate change impacts, other environmental impacts, such as water footprint, toxicity and eutrophication are expected to be decreased due to the reduction in the use of freshwater and chemical products. Specifically, the REDWine system is expected to contribute to reducing GHG emissions (expressed in CO_2 equivalents) by at least 20%. First scenarios have been carried out comparing wine production using conventional technologies (Neto et al., 2012) and using the REDWine system operating in Palmela, Portugal (where will be implemented). The results show that REDWine solutions will provide direct GHG emission savings of about 30%, from 2.45 kgCO2eq to 1.78 kgCO2eq per bottle of wine produced.

***A4F**, **IPS, LNEG,** and **AVIPE** (Portugal), **NOVIS** (Germany), **IDENER, LEITAT,** and **LIPOTEC** (Spain), **ALGAMA** and **COLDEP** (France), and **PERVATECH** (Netherlands) contribute with data for the LCA study.*

Keywords
Life Cycle Assessment, CO2 from fermentation, circular economy, Chlorella, Ecowine

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BIOGRAPHY

Susana Leão holds a PhD in Environmental Engineering (specialised in Life Cycle Assessment) by University of Montpellier, a Master in Ecological Agriculture by University of Barcelona, and an Environmental Engineering Degree by University of Aveiro. Her career has been focused on promoting better environmental performance of products and services through the development and application of environmental assessment methods. She has a wide experience on R&D of Life Cycle Assessment (LCA) studies in national and international projects in different fields (waste and water management, sustainable construction, energy, agriculture). Her scientific interests are sustainability assessment, LCA methodology, water and carbon footprints, eco-design and circular economy. She has presented her work in many different international conferences (posters and oral presentations) and has published different peer-reviewed papers in scientific and technical journals. Currently, she is a Senior Researcher at the Sustainability Area of the Circular Economy Department at the LEITAT Technological Center.

COMPANY PROFILE

Founded in 1906, Leitat is one of the reference entities at state and European level in technology management. It has a team of more than 500 professionals, experts in applied research, technical services and management of technological and innovation initiatives. Leitat provides social, industrial, economic and sustainable value, offering comprehensive solutions in multiple sectors and areas: development of new materials, eco-sustainable production, occupational health prevention systems, revaluation of waste and use of natural resources, interconnectivity and digitization of industry, green energy and maximization of energy efficiency. Leitat is recognized by the Ministry of Economy, Industry and Competitiveness and is one of the main entities participating in the Horizon2020 program of the European Union.

IMPROVING TRANSPARENCY IN LIFE CYCLE ASSESSMENT OF ALGAE SYSTEMS: A CASE STUDY ON THE SPIRALG BIOREFINERY

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ABSTRACT

Life Cycle Assessment (LCA) has been used for more than 20 years to assess the environmental impacts of algae systems. The diversity of algae species, cultivation techniques, and conversion pathways has led to unique algae system designs. In addition, methodological choices made by LCA practitioners in the goal and scope and Life Cycle Inventory stages increase the variability of LCA results, therefore reducing their comparability. This study analyses the influence of methodological choices such as the functional unit, method to handle co-products, and approach to consider carbon capture, storage, and release on the environmental impacts of algae systems. Based on a case study, guidelines to improve the transparency and reproducibility of LCA studies are then provided. The case study focuses on a novel European biorefinery for phycocyanin production from *Arthrospira platensis* (hereafter simply referred to as Spirulina). An environmental LCA was conducted based on real pilot scale data collected in the frame of the SpiralG project (H2020-BBI). Two scenarios were compared to evaluate the effect of mitigation strategies and technological improvements on the environmental impacts of the biorefinery. The databases were built with the Activity Browser while the calculation were performed using the Brightway LCA framework and additional Python scripts. The LCIA method ReCiPe midpoint (H) v1.13 was selected. Our LCA results show the benefits of using waste heat from co-located anaerobic digestion plants for Spirulina cultivation and biomass pre-processing. Technological improvements help further reduce the electricity consumption in the phycocyanin extraction and co-products treatment stages. Combining these novel techniques, we can significantly mitigate the impacts of the SpiralG biorefinery on climate change. To conclude, LCA studies published over the past 20 years have lacked justifications regarding the methodological choices made by practioners. The introduction of guidelines on algae systems is therefore the first step towards improving the transparency and reproducibility of LCA studies.

Keywords
Life Cycle Assessment, Spirulina, phycocyanin, biorefinery, system modelling

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BIOGRAPHY

Léa Braud is a PhD Researcher in the UCD School of Biosystems and Food Engineering and at BiOrbic, Bioeconomy SFI Research Center. Her current research is part of the European SpiralG project (H2020-BBI) and focuses on Life Cycle Assessment (LCA) methodologies applied to algae systems. A key element of her work is to develop computer programs to adapt existing LCA methodologies to the specificities of algal biorefineries. Her research promotes the integration of algae systems into a local and circular bioeconomy.

COMPANY PROFILE

UCD School of Biosystems and Food Engineering is the leading centre for education and research in the application of engineering principles to agriculture, food and renewable resources in Ireland. BiOrbic, Bioeconomy SFI Research Centre is Ireland's national bioeconomy research centre, established to promote and develop Ireland's bioeconomy through excellent research and innovation.

LCA OF COMMERCIAL SCALE *TETRASELMIS SP.* PRODUCTION

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ABSTRACT

Microalgae production is a growing industry due to its increasing application in the food, feed, cosmetic, and nutraceutical sectors. However, there is still debate on the real environmental sustainability of algae production on a large scale compared to other agricultural products. This study tried to answer this sustainability question using Life Cycle Assessment (LCA) to understand what can be done to improve the production process in terms of the environmental impacts' hotspots. *Tetraselmis* sp. was produced at large scale (in a 150-m^3 tubular photobioreactor) at Allmicroalgae – Natural Products S.A. (Pataias, Portugal) [1], representing the largest microalgae production trial data to be used for an LCA, to date, within the literature. LCA methodology was applied to assess the environmental impacts of producing 1 kg of *Tetraselmis* sp. algal powder at the Allmicroalgae production facility following the principles of ISO 14040/44 and using the free open source OpenLCA v.1.10.3 software with the Ecoinvent 3.3 database [2]. A cradle-to-gate approach was adopted with no allocation procedure and ReCiPe Hierarchist Midpoints indicators as impact categories. Results showed that the major impact of microalgae operational production was electricity usage by the pumps during the cultivation. In terms of land and water use, microalgae production is a far better solution than soy-based feedstocks. However, advances in the microalgae production process need to be considered to compete in the other impact categories. Improvements to the current production systems demonstrated the potential to increase the system's sustainability, decreasing the microalgae production environmental impacts and making it more competitive. Some examples analyzed were increased productivity, usage of CO_2 emitted from a cement factory, better infrastructure design (with longer lifetime and more sustainable materials), and use of renewable energy sources (such as solar energy). These modifications in the process and facility design could decrease, for example, the climate change impact by 72% and water depletion impact by 63%.

Keywords
LCA, *Tetraselmis*, tubular PBRs, environmental impacts, large-scale production.

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BIOGRAPHY

Lais Galileu Speranza is the Life Cycle Analysis (LCA) Specialist at GreenCoLab and is responsible for the Sustainability Action Line of the organization that evaluates the sustainability of the algae products and process systems. She holds a Ph.D. in Chemical Engineering from the University of Birmingham (UK) and graduated in Environmental and Urban Engineering and Science and Technology's Bachelor at Universidade Federal do ABC (Brazil) with a one-year exchange program at the University of Nebraska – Lincoln (US). Previously, she worked with environmental analysis, LCA, biofuels policies, use of algal biomass for wastewater treatment, and the use of thermo-conversion and supercritical conditions to produce energy vectors from biomass and waste. Currently, she develops the LCA models of the GreenCoLab partners to quantify the environmental and social impacts of algae production and biomass processing, build techno-economic analyses (TEA) to define future improvements in the production and commercialization pipeline, and applies the circular economy approach to promote a positive impact from algae on the society.

COMPANY PROFILE

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law.

GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology. The GreenCoLab's multidisciplinary team covers expertise across the whole algae value chain; from the lab to large scale production, strain optimization, scale-up, harvesting, biorefining to commercialization.

Session 13: Bioremediation

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COUPLING ANAEROBIC DIGESTION AND MICROALGAL CULTIVATION FOR EFFICIENT NUTRIENTS AND INORGANIC CARBON UPTAKE

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ABSTRACT

Anaerobic digestion (AD) is the biological process where organic matter is converted into biogas through multiple biochemical reactions occurring in the absence of oxygen. The main by-product of AD, also referred as digestate, represents a nutrient-rich substrate containing a large variety of nutrients including ammonium, phosphate and various minerals [1]. In this context, microalgal cultivation appears as a sustainable and promising technique to valorize AD digestate while producing a valuable algal biomass [2]. However, several factors such as high turbidity and high ammonium concentration still limit the utilization of raw digestate without pretreatments requirements (filtration, dilution, etc.) [3].

The aim of this study was to determine the optimal culture conditions in order to enhance *Spirulina* growth and nutrient uptake using AD digestate. Different dilutions factors (10x, 20x and 50x) as well as source of inorganic carbon ($NaHCO_3$ or CO_2) were tested during cultivation assays in 6 L flat panel bioreactors.

At dilution rates of 20x and 50x, *Spirulina* was able to proliferate as soon as a source of inorganic carbon was added to the cultivation media, with different growth profiles depending on the conditions. However, even if $NaHCO_3$ or CO_2 were supplied to the system, a dilution factor of 10x did not promote any algal growth, probably due to the high concentration of ammonium (223 mg/L). At a dilution rate of 20x, the addition of 8 g/L of $NaHCO_3$ generated the highest growth rate of 0.22 d^{-1} while the injection of CO_2 produced up to 1.03 g/L of algal biomass after 15 days of cultivation. Regarding the nutrient uptake, pH regulation to 8.5 with CO_2 injection allowed an ammonium removal of 98.9%, reducing significantly the volatilization of ammonia, as observed in the culture not supplemented with source of inorganic carbon. The experimental finding highlighted the importance of pH regulation to avoid any ammonia loss and promote ammonium assimilation by microalgae.

In conclusion, coupling AD and microalgal cultivation presented the double advantage of reducing the use of chemical-based nutrient while contributing in the bioremediation of excess nutrient and biogenic CO_2 generated by AD units within the concept of circular economy.

Keywords
Anaerobic digestion, Spirulina, Bioremediation, Ammonium, Waste management

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SPEAKER INFORMATION

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BIOGRAPHY

In 2014, Jean-Baptiste received his Master’s degree in process engineering at the Université of Aix-Marseille (France). From 2015 to 2019, he did his PhD at the Biomass Technology Laboratory (BTL) of the University of Sherbrooke (Canada). His research project focused on the phytoremediation of industrial waste streams coming from three industries involved in the production of biofuels (Greenfield Global, CRB Innovations and Enerkem). After his graduation, Jean-Baptiste continued to work at the BTL as a postdoctoral fellow until the end of 2022. In 2023, Jean-Baptiste joined the APESA research center based in Montardon (France), to work as R&D Project Manager in the field of biomass and effluents valorization. His specific area of expertise is algal cultivation for wastewater treatment and green house gas reduction. He also has a strong expertise in other bioprocesses such as alcoholic fermentation and anaerobic digestion for bioethanol and biogas production, respectively. Jean-Baptiste is (co-) author of over 14 refereed journal publications.

COMPANY PROFILE

APESA – French private technology center in environment and risk control carries the values of sustainable development. We offer services and an independent expertise to guide customers towards an ecological transition via a circular economy vision. APESA is organized in four areas: Management, Innovation, Animation and Valorization. The Valorization group is composed of 6 engineers/doctors, 4 experimental technicians, 2 PhD students. Our technical platform is the experimental support of the APESA valorization team. It is as much a laboratory as a test platform. With nearly 15 years of experience in the field of waste recovery and more particularly biomass, APESA uses this tool to carry out experiments aiming to validate the feasibility, to optimize the dimensioning and to help our customers decide of setting up projects and industrial solutions in the fields of energy recovery waste or biomass. From the deposit (waste, biomass, effluents), through the process (anaerobic digestion, composting, wastewater treatment) and up to the quality of the downstream product (digestate, compost, discharges) we are able to test the whole industry.

FILAMENTOUS MICROALGAE AS TERTIARY WASTEWATER TREATMENT

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ABSTRACT

The Norwegian urban wastewater treatment plants are responsible for the disposal of annual 900 tons of phosphorous and 15,000 tons of nitrogen, which play a further role in the eutrophication of the natural water bodies. Despite the low concentration of those nutrients in the treated wastewater, at the final step of the secondary treatment, additional measures can be taken for their depletion. ALGECO project (www.alg.eco) aims to use NORCCA's filamentous algae as a resource for bioremediation of urban wastewater and promote a green shift in the sector. NORCCA, the Norwegian Culture Collection of Algae, hosts more than 2000 algal strains, most collected from the Arctic and other Nordic regions, representing the most valuable biological and chemical resource of the Microalgae Section in the Norwegian Institute for Water Research. Several NORCCA's filamentous algae strains were screened using a high throughput system, and five with the highest growth rates on clean wastewater supplied by the Oslo city treatment plant were identified. Those strains are currently being used to develop a low-cost integrated cultivation platform for later implementation as a tertiary wastewater treatment. Batch experiments ran in 1,000 L raceway ponds have demonstrated a significant nutrient load reduction in the treated wastewater. The operational parameters are currently under optimization in close collaboration with the Oslo city treatment plant to successfully implement the developed system *on site*. Additionally, studies on utilizing the produced biomass as a biofertilizer or soil conditioner will follow to use the available resources fully.

Keywords
Microalgae, Wastewater, Tertiary treatment, Bioremediation, Nutrient depletion

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BIOGRAPHY

Margarida Costa has a strong background in added-value marine compounds. She had previously worked in bioactive marine natural compounds from cyanobacteria in CIIMAR, the Marine and Environmental Research Center, at Matosinhos (Portugal). In 2014, she was granted an ITN Marie Skłodowska-Curie research grant and moved to the University of Iceland for a PhD in sponge natural products chemistry. Besides, Margarida collaborated on other European projects, studying the metabolome of cyanobacterial strains with anti-obesity properties. Later in 2019, Margarida Costa seized the opportunity to enroll an industrial postdoc at Allmicroalgae´s production facility, optimizing microalgae growing conditions and producing compounds of interest at the laboratory, pilot, and industrial scales. At the company, she had also been responsible for project management and funding applications. Margarida is currently working at the Microalgae Section, in the Norwegian Institute for Water Research (NIVA), in Oslo (Norway). She works closely with other companies and research institutes in the microalgae biotechnology field to find scientific and industrial solutions. Margarida counts around thirty peer-reviewed scientific papers, three book chapters, and a patent.

COMPANY PROFILE

The Norwegian Institute for Water Research (NIVA; www.niva.no) is a national research institute organized as a private foundation. The institute is Norway's leading competence centre for environmental and resource issues relating to the field of water. NIVA carries out research, monitoring, innovation, and development work. NIVA plays a vital role in water resources management as a provider of research-based studies and advisory services on water-related issues. NIVA's broad scope of competence, research expertise, and extensive data collections represent an important resource for Norwegian business activities and industries, public administration on a municipal, regional and national level, and for Norwegian interests in the international arena. NIVA has about 290 employees, of which >80% are water professionals. NIVA's headquarters are located in Oslo; with 4 regional offices and a large-scale marine research station. In addition to the foundation itself, the NIVA Group consists of the research and consultancy companies Akvaplan-niva and NIVA Chile as well as the development firm NIVA-tech AS with subsidiaries BallastTech-NIVA and NIVA China.

COMPARATIVE ANALYSIS OF SOIL DEGRADATION AND SOIL STRUCTURE IN CROPLANDS AFFECTED BY EROSION AND SOIL DEHYDRATION TREATED WITH A BIOLOGICAL SOIL CRUST FORMING ALGAL CULTURE

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ABSTRACT

Climate-related land degradation and desertification threaten around half of EU member states. In Hungary, wind and water erosion affects 2.3 million hectares of land. One of the most important indicators of soil degradation is the water retention capacity of the soil. Therefore, the primary objective is to maintain or increase it. Due to their adaptation to extreme enviromental conditions, soil algae can survive in drought-stricken areas. With their contribution, biological soil crusts can be formed on the top layer of the soil. Soil crusts can stabilize the surface of the soils, enhance the water retainment, thus indirectly contributing to the settlement of higher order vegetation. Therefore, soil algal cultures can be applied to inoculate desert, steppe and eroded areas. The basis of our demonstrated soil crust-forming technology is the *Klebsormidium bilatum* filamentous green soil algae isolated by Albitech Biotechnology Ltd. In 2020, we examined the impact of algal inoculation on brown forest soils in sloping arable land. We measured the extent of soil degradation by artificial rainfall simulation, carried out soil moisture, aggregate stability, macro- and microporosity, and soil crust structural tests. According to porosity tests and soil moisture measurements, the formed algae crusts on the soil surface had a beneficial effect on the soil structure. The more favourable micromorphological structure also caused the deeper layers of the soil more aerated, which had a positive effect on the hydrological properties of the soil. The established algae layer and the improvement in soil structure contributed to a reduction in soil losses caused by water erosion. As a result, we also experienced an increase in yields of corn and spring barley in sloping areas treated with soil algae.

The project was supported by MKI-2018-00034 grant and the National Multidisciplinary Laboratory project NKFIH-872 of the National Research, Development and Innovation Office, Hungary.

Keywords
Soil, crusts, *Klebsormidium*, erosion, agriculture

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BIOGRAPHY

PhD student, University of Pannonia, Doctoral School of Chemistry and Environmental Sciences; Laboratory Leader, Albitech Biotechnology Ltd. Awards: Best Young Author Award (International Congress of the Hungarian Society for Microbiology, 2018) Research area: the role of biological soil crusts; cultivation of soil algae; detection of phytohormones from algal cultures.

COMPANY PROFILE

Albitech Biotechnology Ltd. is a dynamically developing Hungarian enterprise established in 2007 aiming at microalgae research and development of microalgae-based products for agricultural purposes. We work with monocultures (only one type of algal strain is present during photofermentation), so no other algae that could change the properties of the culture broth should be included in the product. Our company ensures the constant quality of the products by using closed photofermentation systems and continuous quality control. During our operation, we have collected more than 100 different soil and aquatic microalgae strains. The culture collection provides us the opportunity select the most suitable strains for our goals. Our main research topics are screening algal cultures for biologically active molecules, algae-bacteria co-cultivation experiments, investigating the effect of microalgae inoculation on the soil.

REUSING EFFLUENTS FROM AGRICULTURE TO UNLOCK THE POTENTIAL OF MICROALGAE (REALM)

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ABSTRACT

The recently European-funded research project REALM, which stands for "Reusing Effluents from Agriculture to unLock the potential of Microalgae", aims to transform nutrient-rich drain waters from soilless farms into value. The concept relies on a decentralised production of microalgae at reduced costs while treating hydroponic greenhouse drain water, thus mutually increasing the circularity and profitability of microalgae production and soilless farming.

Previous studies have shown the feasibility of this concept at a small scale, and now REALM aims to demonstrate it at an industrial scale and European level. Accordingly, two validation facilities (>1 m^3) in Northern Europe (the Netherlands and Finland) and two demonstration facilities (~1 ha) in Southern Europe (Portugal and Spain) will be installed. All facilities will be optimised to match the necessities of their operating conditions, including the drain water composition, weather conditions and chosen strains. These facilities will operate under a turbidostat or chemostat regime (continuous harvest) for maximum productivity and minimal nutrient disposal. The demonstration facilities, located next to key drain water inputs (hydroponic crops), will be composed of cultivation and harvesting units powered by photovoltaic energy. An automatic cloud-based control and monitoring system based on predictive models and novel sensors will assist and improve production. Downstream processing units will then use a biorefinery approach to develop microalgae-based products, namely agrochemicals and aquafeed. In addition, a business model will be developed to propose the concept of multiple decentralised microalgae production facilities next to soilless greenhouses serving a centralised processing facility. This concept will offer sustainable water treatment technology to farmers all over the EU by closing the nutrient loop and is expected to reduce microalgae production costs increasing the competitiveness of the proposed microalgae-based products while targeting several objectives of the European Green Deal.

Keywords
Microalgae, Agriculture, Sustainability, Automation, Water treatment.

SPEAKER INFORMATION

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BIOGRAPHY

Mariana Carneiro has completed her Lic. Degree in Environmental Sciences and Technology in 2010, an MSc degree in Marine Biology in 2012, and a PhD in Chemical and Biological Engineering in 2021 from the University of Porto. Her PhD thesis was focused on the assessment and improvement of microalgae biomass productivity, which included collaborations with several universities and institutes, including the University of Santiago de Compostela (USC) in Spain, the Institute of Ecosystem Study (ISE) of the National Research Council (CNR) in Italy, the Centre of Marine Sciences (CCMAR) from University of Algarve (UAlg) from in Portugal, and the Centre Algatech in the Czech Republic. After concluding her studies, she started to work at the microalgae company Necton S.A. in 2021, where she performs as a project manager and oversees process optimisation or innovation needs within the microalgae production scope, among others.

COMPANY PROFILE

Established in 1997, Necton is the oldest company in Europe that specialises in the cultivation and commercialisation of microalgae. The company was set on an ideal location to grow these microorganisms in the natural park of Ria Formosa on the sunny south coast of Portugal. Throughout the years, the company acquired extensive knowledge in cultivating marine, freshwater, and hypersaline species. Necton's current portfolio includes over 30 species, such as *Nannochloropsis oceanica*, *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Tetraselmis chui*, *Porphyridium cruentum*, and *Skeletonema costatum*. To produce the microalgae biomass the facility relies on more than 100 m^3 of horizontal tubular photobioreactors and a 200-m^3 raceway pond.
Necton's growth is supported by a continuous R&D effort that includes participating and managing several national and European-funded research projects. Through this effort, Necton was able to achieve scientific, technological and market knowledge of microalgae cultivation and applications for the aquaculture and cosmetic sectors, among others. The company's history, experience and constant collaboration with universities, research organisations and other companies have made Necton one of the leading European companies in microalgae biotechnology.

OUTDOOR N2O EMISSIONS MEASUREMENTS IN ALGAE-BACTERIA SYSTEMS GUIDED BY MODELLING

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ABSTRACT

Algae-bacteria processes in wastewater are promising for reducing the energetic demand due to oxygenation, since microalgae produce the oxygen supporting the growth of bacteria [1]. Here we present an outdoor experiment carried out in Rennes, France, in raceway ponds treating agricultural digestate under a greenhouse. The objective was to validate the predictions of the numerical ALBA (ALgae-BActeria) model [2], which pointed out conditions of strong competition for CO_2 between microalgae and nitrifiers, despite a pH regulation process [3]. Low inorganic carbon concentrations are known to hinder the process efficiency and to be favourable for N_2O production by nitrifiers [4]. The model highlighted that increasing the medium alkalinity could allow a higher inorganic carbon storage capability, avoiding then this unexpected CO_2 limitation for a pH controlled process [5].

The experiments consisted in testing various level of ammonium/alkalinity ratios and recording the dynamics of the process. The experimental protocol was designed using model simulation accounting for the local meteorology [6]. We developed a dedicated protocol to measure N_2O, based on salt-induced stripping by adding NaBr [7].
The experiments demonstrated that increasing alkalinity allowed to strongly boost the dissolved CO_2 level in the culture, and finally enhancing algal productivity. Moreover, production of N_2O turned out to be triggered in the raceways with the lowest level of CO_2.

These experimental results will contribute to improve the N_2O production sub-model, in order to more proactively avoid conditions of N_2O emissions in industrial scale applications. This approach will offer new insights in algae-bacteria processes, guiding the design of optimal operational strategies, and finally making this process more environmental friendly.

Keywords
N_2O emissions, Alkalinity, Modelling, Inorganic carbon, Wastewater

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SPEAKER INFORMATION

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BIOGRAPHY

Graduated in Environmental Engineering at University of Florence, within her M.Sc. thesis she was in charge of the experiments and the modelling of a granular sludge reactor for wastewater treatment.
From November 2017, she was a Ph.D. student in Environmental and Infrastructure Engineering at Politecnico di Milano, focusing on mathematical modelling of algae/bacteria systems for wastewater remediation. She spent one year at the INRAE (Narbonne), to calibrate and validate the model on long-term datasets. After defending her Ph.D thesis in March 2021, she got a Postdoc position in the INRIA-BIOCORE team, where she worked on the optimization of algae-bacteria systems. Since September 2022, she is a permanent researcher at INRIA-BIOCORE team. She is focusing her research work on experimental design and modelling for N_2O emission estimation from outdoor algae-bacteria cultivation for wastewater remediation, hybridizing different modelling techniques involving Artificial Neural Networks. In October 2022 she was awarded with the price For Women in Science, L'Oréal UNESCO.

COMPANY INFORMATION

The goal of the Biocore team is to contribute to environment preservation, develop new renewable energy sources and avoid water pollution or the use of chemicals for crops. Biocore is an association between Inria, INRA, CNRS and Sorbonne University. Biocore is designing models to understand, optimize microalgae-based processes and assess their environmental impact. We have been working 20 years in association with the Laboratory of Oceanography from Villefranche (Sorbonne University) on CO_2 uptake by microalgae (atmospheric or industrial), their response to a nitrogen deficiency, in particular to stimulate the production of carbon reserves (lipids and sugars). Biocore developed an automated computer-controlled platform from liter to m^3 to grow microalgae in highly controlled conditions and automatically sample, filter and analyze the biomass and remaining nutrients. Biocore has been involved in several national and international projects and coordinated five of them.

Session 14: Biomaterial

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EFFECTIVE CONVERSION OF ATMOSPHERIC NITROGEN INTO CYANOPHYCIN: MUTAGENESIS AND OPTIMIZATION OF CULTIVATION OF *NOSTOC SP.* PCC 7120.

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ABSTRACT

A sustainable, green economy must be created to address global challenges. Nitrogen, the principal component of amino acids and proteins, is currently isolated from the air by the relatively expensive and energy-dependent Haber-Bosch process that converts atmospheric nitrogen into ammonia, a toxic and dangerous compound, for the needs of chemical industry and agriculture[1].
Diazotrophic cyanobacteria, a group of photosynthetic prokaryotes, can naturally fix atmospheric nitrogen, driven by the power of solar energy, and convert it into organic nitrogen-containing compounds. The fixed nitrogen can be deposited in the form of proteins or cyanophycin, also known as CPG (cyanophycin polypeptide granules). Cyanophycin is a non-ribosomally produced amino acid polymer composed of an aspartic acid backbone and arginine side groups. Cyanophycin is insoluble under physiological conditions and is accumulated in the form of granules in the cytoplasm and in heterocysts[2].
We have developed a methodology for the isolation of CPG over-accumulating mutants of the diazotrophic strain *Nostoc* sp. PCC 7120 by selecting them on media containing canavanine, a toxic analogue of arginine[3]. We isolated mutant strains accumulating 1.5 to 2-fold more cyanophycin than the wild type depending on cultivation conditions. The mutant strains demonstrate 30% higher volumetric cyanophycin production in optimal conditions. We sequenced the genomes of the isolated mutants and identified shared and unique mutations in several candidate genes that may be involved in the regulation of cyanophycin accumulation and offer potential targets for genetic manipulation. Overall, we can conclude that we developed a promising platform for the biological conversion of atmospheric nitrogen into a valuable nitrogen-rich compound.

The research is funded by the Israeli Ministry of Science grant 3-1736 and the Italian Ministry of Foreign Affairs and International Cooperation (MAECI) under award protocol number MAE01432042020-12-03, CUP C99C20001980005

Keywords
Cyanophycin, *Nostoc* sp., mutagenesis, nitrogen fixation, arginine

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SPEAKER INFORMATION

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BIOGRAPHY

Marta completed her master's degree in Industrial Biotechnology at the University of Padova in March 2021. During this time, she worked with cyanobacteria applying batch and continuous systems to study the assimilation of different nitrogen compounds. Since October 2021, she has been an internship student at the Ben-Gurion University and part of the “INFINITRO project”, a joint project with the Microalgae Laboratory of the University of Padova, investigating cyanophycin accumulation in diazotrophic cyanobacteria.

OXYGEN PRODUCTION FROM 3D BIOPRINTED LIVING MICROALGAE FOR TISSUE ENGINEERING

Gao F.[1]

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ABSTRACT

Engineered tissues have the potential to be applied as implants to replace damaged or diseased tissue or as models to study diseases or potential treatments *in vitro*. However, it is still a big challenge to create larger 3D living tissues, which is in part due to a mismatch in oxygen levels in the fabricated constructs. Microalgae are unicellular photosynthetic microorganisms, therefore oxygen producers, that can be easily cultured and 3D bioprinted[1-3] and could be a biological solution to solve the mismatch challenge. However, proper microalgae strains that can be used for engineered tissue constructs need to be screened due to the differences between algae and tissue cultivation conditions such as pH, temperature, and osmolarity. In addition, the oxygen level should be monitored and controlled to meet the specific oxygen requirements of different tissues. In this project, 10 microalgal strains, i.e., *Chlorella sorokiniana*, *Spirulina platensis*, *Chlamydomonas reinhardtii* CC124, *Chlamydomonas reinhardtii* CC1690, *Tisochrysis lutea*, *Picochlorum* sp., *Tetraselmis* sp., *Synechococcus* sp., *Leptolyngbya* sp., and *Nannochloropsis oceanica* were cultivated in both algae and tissue (cartilage, liver, and heart) media at 25 and 37 °C, respectively. The oxygen production rates of the microalgal strains that can grow well with tissue media were monitored using a Biological Oxygen Monitor. Following, models were built to predict oxygen production at different light levels in different media. *C. sorokiniana*, *C. reinhardtii*, and *Picochlorum* sp. can grow well in algae and three tissue media at both 25 and 37 °C. *C. sorokiniana* and *C. reinhardtii* are better performers in oxygen production in tissue media, especially in the liver medium. These 10 microalgal strains are being 3D bioprinted by University Medical Center Utrecht and tested for printability, viability, and biological functionality. This project opened new possibilities for using living microalgae in tissue engineering and also shows great potential for other applications in regenerative medicine. For example, we are currently investigating the possibilities to use 3D bioprinted living microalgae for wound healing, i.e., providing oxygen for faster skin-tissue repair. This research is funded by the alliance TU/e, WUR, UU, UMC Utrecht and the NWO Open Competition Domain Science XS (no. OCENW.XS22.1.042).

Keywords
Living microalgae, biological solution, tissue engineering, oxygen production, regenerative medicine

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SPEAKER INFORMATION

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BIOGRAPHY

Dr. Gao is a researcher in Bioprocess Engineering group at Wageningen University, the Netherlands. He holds two PhD degrees, one in Aquatic Products Processing and Storage Engineering obtained at Ocean University of China (2019) and another one in Bioprocess Engineering obtained at Wageningen University (2021).
Currently, he is the project leader of an NWO-XS project (No. OCENW.XS22.1.042) funded by the Dutch Research Council and is involved in a project funded by the Seed Fund Alliance Utrecht University, Wageningen University, TU Eindhoven, and University Medical Center Utrecht, working on oxygen production from microalgae for tissue engineering. He was involved in the European Horizon 2020 project MAGNIFICENT (No. 745754; Wageningen University, Netherlands, 2017-2021) and an exchange project (No. 9055; GreenCoLab, Portugal, 2021) funded by the European Molecular Biology Organization. He obtained the VLAG Research Fellowship to visit University College London and University of Cambridge in the UK in 2022.
He obtained two improved *Tisochrysis lutea* strains at Wageningen and won the European Union-Young Algaeneers Innovation Award (AlgaEurope 2021, Silver Winner, funded by European Commission) and the Silver Medal of the Best Microalgae Awards (AlgaEurope 2020). During the past four years, he gave ~20 talks on Microalgal Biotechnology during multiple large international conferences.

COMPANY PROFILE

Wageningen University is the only university in the Netherlands to focus specifically on the theme ‘healthy food and living environment’. Wageningen is one of the top-ranked universities in the world. According to influential university rankings, the university ranks the world’s best in the field of Agriculture & Forestry.
Bioprocess Engineering chair group (BPE) teaches and develops innovative bio-based processes. We work on a sustainable and healthy future by engineering efficient bioprocesses for high-quality products. We study and develop photoautotrophic and heterotrophic production systems for biobased products, as well as high-quality processes for the production of biopharmaceuticals. BPE operates the biggest algae production and research centre (AlgaePARC) in Europe, with collaborations with more than 50 industrial and 25 academic partners.

PILOT-SCALE CULTIVATION OF *CHLOROMONAS TYPHLOS* IN A PHOTOBIOREACTOR

S. Van Miert

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ABSTRACT

The most studied and cultivated microalgae have a temperature optimum between 20 and 35 °C. This temperature range hampers sustainable microalgae growth in countries with colder periods. To overcome this problem, psychrotolerant microalgae, such as the snow alga *Chloromonas typhlos*, can be cultivated during these colder periods. However, most of the research has been done in the laboratory. The step between laboratory-scale and large-scale cultivation is difficult, making pilot-scale tests crucial to gather more information. Here we present a successful pilot-scale growth test of *C. typhlos*. Seven batch mode growth periods were compared during two longer growth tests in a photobioreactor of 350 L. We demonstrated the potential of this alga to be cultivated at colder ambient temperatures. The tests were performed during winter and spring time to compare ambient temperature and sunlight influences. The growth and CO_2 usage were continuously monitored to calculate the productivity and CO_2 fixation efficiency. A maximum dry weight of 1.082 g L^{-1} was achieved while a maximum growth rate, maximum daily volumetric and areal productivities of 0.105 d^{-1}, 0.110 g L^{-1} d^{-1} and 2.746 g m^{-2} d^{-1}, respectively, were measured. Future tests to optimize the cultivation of *C. typhlos* and production of astaxanthin, for example, will be crucial to explore the potential of biomass production of *C. typhlos* on a commercial scale.

Keywords
Biomass production, greenhouse, CO_2-utilization, microalgae, cold climate

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BIOGRAPHY

Dr. S. Van Miert has a Master in Pharmaceutical Sciences, a Master in Industrial Pharmacy and a PhD in Pharmaceutical Sciences – Pharmacognosy and Phytotherapy. She is the research manager of the research group RADIUS. From 2014-2021 Dr. Sabine Van Miert was a member of the steering committee of BelTox, since 2017 she is a member of European Algae Biomass Association and Strategisch Platform Insecten and since 2019 of IPIFF. From 2020 she is a member of the IOF board of KU Leuven association and since 2022 a member of the 'Vlaams Aquacultuur Platform'. Van Miert has expertise in the use of natural resources as source of (new) compounds and the extraction and purification procedures to obtain (new) chemicals. She is the (co-) author of 45 papers in peer reviewed journals and 2 book chapters.

COMPANY PROFILE

Thomas More is the largest university of applied sciences in Flanders, Belgium and offers more than 30 Dutch-taught and a range of English-taught bachelor degree programmes & postgraduates in the province of Antwerp. Within Thomas More we engage in practice-oriented research and services and develop new knowledge, insights and innovative products or services. We apply scientific knowledge in specific professional situations, thus stimulating innovation in companies and society. RADIUS is one of the research groups and counts 18 researchers with expertise in insects and algae as new sources of high value components.

POSTER PRESENTATIONS
Physiology

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MONITORING *CHLAMYDOMONAS REINHARDTII* GROWTH IN BIOFILM MATRICES

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ABSTRACT

Microalgal cultivation for biotech purposes has an established history of strain optimisation for growth and high-value product development. Historically, many works have focused on developing liquid cultures in various styles of photobioreactors with providing industrial relevant yields. Microalgae are not, however, limited to growth in suspension and many species are commonly found in natural biofilm communities.

Recent evidence suggests biofilms are structurally complex, dynamic living systems where algal strains may express specific growth characteristics within a complex matrix. Microalgal biofilm communities have architectural features that contribute to population heterogeneity and consequently to emergent cell functions. In order to develop a deeper understanding of this phenomenon, we are growing the model organism *Chlamydomonas reinhardtii* in a bench-top mini reactor under a number of matrices. We are testing for example the encapsulation of algal cells in agar hydrogels and the growth of cells confined between membrane layers. Under all tested conditions, cell growth is quantified and developing biofilms are characterised using a number of existing observational techniques (e.g. Fv/Fm measurements, microscopy).

We can also use fluorescent reporter lines to monitor the properties of *C. reinhardtii* cells in biofilm matrices. The mini reactor we are using as part of the Sinergia project and the established hydrogel matrix will allow us to create a nitrogen gradient across two varieties of biofilm. We generated different promoter-mVenus reporter constructs, where mVenus expression is driven by promoters activated under nitrogen-limiting conditions. Different nitrogen-responsive promoters will have distinct absolute expression levels and various dynamic ranges. After testing these reporter lines initially in liquid cultures under nitrogen replete and -deplete conditions, we will also use the lines in the biofilm reactor to allow monitoring of algae cells grown across a nitrogen gradient. We hope in the future to use the generated and characterised reporter lines to section an established biofilm using a microtome and to investigate the genetic changes that occur in cells in a biofilm at different depths by RNAseq. These future experiments could significantly advance our understanding of the emergent properties associated with various biofilm characteristics, including nutrient exchange and lipid accumulation.

Keywords
Chlamydomonas *reinhardtii*, Biofilms, reporter lines, nitrogen response

ASSESSMENT OF SIX MICROALGAE SPECIES GROWTH AND NUTRIENT COMPOSITION WITH POTENTIAL FOR MARINE LARVICULTURE

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ABSTRACT

In aquaculture, fishmeal represents the main source of aquafeeds due to its nutrient content, palatability, and high digestibility. However, there is increasing interest in reducing the dependence on this resource due to its unsustainability, cost, and supply. Microalgae have emerged as a valuable alternative as a feed/feed supplement due to their ability to produce essential high-added-value metabolites. Proteins, carbohydrates, lipids, pigments, and fatty acids - in particular, docosahexaenoic and eicosapentaenoic acids - are important biochemical components present in microalgal biomass that are crucial for the development and survival of many aquaculture species. Generally, microalgae are used as live feed in the early life stages of different marine organisms and also in the enrichment of live feed (rotifers and brine shrimp). However, the high production costs are one of the largest barriers to employing live microalgae as feed. In this sense, it would be cost-effective to create alternatives such as microalgae-based products. In this study, six microalgae species (*Chlorococcum* sp., *Desmochloris halophila*, *Tetraselmis striata*, *Dunaliella viridis*, *Picochlorum maculatum,* and *Halamphora* sp.) with potential traits for marine larviculture were evaluated for their growth and nutritional value. The different microalgae were cultivated in bubble column photobioreactors, in batch, for 9 days at 20 °C, exposed to continuous LED white light (~128 $\mu mol.m^{-2}.s^{-1}$), and aeration. Microalgal growth was monitored through biomass dry weight and optical density. The cultures were sampled on the last day of the exponential growth phase for biochemical analysis and were characterized in terms of proteins, lipids, pigments, and fatty acid composition. The most interesting nutritional profile was obtained for *Chlorococcum* sp. that revealed high protein (27.32 ± 2.59 %), lipid (12.57 ± 0.32 %) and pigment (Chlorophyll *a*: 46.25 ± 1.19 $\mu g.mL^{-1}$; Chlorophyll *b*: 20.54 ± 0.96 $\mu g.mL^{-1}$) contents. Likewise, *T. striata* showed high contents of polyunsaturated fatty acids (PUFA), mainly *n*-3 PUFA and *n*-6 PUFA, along with excellent growth performance. These results offer a helpful starting point for the development of microalgae-based products with a favorable nutritional profile and the design of large-scale production systems.

Keywords
Microalgae; Growth performance; Nutritional value; Feed; Larviculture

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BIOGRAPHY

Ana Gomes holds a degree in Marine Biology and Biotechnology and a Master's degree in Aquaculture attributed by the School of Tourism and Marine Technology of the Polytechnic of Leiria, Portugal. She has conducted research in species diversification, reproduction, and nutrition in aquaculture (mainly sea urchins and other echinoderms) as a research fellow under the Project MAR-02.01.01-FEAMP-0004: Ouriceira Aqua: Aquaculture and Enhancement of Gonad Production in the Sea Urchin (*Paracentrotus lividus*) at MARE-Polytechnic of Leiria (Portugal). She was a researcher in the Project ALG-01-0247-FEDER-069971 - ALLARVAE: New microalgae formulations for marine larviculture at CCMAR, University of Algarve (Portugal) - MarBiotech Research group where her research was focused on microalgae cultivation, nutrient analysis, molecular biology, and species identification. Ana is author and co-author of six scientific articles in peer-reviewed scientific journals ranked in the 1st and 2nd quartile for aquaculture and food sciences and presented three poster presentations at international meetings.

COMPANY PROFILE

The Centre for Marine Sciences (CCMAR) is a multidisciplinary, non-profit research organization promoting research and education on the marine environment. Emphasis is placed on biological interactions and the sustainable use of resources. CCMAR publishes 150-200 articles annually and, in the last five years, had 15 patents approved and established two spin-off companies.

VIBRO FILTRATION FOR HARVESTING, CONCENTRATION AND REFINERY

Hjelmsmark, H.

SANI Membranes A/S

ABSTRACT

Vibro® Technology is based on the principles of crossflow filtration, but where traditional crossflow filtration applies turbulence to the media to keep the membranes clean, Vibro® Technology applies turbulence at the surface of the membrane instead. This approach offers Four distinct advantages: Firstly, with no stress on the media, this technology offers a very gentle filtration and enables new opportunities in filtration of fragile algae. Secondly, with no crossflow pump and no cooling needed, energy consumption can be reduced by more than 90%. Thirdly, by applying turbulence only on the membrane surface, pressure loss is eliminated and higher concentrations can be achieved. Users of Vibro® Technology has for example achieved concentrations of 150g/l Nannochloropsis and 280g/l Chlorella, while sustaining a high flux of clean, permeated, reusable water. Fourth, Vibro® Technology is linearly scalable, meaning that manufacturers are ensured the same result from when they start with a small benchtop filtration unit to when they scale up to a large industrial unit capable of handling multiple cubic meters per hour.

Vibro® Technology replaces centrifuges, hollow fibers, ceramic membranes and other separation techniques with a single, easy-to-use filtration unit. Vibro® Technology can be used for both batch- and continuous micro and ultrafiltration.

Keywords
Concentration, harvesting, refinery, filtration, separation

PRESENTER INFORMATION

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BIOGRAPHY

Henrik Hjelmsmark is a Mechanical Engineer with a background of many years in the process industry. He is the founder and CEO of SANI Membranes A/S, and the inventor of the Free Flow Plate™ and Vibro® Technologies. From starting his career in project management and technical leadership, Henrik moved on to executive and VP positions in multinational process industry engineering companies.

In 2013 he ventured into creating a business for a new energy efficient and highly sanitary membrane module for micro and ultrafiltration applications. He founded SANI Membranes A/S and developed the patented Free Flow Plate™ membrane module and the patented Vibro® Technology.

COMPANY PROFILE

SANI membranes A/S is a Danish cleantech company delivering unique filtration solutions to Algae, Pharma and Biotech related industries.

The Vibro® Technology, is uniquely ideal for the algae industry as it enables producers to handle harvesting, concentration and refinery in one single unit. Similarly, SANI Membranes is also gaining traction in industries related to production of alternative proteins from sources such as pea and grass and has given presentations at conferences throughout Europe on the advances in filtration brought by the recent invention of Vibro® Technology.

SANI Membranes A/S is selling directly from their office in Denmark and through committed system builders, distributors and OEMs.

EVALUATION OF DIFFERENT MATERIALS AS SUBSTRATE LAYER IN A TWIN-LAYER PHOTOBIOREACTOR

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ABSTRACT

Microalgae, diatoms and dinoflagellates are known producers of a wide variety of economically interesting biomolecules [1]. Mass cultures of this type of microorganisms are usually carried out in suspension reactors [2] in which large volumes of culture must be harvested, usually by centrifugation, to obtain small amounts of biomass. Furthermore, the culture of these organisms could be problematic due to hydrodynamic stress caused in suspension-based closed photobioreactors (PBRs). Biofilm reactors could be an alternative to suspension reactors, since harvesting would be simpler and easier and hydrodynamic stress is not generated[3–5]. Twin layer PBR (TWPBR) is a biofilm reactor that consists of two porous layers, the first is the so-called support layer through which the culture medium continuously diffuses and the second layer is the substrate layer in which the microorganisms are adhered[6]. In the present study, different porous non-woven materials (glass microfiber, NYLON, cellulose acetate and printing paper of 100 $g\cdot m^{-2}$) have been evaluated as substrate layer for the cultivation of the marine dinoflagellate *Symbiodinium voratum* and the marine diatom *Entomoneis* in a TWPBR. The time evolution of the dry weight and the fluorescence of the different microalgae substrates layers were used as indicators of growth. The glass microfiber and NYLON were the materials that showed the best results for both strains. In the case of *S. voratum*, the increased of dry weight after 15 days of culture respect to the initial mass were 2.5 and 2.24 times for fiberglass and NYLON, respectively. For *Entomoneis*, the increase in dry weight respect to the initial mass were of 2 times for fiberglass, and 1.67 times for NYLON. On the other hand, the continuous passage of water through the materials, by diffusion, showed that the printing paper did not have good qualities to be a suitable substrate material since this material disintegrated over time. In conclusion, our study allowed us to evaluate and select glass microfiber and NYLON as appropriate materials for use as a support layer in a TWPBR for the cultivation of *S. voratum* and *Entomoneis*.

Keywords
Microalgae; diatom; dinflagellate;Twin-Layer; biofilm.

Acknowledgements
This research was funded by the General Secretariat of Universities, Research and Technology of the Andalusian Government (Bentofilm project, P20_00785), the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund Program (Project PID2019-109476RB-C22), and the Operative Program FEDER Andalucía 2014–2020 Framework (grant UAL2020-BIO-A2078).

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BIOGRAPHY

My research has focused on the cultivation of marine dinoflagellates to increase the production of biomass and bioactive molecules of high value. I have worked on the design, characterization, optimization, and scaling of culture systems for photosynthetic microorganisms. I have experience in the control and automation of photobioreactors as well as in the optimization of the operating parameters of these devices. I have collaborated with other colleagues to optimize biomolecule separation and purification processes. Since 2020, I am affiliated as assistant professor at the Department of Chemical Engineering of the University of Almería.

SOLID SURFACES PRETREATMENT TO PROMOTE THE FORMATION OF MICROALGAL BIOFILMS

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ABSTRACT

Toxins and other products from microalgae have high potential for the biomedical and agri-food industries, especially in the development of drugs and biopesticides. Among them, the benthic marine dinoflagellates stand out due to the high bioactivity that many of their metabolites present[1]. The production of these metabolites is very limited due to the high sensitivity to agitation of the producing species. For an important group of dinoflagellates such as the genus *Gambierdiscus,*there are no farming systems that allow their mass production[2]. Recently, the development of photobioreactors (FBRs) based on the immobilization of microalgae and biofilm formation has been postulated as a promising alternative to overcome this limitation[3,4]. In the FBRs where the cells are immobilized on a rigid support and submerged (S-FBR) in the medium, the interaction between the first layers of microalgae and the surface of the support are decisive for the formation of a stable microalgal biofilm. The key factors that determine this interaction are the nature of the solid material and the surface treatment of this support[5]. In this work we have studied: i) The surface energy of different materials such as; polyethylene terephthalate glycol (PETG), polycarbonate (PC), polymethyl methacrylate (PMMA), glass (GS), polyvinyl chloride (PVC), polystyrene (PES) and polyethylene (PE), (ii) the adhesion energy between microalgal cells and each material. The microalgae studied are the dinoflagellates *Amphidinium carterae*, *Gambierdiscus* sp., *Prorocentrum lima*. *Symbiodinium* sp. and the diatom *Entomoneis* sp., and (iii) different surface treatments in PETG and GS, selected from the initial adhesion experiment results. The surface treatments have consisted in applying a conditioning layer of proteins (BSA), of the polymer poly-L-lysine (PLL) and intra and extra cellular organic matter of the dinoflagellate Symbiodinium sp. (S-MOI), and the diatom *Entomoneis* sp. (E-MOI) from a 50day culture. Fig. 1 shows biofilm associated with the culture of *Symbiodinium* sp. (Fig.1A) and the culture of *Entomoneis* sp. (Fig.1B) from a 50 day culture. The best combination was glass treated superficially with S-MOI.

Fig. 1. Microalgal biofilm on a glass surface on day 50 of a culture of: A) *Symbiodinium* sp. y B) *Entomoneis* sp.

Keywords
Microalgae; diatom; dinoflagellate; *symbiodinium*; biofilm.

Acknowledgements
This research was funded by the General Secretariat of Universities, Research and Technology of the Andalusian Government (Bentofilm project, P20_00785), the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund Program (Project PID2019-109476RB-C22), and the Operative Program FEDER Andalucía 2014–2020 Framework (grant UAL2020-BIO-A2078).

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BIOGRAPHY

My research has focused on the cultivation of marine dinoflagellates to increase the production of biomass and bioactive molecules of high value. I have worked on the design, characterization, optimization, and scaling of culture systems for photosynthetic microorganisms. I have experience in the control and automation of photobioreactors as well as in the optimization of the operating parameters of these devices. I have collaborated with other colleagues to optimize biomolecule separation and purification processes. Since 2020, I am affiliated as assistant professor at the Department of Chemical Engineering of the University of Almería.

MULTIPLE ABIOTIC STRESSOR EFFECTS ON THE PRODUCTION OF BIOACTIVE COMPOUNDS BY THE MICROALGA *CHRYSOCHROMULINA ROTALIS*

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ABSTRACT

The identification of appropriate environmental culture conditions is a prerequisite for maximizing the production of any microalgal biomass and bioactives thereof. The haptophyte *Chrysochromulina rotalis* has been reported to be a producer of carotenoids, such as fucoxanthin[1], polyunsaturated fatty acids, such as DHA[2], as well as other bioactive compounds[3]. In this study an strain of *Chrysochromulina rotalis* was used to firstly optimize the levels of three abiotic factors (temperature, irradiance level and light color) in 2.5 L bubble columns operated in batch mode. In a second step, the optimal levels found for the three abiotic factors were used to culture *C. rotalis* in pilot scale 80 L bubble columns operated in semicontinuous mode at different dilution rates.
All PBRs were artificially illuminated with RGBWW Light Emission Diodes. pH and temperature were controlled. Kx6 medium was used as culture medium. The daily average irradiance (I_{avg}) ranged from 30 to 637 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and temperature from 15 to 35 ºC. The effect of light color (green, red and blue) were also tested both from the beginning of the culture and from the stationary growth phase.
Regarding results, an I_{avg} of 318 $\mu mol \cdot m^{-2} \cdot s^{-1}$ maximized growth reaching productivity values of biomass, carotenoids and fatty acids of 23.6, 0.08 and 0.68 mg/L·day respectively. In addition, at 25 ºC outcomes of 26.0, 0.63 and 0.74 mg/L·day values, respectively. For green light stress, 10.3, 0.16 and 1.3 mg/L·day values for the above-mentioned concentrations were obtained, respectively. In short, best growing conditions (**S1**) were 25 ºC and 318 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Antiproliferative activity against 4 human tumor lines reached 89.5% of growth inhibition with green light shock at stationary phase while control white light obtained a 60.9%.
With these conditions once reached steady state at dilution selected, the illumination color was abruptly changed to green and maintained during 48 hours (**S2**), reaching similar values to those obtained in smaller scale. Within this system 45 g of biomass with a high percentage of compounds of interest was obtained.

Keywords
Microalgae, OSMAC, bioactive compounds, *C. rotalis*, bubble column, green light

Acknowledgements
This research was funded by the the General Secretariat of Universities, Research and Technology of the Andalusian Government (grant: P18-RT-2477) and the Spanish Ministry of Economy and Competitiveness and by the European Regional Development Fund Program (Project PID2019-109476RB-C22).

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BIOGRAPHY

In 2019 I graduated in the first promotion of Biotechnology at the University of Almeria, same year I took a Biotechnology Master, as I started working as a research support technician in the Department of Chemical Engineering, within the project 'MarBiom' (RTC-2017-6405-1). Recently, in 2020 I enrolled in the Master in Bioinformatics Analysis at the Pablo de Olavide University. Currently I´m in the second year of my PhD in Biotechnology and Industrial Bioprocesses Applied to Agri-Food, Environment and Health, under the direction of M.C. Cerón García, researching in 'BIOPROMAR' (P18-RT-2477 - Search and production of bioactive compounds from marine microalgae through sustainable bioprocesses).

OUTDOOR PHOTOACCLIMATION OF TWO *CHLORELLA* STRAINS CHARACTERIZED BY NORMAL AND REDUCED LIGHT-HARVESTING ANTENNAS: PHOTOSYNTHETIC ACTIVITY AND CHLOROPHYLL-PROTEIN ORAGNIZATION

Masojídek J. [1,2]*

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ABSTRACT

The trade-off of the photosynthetic organisms for having large light-harvesting (LH) antennas is the reduced light-saturation irradiance and their higher susceptibility to photoinhibition. Hence, in laboratory cultures of microalgae, a size reduction of their LH antennas appears to be desirable [1, 2, 3]. In outdoor cultures, such a reduction can increase tolerance to high irradiance in the light-exposed layers of cells and it can also extend the light penetration to deeper culture layers improving the photochemical efficiency (and productivity) of microalgae cultures. From the biotechnological point of view, these features are important for increasing the areal biomass productivity of mass microalgae cultures.

In this work photoacclimation of two natural *Chlorella* strains was studied in outdoor bioreactors. The strain g-120 is characterised by reduced LH antennas while the other R-117 has LH antenna of normal size [4]. The trials aimed to correlate the functional and structural changes in the photosynthetic apparatus, i.e. photosynthetic performance and the composition of chlorophyll (Chl)-protein complexes in photosynthetic membranes to culture growth. *Chlorella* g-120 is typical by a low Chl/biomass ratio (< 0.5% of dry weight), which is about four times lower compared to *Chlorella* R-117. Measurement of the effective absorption cross-section of PSII centres showed a markedly reduced functional antenna size in *Chlorella* g-120 as compared to R-117. This was in agreement with the results of the native electrophoretic analysis demonstrating the lack of high molecular mass Chl-binding protein supercomplexes, i.e. PSII and PSI cores associated with LHCs in *Chlorella* g-120. However, this attribute was not reflected by the improved photochemical efficiency of this strain compared with the normal antenna-size *Chlorella* R-117 strain.

We have found that *Chlorella* g-120 represents a typical, natural, reduced antenna-size strain due to its Chl-protein composition. In outdoor trials, *Chlorella* g-120 also showed significantly lower oxygen production and electron transport rate measured *in-situ*, increased futile energy dissipation via non-photochemical quenching and higher respiration rates compared to normal antenna-size *Chlorella* R-117. These data indicate that the potential use of microalgae strains with reduced LH antennas for outdoor mass cultivation may not be as straightforward as anticipated from laboratory experiments.

Keywords
Chlorophyll-protein; Chlorophyll fluorescence; Light-harvesting antenna size; Microalgae; Oxygen-production; Photosynthesis

Funding
This research was funded by the EU H2020 programme, project SABANA (grant no.727874).

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BIOGRAPHY

He obtained MSc. degree in biochemistry at Charles University in Prague; PhD in microbiology at the Institute of Microbiology in Prague; professorship at the Faculty of Science, University of South Bohemia and Doctor of Science recognition at the Czech Academy of Science. He has gained more than 45 years of experience in the culturing, physiology and photosynthesis of microalgae; at present he is a senior researcher and the leader of the Phototrophic cultivation team in the Laboratory of Algal Biotechnology at the Centre Algatech; last 25 years involved in microalgae biotechnology studying microalgae mass cultures with special focus to photosynthesis monitoring by Chl fluorescence techniques which have been used to characterize microalgae strains, optimize cultivation regimes, correlate photosynthetic performance with growth in laboratory and outdoor cultivation systems. He also lectures at the Faculty of Science of the University of South Bohemia in České Budějovice.

COMPANY PROFILE

Centre Algatech Třeboň is a part of the Institute of Microbiology of the Czech Academy of Science, a public research institution with headquarters in Prague. The Centre consists of four laboratories focused on photosynthesis, cell cycles, biotechnology of microalgae and photosynthetic bacteria.
The University of South Bohemia in České Budějovice was established in 1992; it has gained credit in the field of natural science, agriculture and fisheries.

EFFICACY OF A NATURAL MICROALGAE-BASED BIOACTIVE ON COGNITIVE PERFORMANCE OF GAMERS

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ABSTRACT

Considering the important growing development of gaming in the world, this research area has developed considerably over the last few years. Even if it seems to be well admitted that video gamers showed better cognitive functions parameters compared to non-video gamers there is a constant concern for optimizing performance.

Since microalgae can be cultivated from industrial photobioreactors with eco-friendly production processes (Patented technologies by Microphyt), it represents a sustainable alternative as both a large source of almost untapped bioactive molecules and meet societal challenge as the maintain of biodiversity [1]. One of the major carotenoids found in microalgae and well known for its nootropic benefits through it anti-inflammatory and antioxidant activities on different signaling pathways able to cross brain-blood barrier is the Fucoxanthin. GamePhyt™ is a blend of (1) a microalgae-based bioactive (extract from *Phaeodactylum tricornutum*) containing several molecules, in particular Fucoxanthin, clinically shown to improve cognitive function parameters [2] and (2) natural guarana to sustain a high level of energy and reaction time during long gaming experience. Thus, the purpose of this study was to evaluate whether acute and 30-days supplementation with GamePhyt™ would affect cognitive function and gaming performance in experienced video gamers.

In a double-blind, placebo controlled manner, 51 male and 10 female experienced gamers (21.7±4 years, 24.2±3.6 Kg/m^2) were randomly assigned to ingest a Placebo (P); Low-dose (L) or High-dose (H) of GamePhyt™. The Psychology Experiment Building Language (PEBL) was used to administer a cognitive function test battery.

As highlighted in Figure 1a), Gamephyt™ significantly improves short term and working memory evaluated during Sternberg task test performed at baseline visit after 60 minutes of gaming session while limiting task difficulty level effect with better benefits in High-dose group.

On Figure 1b), the data showed a significant improvement of sustained attention and response control during Go/No-Go task after 30 days of supplementation with both low and high doses.

Based on all the data obtained, we can conclude that GamePhyt™ improves several cognitive performance parameters as processing speed, reaction time, accuracy, decision making and mental fatigue of experienced gamers after both only one intake and 30-days of supplementation.

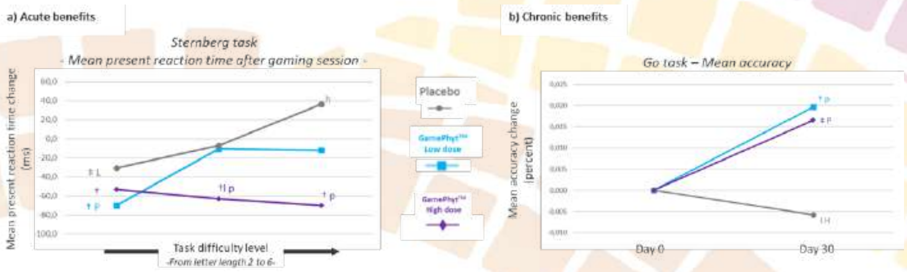

FIGURE 1. Data are means and 95% confidence intervals. a) Mean reaction time change (ms) in Sternberg task after gaming session during baseline visit (only one intake) b) Mean accuracy change (percent) in Go task after 30 days of supplementation. Changes from baseline are shown as † (p<0.05 change from baseline) and ‡ (p>0.05 to p<0.10 trends from baseline). Small case letters indicate p<0.05 differences from placebo (p), low dose (l), or high dose (h) while upper-case letters (P, L, H) indicate trends (p>0.05 to p<0.10). Data were analyzed by General Linear Model (GLM) univariate analyses with repeated measures using weight as a covariate and mean and percent changes from baseline with 95% confidence intervals.

Keywords
Fucoxanthin; Microalgae; Cognition; Gamers; Decision making

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BIOGRAPHY

Jonathan joined the Microphyt company as a clinical research manager in September 2020. Since then, he developed and set up several international pre-clinical and clinical research projects with the main goal to evaluate the efficacy of microalgae-based bioactives in different research areas (cognitive function, sports and nutrition, weight management etc.) meeting marketing perspective applications. Before joining the company, he completed a joint PhD in Human Movement Science (Apr 2012 – Dec 2015), under a CIFRE grant between University of Montpellier PhyMedExp Laboratory INSERM U1046 (University of Montpellier; Supervisors: Pr Maurice HAYOT and Pr Christian PREFAUT) and the Clinique du Souffle La Solane (Fontalvie Group; Scientific referent: Dr Nelly HERAUD), where he investigated the efficacy of antioxidant supplementation during rehabilitation program on muscle performance and exercise capacity in patients with Chronic Obstructive Pulmonary Disease (COPD). After his PhD and a one-year experience as clinical research engineer at Montpellier University Hospital Center, he worked for Mooven company to 1/ develop the clinical research organization specialized in non-pharmacological interventions and 2/ set up clinical research project to evaluate the efficacy of adapted physical activity program using videoconference in disease conditions.

COMPANY PROFILE

At the crossroads of life sciences and bio-processes, Microphyt develops the next generation of natural ingredients for a better living. Studying the physiology of microalgae and the potential of their bioactives, Microphyt can draw on new ways of addressing the key societal challenges with high added-value active ingredients for nutraceuticals and cosmetics, thanks to the amazing diversity of microalgae.
Microphyt have developed and patented the first ever hydro-biomimetic technology for growing microalgae: CAMARGUE photo-bioreactors. Based on a two-phase flow process, they reproduce the natural conditions microalgae need to grow, while optimizing gas-liquid transfer. This ground-breaking innovation makes it possible to cultivate any kind of microalgae including fragile varieties whose cultivation was previously impossible. Microphyt's technology and expertise allowed us to unlock the potential of new microalgae and design new of their kind bioactives ingredients for health, nutrition and beauty.
From the strain identification until the ingredient production and application into human in a safe and efficient way, Microphyt is a leading company for the next generation of natural active ingredients.

ADVANCING THE STATE OF TECHNOLOGY IN ALGAE R&D

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ABSTRACT

Arizona State University's Arizona Center for Algae Technology and Innovation (AzCATI) was established in 2010. Anchored by a world-class algae laboratory and four-acre outdoor testbed, AzCATI has been at the forefront of algal R&D in the United States with funding from the Department of Energy's Bioenergy Technologies Office (BETO) which has a long and successful history of funding an algae R&D portfolio driving progress towards affordable and renewable energy and co-products from algae biomass. A core capability established by BETO was the support of testbed facilities such as AzCATI. An experimental and operational framework to carry out long term cultivation trials to assess the State of Technology (SOT) for algal biomass productivity and road-test new algal technologies and strains has been ongoing since 2015. Since the summer of 2018, the SOT framework established by AzCATI has continued under a BETO funded national lab-led consortium called DISCOVR "Development of Integrated Screening, Cultivar Optimization, and Verification Research." The DISCOVR team constitutes a powerful partnership among four of the DOE's seventeen national laboratories: Los Alamos National Laboratory, National Renewable Energy Laboratory, Pacific Northwest National Laboratory (DISCOVR lead institution), and Sandia National Laboratories. With ASU's AzCATI, they have joined forces to investigate algae strains that could lead to sustainable and clean algal biofuel and continue to generate open and transparent cultivation data sets for use by the algae biotechnology R&D community. In this talk, we will present the latest SOT results from the past 5 years of cultivation. With recent advancements utilizing better cultivars verified thru DISCOVR's pipeline, better crop protection and improved operational conditions, an improvement in biomass productivity and reliability has led to a year over year improvement in annual average productivity from the 2018 baseline of 11.7 g/m2-day to 19.0 g/m2-day for 2022 – a 60% improvement from 2018 thru 2020. We will discuss these results in detail, in particular improvements that led to the year over year increases and highlight where AzCATI and DISCOVR are headed in 2023 and beyond.

Keywords
Cultivation, open ponds, crop protection, process control

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John McGowen is currently serving as Director of Operations and Program Management for the Arizona Center for Algae Technology and Innovation (AzCATI) at Arizona State University and responsible for the overall integration of the academic and industrial collaboration, project communications, personnel management, budget management, and day to day project lifecycle for AzCATI. Research focus is on the identification and implementation of best practices in algae growth and processing, standardization of methodologies and the ongoing implementation of the state of technology experimental framework for conducting long term cultivation trials that was established through the Algae Testbed Public Private Partnership (ATP^3) with funding from the Department of Energy's Bioenergy Technology Office (BETO). Served as Director of Operations and Program Management and Co-PI for ATP^3 from 2013-2019, and currently principal or co-investigator on multiple federal and industry sponsored research and development projects with a continued focus on improving algal biomass productivity and quality in outdoor cultivation, new sensor and production system development, and implementation of crop protection best practices. Dr. McGowen has a PhD in polymer science and worked for Motorola, Amersham Biosciences and GE Healthcare prior to joining ASU in 2005 and has been Director of Operations for AzCATI since its founding in 2010.

COMPANY PROFILE

The Arizona Center for Algae Technology and Innovation (AzCATI) harnesses algae technology to produce renewable energy, food, feed and other valuable products, while performing environmental services to support a more sustainable future for society.

Our core competencies are organized into three main platforms: Research, Education and Training, and Services. These platforms enable AzCATI team members to focus on their strengths and maximize our ability to interact effectively with existing and potential collaborators, and respond effectively to new funding calls from federal, state and industry sources. Strong and competent staff resources under Operations helps to streamline internal processes, raise awareness through communications and respond quickly to internal and external research collaboration, technology and market sector needs.

EFFECT OF NICKEL AND IRON STRESS ON PHOTOSYNTHESIS AND METABOLITE PRODUCTION OF *HETEROCASPA CF. BOHAIENSIS* (DINOFLAGELLATE)

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ABSTRACT

Microalgae are well known to produce valuable compounds in response to abiotic stresses. Among these stresses, metals can influence the production of primary or specific metabolites related to reactive oxygen species formation (Barra *et al.* 2014; Chen *et al.* 2017; Paliwal *et al.* 2017). *Heterocapsa* cf. *bohaiensis,* a newly described dinoflagellate species with haemolytic activity, and associated with shrimp (*Penaeus japonicas*) and crab (*Eriocheir sinensis*) mortalities (Xiao *et al.* 2018; Zhang *et al.* 2019), has been recently isolated in New Caledonian iron and nickel-rich waters (Merrot *et al.* 2022; Pelletier 2006). To induce the metallic stress and the production of metabolites, we exposed *H.* cf. *bohaiensis* to high concentrations (10^{-3}M) of nickel (Ni^{2+}) and/or iron (Fe^{2+}) and investigate its photosynthetic efficiency in continuous culture using Pulse Amplitude Modulated chlorophyll fluorescence. The results indicates that *H.* cf. *bohaiensis* is tolerant to Ni^{2+} but sensitive to Fe^{2+} high concentrations, without cell death. JIP-test suggest that the reduction of photosynthesis in response to Fe^{2+} is due to a disruption in the electron transport chain rather than a defect in the PSII light absorption and trapping which are enhanced by Fe^{2+}(Adamski *et al.* 2011; Redillas *et al.* 2011; Strasser *et al.*). These results highlight the toxic mechanisms of Fe^{2+} in microalgae and help to understand the production of biomass under metallic stress. Studying this species in this particular environment will also bring knowledge in the field of aquaculture and ecotoxicology, owing to the transfer of metals in the food chain (bioaccumulation, biosorption) and to the possible production of toxins. This study will also provide some insights for potential valorisations in the biotechnology and/or cosmetics fields, through the analysis of primary (pigments, fatty acids / antioxidants) and specific metabolites, with interesting activities, produced under metal stress conditions.

Keywords
Dinoflagellate, Nickel, Iron, Metabolomic, Photosynthesis

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BIOGRAPHY

Vincent Meriot is currently a PhD student at the University of New Caledonia working with the Institute of Applied and Exact Sciences (ISEA), Ifremer and Adecal TECHNOPOLE. He is working on the ecophysiology and metabolome of New Caledonian microalgae with a focus on the effect of metallic trace elements. He obtained his Master's degree, in "Applied Blue Biotechnology", at the University of La Rochelle. During his Master, he had the opportunity to do 2 internships in the field of microalgae. These two internships confirmed his interest for microalgae and reinforced his skills in culture and ecophysiology through studies on the effects of environmental parameters on microalgae... He then decided to do a PhD in New Caledonia in order to participate in the improvement of knowledge and in the development of the blue economy in the country where he grew up. Indeed, the PhD is part of a program aiming to develop a microalgae production chain in New Caledonia (Adecal/Ifremer). And thanks to the multidisciplinary of the project, he is acquiring solid skills in photophysiology, ecophysiology, natural substances chemistry and metabolomics.

SUSTAINABLE H_2 PRODUCTION AT VARYING LIGHT CONDITIONS AND AT THE INTENSITY OF SUNLIGHT BY THIN CELL LAYER CULTURES OF *CHLAMYDOMONAS REINHARDTII*

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ABSTRACT

Photobiological H_2 production has the potential of becoming a carbon-free renewable energy source, because upon the combustion of H_2, only water is produced. The [Fe-Fe]-type hydrogenases of green algae are highly active, although extremely O_2-sensitive, which has been the major limitation in exploiting algal H_2 production ever since its discovery.

We developed a sustainable H_2 production protocol for the green alga *Chlamydomonas reinhardtii*, which is based on a short anaerobic incubation in the dark, followed by continuous illumination during which the Calvin-Benson cycle is kept inactive by substrate limitation. Hydrogenase activity is preserved by employing a simple absorbent to remove the evolved O_2. Under these conditions, the cultures remain photosynthetically active for several days, and the electrons feeding the hydrogenases mostly derive from water. The amount of H_2 produced by a commonly used wild type strain (CC-124) is higher as compared to earlier methods and the process is photoautotrophic (Nagy and Tóth, 2017, European Patent Application 17155168.2.; Nagy et al., 2018, Biotechnology Biofuels 11:69).

Our main goal is to make significant progress towards a marketable and economical H_2 producing technology by optimizing cultivation conditions and selecting suitable photosynthetic mutants. We achieved an additional, several fold increase in the H_2 production yield by using thin-layer alga cultures in a 1-L photobioreactor at 350 and 1000 µmol photons $m^{-2}s^{-1}$ light intensities. Under these conditions the *pgr5* photosystem I cyclic electron transport mutant produced ~250 % more H_2 than the CC-124 strain (Nagy et al., 2021, Bioresource Technol 333: 125217).

Recently, we constructed an online gas chromatography system with automated sampling for continuous monitoring of H_2 production in a thin layer photobioreactor. We found that the *pgr5* mutant performs equally well under simulated natural daylight regimes than in continuous light.

Keywords

Hydrogen, photoautotroph, Chlamydomonas, photosynthesis (max.5 keywords)

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Nagy V, Podmaniczki A, Vidal-Meireles A, Kuntam S, Herman É, Tóth D, Kovács L, Scoma A, Tóth SZ (2021) Thin cell layer cultures of *Chlamydomonas reinhardtii* L159I-N230Y, pgrl1 and pgr5 mutants perform enhanced hydrogen production at sunlight intensity Bioresource Technol 333:125217

Nagy V, Podmaniczki A, Vidal-Meireles A, Tengölics R, Kovács L, Rákhely G, Scoma A, Tóth SZ (2018) Water-splitting-based, sustainable and efficient H2 production in green algae as achieved by substrate limitation of the Calvin-Benson-Bassam cycle. Biotechnol Biofuels 11: 69

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BIOGRAPHY

Valéria Nagy studied Biology at the University of Szeged and she obtained her university degree in Biology in 2008. As a PhD student, supervised by Drs. Győző Garab and Tóth Szilvia Zita at the Institute of Plant Biology, Biological Research Centre, Szeged, Hungary, she investigated ascorbate-dependent alternative electron transport in higher plants and the role of ascorbate in the regulation of hydrogen production in *Chlamydomonas reinhardtiii.* She defended her PhD in 2015 and then she started to work as a research associate in the Molecular Photobioenergetics Group (Biological Research Centre, Szeged) and focused on the hydrogen production in green algae. She spent one and half year as a postdoctoral researcher in the group of Assoc. Prof. Yagut Allahverdiyeva-Rinne, at the University of Turku (Finland). Since she returned to Szeged in 2019, she has been working on the development of a sustainable photoautotrophic hydrogen system for green algae.

COMPANY PROFILE

The Biological Research Centre (BRC) Szeged is an internationally acknowledged institution. The four institutes of BRC - the Institutes of Biophysics, Biochemistry, Genetics and Plant Biology - employ about 260 scientists whose work is hall-marked by international scientific publications and patents.

ESTABLISHMENT OF A *FUCUS VESICULOSUS* MATERNITY FOR AQUACULTURE

Pereira, R.[a] (Presenting)

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ABSTRACT

Fucus (brown seaweed) is one of the most commonly used species in Europe. Brown seaweeds are widely used for different applications, including their direct consumption as food or supplements (by animals and humans) or as feedstock for the extraction of alginate and bioactive components[2]. Currently, a large number of high-value components can be extracted from brown seaweed, such as carotenoids, carbohydrates (e.g. fucoidan and hydrocolloids), fatty acids, sterols and phenols, among others.

Despite the interest of the industry, *Fucus* biomass is solely obtained from the exploitation of natural sources in Northern Europe, being one of the most harvested seaweeds in the EU[1]. However, as with all natural resources, a responsible and sustainable exploitation will necessarily mean there is a limit for the naturally available biomass. Adding to that, annual availability of the biomass is always uncertain as well as its chemical characteristics, preventing further developments and applications for *Fucus*.

In order to answer to the increasing needs of a developing EU seaweed industry[3], project Biofábrica was designed, to demonstrate the feasibility of *Fucus* production in aquaculture for the extraction of valuable compounds in a biorefinery.

Due to the very limited capacity of *Fucus* to reproduce vegetatively and grow in a consistent way, we decided that aquaculture production should include a maternity/nursery. This communication reports on the first steps towards that goal, based on induction of gamete release, fertilization, egg manipulation and environmental conditions to promote germlings survival and growth. One of the growth parameters tested, was light intensity, which around 90 PFD (µmol photons m^{-2} s^{-1}) seems to favor germling survival rate (40% higher) compared to higher light intensities. On the other hand, in a subsequent stage germlings at 135 PFD grew on average twice as much (0,88mm/week) when compared to lower light intensity (0,42mm/week).

Biofábrica has been able to successfully and reproducibly produce germlings monthly, over the past 8 months and successfully grow individuals in the lab up to 20cm long (still growing). To the best of our knowledge, this is the first maternity/nursery successfully established for *Fucus*, integrated in a on-shore aquaculture production facility.

Keywords
Seaweed; Macroalgae; *Fucus*; Aquaculture;

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3- Mendes M.C; Sofia Navalho S.; Ferreira A.; Paulino C.; Figueiredo D.; Silva D.; Gao F.; Gama F.; Bombo G.; Jacinto R.; et al. Algae as Food in Europe: An Overview of Species Diversity and Their Application. Foods 2022, 11, 1871.

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BIOGRAPHY

He is since 2020 the Head of Seaweed Division at A4F – Algae for Future, a company with more than 15 years of experience in the field of algae biotechnology. Until 2020 was research director at Algaplus, Lda, a company he co-founded in 2012 and where he occupied different roles.
Academically trained as a Biologist by the University of Porto, with a specialization in scientific research and with a particular interest for applied research, obtained his PhD in 2004, with work developed in between the University or Porto and the University of Connecticut (UConn, USA). The interest for applied research with seaweed started immediately after finishing his BSc and continued until present.
Before the transition to the business world, developed a career as post-doc researcher at the Centre for Marine and Environmental Research (CIIMAR) with passages through the Marine Biotechnology Laboratory at UConn e through the Bridgeport Regional Aquaculture Science and Technology Centre (CT, USA), also teaching and working with young grad and undergrad students.
As researcher and including also the years within the business field, he accumulates the experience of participation in more than 20 national and international projects, published 8 book chapters and more than 40 scientific papers in peer reviewed journals. He also serves as invited reviewer for several of those scientific journals, was member and co-chair of the Expert Working Group on Macroalgae Culture, under the context of project Eklipse and is currently the leader of WG02-Ulva in aquaculture of the Cost Action SeaWheat.

COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our customers' business. We work closely with our customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions, circular economy opportunities and customer goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

PERFORMALGAE: ESTABLISHMENT OF HIGH-PERFORMING INDUSTRIAL MICROALGAE CULTURES FOR THE PRODUCTION OF BIOSTIMULANTS AND FUNCTIONAL FEEDS

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ABSTRACT

PERFORMALGAE is a Portuguese project (PT2020) that joins a multidisciplinary consortium led by Allmicroalgae, with the support of GreenCoLab and the University of Algarve (UALG), which aims to transfer knowledge from academia to the industry and bring innovative products from the industry to the market. The key objective of PERFORMALGAE is to develop microalgae strains with improved performance targeting a high production of relevant metabolites with optimized bioavailability, in order to obtain novel and highly valued products for the markets related to agriculture biostimulants and aquafeed functional ingredients. To this end, PERFORMALGAE relies on establishing microalgal strains whose phenotypes are improved by high-throughput screening techniques (random mutagenesis and flow cytometry) to produce improved productivities of the target metabolites. Another innovative aspect is the increased compound bioavailability through the optimization of cellular disruption and hydrolysis processes to increase algal bioactivities for the proposed applications. The ultimate goal is to develop at least two new commercial products with biostimulant and biopesticide activities. Both products target the plant nutrition market, since the selected microalgae (*Chlorella vulgaris and Scenedesmus obliquus*) are known sources of phytohormones (auxins, gibberellins and cytokinins) among other compounds with biostimulant and biopesticide activities. Simultaneously, the foundations of a novel line of algae-based functional additives, targeting the aquaculture market is also being evaluated. These additives are currently being exploited in strains with known antioxidant and immunostimulant properties to improve the performance and resistance of fish early stages. For this purpose, *Nannochloropsis oceanica* and *Phaeodactylum tricornutum,* with improved contents and availability of high-value nutrients (mainly polyunsaturated fatty acids and pigments) are currently under development. Overall, the results obtained to date led to the isolation of several mutants with improved phenotypes as well as defined protocols that significantly increase the bioavailability of the biomasses for the agriculture and aquaculture sectors.

Keywords
Algae, Biotechnology, Applications, Improved strains, Aquaculture, Agriculture

Funding
PERFORMALGAE project ALG-01-0247-FEDER-069961

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Hugo Galvão Pereira is currently the General Coordinator of GreenCoLab, a novel collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of macro- and microalgae as an essential component for different industries. Previously, he was a research collaborator in the MarBiotech group (Centre of Marine Sciences, Portugal) and in the R&D departments of Necton S.A. (Belamandil, Portugal) and AllMicroalgae (Pataias, Portugal). For his MSc thesis, he worked on the optimization of a novel culture medium for large-scale production of microalgae in photobioreactors at Necton S.A. He completed is PhD at the University of Algarve (CCMAR), which focused on the production of biomass at industrial scale as well as in the establishment of a microalgae-based biorefinery for *Tetraselmis* sp. CTP4 for different biotechnological applications, in collaboration with the National Laboratory of Energy and Geology (LNEG, Lisboa, Portugal), Sparos Lda. (Olhão, Portugal) and AllMicroalgae.

COMPANY PROFILE

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law.
GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology. The GreenCoLab's multidisciplinary team covers expertise across the whole algae value chain; from the lab to large scale production, strain optimization, scale-up, harvesting, biorefining to commercialization.

BENTHIC DIATOMS CULTIVATION IN ARTIFICIAL HYDROGELS

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ABSTRACT

Diatoms contribute to one fifth of earth's photosynthetic production, being responsible for silicon turnover in the oceans and for most of the primary production in intertidal mudflats[1,2]. Different diatom species have found applications in industry: for omega-3 and fucoxanthin production, in medicine as drugs delivers, and thanks to high amounts of triacylglycerols accumulation, they are good candidates for biodiesel production[3,4]. They possess a highly diverse silicon frustule, which has several biotechnological applications: e.g., cleansing agents, filters, defoliants and more recently its properties have been explored to build biosensors and photodetectors[5,6]. Most studies focus on pelagic diatoms, easier to isolate and cultivate, but benthic species properties are still unexplored, being also less available in the algal banks[7,8].

Mudflat benthic species need a physical support to grow on, and once collected are difficult to keep alive, especially big size species (200-400 µm) having a slow growth rate[9]. The growth of these diatoms in natural biopolymer-based hydrogels has already found successful results in Na-alginate[10]. In our study we use carrageenan hydrogel which is more resistant and do not allow cell leakage. Big size cells cultivation (*e.g., Pleurosigma angulatum*, *Gyrosigma balticum*, *Nitzschia spathulata*) was compared in suspension and in hydrogel, resulting in a longer exponential phase and a total higher yield of final biomass in hydrogels. This could be an advantage to isolate new strains and extract new compounds. Currently, diatoms are mainly cultivated in open ponds and closed PBRs, where in the first, easily contamination occurs and the second prevents the light penetration[11]. Additionally, none of these methods would be feasible for benthic species cultivation. Upscale these cultures to large scale using hydrogel photobioreactors would require less water as the gel has a 90% hydration, harvesting would be more energy-efficient as the biomass would be already concentrated and could be collected through the lens tissue method applied for the species collection from the sediment[12]. Lastly, the capability of growing big size diatoms along with a longer growth curve would be advantageous for a higher accumulation of lipids.

Keywords
Diatoms, microphytobenthos, hydrogels, culture system, new compounds

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BIOPROSPECTING FOR BEHAVIOR-INTERFERING BACTERIA IN MIXED CULTURES WITH *CHLORELLA VULGARIS*

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ABSTRACT

The interactions between microalgae and bacteria are complex and specific among microbial species. Because of the vast molecular and biochemical responses, the relationship of a microbial consortium is still poorly understood. However, the coexistence of these microorganisms can lead to the enhancement of traits aimed at a large-scale production of microalgae, such as growth promotion, improved biomass quality, or a more efficient harvesting process. The objectives of the current work were to prepare microalga-bacteria mixed cultures and monitor the behaviour of microalgae that may benefit the industrial production of *Chlorella vulgaris* biomass leading to higher productivities as well as enhanced biomass quality. To this end, *C. vulgaris* grown in industrial tubular photobioreactors were supplied by Allmicroalgae (Pataias, Portugal), and different bacteria were isolated from the existing natural microbiome in the microalgal culture. After that, bacterial isolates were selected from genera previously reported as beneficial to algae and plants and co-cultured with *C. vulgaris* at a laboratory scale. Cultures were prepared for co-inoculation in a microalga:bacteria ratio of approximately 1:10 and maintained in an incubation chamber with controlled conditions of temperature (30 °C), light intensity (100 mmol m^2 s^1) and shaking (180 rpm). Culture performance was assessed by optical density (λ=600, 680 nm), microalgal cell counts, and bacterial colony-forming units (CFU). Microalgal biomass was stored to perform biochemical characterization of protein and vitamin contents and fatty acids profile. Preliminary results show that from the 22 tested bacterial strains grown in mixed cultures, 36% negatively affected microalgal growth, 28% had no effect, and 36% improved microalgal cell concentration compared with an axenic *C. vulgaris* control. One of the best performing bacterial genera was *Acinetobacter*, with strains of this genus improving system productivity by 30%. Conversely, *Aeromonas* bacteria promoted algal death. The reported effects may unravel interactions among naturally occurring bacterial communities and *C. vulgaris*, representing a potential source of growth-enhancing compounds.

Keywords
Microalgae behaviour; Co-culture; Microbial interactions; Biotechnology;

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BIOGRAPHY

Master’s in molecular and Microbial Biology at the University of Algarve (2018/20) with an internship at University College Cork through the ERASMUS+ Program. Graduated in Biological Sciences, with Valedictory Honors, at the University of Vale do Itajaí (2012/16), where she was a member of the research group "Applied Molecular Genetics and Biotechnology" and "Bioprospection of Marine Microorganisms". She has expertise in molecular biology, microbiology and quality control, bioprospecting for bioactive compounds, and bioremediation. She has published 7 congress communications, one of them awarded with a high scientific merit prize on a poster session. She currently has a research fellowship at the MarBiotech Research group (CCMAR/University of Algarve) to select and analyze microalgae strains with high biotechnological potential in aquaculture.

COMPANY PROFILE

The Centre for Marine Sciences - CCMAR - is an independent, non-profit research organization and is one of the main research centers in marine sciences in Portugal. With a multidisciplinary team of about 250 scientific researchers, that aims to promote research and training in the various facets of the marine environment.

Allmicroalgae – Natural Products S.A. one of the largest European producers of microalgae is a leader in premium-quality microalgae biomass, food safety and innovation. The company’s production plant, which employs more than 30 highly specialized people, is located in Leiria, Portugal, from where it delivers microalgae solutions for food & beverage, nutraceutical, feed and agro applications all over the world. Optimising culture conditions to maximize production, biochemical profile and functional activities of its microalgal biomasses are at the core of the company’s R&D activities . Committed to sharing knowledge and technology at the highest safety standards, Allmicroalgae produces microalgae via auto- and heterotrophic pathways where fermenters, flat-panels, tubular photobioreactors and open raceways ponds enable a large-scale production.

FLASHING LIGHT STIMULATES PIGMENT AND PUFA PRODUCTION BUT DOES NOT IMPROVE GROWTH OF MICROALGAE

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ABSTRACT

Phototrophic microalgae use light to produce biomass and high-value compounds, such as pigments and polyunsaturated fatty acids (PUFA), with high relevance for food and feed applications [1]. This work aimed to evaluate the effect of different flashing light conditions on the growth performance and biochemical composition of several microalgae strains (*Nannochloropsis gaditana*, *Koliella antarctica*, *Tetraselmis striata* and *Diacronema lutheri).* Photosynthetic rates of cells under flashing light regimes did not improve compared to those of cells under continuous light. These conclusions were valid either for dilute or concentrated microalgae cultures exposed to flashing light frequencies ranging 0.01 Hz–1 MHz with duty cycles of 0.001–0.7 and time-averaged light intensity of 50–1000 µmol s^{-1} m^{-2} [2]. The flashing light conditions (frequency and duty cycle) at which the photosynthetic performance was similar to continuous light (the so-called *flashing light effect*) depended strongly on cell acclimation, culture concentration, and light intensity. However, flashing light at low frequencies (<50Hz) and short duty cycles (0.05) applied during the final growth stage for 3 days induced high-value PUFA and pigments in various microalgal strains (*N. gaditana*, *K. antarctica*, *T. striata* and *D. lutheri* [3,4]). For example, fucoxanthin, diatoxanthin, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids productivities in *D. lutheri* under the aforementioned flashing light condition increased up to 4.6 fold, while productivities of lutein, zeaxanthin and EPA in *T. striata* increased up to 1.3 fold compared to that of cells under continuous light. On the other hand, these conditions decreased biomass productivity in *D. lutheri* 2-fold while *T. striata* remained unaffected compared to continuous light treatment. In conclusion, artificial flashing light does not improve microalgal biomass productivities in photobioreactors, but low frequencies (f < 50 Hz) are promising to improve PUFA and pigment production when applied at a final growth stage during production. Short-term treatments of flashing light may be promising for industrial algal production as a finishing step to increase biomass value.

Keywords
Flashing light, microalgae, pigments, fatty acids, artificial light

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flashing light in *Diacronema lutheri* and *Tetraselmis striata* CTP4. Bioresource Technology Reports, 19, 101158.

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Peter completed his professional education as an electrician in the field of solar energy, a BSc in Marine Biotechnology from the University of Applied Science in Bremerhaven (Germany), a MSc in Aquaculture from the University of Algarve in Faro (Portugal) and a PhD in Aquatic Biosciences from Nord University in Bodø (Norway). Peter is researcher at the GreenCoLab, representing the partner company Necton S.A. in this collaborative laboratory. During his research career at different research institutions in Europe and Israel, he published multiple peer-reviewed articles on microalgae with a focus on light requirements for optimal growth and biomolecule induction. Being an algal biotechnologist, he has strong skills in experimental design, algal cultivation and photosynthetic measurements. Peter joined the GreenCoLab with the motivation to create jobs in the microalgal sector, advance R&D and develop new technologies that lead to an improved algal production at companies associated with the GreenCoLab. His main tasks are project elaboration, leading the AlgaSynTec workgroup, coordinating the ALGACYCLE and ALGAESOLUTIONS project, training students and the development of artificial light-based algae production prototypes.

COMPANY PROFILE

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law. GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology. The GreenCoLab's multidisciplinary team covers expertise across the whole algae value chain; from the lab to large scale production, strain optimisation, scale-up, harvesting, biorefining to commercialization.

EVALUATION OF LIGHT QUALITY, TEMPERATURE AND NUTRITIVE DEPRIVATION IMPACT ONTO STARCH ACCUMULATION IN *CHLORELLA VULGARIS*

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ABSTRACT

SEALIVE (1) and Nenu2PHAr (2) European projects are both dedicated to produce biodegradable and biosourced plastics. Microalgae can provide material for the manufacture of plastics currently obtained from petrol or food crop (3). Starch is notably used as a natural biopolymer base to be integrated in plastics blends (4), and can also be degraded into monomeric glucose to feed PHA producing bacteria (5).

Several external factors act as signals to redirect the metabolism towards the production, polymerization and storage of glucose (6). Notably, nutrient depletion is known to promote both carbohydrates and lipid pathways. However, in a continuous culture mode, nutrient removal from culture media can be difficult to achieve and other stress inducers might be preferred.

Recently, other types of stress have been shown to lead to starch accumulation in green microalgae (7). Supra-optimal temperatures were shown to trigger starch accumulation in *Parachlorella kessleri* and *Chlamydomonas reinhardtii* (7) (8). The depletion of blue light from the light spectrum was described as a carbohydrates enhancing factor in *C. reinhardtii* (9).

Chlorella vulgaris CCALA924 was identified as a high starch producer relevant for industrial scale cultivation (10). This strain was described to accumulate starch up to 60% of dry weight when submitted to a physiological stress. Here, its ability to accumulate starch under nutrient deprivation, high temperature or blue light free spectra was tested and content of at least 40% of starch was obtained at laboratory scale. Interestingly, starch structure had almost no amylose when produced under blue light free spectra, whereas nutrient deprivation and high temperature conditions lead to 15% of amylose.

These lab results should be tested at pilot scale in order to evaluate the technico-economical relevancy of those new means of producing starch in microalgae.

Keywords
Chlorella vulgaris, physiological stress, nutrient deprivation, supra-optimal temperature, light quality, starch, amylose, bioplastics

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BIOGRAPHY

Junior algaeneer working at CEA, France. PhD student for 2 years, its project focus on starch accumulation in Chlorella for bioplastics applications.

COMPANY PROFILE

CEA is a leading Research and Technology Organization in the field of energy. The microalgae bioprocesses (MAP) platform can produce tailored algal biomass using different cultivation/harvesting technologies, from lab to semi-industrial scale. Biological and chemical characterizations and techno-economic modelling are also performed. MAP is involved in collaborative R&D projects with public and private stakeholders.

EVALUATION OF BIOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY OF 5 SPEACIES OF TROPICAL FRESHWATER MICROALGAE

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ABSTRACT

Microalgae have gained prominence for industrial applications, producing biomass of potential economic value. However, even with its great diversity, just few genera are used industrially, and screening for different species may help increase this number. We aimed at evaluating the production of biomolecules, antioxidant and antimicrobial activity of 5 strains of tropical Chlorophyta microalgae. The organisms were cultivated in BG-11 medium under controlled temperature, optimal light conditions and 12h/12h light/dark cycle until late exponential phase. Biomass was then collected and biomolecule, antioxidant and antimicrobial activity were determined. The highest growth rate (1.06 d-1) and dry biomass (DW) 164.75 mg.L-1 were registered in the microalgae Pediastrum sp., followed by Chlorella emersonii with 0.91 d-1 growth rate and 128 mg.L-1 DW. Total proteins in both Pediastrum sp. and C. emersonii were the highest, with ~ 80 mg.L-1. Considering total lipids content, Pediastrum sp., Tetranephris brasiliensis and Staurastrum pantanale produced 16 – 18 mg/L, the highest among the species investigated. S. pantanale showed the highest carbohydrates content (30.68 mg.L-1), which was at least 3 times higher among the species investigated. Antioxidant activity were highest in Pediastrum sp. (36%) and S. pantanale (32%). All algae showed antimicrobial activity against S. aureus, the highest by D. brasiliensis, with inhibition up to 250 mg.mL-1 extract concentration.
The results we obtained for C. emersonii agree with literature that show high biomass yield and supports the wide application and use of this genera in the industry. However, it was the Hydrodictyaceae Pediastrum sp. the most promising organism. Similar to our results, CALIXTO et al. (2016) and SASSI et al. (2019) observed higher growth rate and proteins content for Pediastrum sp. in comparison to Chlorella sp. (Chlorellaceae). The higher carbohydrates in the Desmidiaceae S. pantanale we obtained is in accordance to the results in Hussain et al. (2020). This study showed important biotechnological potential for Pediastrum sp. due to high proteins and lipids production, antioxidant activity and moderate antimicrobial activity, which altogether makes this an interesting candidate for scale-up and industrial applications.

Keywords
Biomolecules; Food; Physiology; *Chlorella; Pediastrum.*

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PhD student in the Post-Graduate program in Biotechnology of the Federal University of São Carlos. Researcher in a microalgae prospecting project to obtain compounds of biotechnological interest, focusing on biomolecules and products of secondary metabolism. Has experience in the cultivation of eukaryotic microalgae, natural products of microalgae and microbiology.

THE EFFECT OF LIGHT INTENSITY ON PIGMENT CONTENT IN *A.PLATENSIS*

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ABSTRACT

Pigments are valuable antioxidants showing various health benefits. The most valuable pigment of cyanobacteria *Arthrospira platensis* is C-phycocyanin showing antioxidant, anti-inflammation, and immunomodulatory properties and is also demanded as a blue pigment in the food industry. In this study, the light intensity on pigment composition was evaluated. Microalgae was cultivated for seven days at 16:8h light:dark period under white LED light in bubble column PBRs. The quantitative analysis of pigments was performed by UV-VIS analysis of extracts. It was found that light intensity strongly affected pigment composition. The growth of biomass occurred under light intensities 90-160
µmol $s^{-1}m^2$. It was not growing under lower or higher light intensities; trichomes became short and fragile and run through a filter. The content of C-phycocyanin and Chlorophyll *a* rapidly decreased with light intensity increase – three and two times, respectively, showing an almost linear function of light. While carotenoid content slightly increased with light intensity increase. It was found that temperature more affected carotenoid content than other studied pigments. At 25℃ carotenoid content increased from 2.5 to 3.4 mg g^{-1} while at 27℃ it increased from 2.9 to 5.2 mg g^{-1}. Increasing morning temperature from 24.0℃ to 27℃ (max temperature during the day was kept at 30.8℃) biomass growth increased by 30% and optimal light intensity moved from 120 to 143 µmol $s^{-1}m^2$. The obtained C-phycocyanin amount is dependent on biomass amount and C-phycocyanin concentration in it. So obtained C-phycocyanin amount was similar in low light intensities 93-125 µmol $s^{-1}m^2$ because rapidly increasing biomass growth compensated decrease of C-phycocyanin concentration in biomass. However further light increase decreased obtained C-phycocyanin amount for samples with a morning temperature of 26℃ and lower temperature.

Keywords
Arthrospira platensis, spirulina, pigments, light intensity

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BIOGRAPHY

Master's degree in chemical engineering, Ph.D. in material science. Running lab works and lectures on various chemical engineering courses, e.g. Mass transfer, Units of chemical engineering, etc.
Since 2017 working on biotech and microalgae spirulina (*Arthrospira*) topic. PostDoc in EU funded project "Design of artificial lighting for phycocyanin production in bubble column photobioreactor".
Co-founder and business developer of SIA SpirulinaNord since 2019.
My passion is applied research. I enjoy optimization of production processes and scale-up. And even more I love to see that clients return for fresh and tasty spirulina products and report that they have improved their health with our products.

COMPANY PROFILE

Riga Technical University – the largest technical university in the Baltic states. www.rtu.lv
SpirulinaNord – a startup cultivating spirulina by innovative indoor technology designed for the Nordic climate. It sells innovative drinks of fresh spirulina. www.spirulinanord.eu

LESSONS LEARNED FROM THE LAB: CHALLENGES IN UPSCALING MICROALGAE FOR DITERPENOID PRODUCTION

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ABSTRACT

Plant diterpenoids encompass a diverse group of specialized metabolites, which have both significant ecological functions and many industrially relevant applications in pharmaceuticals, nutraceuticals, and the cosmetics industry. The heterologous expression of diterpene synthases in microbial hosts has opened numerous possibilities for the bioproduction of these metabolites. Photosynthetic microbial hosts present a sustainable alternative to traditional fermentative systems. On the other hand, product yields in microalgae have thus far remained lower than in bacteria and yeasts, therefore research to overcome unsolved bottlenecks in industrial scale-up is essential to the success of microalgal biotechnology.

Here we report on the production of the diterpenoid casbene in the model green microalga *Chlamydomonas reinhardtii* in both laboratory and scale-up at the Algal Innovation Centre (AIC) of The University of Cambridge. Our experience of moving through scales of cultivation has revealed challenges and solutions for translation and commercialization of algae biotechnology beyond the lab.

We generated several different strains of Chlamydomonas that produced casbene. At scale, key abiotic factors such as temperature, light, bioreactor volume and type were tested. Furthermore, strain selection identified that the walled strain of *Chlamydomonas,* RSW2, under scale-up batch conditions in the AIC, produced 2.3 times higher amounts of casbene than did cell wall deficient strains CW15 and UVM4. Further investigations under lab conditions found that casbene production was 4.1 times higher in cell walled RSW2 compared to cell wall less CW15 and UVM4. This is important because due to ease of transformation, the latter strains are the most widely used for metabolic engineering.

In addition to optimizing abiotic growth conditions, the role of biotic factors at scale also need to be considered. Preliminary investigations suggest that algae-bacteria co-cultures can improve the production of casbene and highlights the need to investigate the role of microbial communities to improve industrial biotechnology. Further investigations to address scale-up challenges involved maximizing casbene production by using an inducible riboswitch during exponential growth phase to improve the efficiency of growth and the final productivity of the desired product.

Keywords
Industrial biotechnology, diterpenoid, genetic engineering, Chlamydomonas reinhardtii, scale-up

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BIOGRAPHY

I studied a BSc in Marine Biology in Sydney, Australia before moving to the UK to pursue a PhD in Plant Sciences at The University of Cambridge. My current research aims are to test abiotic and biotic parameters to improve the production of high-value diterpenoids in microalgae for industrial biotechnology. I am also particularly interested in how microalgae can be used to serve our sustainability goals and climate concerns.

Outside of my research interests, I am committed to engaging in climate change awareness and action. I love anything involving the outdoors, from diving the deep, sailing the seas and hiking mountain tops. If I’m not in the lab with the algae, you will find me reading or painting.

Food

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SPIRULINA SP. – DIFFERENT DRYING TEMPERATURES AND IT'S BIOLOGICAL ACTIVITY

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ABSTRACT

Spirulina sp. is a widely cultivated cyanobacteria which contains a high amount of bioactive compounds with health-promoting effects and remarkable antioxidant activity due to the abundant presence of polyphenols and carotenoids [1]. The effect of different drying temperatures (room temperature, 40, 50, 60, 80, and 100°C) on the phenolic profile and biological activity of *Spirulina sp.* was investigated. Samples were dried at various temperatures, extracted and then fractioned. Antioxidant activity of methanolic (F3) and dichloromethane (F4) fractions was evaluated using ABTS, FRAP, ORAC and DPPH assays. To determine the toxicity potential of prepared fractions, zebrafish embryotoxicity test was performed [2]. Phenolic compounds in *Spirulina sp.* samples were identified using HPLC, while LC/ MS-Q-TOF screening was used to predict the most probable bioactivities, chemical classes and drug-likeness properties of examined compounds. According to antioxidative assays, F3 fraction had higher ABTS and FRAP activity, while F4 fraction had higher DPPH and ORAC activity. The F4 fraction dried at 50°C showed the highest antioxidant activity using DPPH (687.06 +/- 11.41 mg AAE/g fraction), while ABTS and FRAP assays showed high activity for F3 fractions dried at 25, 30 and 100°C. There was no negative impact on the survival of zebrafish embryos and larvae observed at concentrations lower than 0.02 mg/mL. The most abundant polyphenols were epicatechin, vanillic acid, caffeic acid, diosmin, thymol, apigenin, while the presence of 3 alkanolamines and 25 lipid-like molecules was revealed by QTOF analysis. High antioxidant activity was observed at lower temperatures can be related to the presence of carotenoids and chlorophyll. Antioxidant potential of sample dried at 100°C can be attributed to pigment derivatives [2]. The optimal temperature for preservation of polyphenols was 50 °C. A strong correlation between structural class of detected compounds and highly predicted activities (cardiovascular, anti-inflammatory, anticancer) has been observed.
The results indicate that drying at a lower temperature retains phenols and other components and maintains high antioxidant activity. From the obtained results, it can be concluded that lower drying temperatures better influence the content of phenolic compounds and biological activity of *Spirulina sp.*, when compared to higher temperatures.

Keywords
Spirulina sp., cyanobacteria, drying temperature, biological activity, antioxidant activity

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MICROALGAE LIPID-ENRICHED EXTRACTS FOR PARTIAL FAT SUBSTITUTION IN BAKERY PRODUCTS

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ABSTRACT

Nowadays, there is an increasing interest in reducing the amount of fats present in food as high-fat diets are known to be the cause of heart diseases and several other health problems. Many bakery products present high amounts of fat, especially saturated fats, because of the high amounts of butter/margarine that are used in their production. Therefore, the reduction/replacement of the amount of fat present in bakery products, without affecting their sensory characteristics, is highly desirable. Considering this, the main aim of this work was to obtain lipid-enriched extracts from food grade microalgae for the partial substitution of fat in a bakery product (a *Brioche* bread).
Extracts of three different commercial strains of *Chlorella vulgaris* (organic, smooth and white) and *Tetrasellmis chuii* were produced using solvent extraction (Ethanol), solvent extraction with ultrasound pre-treatment (Ethanol/U), and solvent extraction with a deep eutectic solvent pre-treatment (Ethanol/DES).
Extraction with ethanol after a pre-treatment with the eutectic solvent choline chloride:urea (1:2) resulted in higher extraction yields, with an improvement of up to 61% and 65% in the extractions of organic *C. vulgaris* and white *C. vulgaris*, respectively, when compared with the other extraction methods. Ethanol/DES extracts also had higher fatty acid contents. The analysis of the fatty acid profile of the extracts showed that the main class of fatty acids present in the *Chlorella* extracts is PUFA (polyunsaturated fatty acids), followed SFA (saturated fatty acids) and by MUFA (monounsaturated fatty acids), while in the *T. chuii* extracts their relative content is PUFA > MUFA > SFA. *C. vulgaris* smooth and *T. chuii* extracts have lower contents of saturated fatty acids, which makes them good candidates for food applications. Both extracts were screened for their antimicrobial and antioxidant properties. None of the extracts presented antimicrobial capacity but the *T. chuii* extract revealed antioxidant properties.
The *C. vulgaris* smooth and *T. chuii* extracts obtained with the Ethanol/DES method were further used as partial substitutes of fat in *Brioche* bread. A reduction of the saturated fatty acid content of the *Brioche* bread was attained, without affecting the sensory features of the product.

Keywords
Microalgae; lipids; saturated fat; extraction; deep eutectic solvents

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DHA OIL ENCAPSULATION: OPTIMIZATION OF FORMULATION AND DRYING PROCESS

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ABSTRACT

Omega-3 polyunsaturated fatty acids (PUFAs) present a great interest to human's growth and health, especially eicosapentanoic acid (EPA) and docosalhexaenoic acid (DHA) [1]. These two compounds have attracted attention to food and nutraceutical industries. Moreover, from 2020, DHA must be introduced to infant milk to assure brain and visual maturation [2]. Nowadays, the principal natural source of DHA is from marine oils with a growing interest in algae oils [3]. While algae biosynthesis and oil extraction processes have been developed and optimized in the last decades, the encapsulation by drying process remains a critical step [4]. The two main processes used in industry are freeze drying and spray drying [5]. With low temperatures and vacuum conditions, freeze drying is allowed to assure a good quality of DHA oil encapsulation. However, this technology presents the disadvantages to work in batch and to be energy and time consuming. Spray drying presents the advantage of working continuously with low energy cost. However, high temperatures and oxygen are needed to conduct production that may induce an oxidation of DHA oil. An innovative technology namely electrostatic spray dryer can operate continuously with cooler temperatures (between 35 – 80°C) and with nitrogen [6] to avoid DHA oxidation. The aim of this study is to compare these three drying technologies using three surfactants associated with maltodextrin DE 19: sodium caseinate, methylcellulose and a saponin. The peroxide value of each powder was measured after 2 months at 40°C with oxygen and compared with the relative maximum of peroxide value. The results (Figure 1) show that the saponin formulation presents the lower percentage of oxidation with a maximum value of 22.6 % for freeze drying and minimum value of 7.8 % with electrostatic spray drying. This surfactant can be an interesting ingredient for "green" formulation. For the three formulations, the electrostatic spray dryer presents the lower values of oxidation with an average reduction up to 6.6 times. This study also demonstrates that the electrostatic spray dryer seems to be a promising technology for DHA oil microencapsulation.

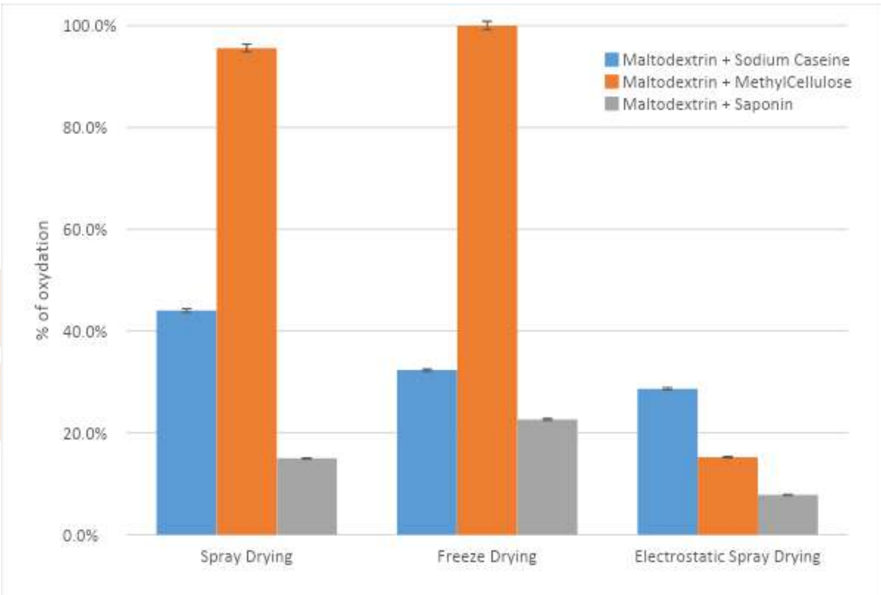

Figure 1: Percentage of relative oxidation comparing drying technologies and formulations

Keywords
DHA oil – drying process – oxidation – microencapsulation – formulation

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COMPANY PROFILE

Fluid Air is a division of Spraying Systems Co., the world leader in spray nozzle technology. Spraying Systems Co. was founded in 1937 and has established its leadership through the development of its extensive global sales force, its global manufacturing capabilities, including twelve manufacturing plants worldwide, and its innovative, solution-oriented approach toward customers.
Founded in 1983, Fluid Air Inc. specializes in solid dosage technology. Fluid Air has always excelled in its ability to develop quality and robust products tailored to its customers' needs. Working together with Spraying Systems Co., Fluid Air now has a strong international presence with best-in-class global service. With an aggressive will to develop new and innovative technology, Fluid Air continues to push the boundaries of what is possible and always aims to satisfy the customer.

ENZYMATIC SYNTHESIS OF ENRICHED-OMEGA 3 STRUCTURED PHOSPHOLIPIDS FROM MICROALGAE WITH NEUROPROTECTIVE ACTIVITY

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ABSTRACT

Microalgae are considered a sustainable and alternative source of interesting bioactive compounds that exerts a multitude of beneficial effects such as polyunsaturated fatty acids (PUFA). Among them, in the last decades great emphasis was put on docosahexaenoic acid (DHA), an ω-3 PUFA necessary for the development and normal function of the brain.Nowadays, the current interest in phospholipids derives from their efficacy in incorporating different fatty acids at the cell membrane level to improve its bioavailability since they are better absorbed than triacylglycerides (TAG). In consequence, structured phospholipids containing DHA are considered the most suitable form to increase its accumulation in brain and seems very useful to prevent neurodegenerative and neuroinflammatory diseases.

In this way, this study aims to obtain structured phospholipids enriched in DHA ω-3 fatty acid extracted from marine microalgae by enzymatic synthesis and environmentally friendly processes. For this purpose, *Schizochytrium sp.* was selected because of its high concentration of DHA.

The extraction of microalgal oil by advanced extraction techniques such as pressurized liquid extraction (PLE) was first optimized using a mixture of hexane:ethanol (1:1) achieving a yield of 24.39 % ± 0.97 and scaled-up up to 10 times. Characterization by GC-MS was studied, and results were compared to commercial oil. Microalgae oil was extracted in form of TAG obtaining 51.90 % ± 0.32 DHA that was used to enzymatically obtain structured phospholipids by esterification with commercial phospholipid glycerylphosphorylcholine (GPC). Two different commercial phospholipases, Lecitase and Quara Low P, were immobilized by hydrophobic adsorption and studied at 45°C reaction temperature, using nitrogen atmosphere to protect phospholipids from oxidation. Different GPC:TAG ratios were studied (1:30, 1:20 and 1:10) and their influence on solvent-free reactions were also compared. Under best conditions, it was possible to obtain DHA enriched phospholipids in their mono and disubstituted form with different selectivity for each enzyme used.

Therefore, the production of structured phospholipids using enriched-DHA microalgae oil, green technology and enzymatic methods was successfully achieved. Future assays will be focused on purification of the final product and bioactivity assays.

Keywords
Schizochytrium, DHA, structured phospholipid, phospholipases, green technology

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Gonzalo Berzal has completed his master's degree in Novel Foods at the Autonomous University of Madrid (Spain). He is a now a second-year predoctoral student in Food Science. He has received several research grants during his studies (Collaboration Grant from the Ministry and Aid for the promotion of Research from the Autonomous University of Madrid) in which he was able to work in different areas, such as green extractions of oils, enzymatic reactions and fractionation of compounds of interest. As a result, he gained experience in HPLC, GC-MS, PLE and Column fractionation.

TISOCHRYSIS LUTEA BIOMASS AS A SUBSTRATE FOR LACTIC ACID FERMENTATION

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ABSTRACT

Microalgae contain high quality proteins, polyunsaturated fatty acids and bioactive molecules endowed with beneficial properties (Lafarga et al., 2021). They are, thus, considered interesting candidates for nutraceuticals and functional foods (Matos et al., 2017). The uses of microalgae in food production include the development of functional fermented foods. Lactic fermentation allows to add the beneficial effects of probiotic bacteria to the useful biomolecules lying in microalgal biomass, also taking advantage of the effects on organoleptic features produced by fermentation. The aims of this work were to evaluate the potential of *Tysochrysis lutea* F&M-M36 lyophilized biomass as a substrate for *Lactiplantibacillus plantarum* ATCC 8014 fermentation, as well as to investigate the effects of the indigestible fraction of the microalgal biomass on bacterial growth, and to verify whether the microalgal biomass could exert a protective role towards the probiotic bacterium during a simulated digestion process. Initially, three lactobacilli were characterized for growth and resistance to simulated digestion, then the best strain (*L. plantarum* ATCC 8014) was selected for the fermentation trial. This latter was set up with *T. lutea* F&M-M36 raw and digested biomasses suspended in two different matrixes. The biomasses obtained at the end of the fermentation trial were analyzed for gross biochemical composition, digestibility and radical scavenging activity and compared with the unfermented biomasses; moreover, survivability of *L. plantarum* ATCC 8014 to in vitro digestion was also evaluated. The higher growth and organic acid production was observed with microalgal raw biomass suspended in diluted organic medium. No protective effect on bacterial survivability was observed after fermentation. Radical scavenging activity of a lipophilic extract was higher in fermented than in raw microalgal biomass and was also higher than in the fermented post-digestion residue. Fermentation seems an interesting process to obtain functional foods from *T. lutea*, although further investigation is needed to optimize the matrix for fermentation so as to improve bacterial growth, and thus fully elucidate the role of fermentation for functionality improvement.

Keywords
Tisochrysis lutea, lactic fermentation, lactic bacteria survivability

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Natascia Biondi, PhD in Microbial Biotechnology, is a researcher at the Department of Agriculture, Food, Environment and Forestry (DAGRI) of the University of Florence. She has more than 20 years of experience in the microalgae field and has participated to several EC funded projects (FP4, FP7, H2020). She is secretary of the Italian Association for the Study and Application of Microalgae (AISAM). Main topics of research: microalgae cultivation and application in the food, nutraceutical and agricultural fields.

REVALUATION OF SWRO BRINE FOR THE PRODUCTION OF CAROTENOIDS FROM A NATIVE STRAIN OF *DUNALIELLA SALINA*: A CASE STUDY IN GRAN CANARIA

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ABSTRACT

Dunaliella salina is a halophile green microalgae cultivated worldwide mainly for the production of *β*-carotene. The commercial demand of this pigment, which has a variety of market applications in the food and feed industry, nutraceutics and cosmetics, is in continuous rise. *Dunaliella salina* BEA 1948B is a species native to the Canary Islands and its ability to grow in contamination-excluding hypersaline conditions, its rapid growth and ability to acclimate to rapid environmental changes makes it an ideal candidate for industrial cultivation purposes. Surrounded by the sea, the Canary Islands (Spain) offer optimal conditions for industrial production of marine microalgae due to their strategic economic location and year-round sunny weather with stable temperatures. The objective of this study was to revaluate the wastewater brine from a sea water reverse osmosis (SWRO) desalination plant to optimize water and nutrient efficiency for the cultivation of *Dunaliella salina* BEA 1948B in a semi-industrial scale scenario. In order to do this, the strain was cultivated in 250-L raceway reactors at four different increasing salinity values of ≃ 40; 70; 80 and 100 ppt. Concentration of chlorophylls *a* and *b* and total carotenoids yielded by the different growth conditions throughout the cultivation course were measured. Total carotenoids in culture reached their highest concentrations on day 26 of cultivation in all salinity conditions with values of 23.5, 39.1, 42.0 and 45.0 g L^{-1} and productivities 0.91, 1.51, 1.62 and 1.74 mg L^{-1} day^{-1} in dry weight, respectively.These findings suggest that SWRO brine could be a promising low cost medium to cultivate *Dunaliella salina* in arid or semi-arid areas where seawater desalination plants are common due to limited freshwater availability. Furthermore, the use of SWRO brine as medium in the cultivation process gives an added value to this wastewater by-product and may also result in the removal of certain residual compounds from the brine, improving its quality before discharge. All of these are key factors to obtain an economic and environmentally sustainable industrial production.

Keywords
Carotenoids, *Dunaliella salina*, Nutrient efficiency, SWRO brine revaluation, Water efficiency

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COMPANY PROFILE

Instituto Tecnológico de Canarias is a public company of the Canary Islands Government (Spain). Its main objective is to foster the industrial development of the region through R&D activities and technical services to SMEs in the field of Renewable Energies, Water Treatment & Management, Environmental Sciences, Medical Engineering and Biotechnology.

MICROALGA *DUNALIELLA* AS AN ALTERNATIVE PROTEIN SOURCE: EXPLORING THE POTENTIAL OF MIXOTROPHIC CULTIVATION

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ABSTRACT

Dunaliella is a genus of halophilic chlorophyte microalgae and is well-known for its ability to cope with extreme environments and to yield bioproducts with industrial relevance, such as carotenoids, lipids, and proteins, which can reach over 50 % on an ash-free dry weight basis [1]. In recent years, interest in the potential of *Dunaliella* to serve as a protein source with a high-quality amino acid profile has gained momentum in response to the increasing demand for alternative food, feed, and health supplement sources. Mixotrophic growth is commonly associated with higher biomass productivity and lower energy consumption. Since cell density and biomass are essential parameters for large-scale production, mixotrophic cultivation might improve biomass accumulation. The combination of such mixotrophic growth of microalgae and nutrient recovery from food industry wastewater offers great opportunity in the context of the circular economy, sustainable and efficient microalgal cultivation, and bioproducts generation. The presented work aimed to evaluate the impact of different carbon sources on growth, protein profile, and nutrient uptake for *Dunaliella tertiolect*a CCAP 19/30. Two experimental sets were conducted to 1) assess the contribution of atmospheric carbon to *D. tertiolecta* growth and its capacity to grow heterotrophically with lactose; 2) evaluate the impact of using six different organic carbon sources on its growth, protein yield and amino acid composition. Our results demonstrated that *D. tertiolecta* could not grow heterotrophically. Cell and optical density, ash-free dry weight, and essential amino acids index (EAAI) were inferior for all treatments using organic carbon compared to $NaHCO_3$. However, despite the carbon source, the EAAI from all treatments were higher (>1.6) than FAO/WHO recommendation (EAAI=1.0) for human consumption. Neither cell nor optical density presented significant differences among the treatments containing organic carbon, demonstrating that organic carbon does not boost *D. tertiolecta* growth. All the treatments presented similar nitrogen, phosphorus and sulfur recovery. Based on the results, *D. tertiolecta* CCAP 19/30 is an obligate autotroph and a good source of high-quality protein for food application.

Keywords
Mixotrophic growth; Wastewater reuse; Protein; Essential amino acids; Dunaliella tertiolecta

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UPSCALING THE AUTOTROPHIC CULTIVATION OF *CHLORELLA SOROKINIANA* TO PRODUCE HIGH-ADDED VALUE MACROMOLECULAS: FROM LAB-SCALE UNITS TO A SEMI-PILOT CULTIVATION SYSTEM

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ABSTRACT

In the emerging field of algal biotechnology, addressing engineering challenges for the prevention of process-product variability and the establishment of the optimal continuous operation of algal production systems, are urgent prerequisites for the inclusion of microalgae-based products in human nutrition. In response to this need, the current study aims at the efficient managing of this problem, in relation to the autotrophic cultivation of *Chlorella sorokiniana,* through an extensive experimental approach at different scales. This includes in particular: the Taguchi experimental design applied at the low scale of 500mL Erlenmeyer flasks, followed by the implementation of the optimal cultivation conditions (derived from the statistical data analysis) at the intermediate scale of a 3L bench-top photo-bioreactor and finally, the appropriate adjustment of the cultivation protocol at the larger scale of a 100L circular pond for further biomass and macromolecules productivity optimization.

Towards the parameterization of *C. sorokiniana* autotrophic cultivation, an L9-Taguchi design of growth conditions (in duplicate) was employed to systematically investigate the synergistic role of three critical process variables: (i) the illumination intensity, (ii) the initial nitrogen concentration and (iii) the initial sodium bicarbonate concentration in the growth medium as supplementary inorganic carbon source. The applied levels of variation of each factor were respectively: 2500,5000, 7500 lux; 250, 750, 1250 mg $NaNO_3$ /L; and 0, 750, 1500 mg $NaHCO_3$ /L. The process performance against the above process variables was monitored in terms of the final concentration of total biomass, lipids, proteins, and carbohydrates. Analysis of the produced experimental data showcased one of the non-tested set of experimental conditions as the most preferable one. Subsequently, the cultivation protocol that was marked out was applied in a 3L photo-bioreactor under well-controlled conditions to further exploit sodium bicarbonate's effect through semi-continuous and continuous pH adjustments. Finally, the cultivation policy co-shaped through the previous lab-scale experimentation was implemented in a 100L open circular pond, whereas a series of pH-adjustment treatments were established for both contamination control and sodium bicarbonate activation. The statistical data analysis and interpretation obtained from the Taguchi-designed experiments was unambiguously contributive towards a substantial improvement of process performance and product stability.

Keywords
Chlorella sorokiniana, Taguchi experimental design, semi-pilot scale, sodium bicarbonate, photo-bioreactor

Acknowledgements
This research has been co-financed by the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH-CREATE-INNOVATE (project code: T2EDK-02279). Project title: Human nutrition, animal and fish feeding on microalgae derived products through sustainable photosynthetic autotrophic cultures.

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INDUCTION OF DIVERSE METABOLITES BY CO-CULTIVATION OF *CHLORELLA VULGARIS* AND *STREPTOMYCES ROMISUS:* DETERMINATION OF BIOATIVE POTENTIAL

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ABSTRACT

Utilization of microalgae-bacteria consortia has revealed a significant impact of the commensal and mutualistic relationships that manifests in enhanced algal growth, increase in the presence of carbohydrates, lipids, and pigments, but also synthesis of new metabolites with a prominent potential for application in the industry sector [1]. In the recent decade, co-cultivation, as a potential replacement of mono-culturing of various microorganisms, gained special interest due to a whole range of desirable characteristics, such as modularity, robustness, predictability, scalability, and stability [2]. However, very little is known about the significance of *S. rimosus* and *C. vulgaris* co-cultures concerning the production of high-value metabolites with anti-bacterial, anti-oxidant, and anti-aging activities. To fill this gap, *C. vulgaris* and *S. rimosus* were co-cultivated under mixotrophic conditions, with a 12:12 (day:night) photoperiod on Tryptic Soya Agar plates for 7 days. Freeze-dried biomass of *C. vulgaris* and *S. rimosus*, as well as their mono-cultures were subjected to a wide range of bioassays. Co-cultivation of *S. rimosus* and *C. vulgaris* effectively improved bioactive compound synthesis which reflected in higher bioactive potential when compared to the one obtained in mono-cultures. In that manner, co-culture extract showed higher antimicrobial activity (against both *S. aureus* (46.06% of inhibition) and *E. coli* (72.28% of inhibition)) when compared to activities recorded on both tested mono-cultures. High antioxidant activity was obtained using the FRAP assay on co-cultivation and algal mono-culture extracts. In terms of anti-aging properties, the highest percent of inhibition of collagenase activity was recorded on *S. rimosus* mono-culture (90.5, 88.8, 87.8 % of inhibition on 3, 1, 0.5 mg/mL of extract, respectively), which appears to be related to oxytetracycline, widely known as Streptomyces product and collagenase inhibitor. Tested extracts in a concentration range from 0.6 to 0.15 mg/mL showed no negative impact on the survival/development of zebrafish *Danio rerio* embryos. Collectively, co-cultivation of macroalga *C. vulgaris* and bacteria *S. rimosus* has shown to have great commercial potential and opens possibilities for the discovery of novel bioactive molecules.

Keywords
Chlorella vulgaris, *Streptomyces rimosus*, co-culture, bioassays, bioactive molecules

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EFFECT OF BIOMASS AND POST-EXTRACTION RESIDUE FROM BRAZILIAN SPIRULINA ON DOUGH RHEOLOGY AND TEXTURE PROPERTIES OF WHEAT BREAD

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ABSTRACT

The use of innovative and sustainable ingredients, such as Spirulina biomass (SB), has been stated as a fact of food products development, mainly to guarantee foods with better technological, nutritional, and sensory quality1, in addition to the positive impact on the environment, natural resources and animals, to ensure sustainable food systems2. Spirulina (Arthrospira platensis) is a filamentous cyanobacterium, popularly known as microalgae, with high nutritional value, as a source of proteins, and bioactive compounds, particularly natural pigments such as C-phycocyanin (C-PC)3. Spirulina has been included as a functional ingredient in crostini (toasted bread) and other bread, breadsticks, cookies, couscous, wheat crackers, pastas4 and dairy products5, to increase the food industry's portfolio, generally including an intense color (typically green) and flavor, but also improving nutritional and technological aspects4. An important aspect to consider regarding the C-PC obtention from organic Spirulina biomass, donated by Fazenda Tamanduá® (Santa Terezinha/Paraíba – Brazil), is the formation of a large amount of organic residue (named as post-extraction residue biomass - RB)6 that has not been exploited in the literature. Therefore, this study aimed to develop wheat bread (WB) with the addition of SB (WB-SB) and RB (WB-RB). The impact of these incorporations on the dough rheological performance (pasting curves and viscoelastic behavior), compared to control WB (WB-Co) was performed, as well as the impact on bread texture, volume and color. The incorporation of 3% of SB and RB in WB was designed using 97% wheat flour adding 2.8% dried yeast and 1.8% salt, in flour basis percent. Water content was adjusted according to the water absorption (WA) value determined from the MicrodoughLab mixing curves (AACC method 54.70-01)7. The results have shown that SB and RB affected the rheology properties of WB doughs, with a slight increase in the percentage of water in SB and RB doughs (WA: 54.0% Co, 54.2% SB and 54.7% RB). Moreover, SB and RB addition have impacted the dough's mixing and pasting parameters and the bread firmness and cohesiveness of WB-SB and WB-RB. Further analyses are necessary for better comprehension of SB and RB effect on WB' sensorial acceptance and proximate composition.

Keywords
Arthrospira platensis; breadmaking; functional food; oscillatory tests; texture.

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PRODUCTION OF MICROALGAE-DERIVEDP ß-1,3-GLUCANS AND THEIR APPLICATION

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ABSTRACT

Water unsoluble ß-1,3-glucans from yeasts or cereals are already added to food and feed as immune boosters. Microalgae form ß-1,3-glucans as well. Diatoms for example produce water soluble ß-1,3-glucans as storage molecule. Due to their various possible applications, ß-1,3-glucans pose an interesting microalgae derived product.
We used artificially-illuminated flat-panel airlift reactors to examine the production of ß-1,3-glucans. Since microalgae accumulate ß-1,3-glucans as (primary) energy and carbon storage under unfavourable growing conditions, such as nutrient depletion, our experiments focused on ß-glucan accumulation during nutrient depletion. We first compared three microalgae species *Cylindrotheca fusiformis*, *Phaeodactylum tricornutum*, and *Monodopsis subterranea* regarding their suitability as production strains. Due to the rapid accumulation of ß-1,3-glucans to a content of more than 30 % w/w, *P. tricornutum* was selected for further experiments.
Further, the influence of different cultivation conditions on the accumulation of ß-1,3-glucans during nutrient depletion was investigated. Our results show that a depletion of nitrogen led to faster accumulation of ß-1,3-glucans than a depletion of phosphorus. Moreover, light conditions had a major influence on the accumulation: A high amount of light per gram biomass (specific light availability) led to a faster accumulation of ß-glucans, but not necessarily to a higher maximally accumulated amount. Higher cell density at the beginning of depletion resulted in slower accumulation, possibly due to self-shading effects in the culture. In all our experiments, in addition to ß-1,3-glucan, the potential co-products fucoxanthin and eicosapentaenoic acid were also investigated.
The algae species we selected, *P. tricornutum*, forms chrysolaminarin, a water-soluble 1,3-1,6-ß-glucan. Together with project partners, we conducted experiments on the application of chrysolaminarin. The results of feeding trials with fish (*Sparus aurata*) indicated that the addition of chrysolaminarin to the diet might pose a countermeasure against gut inflammation [1]. In trials with zebrafish, chrysolaminarin was shown to have a cholesterol-lowering effect, comparable to a commercial drug (Simvastin) [2]. In addition to experiments on animals, experiments regarding plant-strengthening effects were carried out on grapevine. Grapevine plants treated with chrysolaminarin showed a lower frequency and severity of infection with pathogenic fungi compared to the untreated control group.

Keywords
ß-1,3-glucan, chrysolaminarin, *Phaeodactylum tricornutum*, *Monodopsis subterranea*, *Cylindrotheca fusiformis*, flat panel airlift photobioreactors

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Konstantin Frick studied biology at the University of Hohenheim with a focus on botany. He did his master's thesis on starch production using the microalgae species *Chlorella sorokiniana* in outdoor cultivation.

After graduation, Frick joined University of Stuttgart and worked in different projects in the field of microalgae. Since the beginning of 2022, Frick has been working in the research group for microalgae biotechnology at the Fraunhofer Institute for Interfacial Engineering and Biotechnology (IGB). In addition, he holds a position as Principal Investigator at the Institute of Interfacial Process Engineering and Plasma Technology at the University of Stuttgart.

Frick focusses on applied science. His main interest is the cultivation of microalgae and the influence of cultivation conditions on biomass composition. His current research focuses on the production of chrysolaminarin, a ß-1,3-glucan, and its potential application in various fields (food, feed, agriculture).

COMPANY PROFILE

The Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB develops and optimizes processes, technologies, and products for health, sustainable chemistry and the environment.

EXTRACTION OF BIOACTIVE COMPOUNDS FROM *TISOCHRYSIS LUTEA* USING GREEN SOLVENTS AND ADVANCED EXTRACTION TECHNIQUES

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ABSTRACT

The marine microalgae *Tisochrysis lutea* is one of the most common microalgae used as natural food in aquaculture. *T. lutea* is a valuable alternative source of highly bioactive compounds such as carotenoids and polyunsaturated fatty acids (PUFA). Fucoxanthin, the main carotenoid found in this microalga has benefits for human health such as anti-inflammatory activity, antioxidant, or anti-obesity. On the other hand, docosahexaenoic acid (DHA), the main omega-3 PUFA essential in neuronal tissues, has beneficial effects related to brain development as well as its anti-inflammatory action.

Thus, extraction of bioactive compounds from microalgae using environmental-friendly processes and green solvents is essential not only to develop procedures that are in agreement with Green Chemistry but also to preserve its bioactivity.

This work aims to obtain enriched fractions of valuable compounds by advanced extraction techniques combined with green and bio-based solvents from *T. lutea* compared to traditional extraction techniques that uses hazardous organic solvents.

The experimental design to maximize the extraction yield was developed by ultrasound-assisted extraction (UAE), choosing as experimental factors the percentage of solvent (0, 50 and 100% of 2-methyl-tetrahydrofuran (2-methyl-THF) in ethanol), the extraction time (20, 30 and 40 minutes) and the temperature (40, 50 and 60ºC). The highest extraction yield was obtained using ethanol as solvent. Nevertheless, the most interesting extracts based on their chemical composition were obtained when the presence of 2-methyl-THF in the extraction mixture was greater than that of ethanol. Through analytical techniques such as high-performance liquid chromatography and gas chromatography, as well as spectrophotometric techniques, the content of polar lipids, DHA and other fatty acids, total carotenoids and fucoxanthin were quantified. In addition, the antioxidant capacity of different selected extracts was studied, being once again the most interesting those extracted with different amounts of 2-methyl-THF due to its selectivity and enriched composition in compounds with high added value.

Therefore, it is shown the importance of choosing an advanced extraction technique together with the use of green solvents to develop more ecological processes that could extract bioactive compounds of great interest in industry from alternative sources such as microalgae.

Keywords
Tisochrysis lutea, fucoxanthin, DHA, UAE, 2-methyl-THF

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Dr. Paz García is a Postdoctoral Researcher at Universidad Autónoma de Madrid (UAM) where she develops her research in green extraction, purification, and characterization of bioactive molecules from alternative sources, mainly microalgae. Moreover, she is professor of Medical Microbiology at Universidad Francisco de Vitoria.

Nowadays, there is a major awareness in society about the development of greener and more sustainable processes coupled to revalorization of biomass framed in a circular bioeconomy to slow down the environmental impact that human activities have on the planet. Thereby, exploitation of alternative and plant-based materials as new sources of bioactive compounds with applications in different biotechnological industries is the aim of her research. In this sense, microalgae have emerged as a cherished and sustainable source of value-added products.

She applies ecofriendly protocols and green solvents to efficiently extract healthy lipids and antioxidants from microalgae with nutraceutical purposes. She also modifies the bioactives with estabilized immobilized enzymes to improve their characteristics and to create functional foods.

Due to her willingness to apply her knowledge to produce healthy products, Dr. Paz García joined the UAM Emprende program for talented researchers in Food Science last year in which she obtained the Pitch Deck Honourable Mention. In this new stage, she wants to bring science to society in a funny and appealing way through innovation in food design of functional foods while developing a whole process which is in agreement with Global Trends and sustainability within academia.

MICORALGAE AND BACTERIA: A MATCH MADE FOR SPACE TRAVEL?

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ABSTRACT

Vitamin B_{12} is a cofactor for enzymes involved in central metabolism and is therefore essential for good human health. It is a complex molecule that can vary at the upper and lower axial ligand, altering its biological function. Vitamin B_{12} is only synthesised by certain prokaryotes and requires over 20 enzymatic reactions. On Earth, the majority of humans fulfil their vitamin B_{12} requirements through the consumption of meat, eggs and dairy products, which are difficult to obtain on long space missions. The MELiSSA loop is one example of a biological life support system (BLSS) for long-distance space exploration. It contains five compartments, and through compartment IV all the nutritional needs of the astronauts must be met. Compartment IV is composed of two sub-compartments, the higher plants in IV_B and the photoautotrophic cyanobacterium *Limnospira indica* in compartment IV_A. Higher plants do not make, require or store vitamin B_{12}. Furthermore, using a new microbiological bioassay for vitamin B_{12} that we have developed, we have shown that *L. indica* makes pseudocobalamin, a form of B_{12} not suitable for humans. Therefore, the astronauts would be at risk of developing a B_{12} deficiency and the resulting negative health outcomes. The novel microbiological bioassay has also been used to investigate the presence of B_{12} in the other compartments of the MELiSSA loop. *Rhodospirillum rubrum*, a bacterium historically proposed to occupy compartment II, produces cobalamin, the more bioavailable form of the vitamin. As a proof-of-concept approach we have established cocultures of *R. rubrum* and the edible eukaryotic microalgae *Chlorella vulgaris* or *Chlamydomonas reinhardtii,* which could potentially provide a biomass enriched with B_{12}. Both microalgae have featured in space research for decades, but only in monocultures. We use these synthetic cocultures to investigate how environmental conditions found in space, such as higher ionising radiation and microgravity, could alter the dynamics of cocultures, and thereby the B_{12} content of the biomass. As the popularity of vegetarian/vegan diets increases, novel vegan sources of vitamin B_{12} are also required on Earth. Consequently, not only is this useful for improving BLSSs for space travel it may also improve B_{12} provision on Earth.

Keywords
Food production, Vitamin B_{12}, microalgae, space travel, co-cultures

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I studied Marine Biology at the University of St Andrews, and then completed a Mres in Marine Biology at the University of Plymouth studying nutrient sensing in marine diatoms. I moved to Cambridge to study for my PhD in the Department of Plant Sciences.

CHARACTERIZATION OF HALLOUMI CHEESE SUPPLEMENTED WITH ALGAE BIOMASS

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ABSTRACT

The addition of microalgal biomass in dairy products has been of increasing interest to obtain more sustainable and nutritive fermented products. Microalgae are potential sources of bioactive compounds, which can affect the antioxidant properties and the mechanical behavior of food[1,2]. This work aimed to evaluate how the incorporation of algae (*Chlorella vulgaris* and *Algaessence* - a mixture of *C. vulgaris* microalgae, and *Fucus vesiculosus* and *Ulva rigida* macroalgae) affects the mechanical properties, nutritional value and bioactivity of Halloumi cheese[3]. Temperature and frequency sweep tests (oscillatory rheology) were performed at 20 °C and 90 °C, the impact microalgae addition was not reveled for the studied levels (1% to 5% w/w). The cheeses are viscoelastic solids (G´ > G´´) despite a higher frequency or temperature dependence, when microalgae are added. Texture analysis of the cheeses, performed by using a puncture test by a Texturometer, revealed a hardness reduction when algae levels were increased. Nutritional composition (namely lipids and ash) was significantly (p<0.05) influenced by the addition of different algal biomass levels, showing an increase of 6.60 % in lipids and 20.66% in ash for 5% *Chlorella*-fortified cheese, when compared to the control (without algae addition). Regarding the bioactivity of the cheese, antioxidant activity analysis and total phenolic compounds determination were carried out using FRAP and Folin-Ciocalteu methods, respectively. The results indicated an increase (p<0.05) in the antioxidant activity and phenolics, specifically in cheeses fortified with 3% and 5% of *C. vulgaris* (Fig. 1). Sensory analysis of Halloumi cheeses showed that panelists preferred the control and 1% *Chlorella*-fortified cheese, both with an overall acceptability of 4.20 (1 to 5 hedonic scale). In addition, it was observed that 53% and 30% of the tasters "would probably buy" the cheese supplemented with 1 and 3% of *C. vulgaris* respectively, indicating a high probability of consumption of these innovative and hybrid cheeses, made from animal and vegetable sources.

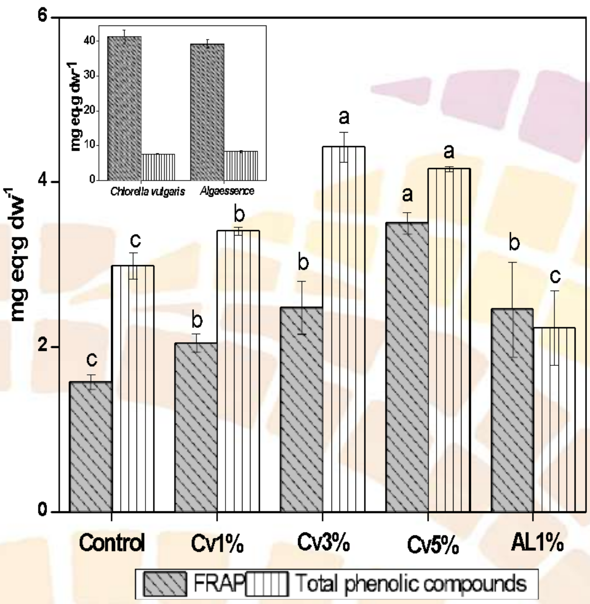

Fig 1. Antioxidant activity and phenolic compound concentration of 1, 3 and 5% *Chlorella*-fortified cheeses and 1% *Algaessence*-fortified cheese.

Keywords
Algae biomass, cheese, rheology, texture, bioactivity.

Funding
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Héctor is graduated in Food Engineering - Universidad de Córdoba - Colombia (2015) and Master's in Food Science and Technology from the Federal University of Viçosa(2018). He is currently a Ph.D student in Food Engineering at the University of Lisbon, integrated in LEAF (Linking Landscape, Environment, Agriculture And Food) Research Unit. He has experience in the field of food science and technology working on obtaining processed cheeses and characterizing protein nanostructures synthesized through the Maillard reaction, as well as determining their technical-functional properties. At the moment, his Ph.D project focuses on the development and characterization of cheeses supplemented with microalgal biomass.

SCREENING FOR MICROALGAL BIODIVERSITY FROM REUNION ISLAND IN SEARCH OF POTENTIAL SOURCES OF ASTAXANTHIN

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ABSTRACT

Since 2018, the Phycored project, which is led by Green Mascareignes Technologies SAS (GMT) in partnership with the Laboratory of Chemistry and Biotechnology of Natural Products (ChemBioPro) of the University of Reunion Island, aims to study the feasibility of producing on Reunion Island, from cultures of local microalgae strains, astaxanthin, a xanthophyll carotenoid valuable in the fields of nutrition and health especially for its antioxidant properties among others. In this context, our project is devoted to the search for strains isolated from local freshwater biodiversity capable of producing and accumulating astaxanthin.

Our work allowed to isolate for 12 strains, from freshwater samples collected all around the island, that showed similar morphological characteristics than *Haematococcus pluvialis* (Chlorophyceae, Chlamydomonadales, Haematococcaceae), a well-known microalgal species that is already cultivated for industrial production of natural astaxanthin [1]. Molecular and phylogenetic analysis [2–4] allowed their genetic identification and revealed that they belong to two different species: *H. pluvialis* and *Dysmorphococcus globosus* (Chlorophyceae, Chlamydomonadales, Phacotaceae). Chemical analysis of pigment extracts using High Performance Thin Layer Chromatography (HPTLC) [5, 6] confirmed that our local *H. pluvialis* strains was able to produce and to accumulate astaxanthin under light and nutritional stress conditions. These results also showed that local strains of *D. globosus* are also able to produce astaxanthin.

To our knowledge, this is the first time that these species have been described in Reunion Island. In a recent study, Zohir et al. [7] have reported that a strain of *D. globosus* from the Himalayan region is able to produce astaxanthin with higher productivity than *H. pluvialis* species and could therefore represent an alternative source of this high valued carotenoid.

In this presentation, we will also show our preliminary results on the biochemical composition of the extracts from our strains and their physiological behaviors in response to local climatic and stress conditions in order to demonstrate their biotechnological interest. Our objective is to verify that their cultivation is appropriate for producing astaxanthin with sufficient yields to develop for a new economically viable industrial activity in Reunion Island.

Keywords
Microalgal biodiversity, Astaxanthin, *Haematococcus pluvialis*, *Dysmorphococcus globosus*, Food Biotechnology

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BIOGRAPHY

Samuel Jannel is a French student who is carrying out a thesis entitled "Exploration of the biotechnological properties of microalgae from Reunion biodiversity" which is led by Green Mascareignes Technologies SAS (GMT – http://green-mascareignes.com) in partnership with the Laboratory of Chemistry and Biotechnology of Natural Products (ChemBioPro – https://chembiopro.univ-reunion.fr) of the University of Reunion Island. After obtaining a master's degree in plant biology and biochemistry at Louis Pasteur University in Strasbourg, Samuel started his professional career as quality controller for Dow AgroSciences in Alsace. He moved to Reunion Island in 2007 and discovered the exciting field of microalgae while he was working for a start-up whose ambition was to industrially cultivate these microorganisms to produce biofuels. After this successful experience, he decided to pursue his career in this field in search for new promising economic opportunities for the territory. Samuel joined GMT in 2020, which offered him the opportunity to carry out this thesis within the framework of the PhycoRed project.

COMPANY PROFILE

Green Mascareignes Technologies SAS (GMT) is a company founded in 2015 by Marc Bermudes, its current president. The company is specialized in the development of original interdisciplinary research and development projects in the fields of environmental biotechnology, the design and marketing of products and processes resulting from research, the exploitation of industrial processes, advices and training. The company is located at Saint Denis, Reunion Island.

The Laboratory of Chemistry and Biotechnology of Natural Products (ChemBioPro) is a research laboratory of the University of Reunion Island (EA 2212) specialized in the chemical and biotechnological characterization and valuation of the terrestrial and marine natural resources from south-west of the Indian Ocean. These resources include notably marine invertebrates, microorganisms and endemic and native plants. The laboratory is located in Saint Denis and Saint Pierre, Reunion Island.

NATURAL ASTAXANTHIN FROM A NOVEL FERMENTATION STRAIN OF *HAEMATOCOCCUS PLUVIALIS*

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ABSTRACT

The first ever US patent granted for a novel strain of *Haematococcus pluvialis* creates new commercial applications for algal astaxanthin. This high-value red-orange carotenoid is used as a human and animal food ingredient for its numerous documented health benefits and pigmentation effects. The novel strain is a stable natural variant - not the result of mutagenesis treatment or genetic engineering – uniquely characterized in part by being yellow during heterotrophic cultivation rather than green. This has the specific benefit of very low chlorophyll for added stability of astaxanthin for extracts derived from subsequently reddened biomass. The strain is carotenogenic under culture stress conditions in complete darkness. It turns red with accumulated astaxanthin being 87% or more of total pigment while being recalcitrant to encystment compared to wild type strains. Chromatography reveals the characteristic astaxanthin ester and isomer chemistry of light-grown *H. pluvialis*, accumulating astaxanthin primarily in the form of monoesters and diesters (97% esterified, 3% free) and with predominance of the 3S,3'S isomer configuration. The remainder of the carotenoid fraction in red cells is largely comprised of lutein and beta-carotene with minor canthaxanthin present. The digestibility of the red non-encysted biomass delivers a product which is highly suitable for feed and food applications without the need for added cell wall breakage after downstream processing. In heterotrophic cultivation, the strain shows very fast cell division for increased biomass (52 times more biomass production over 7 days) compared to the best published rate for autotrophic or mixotrophic growth. Cell reddening can be five times faster in heterotrophic cultivation, taking just 40 hours to reach commercial levels. *H. pluvialis* is allowed under EFSA as whole cell for food applications. Extracts produced from dark-grown biomass fall within FDA and EFSA chemical specifications for astaxanthin-rich extracts from *H. pluvialis* for use as a novel food ingredient. Combination of this novel *H. pluvialis* strain, together with efficient cultivation, enables lower cost manufacture of natural astaxanthin-rich algal biomass and extracts. This break-through opens up a multitude of new opportunities for the use of this proven high value antioxidant in human and animal food and feeds.

Keywords
Haematococcus, astaxanthin, heterotrophic, food, aquaculture

NITROGEN FIXATION IN CYANOBACTERIA TOWARDS NEW BIO-BASED INDUSTRIAL APPROACHES: EXPERIMENTS AND KINETIC MODELING

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ABSTRACT

The economic development of the current society is supported by hefty nitrogen (N) inputs. Since the early 20th century, nitrogen production relies almost entirely on Haber-Bosch chemical N-fixation process[1]. The development of atmospheric N_2 conversion processes into forms that can support food production has allowed the world's population to increase to unprecedented levels[2], but has also had a strong negative impact on the environment[1].
The need for more sustainable N-based processes could find a suitable solution in the cultivation of diazotrophic cyanobacteria, which are able to directly fix atmospheric N_2 into bioavailable forms[3]. As a matter of fact, it is estimated that if cyanobacteria were cultivated on agricultural scales, cultures could fix nitrogen on a level equivalent to global ammonia demands[3].
The industrial applicability of the cultivation process relies on overcoming technological limits and achieving high biomass productivity. Continuous cultivation systems could meet large-scale production, achieving higher productivity and ensuring constant product quantitative and qualitative properties over time[4]. By applying a mathematical model based on mass balances, it is possible to find the conditions under which biomass productivity and nitrogen fixation are maximized, at steady-state.
For this purpose, suitable kinetic models describing biomass growth and N-fixation rates as function of the main process operating variables are needed. Although cyanobacterial kinetic growth models are largely studied, N-fixers modelling is challenging because growth is controlled not only by the light intensity, but also by the availability of atmospheric N in the liquid medium[5].
In this work, *Nostoc* PCC 7120 has been cultivated in continuous systems to investigate the role of main operating variables on growth rate, such as light intensity and residence time, and to retrieve the value of the maximum specific growth rate for this species. By applying respirometric tests, the kinetic parameters for light intensity and temperature were measured. Respirometry outcomes for temperature, gained both in N-fixing conditions and in presence of nitrate, suggested that temperature effect on nitrogen solubility should also be considered for kinetic growth modelling. Parameters thus obtained have been then used to develop a more suitable kinetic model describing N-fixers autotrophic growth.

Keywords
Diazotrophic cyanobacteria, *Nostoc* PCC 7120, Kinetic growth model, Droop model, Continuous cultivation

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BIOGRAPHY

Veronica Lucato accomplished cellular and environmental biotechnology formation during her studies carried out at the University of Padua. Graduated in Industrial Biotechnology in 2020 with full marks working on a thesis on the production of high-value compounds by exploiting the continuous cultivation of cyanobacteria, she continues to carry out research activities in the field of optimization of microalgae cultivation processes with particular attention aimed at the cultivation of nitrogen-fixing cyanobacterial species. In 2021 she was committed to a project concerning the reduction of climate-altering gases from combustion flue gasses using a prototype pilot photobioreactor. She is currently involved in a PhD project, aiming at producing biomass with high protein content, at the industrial engineering department of Padua, attending the chemical and environmental engineering curriculum.

EFFECT OF ULTRASOUND DISRUPTION ON LIPID EXTRACTION FROM THE MICROALGA *NANNOCHLOROPSIS SP.*

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ABSTRACT

Photoautotrophic microalgae are a novel source of biomass rich in lipids containing nutritionally interesting n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA). However, microalgae incorporated into foods as whole biomass may lead to limited n-3 LC-PUFA uptake by the human body. Incorporation of microalgal oil instead of whole biomass is an interesting alternative. The extraction efficiency of these lipids can be enhanced by adding a cell disruption step during lipid extraction. Ultrasound assisted extraction (UAE) to enhance lipid recovery from microalgae has been covered in recent literature. UAE is used to speed up lipid extraction for analytical purposes or for industrially applicable lipid extraction of microalgae. In addition, the ultrasonication and lipid extraction by organic solvents of microalgal biomass has been performed in different set-ups: simultaneous ultrasonication and lipid extraction or ultrasonication and subsequent lipid extraction, ultrasonication of wet biomass or dry biomass. Often, no reference method is included to which the lipid extraction efficiency using UAE is compared and the total lipid content of the biomass is not always specified making it impossible to calculate the extraction efficiency. Therefore, the effectiveness of this cell disruption technique for industrial microalgae processing has not consistently been proven in literature.

This study investigates the effect of UAE of Nannochloropsis sp. biomass on the lipid extraction efficiency and the lipid quality, expressed as free fatty acid content and peroxide value. The effect of UAE on wet biomass in presence of organic solvents is compared to control extractions at room temperature and elevated temperatures corresponding to temperatures reached during ultrasonication. The effect of ultrasonication power and ultrasonication time is studied.

The results show that the lipid extraction efficiency increases after UAE when ultrasound was applied at high power. Control extractions at elevated temperatures, mimicking the temperature profile observed during UAE, result in similar lipid extraction efficiencies as observed after UAE.

This study is funded by Flanders’ Food and VLAIO in the framework of the EffSep project (Grant number HBC.2019.0012)

Keywords
Lipids, Nannochloropsis sp., Cell disruption, Ultrasound

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Esther Mienis has obtained her Bachelor and master's degree in food technology at the Wageningen University, The Netherlands and is currently working as PhD researcher at the Laboratory of Food & Lipids at the KU Leuven, Belgium under the supervision of Prof. Imogen Foubert. She is studying the effect of (combinations of) cell disruption techniques on the extraction of lipids from the microalga *Nannochloropsis* sp. Specific attention is given to the quality and stability of the extracted lipids. This research is funded by Flanders' Food and VLAIO in the framework of the EffSep project (Grant number HBC.2019.0012). Esther is passionate about novel food sources, sustainable processing and effects of processing on lipid quality.

COMPANY PROFILE

The laboratory Food & Lipids concentrates her research on the lipid (fat) fraction of food stuffs. On the one hand, lipids are often under pressure from a health conscious point of view but on the other hand they are essential for the sensory (e.g. taste), nutritional (e.g. essential fatty acids, fat soluble vitamins) and technological (e.g. spreadability) properties of fat rich food products. The mission of the laboratory is thus to conduct research facilitating the production of healthy/ier fat rich food products without compromising the taste or the technological functionality.

ASSESSING THE GROWTH AND NUTRITIONAL CHARACTERISTICS OF *CHLORELLA VULGARIS* WHEN CULTIVATED IN A COST-EFFECTIVE WAY, INTENDED FOR COMMERCIAL PRODUCTION IN MADEIRA ARCHIPELAGO

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ABSTRACT

Chlorella vulgaris is a well-known green eukaryotic microalga accepted in the EU for direct food consumption. The main restraints to industrialize the production is the access of an expert's team, implement low cost photobioreactors in sufficient area to make it feasible, unlimited access to water and optimal fertilizers to achieve the expected cultivation biomass and biochemical traits. Several parameters were assessed in this study including growth rate (d^{-1}), biomass productivity ($mg.L^{-1}.d^{-1}$), harvest cell density (x10^6 cells mL^{-1}) and cell dry weight (pg $cell^{-1}$). Various experiments were performed including 4 commercial fertilizers, iron fertilizer, and BBM formulation for comparison. In addition, the 3 formulations performed with the commercial fertilizers were supplemented with the same micronutrients used in BBM formulations. Biochemical assessments were performed to the dehydrated *Chlorella vulgaris* biomass, proteins concentration were between 188.99 ± 16.06 $mg.g^{-1}$ and 614.73 ± 65.34 $mg.g^{-1}$. The remaining growth medium was also assessed for total nitrogen to determine the microalgae consumption rates which varied from 56.44 ± 12.50 µg $NO_3.mL^{-1}$ and 381.97 ± 2.55 µg $NO_3.mL^{-1}$. Lipids ranged from 57.62 ± 10.92 $mg.g^{-1}$ to 125.19 ± 2.99 $mg.g^{-1}$, minerals from 134.64 ± 3.69 $mg.g^{-1}$ to 364.31 ± 0.64 $mg.g^{-1}$, chlorophyll *a* from 6.23 ± 0.49 $mg.g^{-1}$ to 21.72 ± 0.74 $mg.g^{-1}$, chlorophyll *b* from 0.81 ± 0.4 $mg.g^{-1}$ to 7.60 ± 0.73 $mg.g^{-1}$ and carotenoids from 0.50 ± 0.2 $mg.g^{-1}$ to 3.37 ± 0.26 $mg.g^{-1}$.
Overall conclusion is that using fertilizers optimization, the values obtained with BBM cultivation are almost reachable, reducing the production costs. *Chlorella vulgaris* cultivated exclusively with BBM makes this business model unprofitable and/or unachievable. Reducing production investment is the "golden egg" for this industry, producing more for less and turning this biomass accessible.

Table 1 – Comparing BBM with the best fertilizer recipe (% of difference with BBM)

	BBM	Fertilizer recipe variation B
Protein ($mg.g^{-1}$)	614.73	391.31 (- 36 %)
Lipids ($mg.g^{-1}$)	111.02	125.2 (+13 %)
Minerals ($mg.g^{-1}$)	134.64	210.24 (+56 %)
Chlorophyll *a* ($mg.g^{-1}$)	21.72	13.07 (- 40 %)
Chlorophyll *b* ($mg.g^{-1}$)	7.6	4.04 (- 47 %)
Carotenoids ($mg.g^{-1}$)	3.37	1.43 (- 58 %)

Keywords
Chlorella vulgaris; Protein; Lipids; Photobioreactors; Madeira archipelago

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Nuno Nunes completed his PhD in Biological Sciences in 2020 by University of Madeira, Faculty of Life Sciences, has a master's degree in applied Biochemistry obtained in 2011 and a graduation in biochemistry, finished in 2008 in the same University. Published 12 articles in peer reviewed journals. Participated in 13 events and has been involved in 8 research projects. Nowadays is a post-Doc researcher in the ISOPlexis Centre of sustainable agriculture and food technology, working on the "Use of biorefinery as a strategy for processing algae and obtaining new bio-based products, increasing the sustainability of the local economy".

COMPANY PROFILE

The PhytoAlgae company (https://phytoalgae.pt/) currently, is strongly dedicated to the production of premium food supplements with unique characteristics, rich in various bioactive compounds and protein, with a focus on preventing and improving the health of its consumers through biological and bio-sustainable nutrition. Bearing this in mind, the company bases its activities on a rigorous and detailed R&D plan, the objective of which is the constant search for optimal crop production conditions, the exploration of new species of microalgae with unique traits, and the elaboration of metabolic profiles of the species in production, searching for new natural biomolecules present in the biomass with unique biological functions.

IMPROVING THE SENSORY QUALITY OF MICROALGAL FOOD INGREDIENTS

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ABSTRACT

The use of microalgae as a valuable food ingredient has deserved special attention in recent years, considering its ecological sustainability and nutritional richness (1,2). Although consumer appeal for microalgae is increasing, there is still a long way to go before microalgae-based food products become mainstream in Europe. One of the main drawbacks for the industrial scale-up is related to the new challenging colours, flavours and tastes present in microalgae-containing formulations (3).
There are some microalgae in the market ingredients with less pronounced colors and flavors, produced in heterotrophic conditions, but their biochemical composition is different from autotrophic microalgae, mainly in terms of scavenging activity, with heterotrophic growth showing lower free radical and superoxide anion activity (2). Other technologies have already been applied to microalgae, such as the extraction with ethanol and a reduction in the intensity of flavor and color of bread was verified, but with a negative impact in bioactive properties (4). Therefore, it is essential to bet on clean technologies, which in the future can be used by microalgal biomass producing companies to produce sustainable food ingredients.
YUM ALGAE aims to improve the overall sensory quality of microalgal ingredients by using enzymes, which will target two of the major organoleptic bottlenecks: the fish-like smell associated to volatile compounds and the green dark color of the microalgal pigments. Microalgae subjected to the enzymatic process developed within the project can be used as natural, innovative and sustainable ingredients to nutritionally enrich food products. Two different food matrices, bread and cheese will be studied to achieve higher levels of algae incorporation and good acceptance by the consumers, maintaining high functionality, nutritional quality and bioactivity of the food end-products.
The results of the nutritive and sensory profile of the microalgae biomass and microalgae-added bread and cheese products, before the enzymatic treatment of the microalgae biomass, will be presented, and the compounds responsible for the flavour will be identified by chromatography and sensory techniques. In the future NORCE/Norway will conduct enzyme purification techniques to produce sensory enzymes and IST/Portugal will optimize the enzymatic processing of the microalgal ingredients for each product case.

Keywords
Microalgae, sensory profile, enzymes, bread, cheese.

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BIOGRAPHY

Maria Cristiana Nunes holds a 5 year graduation and a PhD in Food Engineering from the School of Agriculture (ISA)/ University of Lisbon (PT). She has currently been invited Assistant Professor at the Lisbon School of Medicine/ University of Lisbon (Food Quality Management), and Assistant Professor at Lusófona University (Food Technology). She has a research position at ISA in the Project YUM Algae: enzYmes for improved sensory qUality of MicroALGAE ingredients in foods, supported by the EEA Grants (PT-INNOVATION-0075). She participates in several national and international research (ORCID: 0000-0002-1281-8606) as a member of the LEAF Research Unit (UI/AGR/04129). Supervisor of 3 PhD theses and of 15 MSc theses, collaborating in other 7 PhD theses and several MSc theses.

Research areas of interest: main work focused on the use of poorly exploited food sources (e.g., algae biomass and food industry by-products) for the development of high added value products; functional properties of macromolecules - proteins and polysaccharides; development of new food products; evaluation of the rheology behavior of different food matrices. Many of the research projects are done in collaboration with the food industry, and she has an award in the area of innovation - “Born from Knowledge” prize granted by the National Innovation Agency in 2021.

GENERATION OF IMPROVED *CHLORELLA SOROKINIANA* UV MUTANTS WITH ENHANCED LIPID ACCUMULATION CAPACITY

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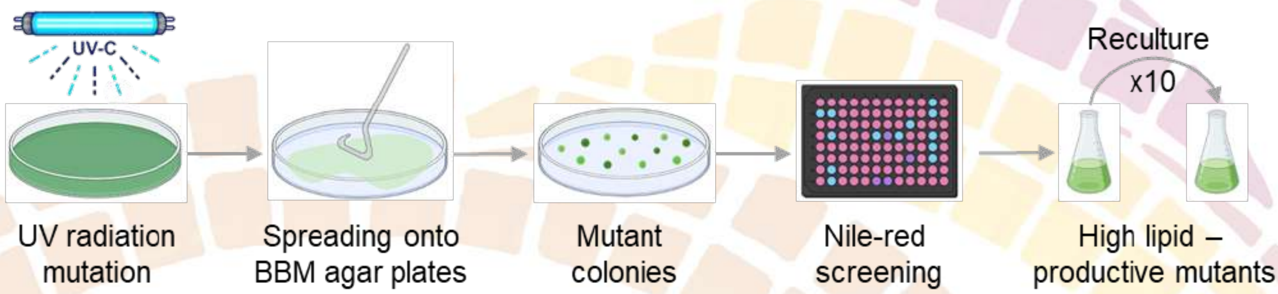
ABSTRACT

Microalgae are a prominent natural source of macronutrients and they have mobilized a massive interest in getting them enlisted in the catalog of human nutrition products. However, due to their low production capacity, strain improvement is the key to increasing biosynthesis of targeted biomolecules. Random mutagenesis causes genetic alterations that can potentially lead to the enhanced accumulation of desired intracellular components such as lipids [1]. Such gene modifications can be evoked by chemical or physical mutagens, like ultraviolet (UV) radiation [2]. This research assesses UV-induced mutations of *Chlorella sorokiniana* as a means of enhancing lipid synthesis aiming at the development of valuable strains for the food industry. The mutagenesis protocol comprises culture exposure to UV-C light for sufficient time to attain cell fatality higher than 50%, followed by a relief period of 24-h in the dark. Attainment of the desired cell fatality was monitored by $O.D._{750nm}$ measurements of the mutated strains and comparison against wild type (same treatment without UV exposure). Upon determination of the appropriate time of UV treatment, the protocol was applied on fresh *C. sorokiniana* cells. After the 24-h dark period, the cells were spread onto BBM (Bold's Basal Medium) agar plates and incubated until algal colonies appeared. The biggest and greenest mutant colonies were inoculated in 5 mL of fresh BBM in 25-mL Erlenmeyer flasks and cultivated for 5 days at 25 °C under diel cycle of 16/8 hours light/dark. The success of the triggered mutagenesis, in terms of intracellular lipid content, was assessed by staining the cells of the cultures with Nile red [3] and comparing against the wild type . The mutants exhibiting more intensive fluorescence than the wild type were selected for subsequent lipid quantification with the sulfo-phospho-vanillin method [4]. The most productive mutants were cultivated for 10 cycles of 12 days each to ensure that their growth and lipid accumulation rate remained stable. This work resulted in the generation of 5 stable *C. sorokiniana* mutants with lipid productivity considerably higher than that of the wild type strain.



Keywords
Chlorella sorokiniana, random mutagenesis, UV radiation, strain improvement, lipid accumulation

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Acknowledgements

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BIOGRAPHY

Georgia Papapanagiotou is a Ph.D. Candidate at the Department of Chemical Engineering (School of Engineering, Aristotle University of Thessaloniki - AUTH). Her research deals with developing an innovative autotrophic microalgae cultivation system for the sustainable production of high-value nutraceutical products through targeted bio-refinement of the produced biomass. She holds an MSci degree in Food Science and Technology from the School of Agriculture of AUTH, an MSc degree in Hydrobiology and Aquacultures (School of Biology, AUTH), specializing in the polyphasic characterization of commercially cultivated microalgae, and an MSc degree in Total Quality Management (School of Science and Technology, Hellenic Open University – HOU), specializing in the parameterization of microalgae cultivation through the application of fractional factorial experiments. Her research interests focus on the commercial exploitation of microalgae for food and nutraceutical products and the applications of fractional factorial experimental design in the broad area of microalgae biotechnology. She is an accredited agricultural advisor and an external research associate of AUTH and the Hellenic Statistical Authority (ELSTAT).

HARVESTING OF MICROALGAL BIOMASS BY OIL FLOTATION AND SEDIMENTATION

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ABSTRACT

This work deals with the harvesting of the green alga *Chlorella vulgaris* using a novel flocculating agent prepared from a solution of hexadecyltrimethylammonium bromide at a initial concentration of 1 g/L and a rapeseed oil-in-water emulsion. The hexadecyltrimethylammonium bromide (CTAB) molecules bind to the surface of the oil droplets, giving a positive charge to the emulsion particles formed. The emulsion droplets thus stabilized interact very well under appropriate conditions (pH, dose of flocculating agent) with negatively charged algal cells, causing floc formation and sedimentation or flotation. In experiments, algal suspension in mineral medium was tested at different pH values and flocculation reagent doses. From the results, it was found that the pH and the amount of flocculating agent had a significant effect on the efficiency of the algal biomass separation process, which reached high values above 95% at pH 10 and 12, already at a dose of 1.75 ml of flocculating agent. Moreover, the biomass obtained had a relatively low concentration of CTAB in the case of an efficient process, which would not preclude subsequent food use. After the initial experiments, the CTAB content of the reagent was reduced and to evaluate the effect of the mineral medium itself, the experiments were repeated in a NaCl model medium of the same ionic strength. In addition to rapeseed oil, sunflower, olive and peanut oils were also tested.

Keywords
Cetyltrimethylammonium bromide; *Chlorella vulgaris*; cooking oil;
sedimentation; flotation

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Presenting author is employee of Department of Biotechnology at University of Chemistry and Technology, where he is also doctoral student of study program biotechnology. His main topic dealing with new methods of flocculation and flotation of microalgae. Author has also experience with lab scale bioreactors and with interaction of microorganism with solid surfaces.

COMPANY PROFILE

The University of Chemistry and Technology, Prague is a centre of first-rate study and research in the area of chemistry in Czechia and is one of the country's largest educational and research institutions focused on technical chemistry, chemical and biochemical technologies, material and chemical engineering, food chemistry, and environmental studies.

MICROALGAE BIOMASS AS A CLEAN LABEL FOR VEGAN MAYONNAISES

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ABSTRACT

Being intrinsically linked to consumer trends, one of the main challenges of Food Industry is its constant need of changing to adapt to these trends. Currently, consumers are eager for food solutions that promote one-health[1], simultaneously assuring physical and mental health, while maintaining a strong environmental and ethical consciousness, hence pressing for sustainable and transparent supply chains. Furthermore, the clean label trend arises, as consumers are looking for simpler formulations, with less ingredients and easily recognizable origins[2]. Companies must invest in reinventing traditional products and adapting them to these trends.

This work, part of the cLabel+ project, aimed to evaluate the potential of microalgae biomass as clean label ingredient to replace common preservatives, antioxidants and colorants of Casa Mendes Gonçalves' vegan mayonnaises.

Five types of *Chlorella vulgaris* were tested – A2F, Organic, Smooth, Honey and White. A2F-*Chlorella* was obtained from a certified producer from Norway. The remaining *Chlorella sp.* were supplied by Allmicroalgae, who used random mutagenesis and different culture conditions to obtain different compositions and pigmentations[3].

The antimicrobial potential of *Chlorella sp.* was tested using the Drop-Test method. The *Chlorella sp.* presented different inhibitory effects depending on the strain of microorganism, microalgal biomass concentration and algae species. Considering that antimicrobial activity is often correlated with the content in bioactive compounds, total phenolic content (Folin-Ciocalteau method) and antioxidant activity (DPPH and FRAP) were also assessed.

After preliminary tests of microalgal biomass incorporation, considering color and flavor, only Honey *Chlorella vulgaris* was considered adequate for vegan mayonnaise development. Crude protein (Dumas method) and crude fat (Soxtec method) were determined for the tested microalgae. Honey *Chlorella* was incorporated in different proportions in fava bean and lupin stabilized mayonnaises, and its effect on texture (TPA), viscoelastic properties (SAOS), flow behavior, droplet size distribution and microstructure (SEM) was assessed. The color of the produced mayonnaises (CIELAB) was compared with traditional mayonnaise and plant-based mayonnaise dyed with conventional colorants. A preliminary sensory assay was also performed.

From the results obtained, it is possible to conclude that *Chlorella sp.* can be used as a clean label ingredient, originating products with better nutritional characteristics than the original mayonnaises.

Keywords
Food Ingredients; New product development; Healthy Foods; Clean label foods; Plant-based foods

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Acknowledgements
This work was supported by Agência Nacional de Inovação (Portugal) through the project cLabel+ (PO CI/ANI/46080/2019) and by FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit. We would like to acknowledge Cristiana Duarte (ISA – ULisboa) for the SEM analysis and Prof. Cecilio Carrera Sanchez for the DSD analysis.

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BIOGRAPHY

BSc and MSc in Food Science and Engineering by the School of Agriculture of University of Lisbon. PhD student in the same institution, in Uso Sustentável da Terra – Food Science and Engineering. Fellow researcher in LEAF Research Centre, her focus is on new product development using innovative ingredients, as underexplored food sources and fermented foods.

INFLUENCE OF PRE-TREATMENT AND STORAGE CONDITIONS ON STABILITY OF NUTRIENT CONTENT IN *CHLORELLA VULGARIS* AND *TETRASELMIS CHUI* BIOMASS

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ABSTRACT

Microalgae contain many different valuable components, with a significant nutrient value. Each component, or a combination of these, will have their specific characteristics and possible use in a commercial framework. For some of these components, stability during storage can be a problem. On the other hand, given the high concentration of antioxidants and antimicrobials in microalgae, these may also be used as preservatives, inhibiting food waste.

Storage of biomass from *Tetraselmis chui* and *Chlorella vulgaris* was explored in respect to nutrient content, under seven different combinations of pre-treatments and storage temperatures. The three different pre-treatments that were compared, were 1) centrifuged biomass paste with a water content of 40-50 %, 2) freeze dried biomass and 3) biomass that was freeze dried followed by bead milling with 99% efficiency. The storage conditions were -20ºC, 4ºC and 22ºC in darkness and with a small headspace of air. The nutrient components included in the study were chlorophyll *a* and *b*, carotenoids, polyphenols, fatty acids (FA) including saturated FA (SFA), monounsaturated FA (MFA) and polyunsaturated FA (PUFA). The study included 6 sampling points, up to ~2,5 years of storage.

Results showed that carotenoids in particular were quite unstable during longer term storage. For example in the case of milled biomass from *T. chui* stored at room temperature, the content showed significant degradation after only 1 month, and was non-detectable after one year. Chlorophyll *a* showed also some degradation during storage, while Chlorophyll *b* remained relatively stable. PUFAs were unstable during storage in *T. chui*, while being more stable in *C. vulgaris* during the first year.

In most cases, there was a clear difference between the two species. For *T. chui*, milling had a more negative effect on the storage stability than for *C. vulgaris*. Freezing temperature did not in all cases prevent degradation. The study concluded that it is in general important to store the biomass in non-disrupted form, at all the storage temperatures included in this study.

Keywords
Storage, nutrient stability, *Tetraselmis, c, Chlorella*

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BIOGRAPHY

Kari has a PhD in microalgae technology from University of Bergen, with main focus on cultivation technology. She has been employed as a researcher at NIBIO since 2008. Her research has emphasis on microalgae for food and feed, where cultivation methods and stress physiology is used as a tool for obtaining algae biomass with composition adapted to specific products. Microalgae as a source of proteins, unsaturated fatty acids and pigments are important elements. NIBIOs pilot facility for microalgae production in Ås with large scale photobioreactors, is used for research on upscaling of cultivation methods developed in lab scale, and for production of algae biomass for various microalgae product developments. In addition, she studies psychrophilic extremophiles and other microalgae adapted to low temperatures, as a source of bioactive compounds through bioprospecting, with a focus on cancer inhibitors.

COMPANY PROFILE

NIBIO is a research institute that contributes to food security and safety, sustainable resource management, innovation and value creation through research and knowledge production within food, forestry and other biobased industries. NIBIO is owned by the Ministry of Agriculture and Food as an administrative agency with special authorization and its own supervisory board. NIBIO has algae research activities in three different divisions, located in Ås and Bodø.

FUNCTIONAL FOODS WITH MICROALGAE: IMPACT OF CONCENTRATION AND DRYING METHODS ON PASTA

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ABSTRACT

WHO recognizes *Arthrospira platensis* as a "superfood" due to its nearly 70% of protein contents and a source of vitamins (e.g., B complex) and fatty acids (including ω3 and ω6). Alike *A. platensis, Chlorella vulgaris* has antioxidant, anti-inflammatory and immunomodulatory properties, while having a similar nutritional and bioactive composition to *A. platensis*, with vast quantities of natural pigments, namely carotenoids[1].

Microalgae and cyanobacteria have a relevant positive environmental impact for their higher protein yield comparing to soy and livestock, and, unlike terrestrial crops, they don't require arable soil.

Previous studies of microalgae-fortified food products have resulted in a nutritionally enhanced pasta, without affecting its cooking and texture quality properties[2]. In addition, different drying methods result in distinct nutritional composition, bioavailability, sensory profile, and functional quality of the biomass. While solar drying is less aggressive on bioactive compounds and is cheaper, it takes a longer time and need a larger area, depending on climate conditions. On the other hand, spray drying is commonly used, as it is faster, flexible and can be used in continuous operation. Disadvantages stem from high installation and energy costs, and being unrecommended for thermosensitive ingredients[3,4].

The effect of adding spray-, solar-dried and non-dried (frozen paste) *Chlorella vulgaris* and *Arthrospira platensis* on the technological, nutritional, and bioactive properties of fresh wheat pasta was investigated.

Samples were characterized in terms of rheology (viscoelasticity), texture (TPA, firmness, stickiness, and tensile strength), and cooking quality (water absorption, cooking loss, and swelling index) tests to better understand the impact of microalgae addition in the pasta's properties. Furthermore, bioactivity tests evaluating the sensitive compounds like the total phenolic content, antioxidant activity (DDPH and FRAP) and colour were performed. A sensory analysis was also performed.

Results pointed out to an optimum of 4% level of microalgae incorporation. Differences in results from different drying methods have been demonstrated. Non dried and solar dried samples have a larger bioactivity and different technological properties. However, spray dried microalgae were able to either maintain or reinforce pastas' internal structure, demonstrated by almost all parameters, reenforcing the value of a more intensive drying, to produce a quality product.

Funding

This work was supported by Agência Nacional de Inovação (Portugal) through the project cLabel+ (PO CI/ANI/46080/2019) and by FCT – Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.

Keywords

Chlorella vulgaris; *Arthrospira platensis, frozen paste,* solar & spray drying, *wheat pasta*

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Pioneered the studies on Food Texture & Rheology in Portugal, set up the Food Rheology and Cereal Technology Labs to support research.
Her work is related with sustainability and efficiency, involved in projects with the Industry (e.g. food product development including functional ingredients and foods as well as by-products and underexplored food sources, with strong impact on consumer's wellbeing and health).
Participation in several European projects.
Reaching 100 ISI publications and several book chapters in Applied Food Rheology and Functional Foods.
Expert evaluator for: i) the Eurostar/Eureka Secretariat; ii) the Research Executive Agency of EU and iii) the Danish Agency for Science Technology & Innovation – Innovation Fund Denmark (IFD).

WASTEWATER MICROALGAE AS BIOFERTILIZER: IMPACT ON LETTUCE GROWTH AND COSUMTION SAFETY

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ABSTRACT

In recent years, the number of studies related to the use of microalgae as biofertilizer have increased, showing promising results (Ronga et al., 2019). In a circular bioeconomy context, wastewater can be seen as a low-cost source of nutrients for microalgae growth. However, when this biomass is used for agricultural purposes, the presence of pathogens, heavy metals and emerging organic contaminants (EOCs) in the biomass should be monitored. The aim of this study was to perform an experiment in greenhouse using microalgae biomass grown in wastewater as biofertilizer for lettuce production. In order to guarantee human health, pathogens, heavy metals and EOCs were monitored in wastewater, microalgae biomass and lettuce leaves. In this agronomic assay, lettuce growth was tested under different conditions: (C-) negative control with no fertilizer; (C+) positive control with full dose NPK fertilizer; (T1) microalgae biomass+50%N fertilizer+100% PK fertilizer; (T2) microalgae biomass+100% PK fertilizer. C+ and T1 presented similar results, characterized by the highest fresh shoot weight. Concerning pollutants, the concentrations of pathogens and heavy metals found in microalgae biomass were below the threshold of the European regulation (EC) 2019/1009 and heavy metals were not reported in lettuce leaves. EOCs were found in the domestic wastewater. Although only caffeine and hydrocinnamic acid was reported in small quantities in the biomass, these EOCs were not detected in lettuce leaves samples. This study revealed that wastewater grown microalgae can be used for agricultural purposes to reduce the dose of mineral nitrogen without compromising human health.

Keywords
Microalgae; wastewater; heavy metals; pathogens; organic emerging contaminants; biofertilizer.

References

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BIOGRAPHY

Dr. Enrica Uggetti is graduated in Environmental Engineering from the Politecnico di Torino (Italy) in 2007, and she received the Ph.D in Civil Engineering from the Universitat Politècnica de Catalunya (Spain) in 2011. Between 2011 and 2013 she has been hired as postdoctoral researcher by the French National Institute for Agricultural Research (LBE-INRA) in Narbonne (France). Since 2013, she is working as senior researcher at the Universitat Politècnica de Catalunya within the Environmental Engineering and Microbiology Research Group (GEMMA).
She is internationally recognized for her expertise in the field of nature-based solutions for wastewater treatment, with special focus on constructed wetlands and microalgae-based treatment. She has been involved in more than 25 projects, leading 5 of them. Co-author of more than 50 research papers, she has also written 1 book and 5 books chapters. During the last years, her research interest has focused on resource recovery from wastewater under a circular economy approach. Within the GEMMA group, she is currently leading the research line focusing on the production of biofertilizers and biostimulants from microalgae grown in wastewater. She is also involved in different projects dealing with agricultural application of sludge obtained from domestic and industrial wastewater treatment.

COMPANY PROFILE

The Group of Environmental Engineering and Microbiology (GEMMA) is part of the Department of Civil and Environmental Engineering (DECA) of the Universitat Politècnica de Catalunya. BarcelonaTech (UPC).
The Group is dedicated to interdisciplinary research, innovation, knowledge transfer and education in environmental engineering; particularly in the fields of environmental biotechnologty, water supply, wastewater and solid waste treatment, and bioenergy generation. GEMMA was founded in 2005, since then the group has played a remarkable role promoting ecoinnovation and biotechnologies for efficient wastewater and sludge treatment and reuse.
GEMMA aims at contributing to sustainable development by means of fundamental and applied research activities, and through innovation in the water and energy fields.
The goal of our research activities is to develop and promote sustainable and innovative water, wastewater and sludge treatment biotechnologies, capable of removing a wide range of contaminants, including emerging pollutants.

IMPACT OF DRYING ON HETEROTROPHIC *CHLORELLA VULGARIS*: A PROTEIN QUALITY ASSESSMENT

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ABSTRACT

Although biomass drying is a critical step in the microalgae value chain for preservation and transportation purposes, limited knowledge exists on the effect of drying on protein techno-functional properties and bioavailability. In this study, we subjected heterotrophically grown *Chlorella vulgaris* biomass to 2 industry standard (spray drying (SD), freeze drying (FD)) and 3 innovative (solar drying (SolD), agitated thin film drying (ATFD), pulse combustion drying (PCD)) drying methods. We hypothesized that higher drying temperatures and mechanical forces could disrupt the *C. vulgaris* cell wall, releasing proteins and other intracellular components. Moreover, methods operating at higher temperatures could denature heat-sensitive components such as proteins as well as pigments (chlorophyll, antioxidants and vitamins), resulting in altered appearance, nutritional value and techno-functional properties. To challenge this hypothesis, we tested the dried powders for several techno-functional parameters (protein solubility, water/fat binding, foaming, emulsifying and gelling properties, solubility, color) as well as the degree of protein denaturation (differential scanning calorimetry, DSC) and sensorial properties (smell and taste attributes). In addition, microbial safety and the *in vitro* digestibility (INFOGEST 2.0) were assessed. Our results demonstrate a differential impact of the drying methods on techno-functional properties. Freeze dried powder performed best in fat-related functional properties (emulsifying capacity, oil holding capacity), whereas ATFD treated powder showed significantly higher protein solubility, gelling capabilities and water holding properties. Treatment with PCD then again resulted in significantly increased foaming properties compared to the other treatments. Determination of denaturation enthalpy, using DSC, revealed significantly reduced enthalpy after ATFD treatment, indicating increased protein denaturation. *In vitro* digestion trials with subsequent nitrogen (N) solubility determination (a measure for digestible protein) showed significantly increased N-solubility for the ATFD treatment. Sensorial analyses using a trained panel revealed observable differences in sensorial attributes, although non-significant. All dried powders could be considered microbiologically safe, with total microbial count ranging from 10^3 (FD, SolD, ATFD) to 10^5 CFU/g (PCD, SD). In conclusion, different drying methods differentially affect the release and denaturation of *Chlorella vulgaris* protein, resulting in distinctive techno-functional properties and bioavailability.

Keywords
Chlorella vulgaris, drying, protein denaturation, techno-functional properties, digestibility

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COMPANY PROFILE

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MICROALGAE CULTIVATION IN CLOSED PHOTOBIOREACTORS, INTEGRATED INTO A CIRCULAR ECONOMY

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ABSTRACT

Climate change, a globally rising population, a lack of land and water as well as the limitations of the classical agricultural industry create the urgent need for new solutions. Clearly, previous solutions have shown insufficient to solve existing and upcoming global problems. Most of the fossil energy used by mankind was created from microalgae. Thus, why not use microalgae's capabilities to overcome future problems? For some time now, the cultivation of microalgae and cyanobacteria has been well researched. Through novel technologies, novel biologic strands and refinery technologies, a wide range of high-quality ingredients may be produced with microalgae and cyanobacteria. Basically, the production of high-quality microalgae biomass needs only a minimal amount of water, no pesticides, and CO2.

Yet, the processes to cultivate microalgae and cyanobacteria are complex and require a well-thought-through multidisciplinary approach. By providing innovative engineering solutions, Algoliner helps to unleash the full potential of microalgae and cyanobacteria cultivation. The Algoliner method makes microalgae cultivation more sustainable and versatile for a fraction of the costs of conventional cultivation methods. We care for the environment. Thus, responsible raw material usage to us is more than just a means to cut costs. Our method allows us to build photobioreactors (PBRs) with less than half the raw materials necessary compared to conventional PBRs. We further took the entire lifespan of our systems into consideration when designing the Algoliner PBRs. We have thus chosen materials suitable for both direct and chemical recycling.

Further projects we have realized or are still realizing are novel cleaning and sterilization methods as well as the incorporation of additional CO2 in our cultivation. The Algoliner approach to cleaning and sterilization drastically reduces the need for chemical sterilization and thus allows for a fully automated cultivation process. In regard to the usage of additional CO2 to enhance the productivity of microalgae cultivation, Algoliner envisioned a CO2 negative solution in which exhaust CO2 of biogas plants (and other large CO2 emitters) are (re)used for the microalgae cultivation.

Keywords
Circular economy, Efficiency of photobioreactors, carbon capturing, precision of process parameters, efficient use of CO2 as a source

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BIOGRAPHY

Hans has a degree in economics (staatl. gepr. Betriebswirt) and a university degree as an engineer in the field of processing polymeric materials (Dipl. Ing. (FH)). During almost 35 years he completed projects in the international automotive industry where he substituted metal parts with polymeric components to reduce both weight and the costs. Putting all his know-how and skills together allowed him to create a novel technology to realize photobioreactors where the pipes are being produced on site by a mobile factory.

COMPANY PROFILE

Algoliner was founded in 2013 by Hans Vaeth to realize his idea to reduce effort and costs for the creation of large scale photobioreactors. Today, Algoliner is realizing photobioreactors in all scales with their own specific components. The novel technology also allows for new process technologies.

Feed

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NEW MICROALGAE PREMIUM DIET FOR ROTIFERS: EFFICIENCY AND DOSAGE OPTIMIZATION OF PHYTOBLOOM® ELITE FORMULA

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ABSTRACT

The development of new microalgae products for rotifers (*Brachionus plicatilis*) nutrition with improved efficiency, specifically adapted to their production modes (batch and semi-continuous) and culture systems, is essential for aquaculture industry. The aquaculture sector expansion in recent years led to rotifers production intensification. *Nannochloropsis* sp. is an excellent microalga for rotifers nutrition. Thence, the use of industrially produced *Nannochloropsis* sp. liquid concentrates for rotifers nutrition is expanding, as it avoids the constraints of in-house microalgae cultivation. However, these diets still require improvements regarding precipitation, agglomeration and microbiological quality, which are detrimental for rotifers production. Besides, rotifers nutritional requirements are still poorly understood, which difficult products development and dietary protocols optimization. This study aims to investigate the optimal dosage of Phytobloom® ELITE Formula (ELITE) for rotifers batch production mode in comparison with a commercial product (Phytobloom® Green Formula - GF). Different dosages of GF were tested and the most successful was selected and applied in rotifers culture fed with ELITE. Diets efficiency was compared using a dosage of 3.2mL/million of rotifers in the first day and 2.5mL on the following days. Rotifers were cultured in cylindroconical tanks in 4 batch days, under standard environmental conditions (28ºC, pH 7.5-8 and 20 ppt). Relevant abiotic parameters such as ammonia and dissolved oxygen were regularly monitored. Rotifers population growth and amitic females' percentage were daily monitored. This work was performed under ALLARVAE project targeting the development of new microalgae products to boost live feed and marine larvae biological performance. ELITE showed significantly lower number and dimensions of microalgae agglomerates and total bacteria compared to GF. ELITE produced significantly higher growth and number of rotifers when compared to GF. In the 4th batch day, ELITE also showed significantly higher number of rotifers with eggs compared to GF. These results suggest that microalgae agglomeration reduction and bacterial load achieved with ELITE allowed higher feed availability in the water column and improved water quality, enhancing rotifers production. This work not only allowed to optimize rotifers feeding protocol with GF, but also supported the enhancement of rotifers production efficiency through the development of an ELITE premium rotifers diet.

Keywords
Microalgae; Product development; Rotifers; *Nannochloropsis* sp.; Feeding protocol

Acknowledgments
Supported by Project ALLARVAE - 069971 supported by CRESC Algarve, Portugal 2020 and European Union.

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BIOGRAPHY

Gonçalo Bastos is graduated in Marine Biology and Biotechnology with a Master's degree in Marine Resources Biotechnology by the School of Tourism and Maritime Technology (Polytechnic Institute of Leiria) since 2019. Currently, he is a PhD candidate at the microalgae-producing company Necton S.A., in cooperation with the University of Algarve (UAlg) and the Portuguese Institute for Sea and Atmosphere (IPMA), whose project will focus on using and imputing value to Necton's industrially produced algal biomass to develop new diets for bivalves in aquaculture. Starting by developing an interest for macroalgae since his Bachelor, which continued to Master's and having resulted in the publication of his thesis regarding the optimization of macroalgae culture conditions to improve the content in phycobiliproteins, he now turned his attention to the microalgae realm.
With an experience of two years as a R&D Technician at Necton, he has acquired knowledge in the employment of an industrial pipeline for microalgae production (microalgae culture techniques, inoculum maintenance, scale-up) and essentially on the development of new microalgae-based products for bivalve aquaculture and marine fish larviculture. At Necton he has collaborated with some P2020 projects such as ZEBRABLOOM and ALLARVAE focused on the development of microalgae commercial products for zebrafish nutrition and for marine larviculture, respectively.
Gonçalo is endowed with good communication skills, proactivity in investigation, ability to work as a team member and he is always highly motivated for new challenges.

COMPANY PROFILE

Established in 1997, Necton is the oldest company in Europe that specialises in the cultivation and commercialisation of microalgae. The company was set on an ideal location to grow these microorganisms in the natural park of Ria Formosa on the sunny south coast of Portugal. Throughout the years, the company acquired extensive knowledge in cultivating marine, freshwater, and hypersaline species. Necton's current portfolio includes over 30 species, such as *Nannochloropsis oceanica*, *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Tetraselmis chui*, *Porphyridium cruentum*, and *Skeletonema costatum*. To produce the microalgae biomass the facility relies on more than 100 m^3 of horizontal tubular photobioreactors and a 200 m^3 raceway pond. Necton's growth is supported by a continuous R&D effort that includes participating and managing several national and European-funded research projects. Through this effort, Necton was able to achieve scientific, technological and market knowledge of microalgae cultivation and applications for the aquaculture and cosmetic sectors, among others. The company's history, experience and constant collaboration with universities, research organisations and other companies have made Necton one of the leading European companies in microalgae biotechnology.

DUNALIELLA: BIOPROSPECTING FOR NOVEL STRAINS AND THEIR PHYLOGENETIC AND BIOCHEMICAL ANALYSIS

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ABSTRACT

Over the years, microalgal biomass has shown to be increasingly attractive as a resource for biotechnological products. As a result of the current technological developments, the prospects and applications of microalgae-based goods are rising daily. Nevertheless, microalgae yield is challenged by the need for specialized know-how to develop high-quality biomass and products. *Dunaliella* form a halotolerant genus, showing highly adaptability to harsh environments. In addition, species like *Dunaliella salina* are often used to produce high-quality molecules such as *β*-carotene in its different biotechnologically relevant isomers (all-*trans* and 9-*cis*), unlike the produced by the pharmaceutical industry, which only yields the all-*trans* isomer. Moreover, other species, like *D. bardawil* and *D. viridis*, are reported to have high concentrations of other natural products, e.g., lutein, lipids, fatty acids, proteins, and amino acids. Recently, the Marine Biotechnology Group has isolated a new strain of *D. viridis* (TAV01) from a salt pond in the Algarve. The identification was made using primers developed to infer regions at the 3' end of the 18S and the 5' end of 28S genes as is usually done for most *Dunaliella* strains with the NCBI GenBank database. This primer pair was designed to amplify and sequence the ITS1 and ITS2 loci as well as the intervening 5.8S region. The phylogenetic analysis was made using Qiagen CLC Genomic Workbench for aligning these sequences with those of other closely related homologues. The alignments produced were analysed by Maximum-Likelihood and Bayesian phylogenetic inference. The ITS2 secondary structure was modelled and aligned with highly similar (>99%) *D. viridis* ITS2 sequences to further improve the phylogenetic analysis. Compared with *D. salina*, the new isolate showed a lower lutein content. However, it displayed a 6- and 3-fold increase in the lipid and protein contents, respectively. Additionally, the fatty acids and amino acids profiles showed a more diverse composition when compared to those of other *Dunaliella* strains. The presence of high-quality natural products in *Dunaliella viridis* TAV01 offers a possibility of turning this new strain into a valuable biological resource for novel feed or food products as ingredients or supplements.

Keywords
Biotechnology, Natural Products, Pigments, Phylogeny.

Funding
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BSc's Oceanography (2017) by University of Vale of Itajaí (Brazil), MSc in Marine Biology at the University of Algarve. Algologist technician working at GreenCoLab carries out bioprospection, identification, and isolation of novel microalgae species, control of culture properties, and participates in experiments for selection and improvement of already established strains for consumption. He also has experience in Harmful Algal Blooms analysis, identification of microalgae, and toxin monitoring.

COMPANY PROFILE

The Centre for Marine Sciences (CCMAR) is a multidisciplinary, non-profit research organization promoting research and education on the marine environment. Emphasis is placed on biological interactions and the sustainable use of resources. CCMAR publishes 150-200 articles annually and, in the last five years, had 15 patents approved and established two spin-off companies.

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law.

GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology.

EFFECTS OF PRE-TREATMENTS COMBINED WITH PEPTIDASES ON *ARTHROSPIRA PLATENSIS* PROTEIN SOLUBILITY

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ABSTRACT

Arthrospira platensis has emerged as a novel protein source for monogastric animals. However, this microalga has a rigid peptidoglycan cell wall that interferes with the bioaccessibility of proteins [1]. In addition, algal proteins can form a viscous matrix trapping valuable compounds [2,3]. The present study aimed to evaluate the *in vitro* effect of six pre-treatments (bead milling, extrusion, freeze-drying after congelation at -80 ºC, heating at 70 ºC, microwave and sonication) combined with trypsin or pancreatin on the solubility of algal proteins.
The microalga suspension with phosphate buffer (20 mg/mL) was incubated for 16 h, in triplicate, with peptidases (20 µg/mL), followed by centrifugation. The concentration of total protein and peptides released to the supernatant was quantified using Bradford and *o*-Phthalaldehyde (OPA) methods, respectively. The protein solubility was evaluated by 14% SDS-PAGE gel electrophoresis through quantification of total protein content and the amount and proportions of the two most predominant proteins (40 < protein 1 < 48 kDa; 18.5 < protein 2 < 26 kDa).
Total protein concentration decreased ($p < 0.001$) with all pre-treatments, particularly extrusion and microwave, combined with trypsin relative to control (no-pre-treatment, NoP), except with sonication. Similar results were found with extrusion and microwave for the amount of total protein, protein 1, protein 2 and other proteins in the gel ($p < 0.001$). However, no significant differences were observed for total peptides measured with OPA reagent.

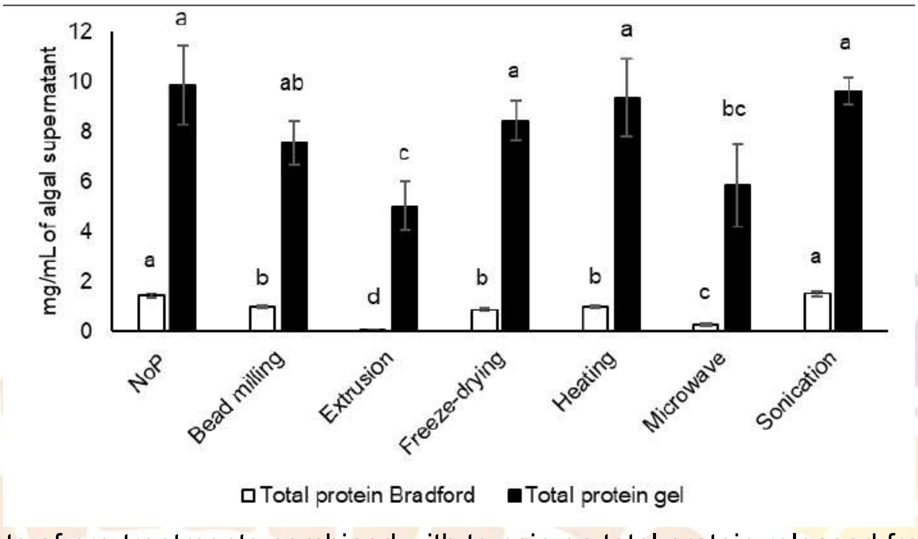

Figure 1. Effects of pre-treatments combined with trypsin on total protein released from *A. platensis* biomass (means ± standard deviation).

Moreover, a reduction of total protein concentration, as well as a decrease of total protein, proteins 1 and 2 and others and proportion of protein 2 in the gel, was obtained with extrusion combined with pancreatin compared to control ($p < 0.001$).
Overall, extrusion combined with enzymatic treatment decreased the solubility of *A. platensis* proteins, possibly due to an aggregation process that proteins undergo when denatured at extrusion conditions [4]. Further *in vivo* studies are expected to improve the understanding of how these results affect protein bioaccessibility and digestibility in monogastric animals.

Keywords
Arthrospira platensis; Pre-treatments; *In vitro*; Protein; Solubility.

Funding
Fundação para a Ciência e a Tecnologia (Lisbon, Portugal) grants (UIDB/00276/2020 to CIISA, LA/P/0059/2020 to AL4AnimalS and UI/BD/153071/2022 to M.P.S.) and Portugal2020 project (P2020/17/SI/70114/2019 and associated researcher contract to M.M.C.).

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BIOGRAPHY

M. Costa accomplished the Master in Veterinary Medicine in 2010 in the Faculty of Veterinary Medicine, University of Lisbon (FMV-UL), and, afterwards, she worked as a research fellow on Animal Nutrition in the same institution. The PhD in Veterinary Sciences, Specialty of Animal Production, was done between 2013 and 2017 in FMV-UL, with the maximum classification (distinction and laud). Then, she worked as a University Professor in Coimbra and a post-doc researcher in Interdisciplinary Centre for Marine and Environmental Research and in School of Medicine and Biomedical Sciences (ICBAS), University of Porto. Nowadays, M. Costa is working as a Researcher in the Centre for Interdisciplinary Research in Animal Health (CIISA), FMV-UL, performing *in vitro* and *in vivo* studies on macro- and microalgae for the last 4 years. In addition, she was a visiting researcher in the Agriculture and Agri-Food Canada Research Centre, Alberta and in the Department of Agri-Food Science and Technology (DISTAL), University of Bologna, Italy. M. Costa published 30 papers in international journals with high scientific impact (Q1) and 21 conference abstracts, most of them in international scientific meetings.

NANNOCHLOROPSIS OCEANICA CULTIVATION IN SUCCESSIVELY REUSED WATER – VIABILITY ASSESSMENT

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ABSTRACT

Microalgae have many different applications, but are still mainly commercialized in high-value markets, due to high production costs, which prevent their commercialization as commodities [1]. Large-scale cultivation is crucial to decrease costs; however, the need for large amounts of water negatively impacts economic viability and environmental sustainability [2]. Reusing water from growth media contributes to reducing water use, as well as salt/nutrients expenditure and wastewater treatment costs [3]. Nevertheless, it is a very complex process since many factors need to be considered to maximize culture productivity, namely stage of growth for harvesting, spent medium clean-up, number of reuse cycles, and the nutrient supply regime [3][4].

In this work, *Nannochloropsis oceanica* was cultivated in four 2.6-m^3 tubular photobioreactors (PBRs), in successive batches. In three of the PBRs, the cultures from one batch were centrifuged and the centrates were used for the next cultivation run. On the fourth PBR (control), new saltwater was used, upon culture renovation. Besides, after each batch, the culture from this PBR was used as inoculum for all four PBRs in the next batch. The whole procedure was performed seven times, over 44 days.

Table 1 – Ratio of global productivities (relative global productivity) of cultures grown in successively reused water over new saltwater and specific accumulated water consumption throughout the experiment for both conditions.

	Relative global productivity ($g\ L^{-1}\ d^{-1}/g\ L^{-1}\ d^{-1}$)						
Reutilization	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**
Ratio water reuse/new saltwater	0.86±0.04	1.01±0.08	0.99±0.07	0.99±0.01	1.16±0.03	1.16±0.03	1.22±0.16
	Accumulated water consumption per biomass produced (m^3/kg)						
New saltwater	3.99	4.38	4.19	4.14	4.13	4.17	4.29
Water reuse	1.99±0.07	1.38±0.04	0.97±0.02	0.75±0.01	0.60±0.01	0.51±0.01	0.45±0.01

Results suggest that reusing water does not impair biomass productivity (Table 1). Instead, increase in relative global productivity above 1 in the last batches, suggests that there is even enhanced growth. Furthermore, this is achieved with a lower environmental footprint concerning water (0.45±0.01 vs 4.29 m^3/kg), salt (11±0 vs 104±3 kg/kg), and magnesium supplement (0.015±0.000 vs 0.062±0.002 m^3/kg). However, the biomass biochemical profile will be analyzed to assess if the successive reutilization of water does not affect the biomass quality.

Keywords
Nannochloropsis oceanica; water footprint; biomass productivity, environmental sustainability, economic viability.

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BIOGRAPHY

Pedro Cunha is currently in the 1st year of his Ph.D. at the University of Algarve in collaboration with Allmicroalgae Natural Products S.A., Centre of Marine Sciences (CCMAR), and GreenColab – Associação Oceano Verde. Currently, he is testing different solutions, at a pilot-scale, to improve the sustainability and economic viability of *Nannochloropsis oceanica* cultivation. The final goal of his Ph.D. thesis is to set the basis for the production of a sustainable vegan eicosapentaenoic acid supplement.

Previously, he completed his master's degree in Bioengineering with a specialization in Biological Engineering at the Faculty of Engineering of the University of Porto. His master thesis was focused on the optimization of *Nannochloropsis oceanica* cultivation in pilot-scale raceway ponds with different structural designs and on the comparison of both photobioreactors. Thereafter, he integrated a master grant into the COMPETE2020 project AlgaValor, related to microalgae integrated production and biomass prospecting.

COMPANY PROFILE

Allmicroalgae provides excellence in microalgae biomass production. Microalgae are cultivated autotrophic and heterotrophically in different types of reactors: tubular, raceway pond, and thin-layer cascade photobioreactors and fermentation tanks. The company is very active in R&D, being involved in three H2020, three P2020, and one EEA grant projects and collaborating with more than 30 Portuguese and 50 international institutions, which allows accomplishing its high-quality standards.

MICROALGAE CULTIVATION AS A KEY ENABLING TECHNOLOGY FOR CIRCULAR GREEN BIOREFINERIES

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ABSTRACT

Green Biorefineries aim to sustainably produce chemicals, materials, proteins and energy by processing green biomass into a solid fraction (fibers) and a liquid fraction (juice) for further refining (Mandl, 2010). While most Green Biorefineries rely on cultivated biomass, such as alfalfa, clover or ryegrass, the use of residual streams, such as roadside grass clippings, could further improve the sustainability of the process and enable the establishment of Circular Green Biorefineries. However, the green juice produced from residual streams might have a lower nitrogen (N) content and result in an economically unviable process for protein production in the current Green Biorefinery configuration (Muneer et al., 2021; O'Keeffe et al., 2012). To address this, microalgae cultivation could be a more robust alternative for protein production from low N biomass. Here, we studied if the liquid fraction of residual grass clippings (grass juice) can be used as a nutrient source for microalgae cultivation, and if the produced algal biomass meets the safety requirements for animal feed applications. *Chlorella sorokiniana* and *Acutodesmus obliquus* were cultivated on multiple dilutions and after different pre-treatments of the grass juice. An initial experiment resulted in comparable growth to mineral commercial medium when algae were cultivated in 10% grass juice after a sedimentation step followed by 0.2-µm filtration and pH adjustment to 7. Sedimentation was necessary to reduce the presence of particles and improve the light penetration of the medium, but the microbial load of contaminants was still high even after the 0.2-µm filtration. Therefore, other combined treatments were tested, i.e., pH adjustment to 8.5 to inhibit fungal growth in association with 5-µm filtration and heat treatment. The addition of the 5-µm filtration and heating steps to reduce the microbial load did not increase algal productivity, while the sole increase of pH to 8.5 resulted in a significant reduction of yeast contaminants and promoted good algal growth. The produced biomass had a 41% protein content, and most microorganisms quantified complied with safety norms for feed production. Overall, these findings offer new perspectives to sustainably manage plant waste and convert it to a protein source in a Green Biorefinery.

Keywords
Novel protein, Feed, Grass Juice, Green Juice, Bioeconomy

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BIOGRAPHY

Dr Marcella Souza is a chemical engineer with a PhD in Biochemical Processes from the Federal University of Rio de Janeiro, Brazil. Her PhD research focused on the cultivation of green microalgae for starch accumulation and the processing of the produced biomass for obtaining sugars, pigments and a protein-rich residue. She is currently working as a postdoctoral researcher in the Laboratory for Bioresource Recovery (RE-Source Lab), Ghent University, Belgium. Her research line focuses on growing novel sources of protein, namely microalgae and duckweed, on residual streams from agriculture and landscape management. Her other research interests include nutrient recycling, biomass processing in a biorefinery, bioeconomy and circular economy.

SCALING-UP OF SPIRULINA MAXIMA CULTURES UNDER EXTREME ENVIRONMENTAL CONDITIONS IN THE SAUDI ARABIAN DESERT

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ABSTRACT

Spirulina is a cyanobacteria known for its high protein content, which can be used for feed for animals. *Spirulina (Arthrospira) maxima* was cultivated initially for two weeks under indoor controlled conditions (25 ±2 °C; 235 ±10 μmol·m^{-2}s^{-1}; 12:12 light:dark) using Jordan medium in 4-L flasks with aeration. The salinity of the media was increased step by step (at every inoculation to a higher volume) by mixing freshwater and sea water to have a final salinity of 25 ISU at indoor conditions. The analytical-grade salts of Jordan medium were replaced by industrial/agricultural grade salts, to adapt the cultures to a cheaper alternative medium, for instance N source was changed (sodium nitrate was substituted by ammonium sulfate). The adapted inoculum was resuspended in 20-L carboys at outdoors conditions in an algae pilot plant (1,000 m^2; 100 m^3 volume capacity) at KAUST, Thuwal, Saudi Arabia. The outdoor cultures were under a period of adaptation of four weeks (8 x 20-L carboys), and then they were used as inoculum for a raceway with a capacity of 1,000-L. The cultures were maintained for four weeks (3 x 1,000-L raceways; averages: temperature 35 ±6 °C; light intensity 1,850 ±300 μmol·m^{-2}s^{-1}, pH 9.0) and then the raceways cultures were used as inoculum for a 10,000-L production raceway filled at the beginning with 50% capacity. After two weeks, the volume was increased regularly, and three more subsequent 10,000-L raceways were filled with this inoculum, and finally a 25,000-L raceway was filled, for a total of 65 m^3 of culture. Simultaneously, the salinity was increased gradually from 25 to 42 ISU (Red Sea salinity). The production parameters at the peak of the summer season (ambient temperature 42°C; cultures temperature 38°C; and light intensity above 2,500 μmol·m^{-2}s^{-1}) were as average 0.681 g·L^{-1}; 163 g·m^{-2}; 16.8 g·m^{-2}·day^{-1}. Due to harsh culture conditions (high salinity, temperature, and pH) virtually no contamination was detected after two months of continuous culture. This new natural *Spirulina* adaptation approach to high salinity allows us to predict a more sustainable production of this strain along the year, helping to sort out the current worldwide drought issues.

Keywords
Arthrospira, Salinity, Sustainable, Large scale

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BIOGRAPHY

A chemical engineer with 13 years' experience in environmental and algal biotechnology, interested in bioprocesses and bioremediation. Currently working at KAUST Research & Technology Park in the project: "Development of Algal Biotechnology in the Kingdom of Saudi Arabia"

COMPANY PROFILE

King Abdullah University of Science and Technology (KAUST) has stablished the project *"Development of Algal Biotechnology in the Kingdom of Saudi Arabia"*, which has been funded by the Ministry of Environment Water and Agriculture (MEWA, project number: 52000003916), with the main objective of assess the potential of algae biomass production for animal feed using different side-stream effluents from industries. The project is under the direction of Dr. Claudio Fuentes-Grünewald.

BIOACTIVES FROM MICROALGAE MICROBIOMES FOR AQUACULTURES

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ABSTRACT

Aquaculture takes a significant role in food supply for the growing world population. However, its productivity is dependent on appropriate health management [1]. Pathogenic microorganisms such as *Flavobacterium psychrophilum, Aeromonas* and *Pseudomonas* sp. Are causing diseases in aquacultural systems and an efficient treatment or even prevention of such diseases is crucial to guarantee stable and high productivities [2, 3]. Microalgae and their microbiomes synthesize a range of bioactive substances that can be used as sustainable preventive or curative agents [3].

The main objective of the AquaHealth project Is to explore microalgae and their associated microbiome for novel bioactive molecules that can be used to treat diseases in aquaculture. Multiple microalgal strains were examined for their ability to inhibit biofilms and the growth of bacteria. For this, the inhibitory effect of the microalgae on several pathogenic and biofilm-forming bacteria was investigated in screening approaches established by SINTEF AS, the Aalborg University and the University of Hamburg. To pave the way toward commercial production of microalgae, promising strains for the application in aquaculture were selected and the production was scaled from laboratory to pilot scale by the Hamburg University of Technology and Sea & Sun Technology GmbH. Different outdoor cultivation systems were compared regarding the influence on the productivity as well as the cellular composition of the selected microalgal strains. In addition, methods for cell disruption and drying were investigated in order to develop a suitable downstream process for the microalgae. Finally, the influence of the selected microalgae will be investigated *in vivo*. For this, the produced and processed microalgae will be integrated into fish feed to examine the effect on the growth of rainbow trout.

Overall, the AquaHealth project delivers new approaches in biotechnology, screening techniques as well as cultivation and processing of microalgae. Economic, environmental and social impacts of novel product candidates are assessed, as well as routes toward commercialization of identified bioactive molecules and technologies.

Keywords
Microalgae, bioactive molecules, blue biotechnology, aquaculture.

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BIOGRAPHY

B.Sc. and M.Sc. degree in bioprocess engineering (Hamburg University of Technology (TUHH), Germany, Instituto Tecnológico y de Estudios Superiores de Monterrey, México). Work experience in the industry (Beiersdorf AG, Fraunhofer Institute for Environmental, Safety, and Energy Technology UMSICHT) and academic (Institute of Wastewater Management and Water Protection, TUHH). Since October 2018, research associate at the group of Sustainable Resource and Waste Management of the TUHH. Dedicated to microalgae cultivation and downstream processing. Vice coordinator of the ERA-Net BlueBio project "AquaHealth - Microalgae Microbiomes - A natural source for the prevention and treatment of aquaculture diseases".

COMPANY PROFILE

The research group Sustainable Resource and Waste Management is involved in the research centers "Climate Protecting Energy- and Environmental Engineering" and "Integrated Biotechnology and Process Engineering". The research encompasses technical, economic and environmental considerations in e.g. provision processes renewable energy and material sources, in production and technology-induced environmental processes.

THE EFFECT OF NITROGEN DEPLETION ON MICROALGAE FOR *DANIO RERIO LERVAE* NUTRITION

Pinheiro F.[1]

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ABSTRACT

Microalgae provide numerous benefits when used in larval culture of different fish species, which include improved gastrointestinal maturation, nutrition, and a proper environment for larval growth when used in “green water” formulations. Although rich in antioxidants, vitamins, minerals, proteins, and lipids that often contain polyunsaturated fatty acids, crucial for larval development, some microalgae require exposure to stress for accumulating protective compounds that that are essential for larval development. Zebrafish (*Danio rerio*) is the second most used model animal in scientific research, being also used as a model for aquaculture. However, zebrafish is usually fed with non-standard feeds, leading to biological variance of experimental results obtained by different research groups. This work aimed to model the nutritional profile of four microalgae species (*Nannochloropsis oceanica, Tetraselmis verrucosa, Phaeodactylum tricornutum* and *Nanofrustulum shiloi*) to evaluate the performance of *D. rerio*. Microalgae were cultured in 1-L bubble columns at laboratory scale using Nutribloom® Plus (NB+) as culture medium at two nitrate concentrations (2 and 10 mM). The cultures were subsequently used to enrich rotifers that were fed to zebrafish larvae for 30 days, using a commercial feed as control. Fish weight and length, skeletal deformation rate and midgut villi length were measured at the end of the experimental feeding trial. Protein concentration increased in all microalgae species when NB+ with 10 mM nitrates was used. The survival of larvae fed with rotifers improved by 89%, whereas the survival rate of larvae fed with commercial feed was only 59%. Zebrafish larvae fed with rotifers enriched with *T. verrucosa* at NB+ with 10 mM nitrates showed the lowest incidence of skeletal deformations (40%), with abnormalities only observed in the precaudal and caudal fin vertebrae. The same treatment promoted the development of the midgut villi, with larvae presenting a villi length of 60 µm as compared to 40 µm in control-fed larvae. In conclusion, NB+ with 10 mM nitrates offers very promising results both in terms of nutrition and larvae quality. *T. verrucosa* grown in this medium showed to be an effective microalga for rotifer enrichment to produce healthy zebrafish larvae.

Keywords
Microalgae, *Tetraselmis,* Lipids, Proteins, Zebrafish

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BIOGRAPHY

Filipa Pinheiro is currently working as a research assistant in the scope of the Zebrabloom project (commercial product development formulated with microalgae to enrich live zebrafish prey), whose main goal is to produce microalgae with distinct biochemical profiles that meet the nutritional needs of zebrafish. She has also been tasked with characterizing the biochemical profile of microalgae and rotifers that are used to feed the larvae. She graduated in Biology from the University of Algarve and is currently a master's student in Aquaculture and Fisheries at the same university.

COMPANY PROFILE

University of Algarve is a higher education institution with 7,751 students, 1,604 of which following 56 postgraduate courses, including 45 and 11 MSc and doctoral programmes respectively. Teaching and research are focused on Life/Health Sciences and Technology, in particular Marine/Aquatic Sciences, often in collaboration with Centre of Marine Sciences (CCMAR). The Centre for Marine Sciences (CCMAR) is a multidisciplinary, non-profit research organization within the University of Algarve focused on promoting marine environment based research and education. Emphasis is placed on biological interactions and the sustainable use of resources.

AMINO ACID PROFILES OF MICROALGAE FOR USE IN CHICKEN FEED

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ABSTRACT

Because of cold climate and short growth periods, production of protein rich plants like canola, faba beans and peas is very limited in Norway. As much as 95% of protein rich feed ingredients for broiler chicken feed are imported. Great efforts are being made to identify novel protein sources, and one potential protein source is based on cultivation of microalgae.

In addition to being a protein source, microalgae may contain a high number of compounds useful for poultry nutrition, such as unsaturated fatty acids, antioxidants, vitamins and trace elements, which may give added value to the feed product.

Protein content, amino acid (AA) profile and -digestibility of any novel protein source is crucial for its value as a protein source. We will use a feed formulation program to evaluate different microalgae, and establish how they fit into formulation of a chicken feed. The chicken feed requirements and ideal AA profile are sufficiently well known based on previous data (National Research Council 1994) as well as data from commercial breeding companies.

AA profiles of microalgae vary significantly between species, and it is important to choose the most suitable species for the intended feed purpose. In addition, AA profiles of the same species vary between different studies, indicating that both different strains and cultivation conditions may influence the AA composition.

In the current study, several species of microalgae have been studied with respect to AA profiles for use in chicken feed. Results from comparisons between species and amino acid profiles at different culture growth stages will be presented.

Keywords
Chicken feed, nutrition, proteins, amino acid profile

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BIOGRAPHY

Kari has a PhD in microalgae technology from University of Bergen, with main focus on cultivation technology. She has been employed as a researcher at NIBIO since 2008. Her research has emphasis on microalgae for food and feed, where cultivation methods and stress physiology is used as a tool for obtaining algae biomass with composition adapted to specific products. Microalgae as a source of proteins, unsaturated fatty acids and pigments are important elements. NIBIOs pilot facility for microalgae production in Ås with large scale photobioreactors, is used for research on upscaling of cultivation methods developed in lab scale, and for production of algae biomass for various microalgae product developments. In addition, she studies psychrophilic extremophiles and other microalgae adapted to low temperatures, as a source of bioactive compounds through bioprospecting, with a focus on cancer inhibitors.

COMPANY PROFILE

NIBIO is a research institute that contributes to food security and safety, sustainable resource management, innovation and value creation through research and knowledge production within food, forestry and other biobased industries. NIBIO is owned by the Ministry of Agriculture and Food as an administrative agency with special authorization and its own supervisory board. NIBIO has algae research activities in three different divisions, located in Ås and Bodø.

Process - Automation

ALGÆUROPE2022

13 - 15•DECEMBER•ROME

LIGHT CONTROL METHOD BASED ON ENGINEERING PARAMETER TO MANAGE MICROALGAE AND CYANOBACTERIA CULTURE

J. BILLY

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ABSTRACT

Nowadays, food and health industries are facing the challenge of finding new sustainable resources. Microalgae and cyanobacteria are promising microorganisms that could potentially fulfill the needs of a constantly growing population in a foreseeable future.
However, successfully producing these photosynthetic microorganisms at industrial scale requires a thorough understanding of their behavior regarding physical phenomena such as light [1].

The aim of our study is to develop a universal and simple culture management methodology, based on strains behavior regarding light in order to keep the cultivation system in optimal growth conditions and finally go to the automation of the culture. This presentation will discuss the validation of this culture management methodology on a known microalgae strain (*Chlorella vulgaris*) and apply the method on a new cyanobacteria strain, which is not widely studied.

For several years, we have developed an engineering parameter for this purpose: the mean rate of photon absorption by the biomass or MRPA [2]. For a concentrated culture, this parameter depends on the incident photon flux density (PFD), the specific illuminated surface ratio of the photobioreactor (a_{light}) and the cell concentration. This parameter is an engineering indicator, easy to calculate, that provides key information on the stability and viability of a production system.

A previous study [3] using *Chlorella vulgaris* was conducted to precisely determine the correlations between productivities, photon conversion efficiency by the biomass and MRPA. This work has shown that for *Chlorella vulgaris* the MRPA should not exceed 20 $\mu mol.g^{-1}.s^{-1}$. Beyond this critical MRPA value, the risk of culture loss is considerable.

For the new strain, the range of MRPA adapted to the cultivation of the strain was determined by characterizing the light sensitivity and evaluating the growth performance for different light conditions. This innovative and robust culture management methodology allows controlling the culture in photobioreactor from the beginning of the culture and avoiding the risk of culture crash. This also enables the cultivation system to maintain optimal conditions for high production efficiency. In light of these results, the developed method seems to be an effective tool for the control of microalgae and cyanobacteria cultivation.

Keywords
Light transfer, Cultivation Methodology, Photobioreactor, Mean Rate of Photon Absorption, Bioprocess

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BIOGRAPHY

She is a PhD student in bioprocess engineering working on photosynthetic organisms specifically on a new cyanobacteria strain. Her PhD consists in collaboration between the GEPEA laboratory and the company AlgoSource both located in Saint-Nazaire. Prior to her current position she undertooks a Master's degree in microbial bioprocess engineering.

She has done several internships in academic and industrial settings, during those she worked on bacteria, fungi and microalgae culture and gained experience in many laboratory techniques like bacterial transformation, mass-spectroscopy, microalgae cultivation…

Aware of the challenges ahead for the next few years, she is now working in a sustainable sector for the future: microalgae.

MONITORING PRODUCTIVITY AND STATUS OF *PHAEDACTYLUM TRICORNUTUM* CULTURES USING SPECTROSCOPY AND MACHINE LEARNING

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ABSTRACT

Microalgae industrial cultivation requires optimal monitoring and control to achieve stable performance and prevent situations of a significant reduction in productivity [1,2].The standard methods used for analysing biomass content are accurate, but they are time-consuming and cannot be applied neither *in situ* nor online, not to mention being expensive [3,4]. Optical sensors based on spectroscopy are rapid and may be applied *in situ* and online, providing data instantly that is characteristic of the process without interfering with it [5–7]. In fact, spectroscopy data may be utilized as biosystem fingerprints, being informant on its status and content [8–11]. This is possible through the use of Machine Learning (ML) tools, which provide the ability to select the relevant information while overcoming noise and non-linear dynamics [12,13]. In this work, fluorescence spectroscopy and absorbance spectroscopy are shown to provide two kinds of important information on *Phaeodactylum tricornutum* cultures: culture content, namely Cell Counts per volume (CC) and Fucoxanthin (F) and Chlorophyll a (Chl a) concentrations, and culture status, i.e., if the culture has a crash tendency or not. This was achieved by applying ML algorithms Projection to Latent Structures (PLS) and Convolutional Neural Networks (CNN), along with spectral variable selection algorithms such as Moving-Window (MW) and Variable Importance to Projection (VIP). Both ML algorithms were used in their regression (PLSR, CNNR) and classification (PLS-DA, CNN) forms. The results of this work consist of ML models that take either absorbance or fluorescence spectroscopy data as input for predicting CC, F and Chl a with $R^2 > 0.8$ of validation accuracy, and for predicting culture crash with 90% accuracy and 80% precision and recall. Moreover, these models only require up to 25% of the original spectroscopy data.

Keywords
Machine learning, microalgae cultivation, monitoring, *Phaeodactylum tricornutum*, fluorescence spectroscopy, absorbance spectroscopy

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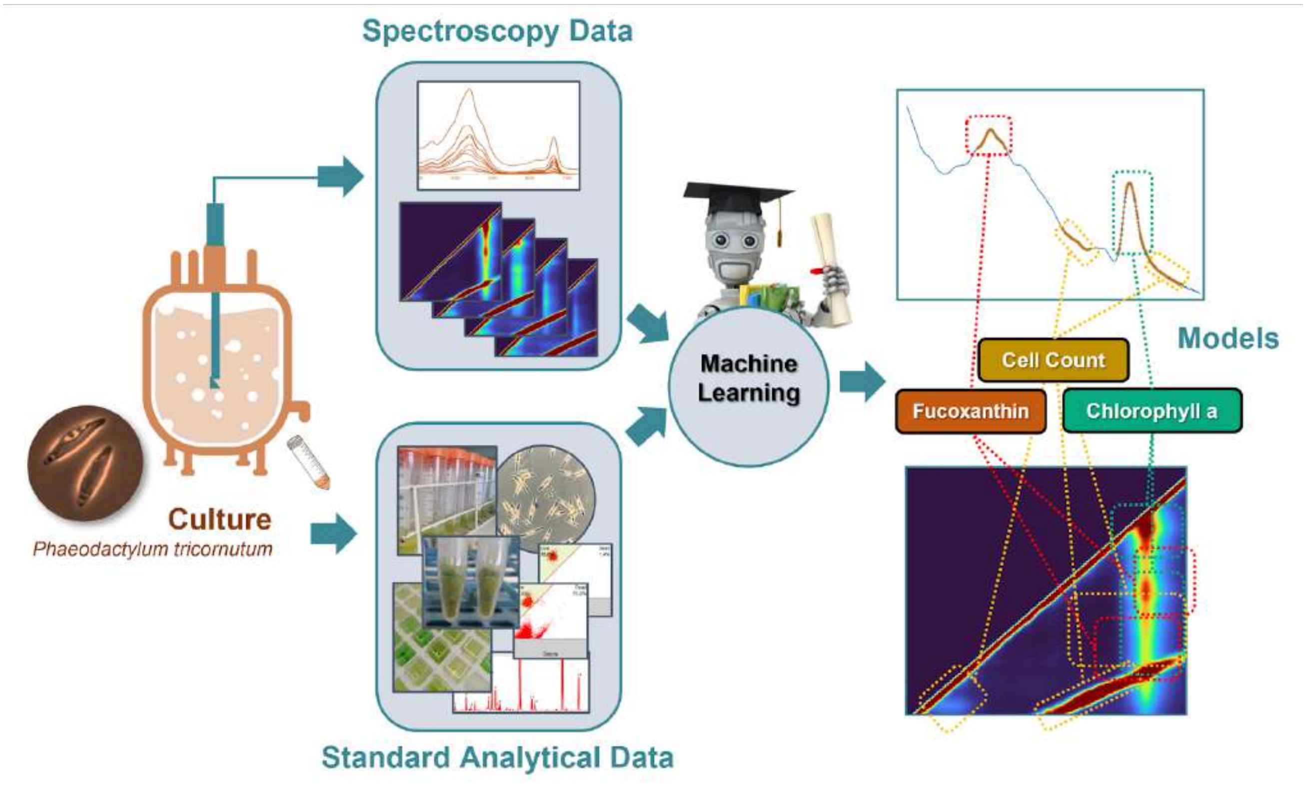
Graphical Abstract



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BIOGRAPHY

Pedro Reynolds Brandao completed the Integrated Master in Biological Engineering in 2018 at Instituto Superior Técnico (University of Lisbon, Portugal). He is currently a PhD student at the Nova University de Lisbon, hosted both by the Experimental and Technologic Biology Institute (iBET) and by the Department of Chemistry of NOVA School of Science and Technology (DQ-FCT); his studies are in the context of the Doctoral Program in Chemical and Biochemical Engineering and focus on multivariate statistical modelling (i.e., Machine Learning) for calibration of optical sensors for monitoring microalgae bioprocesses. He is co-author of 2 articles in specialized journals and has produced a master's thesis on optimizing bioprocesses with microalgae. In addition, he has held positions in both academic and professional fields such as research and development of microfluidic devices with application in physiology and operational assistance in sales, marketing, and customer satisfactory statistics of an herbal nutraceutical product.

COMPANY PROFILE

iBET works in a broad range of fields that encompass multidisciplinary approaches ranging from basic, technological to clinical and translational research, from the development of complex biopharmaceuticals and novel therapies to areas in food and health, including nutraceuticals development and agroforestry.
DQ-FCT is a permanent organic unity devoted to teaching, to scientific research and to provide services in areas of Chemistry, Biochemistry and Chemical Engineering. DQ- FCT was founded in 1981 and has recognized market implementation in education and employment, particularly so because of the novelty and specificity introduced by the course in Applied Chemistry with which teaching at the School of Chemistry began.

HOW TO GUARANTEE THE FIRST GRAMS OF CULTURE AND MAINTAIN THE INOCULUM SAFE FOR PRODUCTION

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ABSTRACT

As of today, the microalgae market is blooming. The volume of production is rising alongside new technologies like photobioreactors to answer needs, no new process covers the very first step of cultivation in a reliable way. The tedious manipulations to start a culture in Erlenmeyer or balloon and the scale up for inoculation is still a time-consuming and hard process.

From these observations we have developed a unique and patented technology (S.A.L.T.) that reaches high cell density while avoiding biofilm formation. The system creates a vertical spiral movement with an airlift (by air injection) in a vertical tubular loop. This agitation facilitates gas exchanges (carbon input and degassing in O_2) and favors light exposure while minimizing shear stress on the cells of fragile microalgae strains. For example, with *Isochrysis affinis galbana Tahiti*, the technology reaches up to 130 million cells per mL, compared to 5-10 million cells per mL in traditional systems like plastic bag. This smooth homogenization allows the culture of a wide variety of microalgae,

SALT technology is essential and works with an automated regulation system. All of our photobioreactors have an identical control system to automate the essential needs : we are able to manage light intensity, temperature, pH, gas injection, medium injection and harvesting methods as batch, semi-continuous or continuous. This automation reduces working times, optimizes consumption and maintains cultures in continuous production over several weeks or months. It also eliminates any problems while scaling up with Erlenmeyer and balloons.

First grams of culture are difficult to get, fragile and important for the next steps. We can not rely for several tones of production on a non safe inoculum. We must use technology that grows inoculum as it prevent both human and material issues often found with small lab tools.

Keywords
Photobioreactors, automatization, SALT technology, High-productivity, Bio-security

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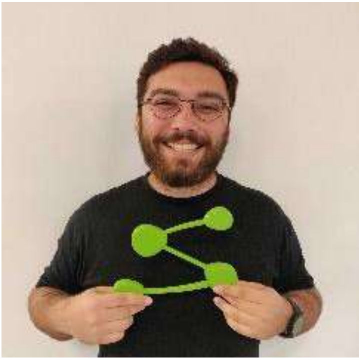

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BIOGRAPHY

Biotechnology engineer specialize on marketing and international business, I graduate from the French Institute of Biotechnology of Paris and from the Instituto Tecnologico de Monterrey, I used to work in the food industry, first at LISAqua : a French company producing fresh and local shrimp ; and then in the start-up Independent Living Base which develop urban farms fully autonomous and automatize. Then I have joined Synoxis Algae as technical-sales engineer to develop the activity in France and worldwide.

COMPANY PROFILE

Synoxis algae designs and markets a wide range of automated high density algal cultivation systems: photobioreactors, tanks, raceways. From microalgae to macroalgae, we provide different solutions to algal culture experts worldwide from different sectors such as food, cosmetics, research, green chemistry and aquaculture… As a spin-off of the plastics manufacturer Synoxis, in existence since 1981, Synoxis algae has twenty years of experience in the field of aquaculture. In 2003, Synoxis participated in Erell Olivo's thesis entitled "Design and study of a photobioreactor for continuous production of microalgae in aquaculture hatchery", in partnership with IFREMER and the University of Nantes. This led to several years of research and development on algal culture systems and algal biotechnology. The range of microalgae photobioreactors consists of three models: a small benchtop model NANO (3 L) for research laboratories, an intermediate nomadic model LUCY (16 L) for inoculum production and an industrial model JUMBO (285 L) for mass production. Today, Synoxis algae has 70 installations in France and abroad in all sectors of activity.

ACOUSTIC PROCESSING OF MICROALGAL CELLS FOR MULTIPRODUCT BIOREFINERIES

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ABSTRACT

Microalgae convert carbon dioxide into functional proteins, sugars, oils, pigments and platform chemicals. However, current harsh and energy dense processes limit the industrial viability of microalgae biorefineries and call for the development of milder multiproduct biorefinery approaches if economic feasibility is to be achieved. Acoustic fields for instance allow for highly tunable mild cell disintegration. Moreover, when combined with microfluidic channels, the precise and energy efficient manipulation of their released components can be achieved. Thus, the present research employs acoustic pulsing ultrasound waves to gently open the microalgal cells, releasing their components, followed by standing wave induced acoustophoresis within a microfluidic environment to separate them based on their size and acoustic properties (density and compressibility). Precisely manufactured microfluidic chips using state of the art SLA 3D printing ensure the complete fractionation of these components. Harnessing these forces could impact bioprocess applications for the development of drugs and novel food ingredients.

Keywords
Algal multiproduct biorefinery; External fields; Acoustophoresis; Microfluidics; Stereolithography

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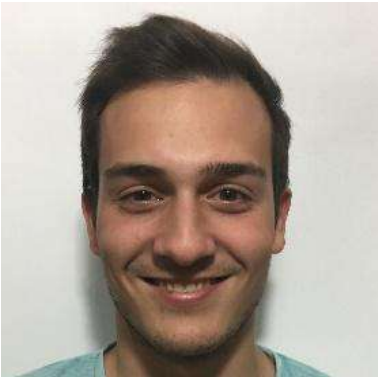

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BIOGRAPHY

Born in '96 in (very) rural Luxembourg, I have been intrigued by nature and technology throughout my life. This fascination led to pursuing a bachelor's in life science at the University of Luxembourg in 2016, including an Erasmus at the University of Aveiro where I focused on ecological subjects and plant biotechnology. After my bachelor's, I decided to pursue two master's in 2020, Conservation and Restoration Ecology at Radboud's University and Biotechnology at Wageningen University. After completing the theoretical courses in both, I made the decision to solely follow a career in Biotechnology due to a new discovered passion for engineering as well as an ever-growing interest in technology. Through my future work, I wish to impact and strive forward the current biotechnological progress towards a more environmentally oriented economy.
Outside of my studies, I am hugely into sports, particularly cycling, running, e-sports and more recently rowing. I am a former cyclist for the Luxembourgish national team in 2014-2015 as well as part of the Luxembourgish E-sports organization "RelyOn". Nowadays, I row for the Wageningen student rowing association "Argo" and lately became a big fan of 3D printing.

DEVELOPMENT OF A CLOUD-BASED AUTOMATIZATION SYSTEM FOR BIOREMEDIATION OF DRAINWATER USING MICROALGAE

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ABSTRACT

Freshwater availability represents an immediate concern, not only for human consumption but also for several industries, including the agricultural sector. The amount and quality of ground and surface freshwater is a major concern in Portugal, particularly in the Algarve region, which is suffering severe drought, with the perspective of becoming worse. This region is known for mild weather and high irradiation, optimal conditions for the establishment of greenhouses for agriculture production. A growing number of greenhouses use hydroponics (soilless) cultivation, a method easily controlled, resulting in higher crop yields, early harvests, and lower water requirements. Nonetheless, there is a substantial outflow of nutrient-rich drainwater, that can give rise to environmental problems if not properly treated. This brings the possibility of converting waste into value, by growing microalgae in drainwater to decrease nutrient concentration and production costs, while obtaining high-value products, e.g., biostimulants and aquafeeds. However, microalgae production is still a labor-intensive process that contributes to more than 50% of the total production cost. Thus, for an economically feasible production of biomass, an automatization control for cultivation was developed. This central-control system integrates and manages data generated by different sensors, from turbidity, pH, dissolved oxygen, nitrate, and phosphate, to monitor in real-time these parameters and circumvent human intervention for nutrient addition and culture harvesting. The most important parameter controlled by the automatization is the drainwater feed that is supplied to the reactor, using the nitrate concentration and turbidity as key variables. The nitrate set-point concentration was defined at 50 mg/L, which is the legal limit for discharge of water streams in Portugal. Below this concentration, fresh drainwater is introduced into the microalgal production system, while the same volume is diverted into a harvesting tank. The development of this system was essential for cost reduction and growth optimization for effective bioremediation of the drainwater, making it a more sustainable process.

Keywords
Microalgae, automatization, bioremediation, biomass, drainwater

PRESENTER INFORMATION

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BIOGRAPHY

João Navalho holds a BSc on Marine Biology and an MSc on Aquaculture, both from the University of Algarve, Portugal. He co-founded Necton S.A., where he is the current President of the Board of Directors and manages the R&D department. His MSc thesis on the development of technologies to grow Dunalliela salina for the extraction of beta-carotene was carried out under the umbrella of one of the earliest Necton's research projects. Thereafter, back in 1998, he became the team leader for the development and market introduction of Necton's microalgae concentrates for aquaculture applications. João has participated in several national and European research projects, holding more than 20 years of experience in microalgal biotechnology, from strain selection and isolation to intensive industrial cultivation in closed and open systems, downstream processing and product development. Co-founder of A4F in 2008, a Necton's spin off at the time, he was CTO for this company until 2014. Since 2015, he has been the consultant of Algafarm, the largest European industrial microalgal production unit, held by the Secil group. João is currently the Innovation Manager of the Magnificent project (BBI-2016-R09) and President of PROALGA, the Portuguese Association for Algae Producers.

COMPANY PROFILE

Established in 1997, Necton is the oldest company in Europe that specialises in the cultivation and commercialisation of microalgae. The company was set on an ideal location to grow these microorganisms in the natural park of Ria Formosa on the sunny south coast of Portugal. Throughout the years, the company acquired extensive knowledge in cultivating marine, freshwater, and hypersaline species. Necton's current portfolio includes over 30 species, such as *Nannochloropsis oceanica*, *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Tetraselmis chui*, *Porphyridium cruentum*, and *Skeletonema costatum*. To produce the microalgae biomass the facility relies on more than 100 m^3 of horizontal tubular photobioreactors and a 200-m3 raceway pond.

Necton's growth is supported by a continuous R&D effort that includes participating and managing several national and European-funded research projects. Through this effort, Necton was able to achieve scientific, technological and market knowledge of microalgae cultivation and applications for the aquaculture and cosmetic sectors, among others. The company's history, experience and constant collaboration with universities, research organisations and other companies have made Necton one of the leading European companies in microalgae biotechnology.

EFFICIENT SELECTION OF ALGAL STRAINS USING A HIGH-THROUGHPUT SCREENING PLATFORM

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ABSTRACT

Microalgae offer renewable alternatives to current commercial products such as animal proteins, fish oils and chemicals (e.g., food colourings). In light of the many challenges facing humanity, such alternatives are more necessary than ever. Environmentally conscious customers are eager for new products with a lower impact, leading the microalgal industry to constantly look at optimizing strains to increase performance, discover new molecules and develop new products. Generally, those strains are improved using evolutionary tools such as mutagenesis or selective pressures. Regardless of the mutagenesis method applied (targeted, random or selective pressure), the screening of generated strains showing the phenotypes of interest is often challenging. In this presentation, we describe the use of a high throughput screening (HTS) platform for the optimization of microalgae strains with improved phenotypic traits of industrial interest. We discuss the advantages and challenges of running such platforms. While HTS platforms are being used routinely with well-known biotechnological strains (e.g., yeast and bacteria), their use in the field of microalgae is still in its early stages. HTS platforms designed to work with microalgae hold great potential for the applications and future success of this industry.

Keywords
Automatisation; High throughput screening; Microalgae; Phenotyping, Fermentalg.

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BIOGRAPHY

Rodrigo Rangel is currently leading the microbiology platform at Fermentalg in France. In this role Rodrigo leads a team of experts in microalgal biotechnology working on R&D projects for the improvement of microalgae of industrial interest. Rodrigo is an experienced scientist with extensive know-how of the biotech industry. He held several positions as R&D manager and scientist in different companies in an international environment (France, China, US and Mexico). During his career, Rodrigo has participated in the development and industrialization of biotech derived solutions such as recombinant proteins, enzymes, fermentation nutrients, metabolites and biopharmaceutical products. Rodrigo holds a PhD in cellular biology and a Master's degree in biotechnology, both obtained at the University of Grenoble in France, he holds an engineering degree in biotechnology obtained at the "Instituto Politecnico Nacional" in Mexico.

COMPANY PROFILE

As an expert in research and bioindustrial exploitation of microalgae, Fermentalg's objective is to offer sustainable solutions and innovative products contributing to the elaboration of healthy, natural and efficient products. Our business: the development, production and marketing of sustainable solutions and active ingredients from microalgae for nutrition, health and the environment. Nutritional lipids, alternative proteins, natural food coloring and innovative environmental solutions make up our company's current and future offers.

EFFECT OF LIGHT ABSORPTION RATE AND NITRATE CONCENTRATION ON ASTAXANTHIN ACCUMULATION AND PRODUCTIVITY OF *HAEMATOCOCCUS PLUVIALIS* CULTURES GROWN IN CHEMOSTAT MODE

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ABSTRACT

This study aims to elucidate the role of light absorption rate and nitrate concentration on astaxanthin accumulation and productivity in nitrogen limited *Haematococcus pluvialis* cultures grown in continuous chemostat mode. In batch operated nitrogen starved cultures the cells accumulate large amounts of astaxanthin at the expense of biomass growth leading to severe decrease in cell division rate promoting eventual cell death. In photobioreactors (PBRs) operated in continuous mode cell multiplication can persist in nitrogen limited conditions with simultaneous astaxanthin accumulation. *H. pluvialis* cultures were grown in a flat-panel PBR with constant dilution rate and different light intensities and feed medium nitrogen concentrations. The steady state biomass, pigment and astaxanthin concentrations as well as the spectral absorption cross-section of the microalgae were measured for each culture. These were used to estimate the specific mean rate of photon absorption (MRPA) in the PBR. A parabolic relationship between MRPA and astaxanthin production rate was obtained. A maximum astaxanthin productivity was reached for the culture featuring MRPA equal to 9000 $\mu mol_{h\nu}.kg_x^{-1}.s^{-1}$. This indicated that astaxanthin synthesis was limited by the MRPA in the PBR. Consequently, the MRPA in the PBR must be carefully controlled to maximize astaxanthin productivity. Finally, our results show that astaxanthin productivity can be further increased by optimizing the mean rate of photon absorption through adjusting incident light intensity, feed medium nitrate concentration or culture dilution rate. Altogether, the results confirm that a large quantity of astaxanthin can be produced in continuous mode under indoor conditions in closed PBRs, according to the one-stage strategy which may obviously be advantageous because it would require only one step. Moreover, the astaxanthin extraction process would be facilitated in these thin-walled cells obtained, which lack the hard cell wall that characterize the cysts (obtained generally in the two-stage culture system) [1–5].

Keywords
Haematococcus pluvialis, astaxanthin, light transfer, chemostat, one-stage approach.

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PRESENTER INFORMATION

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BIOGRAPHY

Khadija Samhat was born in Lebanon in 1995. She completed her Master degree in food sciences and technologies engineering in 2018 from Lebanese University. She has started her PhD thesis in cotutelle between the Lebanese University and Nantes University on April 2019, on the topic "Contribution à l'optimisation de la production d'astaxanthine à partir de la microalgue *Haematococcus pluvialis*". This doctoral thesis is taking place at GEPEA laboratory Saint-Nazaire, and is being supervised by Pr. Jeremy PRUVOST, Pr. Ali ISMAIL, Dr. Hosni TAKACHE and Dr. Antoinette KAZBAR. Her PhD thesis is funded by the Lebanese University.

STATIC OPTIMIZATION AND DYNAMIC MODELLING OF MICROALGAE PRODUCTION IN PHOTOBIOREACTORS

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ABSTRACT

Of vital importance towards the production of photosynthetic microalgae biomass at an industrial scale is the modeling of the photobioreactors where the microalgae are grown. This project proposes a framework for the use of previously obtained process data to develop flexible and robust models for outdoor closed photobioreactors. From the analysis of data obtained from previous cultivations, the model is aimed at predicting biomass concentration and nutrient consumption of future cultivations of the same microalgae species under varying light and nutrient conditions. With this data, it will be possible to better estimate the productivity of a given culture in different weather conditions and locations, as well as better monitor the culture.

The microalgae species first analyzed was a strain of *Nannochloropsis sp* grown in a horizontal uni-layer tubular photobioreactor. The data used as input for the construction of the model were the biomass concentration (measured indirectly as Optical Density), solar irradiation, volume of the culture, nitrate concentration, temperature of the reactor, and the quantity of nutrient medium added throughout the cultivation. The PBR was operated in a semi-continuous mode with periodical renovations of the culture, where a part of the culture volume is removed and replaced by fresh medium; the renovation percentage and frequency is also used as input for the model. Various first principles models based on previous work found in the literature were assessed, with the overall best model being based on a Monod-like equation with the solar irradiation as the parameter, with growth inhibition due to biomass and nitrate; this model had a coefficient of determination (R^2) of 91.42% and that resulted in the fitting shown in Figure 1.

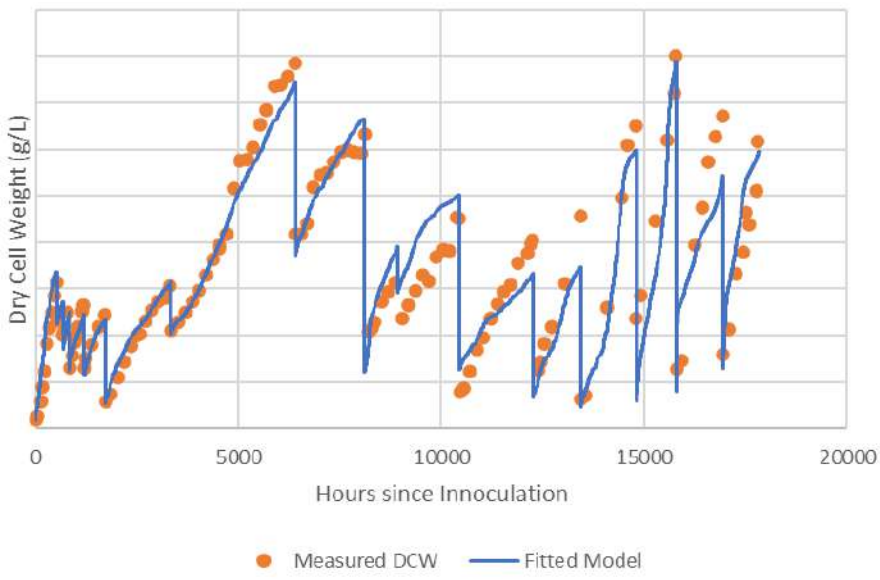

Figure 1: Measured and fitted dry cell weight for the *Nannochloropsis sp* cultivation. The sudden drops in measured dry cell weight correspond to renovations of the culture, where a significant portion is removed and replaced with fresh medium.

Future work involves correlating this model with other cultivations of the same species to determine its validity, analyzing cultivations of different species, and applying these models to real life cultivations in similar photobioreactor configurations.

Keywords
Microalgae, Photobioreactors, Modelling, Optimization, Productivity

PRESENTER INFORMATION

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BIOGRAPHY

Tiago Taborda finished his MSc degree in Biological Engineering at Instituto Superior Técnico in 2019. He is currently developing his PhD in Refining, Petrochemistry and Chemistry Engineering at the EngIQ program, at Instituto Superior Técnico, Universidade de Lisboa and Faculdade de Engenharia da Universidade do Porto, with the PhD grant awarded by Fundação para a Ciência e Tecnologia (FCT), Portugal, with the collaboration of A4F – Algae for Future. His PhD involves the modeling and optimization of closed photobioreactors.

COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions and customer characteristics and goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

Process - Bioactivity measure

ALGÆUROPE2022

13 - 15·DECEMBER·ROME

ALGAE4IBD SEARCHING FOR IBD TREATMENT IN ALGAL DIVERSITY (HORIZON 2020)

Avni D

Avni D[1,2], Shagug N[1,2], Khatteb H[1,2], Barreira L[3,4], Fernandes Rodrigues da Costa D[3,4] Viegas C[3,4], Carneiro M[5], Marzochella A[6], Pollio A[6], Monti D[6], Quéguineur B[7], Dehail M[7], Hennequart F[7], Hrouzek P[8], Hayes M[9], Sedlák D[10], Ughy B[11], Bar-Gil A[12], Marco P[12] Viswanath K[13]

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ABSTRACT

Algae4IBD's mission is to develop commercial products for Inflammatory Bowel Disease (IBD) prevention and treatment using aquatic natural biological resources. With the emerging developments in natural product, notable success has been achieved in discovering natural products and their synthetic analogs with anti-inflammatory activity. Micro and macroalgae, found in marine and freshwater, have been identified as promising sources of bioactive compounds including small molecules and secondary metabolites with a wide range of bioactivities such as antioxidant, anti-inflammatory, and cancer preventive. Those offer enormous potential for developing commercial products with public health benefits. Consumption of algae could, therefore, provide defense against chronic inflammatory diseases such as IBD, that to date have no effective cure. This project offers nature to bedside approach, using an entire development along the value chain for a new biodiscovery therapeutic approach by developing and examining algae-based compounds for IBD patients while guaranteeing algae's biodiversity preservation. We propose innovative solutions for increasing the use of algae-based ingredients and ensuring the science-based improvement of nutritional quality and its effect on public health. The researchers, companies, and hospitals involved in the different stages of the project use the biodiversity of algae, as a source for bioactive compounds. Algae4IBD concorcituom has established a unique algal bank that includes more than 1000 species of microalgae, macroalgae, and cyanobacteria. 150 species were chosen from Algae4IBD's bank to be explored for their anti-inflammatory, pain relives, and prebiotic potential.

Algea4IBD uses state-of-the-art cultivation and extraction technologies to obtain sufficient amounts of active molecules. Those extracts have already generated anti-inflammatory bioactivity hits in our cell-based /in-vitro/ cytokines promoters platforms, which will result in novel algal-based, high-quality bioactive compounds at GMP grade and lower costs for dual purposes – IBD prevention and treatment in relevance to the food as well as the pharmaceutical industries.

Keywords
Algae, extraction, active compounds, RAW264.7, THP-1, Inflammation, cytokines

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BIOGRAPHY

In the last 20 years, I focused on inflammatory process and its role in inflammatory-related disease and cancer. My scientific career has equipped me with tools in biochemistry, immunology, molecular biology, metabolomics, and pre-clinical development, enabling me to establish applied research in my lab at MIGAL. My research interest focuses on immune modulation and immune cells' functionality. We study the immune system's role in Inflammatory-based diseases and myeloid cell biology, also looking at myeloid cells as targets and biomarkers. We research sphingolipids and natural sources, such as algae, to find novel active molecules that affect the activity of the immune cells. By biomass extraction, bioactive compound identification, and isolation we explore the potential of novel compounds for the prevention and treatment of human diseases.

Our research comprises both basic and translational programs focused on chronic inflammation and cancer underlying unmet diseases such as inflammatory bowel disease, Our laboratory specializes in in-vitro, ex-vivo, and pre-clinical models, and we use immunologic, genomic, and metabolomic approaches to study our open questions. Thus, transform complex molecular information into applicable biomarkers, which might improve the well-being of patients with currently no effective care.
Additionally, I lead, as a coordinator, the Algae4IBD consortium (HORIZON2020,) which aims to use algae as a source for novel bioactive compounds for the prevention or treatment of inflammation, pain, and IBD. Algae4IBD is a multidisciplinary consortium that includes 21 partners across Europe in order to elucidate the potential of algae and connect it to a translational approach.

COMPANY PROFILE

MIGAL Galilee Research Institute is an internationally-recognized and multi-disciplinary applied research institute. MIGAL specializes in biotechnology and computational sciences, plant science, precision agriculture and environmental sciences as well as food, nutrition and health

BIODIVERSITY OF MICROALGAE IN HYDROTHERMAL WATERS FORM EXTREME ECOSYSTEMS IN TUNISIA

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ABSTRACT

Microalgae are gradually getting recognition as sustainable alternatives to plant and animal-based resources as they are considered as a promising source of high added value molecules. This has been reflected in the growing interest of industries in cultivating and exploiting their benefits in multiple sectors. Also, these microorganisms are characterized by their biodiversity and their ability to grow in various ecological niches. Despite that the Mediterranean ecoregion is considered as a perfect climate zone for microalgae cultivation, in Tunisia only few limited efforts have focused on identifying the naturally available species and their ecological distribution.

The aim of this research is to assess the biodiversity of Microalgae in different Tunisian hydrothermal waters from arid and desert ecosystems. Hence, water samples and algal mats were collected from 6 fresh water and hydrothermal water sources located at the northern, central and southern regions in Tunisia. Water samples temperatures ranged from 18°C to 51.2°C whereas pH varied from 7.2 to 8.3. Isolation and cultivation of microalgae was performed on BBM medium with an incubation at 25-40°C for 30-40 days under 16:8 light/dark photoperiods. In total, 30 cultures were obtained. Serial dilution technique and streak plate method were used for single-colony isolation of microalgae. A combined approach using light microscopy, colony PCR, ITS rRNA gene markers and amplicon sequencing (NGS) was applied to deeply analyze the algal diversity. Microscopic observation showed the presence of both unicellular and filamentous cells. Molecular identification demonstrated that Chlorophyta was the most prevalent phylum with an important relative abundance (46–99%). At the class level, both, single-cell isolation and NGS showed a major affiliation of species to Chlorophyceae (6–46%) and Trebouxiophyceae (3–15%) which were commonly detected in most samples. Overall, the microalgal community composition varied according to the geographic origin of the sample with the ubiquitous presence of *Dictyosphaerium* and *Tetradesmus* and the specific occurrence of *Sitchococcus*, *Choricystis* and *Chlorella* in the northern region water source (0.883%, 0.9% and 1.281%, respectively).

Our results revealed the huge diversity of algal communities in arid and desert water ecosystems, which constitute a valuable reservoir for biotechnologically valuable microalgae species.

Keywords
Microalgae, Biodiversity, Isolation, NGS, Biotechnological potential.

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BIOGRAPHY

Olfa BBOUSSELMI, graduated with Master's degree in Biotechnology and Industrial development of health products, member of the research laboratory "Biotechnology and Bio-Geo Resources Valorization". Olfa holds several certificates in entrepreneurship and project management and is one of the inventors of a process for obtaining natural Lycopene concentrate. Since 6 years Olfa has been project manager and member of the research laboratory « Biotechnology Research and Valorization of Bio-Geo Resources », at the Higher Institute of Biotechnology of Sidi Thabet-University of Manouba. Since entering the professional world, she has been interested in promoting the results of its research, particularly in terms of extracting active biomolecules from food waste, microalgae and other natural materials. This has earned her several national awards in the field of entrepreneurship.
Apart from her experience in the field of research, Olfa has been co-founder and biotechnologist since 4 years at « Water Spirit », a Tunisian company specializing in the cultivation of microalgae and the extraction of their biomolecule.

COMPANY PROFILE

The Laboratory of Biotechnology and Bio-Geo Resource Valorization (BVBGR), Higher Institute for Biotechnology - University of Manouba, is a governmental Institute for education and research located at Sidi Thabet city, Tunisia, which offers academic programs that respond to both multiple aspects of applied biotechnology and multidisciplinary skills needs of the industry. Besides the education role, ISBST/UMA includes many research structures which develop the management and use of bioresources methods. The BVBGR team activity is mainly devoted to the microbial and molecular resources management in the areas of (i) bioremediation (industrial effluents and pollutants, wastewaters) and biological control; (ii) extremophile communities' diversity and functionality, and (iii) risk assessment and evaluation of selected molecules and biotech microorganisms.

PHOTOSYNTHETICALLY CONTROLLED SPIRULINA, BUT NOT SOLAR SPIRULINA, INHIBITS TNF-α SECRETION: POTENTIAL IMPLICATIONS FOR FUNCTIONAL FOOD AND PHARMA

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ABSTRACT

Along with the development of marine biological pharmaceutical research, high-effective and low-toxic drugs and functional foods isolated from marine organisms have become a growing interest. The highly nutritional and eco-friendly Spirulina (*Arthrospira platensis*) contains functional compounds, such as phenolics, phycocyanin's, and polysaccharides, with antioxidant and immune-modulation effects. It is also known that using different cultivation conditions could affect algae’s second metabolite composition. Recognizing the therapeutic potential of Spirulina blue-green algae (*Arthrospira platensis*), in this study, LPS-activated macrophages and monocytes were treated with aqueous extracts of Spirulina, cultivated in either natural or controlled light conditions. We report that an extract of photosynthetically controlled Spirulina (LED Spirulina), at a concentration of 0.1 μg/mL, decreases macrophage and monocyte-induced TNF-α secretion levels by over 70% and 40%, respectively. It should be noted that the extract from the LED-controlled cultivation process was significantly more effective at suppressing the release of TNF-α. To date, no natural compound has been proven to suppress specifically TNF-α. Therefore, LED Spirulina could pave the way for novel algae-based bioactive compounds as anti-TNF treatment or as a functional ingredient for smart food.

Keywords
RAW264.7, THP-1, Inflammation, Spirulina, *Arthrospira platensis*, TNF-α.

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PRESENTER INFORMATION

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BIOGRAPHY

Research assistant in Sphingolipids, Active Metabolites, and Immune Modulation Laboratory. Our research focuses on the identification and isolation of bioactive natural compounds for the prevention and treatment of inflammatory-based disease. One of our main projects, as part of Algae4IBD (Horizon2020), explores algae and their potential activity to reduce inflammation and inflammatory based-diseases. We use immunology, biochemistry, and analytical approach to discover new algae-based small molecules with the anti-inflammatory potential to be used as functional food ingredients or drug-based for the pharma industry.

MSc student (Tel Hai College, Israel).

COMPANY PROFILE

MIGAL- An internationally-recognized and multi-disciplinary applied research institute, MIGAL specializes in biotechnology and computational sciences, plant science, precision agriculture and environmental sciences as well as food, nutrition and health.

BIOGAS PRODUCTION FROM HALOTOLERANT MICROALGAE BIOMASS: ADAPTATION OF ANAEROBIC DIGESTION INOCULUM TO HIGH SALINITY

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ABSTRACT

Recently, halotolerant microalgae have gained interest as they avoid fresh water use in a growing context of water scarcity. Biogas production from saline biomasses (i.e microalgae) may be limited due to the sensitivity of the methanogenic population to high salinities during anaerobic digestion. Some studies have demonstrated the possibility to acclimate an anaerobic digestion inoculum by gradually increasing the salinity [1]. Semi-continuous experiments were carried out with two 2 L mesophilic pilot reactors (working volume 1.5 L) for a period of 3 months (feeding once a week, HRT = 3 weeks). While the first reactor was fed only with wastewater treatment sludge, the other reactor was fed with an 80/20 %w/w mixture of wastewater treatment sludge and halotolerant green microalgae grown at 70 $g \cdot L^{-1}$ salinity. The final salinity reached in this reactor was 14 $g \cdot L^{-1}$. In the co-digestion reactor, the amount of biomethane produced after each feeding (183 ± 18 NmL $CH_4 \cdot gVS^{-1}$) was on average 16% lower (p-value < 0.05) than in reactor fed with sludge alone (220 ± 36 NmL $CH_4 \cdot gVS^{-1}$) operated at a salinity of 3 $g \cdot L^{-1}$, due to the increased salinity and/or the potentially lower digestibility of microalgae. While biogas from co-digestion reactor had a higher H_2S concentration than biogas from digestion of sludge (approximately 5600 and 800 ppm, respectively), biomethane percentage in biogas was not impacted by co-digestion (73 ± 3 % of CH_4 in both reactors). In addition, the activity of the methanogenic archaea was determined at salinities up to 30 $g \cdot L^{-1}$ (batch tests) at the beginning and at the end of the acclimation to observe the potential microbial communities shift due to higher salinities. Biogas production rates were fitted with Hill Model [2]. The effective concentration reducing 50% of the methanogenic activity was 10 $g \cdot L^{-1}$ of sea salts at the beginning of the experimentation and increased up to 27 $g \cdot L^{-1}$ after the 3 months of acclimation (Figure 1), confirming the adaptation of the inoculum to brackish salinities. Metagenomic analyses are currently underway to observe the shift in populations.

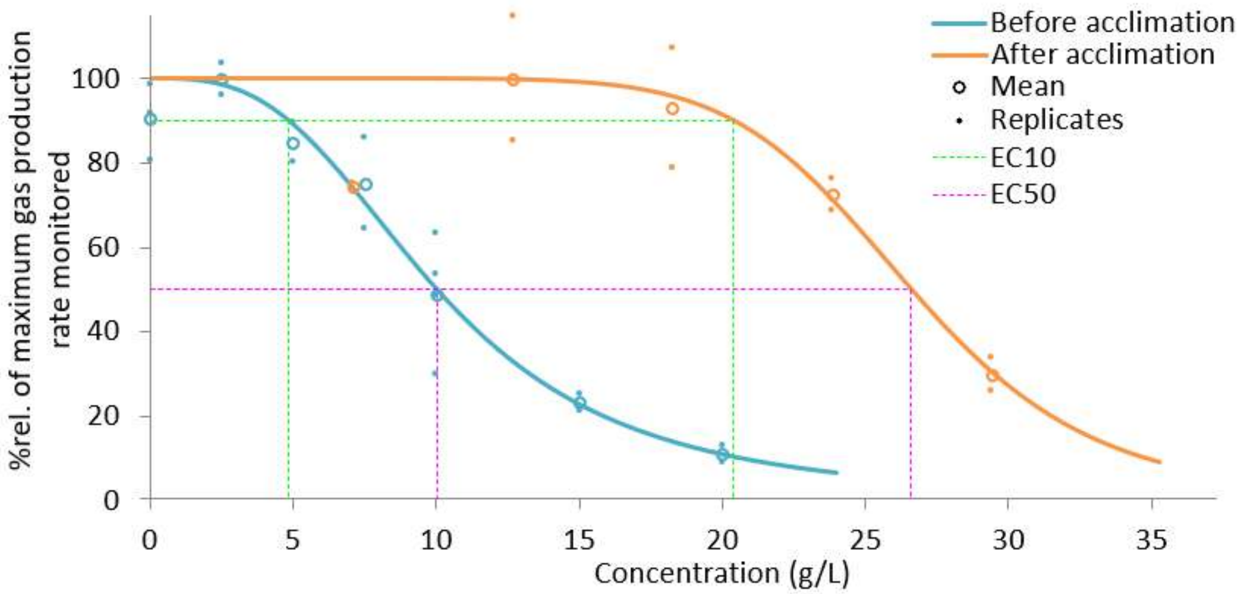

Figure 1: Methanogenic activity of anaerobic digestion inoculum before and after 3 months of salinity acclimation.

Keywords
Anaerobic co-digestion, Biogas, Methanogenic activity, Microalgae, Salinity acclimation

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PRESENTER INFORMATION

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BIOGRAPHY

I'm a French PhD student working on microalgae cultivation and valorisation processes at TotalEnergies since April 2018. In February 2020, I also joined the University of Pau to start my thesis. Since 2018, I have had the opportunity to publish 3 papers on my results and to participate in 3 international conferences. Today, I am working more particularly on the cultivation of microalgae in saline industrial effluents, and on the valorisation of biomass by anaerobic digestion in saline conditions.

THE ANTI-INFLAMMATORY PROPERTIES OF MARINE MICROALGAE IN LPS-INDUCED MACROPHAGES

Shagug, N

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ABSTRACT

Inflammatory diseases such as inflammatory bowel disease (IBD) have become one of the leading causes of health issues throughout the world, having a considerable influence on healthcare costs. Existing treatments for IBD are not effective and emphasize the need for a new biologic approach to improving IBD symptoms. Inflammation is mediated by cytokines produced by stimulated immune cells such as macrophages. Targeting pro-inflammatory cytokines such as Tumor necrosis factor-α (TNFα) often reduces the disease processes by influencing immune cells. Marine microalgae have been identified as an underexplored reservoir of unique anti-inflammatory compounds, which could provide defense against the pathophysiology of many chronic inflammatory diseases such as IBD. With further investigation, microalgae anti-inflammatory phytochemicals have the potential to be used as therapeutics with profound anti-inflammatory activity with reduced side effects. In this study, the anti-inflammatory potential of microalga was assessed in lipopolysaccharide (LPS)-stimulated murine RAW264.7 macrophages. Extraction was prepared using a solid liquid of ethyl acetate or Ethanol: H_2O (70:30%). We have found that the ethyl acetate extract was the most effective in reducing inflammation. From this extract, we isolated and identified the active fraction using a series of chromatographic steps and analytical methods. Our results showed that pretreatment of RAW 264.7 cells with a crude extract from microalgae significantly inhibited the secretion of TNFα by 50-60% ($p < 0.001$). Furthermore, the fractions effectively inhibited the LPS-induced TNFα similar to the effect obtained by the crude extract. Interestedly, some fractions contained omega 3 and omega 7 which are known to have anti-inflammatory properties. Our findings suggest, that the microalgae might become a natural source for new anti-inflammatory treatment and lead to a promising route of treatment for chronic inflammation-linked diseases.

Keywords
RAW264.7, Inflammation, Microalgae, Omega 3, Omega 7

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BIOGRAPHY

Research assistant in Sphingolipids, Active Metabolites, and Immune Modulation Laboratory. My research focused on algae extraction and immune cell-based assays as part of the Algae4IBD project (Horizon2020) that aims to identify and develop novel small molecules derived from macro- and micro-algae to provide a solution for the prevention and treatment of Inflammatory Bowel Disease (IBD). Our lab specialized in immunological, metabolomics and biochemistry approach.

MSc student (Tel Hai College, Israel).

COMPANY PROFILE

MIGAL- An internationally-recognized and multi-disciplinary applied research institute, MIGAL specializes in biotechnology and computational sciences, plant science, precision agriculture and environmental sciences as well as food, nutrition, and health.

COMERCIALLY PRODUCED MICROALGAE AS SOURCES OF BIOACTIVE COMPOUNDS FOR FUNCTIONAL FOODS AND PHARMACEUTICALS

Mélanie S.

Farah K., Sisay U., Ismael-Mohammed K., Carla V., Dina S., Katkam G., João V. and Luísa B.*

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ABSTRACT

Microalgae are largely untapped resources that have been highlighted from a biotechnological standpoint for a variety of applications, including functional foods and pharmaceuticals for disease prevention and/or treatment due to their composition in unique compounds with promising bioactive properties.

In this study, ethanol, ethyl acetate and water extracts from nine commercially available microalgae species (*Porphyridium* sp., *Nannochloropsis* sp., *Tetraselmis striata* CTP4, *Isochrysis* sp., *Phaeodactylum tricornutum*, *Skeletonema* sp., *Spirulina* sp., *Haematococcus pluvialis*, and *Tetraselmis chui*) were evaluated for their radical scavenging activity, metal chelating potential against copper, iron and calcium ions, anti-tumoral activity (assessed by *in vitro* cytotoxic act

ivity against human hepatocarcinoma (HepG2) and human monocytic leukemia (THP-1) cells) and anti-inflammatory properties (determination of Tumor Necrosis Factor alpha production (TNF-α) in liposaccharide (LPS) stimulated human Macrophages (Mac THP-1 cells). Whenever extracts showed promising results, fractionation was performed to further narrow down the promising compounds responsible for the observed activity.

Best results were obtained with hexane fraction from *Tetraselmis striata* CTP4 with anti-inflammatory activity (99.0 ± 0.8% reduction in TNF-α production in LPS stimulated Mac THP-1 cells at 50 µg/mL), dichloromethane fraction of *Phaeodactylum tricornutum* with cytotoxicity towards cancerous cell lines (IC_{50} = 22.3 ± 1.8 µg/mL and 27.5 ± 1.6 µg/mL for THP-1 and HepG2, respectively) and hexane fractions of *Porphyridium* sp. and *Skeletonema* sp. with good chelating activity for iron, copper and calcium (IC_{50} = 0.047, 0.272, 0.0663 mg/mL and IC_{50} = 0.055, 0.240, 0.0850 mg/mL, respectively).

After derivatization, these four fractions were chemically characterized by GC-MS, and fatty acids (with varying degrees of unsaturation) were found to be the most abundant compounds.

Some of the analyzed microalgae species/extracts showed to be sources of compounds with antioxidant, metal chelating, anti-tumoral, and anti-inflammatory properties, thus making them attractive for potential applications in the functional food and pharmaceutical sectors.

Keywords

Microalgae; Health benefits; Bioactive compounds; Anti-inflammatory activity; Anti-tumoral activity.

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PRESENETER INFORMATION

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BIOGRAPHY

Mélanie Silva holds a bachelor's in Nutrition and Dietetics from the University of Algarve, where she graduated at the top of her class in 2018.
She was invited to perform and organize public speaking sessions on nutrition and accomplished her training as a public speaker (obtaining the Certificate of Pedagogical Skills with the final classification of excellent). She also passed the Cambridge Advanced Exam in English with the highest score of C2 (proficiency).
Alongside working as a nutritionist, she was doing her MSc in Food Technology at the University of Algarve. Her MSc dissertation was developed at the Marine Biotechnology lab by participating in the European project Algae4IBD. In this thesis, she produced extracts from algal biomass, performed various antioxidant and anti-inflammatory in vitro assays, identified some phytochemical compounds, and assessed the proximal composition and mineral profile of algal biomass. She graduated at top of her class in 2021.
She was then offered a place as a Research Technologist, where she continues to work on the ALGAE4IBD project. On the side, she also applied for the MSc in Management of Healthcare Units at the University of Algarve where she was awarded scholarships for academic excellence and is expected to graduate at top of her class until end of 2022. She is currently a PhD candidate in Biotechnological Sciences at University of Algarve, where she will continue developing her work as part of the ALGAE4IBD project with focus on the biotechnological potential of algae.

COMPANY PROFILE

The Algarve Centre of Marine Sciences (CCMAR-Algarve) - is one of the foremost marine science research centers in Portugal, gathering experts in the fields of marine biology, ecology, oceanography, environmental sciences, biotechnology, fisheries and aquaculture.
Located in the South of Portugal, CCMAR-Algarve is an independent non-profit research organization within the University of the Algarve. It has the Portuguese Institute for Sea and Atmosphere as a strategic partner and, together with the Interdisciplinary Centre for Marine and Environmental Research, constitutes the Associate Laboratory CIMAR. CCMAR is the lead partner in the Portuguese component of the European Marine Biological Resource Centre (EMBRC).
Dedicated to R&D in marine sciences, CCMAR-Algarve aims to promote multidisciplinary research and education related with the marine environment. With a multidisciplinary team of about 250 scientific researchers, well-equipped marine facilities and laboratories and facilitated access to important marine and coastal ecosystems, CCMAR-Algarve develops activities that fall into five different core areas: Research, Training, Business, Society and Collaboration.
The Marine Biotechnology research group is currently ongoing active research in different fields of microalgal biotechnology, as well as in the exploitation of different marine organisms as sources of novel bioactive compounds.

CHEMOSTAT OPERATION AND PRODUCTIVITIES IN A 25 L TUBULAR SYSTEM AT DIFFERENT LIGHT INTENSITIES AND DILUTION RATES

Jasper Sohier on behalf of:

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Lgem/Synalgae B.V., Achterweg 65, 1424 PP, De Kwakel, The Netherlands

ABSTRACT

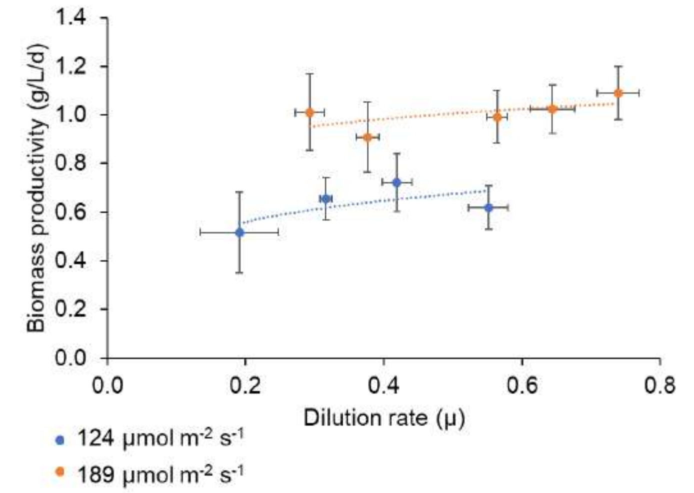
Using our proprietary Lgem photobioreactor (PBR) systems, we can cultivate fragile microalgae species with high efficiency and reliability. We present the data on small-industrial scale production of the fragile algae *Rhodomonas sp*. We investigated the effect of light intensity (124-189 μmol m^{-2} s^{-1}) and dilution rate (D_L = μ; 0.19-0.74 d^{-1}) on the biomass productivity (r_x) and biomass yield on light ($Y_{x/ph}$), during continuous cultivation using light emitting diodes (LEDs) in the Lgem LAB-25 tubular PBR.

The maximum r_x (1.09 g L^{-1} d^{-1}) and $Y_{x/ph}$ (0.53 g mol^{-1}) were obtained at a D_L of μ = 0.74 d^{-1} under high light (189 μmol m^{-2} s^{-1}). Under low light (124 μmol m^{-2} s^{-1}) and a D_L of μ = 0.42 d^{-1}, an r_x of 0.72 g L^{-1} d^{-1} and an $Y_{x/ph}$ of 0.54 g mol^{-1} were achieved. The r_x increased with higher light intensities (except at ~μ = 0.40 d^{-1}), but was not significantly affected by D_L (see Fig. 1a). To reduce the costs of indoor production using artificial light, a high $Y_{x/ph}$ is required. All tested conditions resulted in a high, but not significantly different, $Y_{x/ph}$ (Fig. 1b). The high $Y_{x/ph}$ reveals the efficiency of the Lgem PBRs in cultivating microalgae at pilot scale. Lower D_L (μ = 0.29 d^{-1}) results in a 2.3-fold higher biomass concentration (3.42 g L^{-1} DW) compared to high D_L (μ = 0.74 d^{-1} at 1.48 g L^{-1} DW) while maintaining similar r_x, decreasing water usage and pumping requirements.

In this study, >200 days of operation were achieved without the formation of fouling or downtime whilst maintaining high $Y_{x/ph}$ under all tested conditions (see Fig. 1b).

Results

a)



b)

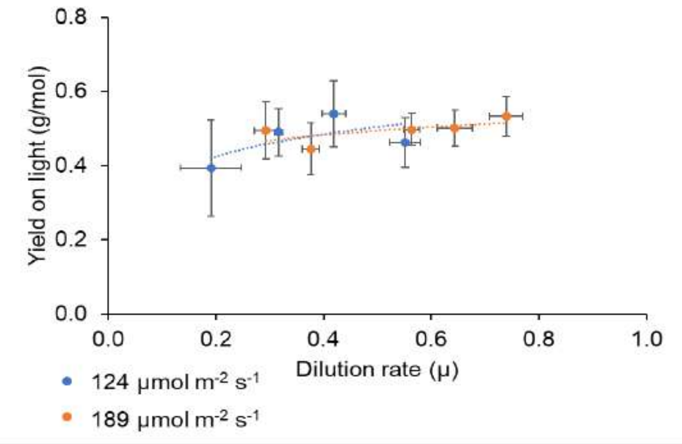


Fig. 1. a) r_x (g L^{-1} d^{-1}) and b) $Y_{x/ph}$ (g mol^{-1}) versus D_L under chemostat operation in an Lgem LAB-25 tubular PBR (32 mm tube diameter) under red:white LEDs in operation for >200 days. Steady state was defined as at least 3 x culture volumes or a minimum of 9 days for $D_L < \mu = 0.2$ d^{-1}. Steady state was obtained when variations in biomass concentration and D_L revealed < 15 % variation.

Keywords

Rhodomonas sp., chemostat operation, process optimization, biomass productivity, tubular photobioreactor.

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Biostimulant, Biocontrol

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SCREENING OF MICROALGAL STRAINS SELECTED FROM FRESHWATER GREEN MICROALGAE COLLECTION FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

We performed the biological screening of organic solvent extracts of single-cell freshwater green microalgal cultures. The microalgae collection of Albitech Biotechnology Ltd. consists of its own isolates. The isolates were collected from two different locations in Hungary: Lake Velencei (a saline lake) and Lake Kőhegyi. The collected samples were diluted and streaked on a solidified enrichment medium. The results of the appropriate dilution were single cell cultures, which were selected by visual inspection and microscope. The axenic cultures were identified in the Department of Microbiology of Eötvös Lóránd University. Identification was carried out using molecular biological methods based on the 18S rRNA gene and Greipel et al. succesfully described a new species - *Chlorococcum szentendrense* – collected from Lake Kőhegyi, a periodic freshwater lake. These microalgae strains received a strain code and they are maintained in active and cryopreserved forms. The purpose of our study was to assess whether the selected microalgae produce metabolites that may inhibit the growth of widely occurring human facultative pathogens. There are many microalgae, e.g. *Chlorella* sp., *Scenedesmus* sp., and macroalgae, e.g. *Ulva rigida* with proven antibacterial effects presumably associated with polyphenols, alkaloids, terpenes, polysaccharides, fatty acids, sterols, lactones, and proteins. We examined the antibacterial effect of the selected algal extracts against the following facultative pathogenic bacterial strains: *E. coli* NCAIM: B.01992, *S. aureus* NCAIM: B.01055 and *P. aeruginosa* NCAIM: B.01057. Two different organic solvents – ethanol and diethyl-ether – were used to create the extracts from the lyophilised biomass. The antibacterial effect of the extracts was determined using the agar gel diffusion method. The minimum inhibitory concentration was measured by broth microdilution assay in 24-well cell culture plates. As a positive control, we used antibiotics to compare the results semi-quantitatively. The *E. coli* strain was the least susceptible to treatments and *S. aureus* was the most sensitive. Among the solvents used, ethanol was the most suitable for the extraction of bioactive molecules of microalgae. In summary, we have confirmed the antibacterial effects of four single-cell freshwater microalgae strains against facultative pathogenic bacteria. This work has been supported by the 2019-1.1.1.-PIACI-KFI-2019-00228 grant of the National Development Agency, Hungary.

Keywords
Strain collection, Solvent extraction, Antibacterial activity, Agar gel diffusion method, Minimal inhibitory concentration

A PHENOTYPING PIPELINE FOR A MICROALGAE-BASED PLANT BIOSTIMULANT

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ABSTRACT

Eutrophication of natural waters poses serious challenges to marine and freshwater life. Only in the Baltic Sea, agricultural wastewaters are responsible for over 45% of the nitrogen and more than 30% of the phosphorus loads (2018). Greenhouse effluents contain still high concentrations of nutrients that can be reutilized by phototrophic microorganisms, microalgae, that provide biomass for various products including plant biostimulants (PB). PB facilitate nutrient uptake, improve crop performance, physiological status, and tolerance to abiotic stress. Interest in PB is growing exponentially, because they can have a key role in addressing sustainability challenges and reduce dependency on synthetic fertilizers and plant protection products. The size of the market with PB was valued at 2.80 billion USD in 2021 and is projected to reach 6.90 billion USD by 2030, growing at a CAGR of 10.2% from 2022 to 2030. Several microalgae (MA) extracts have been reported to stimulate germination, seedling growth, shoot, and root biomass in several crops such as lettuce, pack choi, tomato, and pepper. However, the associations between the model plant, MA strain, and utilized extraction method are rather ambiguous. In our research, we have designed a phenotyping pipeline composed of a rapid *in vitro* bioassay and a medium-term *in situ* assay for screening plant growth promoting effects of microalgal extracts in two different plant models. By comparing two extraction methods for six Nordic MA strains, we found that bead-milling may not render the extracts effective enough. On the other hand, acid hydrolysis produced extracts with significant increase of both, Arabidopsis root length and lettuce biomass. Our experimental design provided formidable datasets as a stepping point for in-depth research of PB. Utilizing this pipeline for a wider array of MA, we will select the most suitable candidates from the Nordic collections for further research, that will aim to determine the affected metabolic and regulatory pathways in the model plants. This knowledge will lay theoretical grounds for development of credible formulations of PB and, finally, advocate a more efficient and sustainable circular-economy model for agricultural systems in the Nordic area.

Keywords
Plant biostimulant, microalgae, greenhouse effluents, phenotyping, crop improvement

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BIOGRAPHY

Dr. Erik Chovancek completed his academic studies at the Slovak University of Agriculture in Nitra. He acquired a bachelor`s degree in Applied Biology (2013) and a master`s degree in Plant Physiology (2017). The topic of his doctoral research was `Evaluation of physiological traits and environmental plasticity of genetic resources of wheat using phenomic approaches` (2017-2020). During his studies, he broadened his expertise and knowledge in Plant Molecular Biology while staying in different research groups; at the University of Helsinki, Shandong Agricultural University (China), and the University of Münster (Germany). At the University of Turku (Finland), he is screening microalgal strains from the Nordic collection for potential stimulating effects on plant growth and development. He designed and tested a pipeline of bioassays for phenotypic screening of algal extracts. The targeted biostimulants present a unique opportunity for a sustainable alternative in agroindustry. Comprising a wide range of compounds, they can promote plant growth, optimize nutrient uptake, and support plant tolerance to abiotic stress factors. However, the exact composition and their mode of action are still rather elusive.

COMPANY PROFILE

(PHOTOMICROBES: www.utu.fi/photo-microbes**)**
Cyanobacteria and microalgae are model organisms for the study of oxygenic photosynthesis and are also promising feedstocks for blue biorefineries. Our team is focused on identification of 'waste' points in photosynthetic electron transport leading to the loss of productivity. We are also studying the biodiversity of Nordic microalgae for production of biofuels, high-value products and wastewater remediation and developing immobilization techniques to increase light-to-product conversion efficiency.

PRESICTING TEMPERATURE DYNAMICS IN OUTDOOR PHOTOBIOREACTORS

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ABSTRACT

The dynamics of microalgae cultivated in outdoor systems is strongly influenced by meteorology. The solar flux, together with air temperature, air humidity and wind velocity directly impacts temperature and light in the cultivation medium [1]. As a consequence they are the main drivers of the growth rate of microalgae and further of the process productivity. A numerical model is a very useful tool in order to monitor and on-line optimize productivity, and adapt the growth conditions (dilution rate, shadowing) to the present and future weather conditions. In this work, we propose a generic dynamical model to catch temperature dynamics in realistic operational conditions.

The main objective is to achieve a temperature prediction model, which is highly flexible, and which can be easy calibrated in a broad range of complex situations. There are several thermal models which were developed for simple reactor geometries (typically for raceway ponds), based on heat transfer modelling [2,3,4]. These physical models of the temperature in a photobioreactor have proven to be highly accurate, yet they require many physical constants and a time consuming procedure to set up the model. The objective of our work is to propose a nonspecific heat transfer model, which can tailored to any photobioreactor after a dedicated calibration phase involving a few weeks of data. The model structure is a generic nonlinear system more suitable for control purposes. Then, the crucial step consists in calibrating the new model using different available sets of experimental data/measurements. The model was successfully calibrated using the data collected for three experimental devices. A raceway pond, a tubular photobioreactor under a greenhouse and a V shaped outdoors panel photobioreactor [5,6]. For the three cases, 4 days of data were required to calibrate the model for accurate temperature predictions over several weeks. Our next objective is to combine this temperature model to a light model to predict productivity and perform model predictive control [7].

Keywords
Modelling, Temperature, Heat transfer, Outdoor processes, Process control

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He obtained a bachelor's degree in mechanical engineering and a master's degree in automotive engineering at the RWTH Aachen in Germany. During his studies in Aachen he worked as a student research assistant in the field of control and optimization of automotive systems. He started at Inria under the supervision of Prof. Olivier Bernard as a part of the Biocore team in October 2021. The PhD position is dedicated to the ITN Marie Curie project called Digitalgaesation. In summer 2022 he spent 2.5 months of his PhD at the University of Wageningen (Bioprocess Engineering group) under the supervision of Prof. Janssen.
The goal of his PhD is to develop a model-predictive control of microalgae processes accounting for future meteorology. The base of his work is developing a model that describes the growth of microalgae in processes in outdoor photobioreactors and can be used for model predicitve control purposes.

COMPANY PROFILE

The goal of the Biocore team is to contribute to environment preservation, develop new renewable energy sources and avoid water pollution or the use of chemicals for crops. Biocore is an association between Inria, INRA, CNRS and Sorbonne University. Biocore is designing models to understand, optimize microalgae-based processes and assess their environmental impact. We have been working 20 years in association with the Laboratory of Oceanography from Villefranche (Sorbonne University) on CO_2 uptake by microalgae (atmospheric or industrial), their response to a nitrogen deficiency, in particular to stimulate the production of carbon reserves (lipids and sugars). We have developed an automated computer-controlled platform from liter to m^3 to grow microalgae in highly controlled conditions and automatically sample, filter and analyze the biomass and remaining nutrients. Biocore has been involved in several national and international projects and coordinated two of them. Biocore contracted with private companies for project associated with microalgae, etc.

PIG 2 WHEAT – MICROALGAE CLOSING THE CYCLE

Gouveia L[1,2]

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ABSTRACT

Pig farms are spread all over the world, not only because pork is one of the most widely consumed meats worldwide, but also due to smaller investment costs and fast economical return to farmers, which is associated to high fecundity, better-feed conversion efficiency, early maturity, and short generation interval (DAHD, 2022). Conventional wastewater treatment is highly expensive, energy-demanding, generates hazardous gaseous, and sludge wastes. Microalgae are promising tools for establishing a circular economy connecting wastewater treatment with agriculture in a sustainable approach. As piggery wastewater (PWW) is highly pollutant in ammonia and organic matter, a pre-treatment is needed to avoid dilution with a spence of fresh water (Ferreira et. 2022).

On the other hand, current agricultural research is focused on ways to achieve sustainable food security amidst an ever-increasing global population and climate crisis. Microalgae meet the criteria of being natural, renewable, promoting plant growth, improving soil fertility, and providing protection against pests and pathogens (Stirk 2021).

Two approaches are highlighted – a physico-chemical one (Photo-Fenton – ALGAVALOR project) followed by microalgae cultivation, and biological one (Constructed Vertical flow Wetlands (CW), microalgae cultivation and Microbial Fuel Cell– WCAlgaeKIT$^+$ project).

In ALGAVALOR project, the biomass produced in PWW was shown to promote seed germination (11-87%), while the respective supernatants had a bigger effect on wheat growth and cobs produced (Fig.1a, b, c).

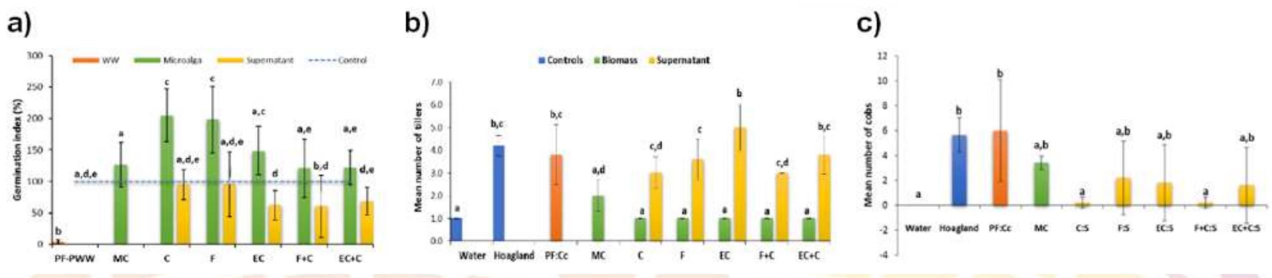

Figure 1. a) Germination index (5 days), b) Mean number of tillers (82 days), and c) Mean number of cobs (182 days) of wheat treated with *Tetradesmus obliquus* from: direct culture (MC), biomass (green) and supernatants (yellow) after harvesting by centrifugation (C), flocculation (F), electrocoagulation (EC), flocculation combined with centrifugation (F+C) an electrocoagulation combined with centrifugation (EC+C). PF-PWW and PF:Cc are respectively the photo-Fenton pre-treated piggery wastewater and concentrate prior to microalga-based treatment (orange). Different letters indicate significant differences among treatments (Tukey's test, $p < 0.05$).

In WCAlgaeKIT$^+$ project will first be developed on a laboratory scale as a proof of concept, to be later developed on a pilot scale for implementation in small (<100) pig farms, existing in rural areas of Portugal and India.

Keywords
Bioremediation, Piggery wastewater, *Tetradesmus obliquus*, Biostimulant, Biofertilizer

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BIOGRAPHY

Luísa Gouveia (LG) has a PhD in Biotechnology. She has 35 years of experience in microalgae biotechnology, 107 peer-reviewed papers author or co-author, 1 book, 17 book chapters (h-index=43), participated in a total of 41 projects and coordinated 10, all directly related to microalgae, wastewater treatment, CO_2 mitigation, biofuels, biofertilizers/stimulants, food, feed, and bioactive compounds. She was Vice-chair and National delegate of the COST Action 1408 EUALGAE-European network for algal- bioproducts (2015-2018). LG is Vice-coordinator of Ibero-American Net for wastewater treatment Red CYTED P319RT0025 - RENUWAL. Portuguese coordinator of Bilateral projects Portugal- Servia 5554/2020 – Production of high-value products from novel marine microalgae using green technologies (2020-2022) and Portugal-India – WCalgaeKit$^+$ Combination of Vertical Wetlands, Microalgae Photobioreactor and Microbial Fuel Cell (KIT) for wastewater treatment in small pig production farms (2022-2024), Portugal-Armenia Development of a New Multifunctional Biotechnological Preparation as a Biofertilizer, Biostimulant, Biopesticide - Armenia Science Committee of the Ministry of Education, Science, Culture and Sport RA (2022-2024).
Associate Editor of Biotechnology for Biofuels journal. Guest Editor of Special Issues of Algal Research, Molecules, Energies. Regular referee (more then 100 scientific journals) and member of R&D project evaluation panels. Supervisor and co-supervisor of more then 50 MSc and PhD students. Participated in 117 Congresses/conferences and 42 Oral presentations by invitation.

COMPANY PROFILE

Laboratório Nacional de Energia e Geologia, I.P (LNEG), the Portuguese National Laboratory of Energy and Geology, is a State Laboratory attached to the Ministry of Environment and Climate Action, which carries out advanced R&D work. Its mission is to promote technological innovation in the fields of energy and geology focused on science and technology, with the overriding objective of raising company competitiveness within a framework of sustainable economic progress.
The Bioenergy and Biorefineries Unit of LNEG is a recognised R&D Centre with expertise in the bioenergy field. It has 39 staff members (28 of which with PhD) and about 25 students and grantees. It has 5 R&D Areas: Biomass: Resources and Valorisation Potential, Biorefineries for Bioenergy, Biorefineries for Bioeconomy, Renewable synthetic fuels, and Sustainability of Biofuels and Biorefineries.

SCREENING AND EXTRACTION OF BIOPESTICIDES FROM NORDIC MICROALGAE

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ABSTRACT

The current exploitation of Earth´s natural resources and the creation of excess waste lead to many ecological and societal problems and creates the need to rethink the present economic model towards an innovative sustainable bioeconomy. Strengthening the bioeconomy sector in Nordic countries could ease economic, ecological, and societal problems. Microalgae, which use the energy from sunlight to fix carbon and create biomass, comprise a still underused natural resource. In the past decade, the utilization of microalgae as biofuel feedstock has been a major focus of research and industrial investments. However, the cost-efficiency of these processes is low. Thus, recently microalgae regained interest as a source of essential high-value compounds for food, feed, agriculture, cosmetics, and pharmaceuticals.

Especially, the use of microalgae as biopesticides for more sustainable conventional and organic farming came into focus. We screened ~25 Nordic microalgae strains for their anti-fungal activity against several plant-pathogens (i.e., *Fusarium oxysporum*, *Alternaria solani*, *Verticillium albo-atreum*, *Pythium ultimum*, etc.) and could identify some possible candidates for further analysis. We tested different pre-treatments (fresh vs. freeze dried biomass) and extractions (aqueous vs. acetone) for optimal biopesticide activity. Together with our other microalgae research projects concerning biostimulants and wastewater treatment, this research aims for a closed-loop circular approach for a sustainable Nordic agriculture.

BIOSTIMULATING AND BIOPESTICIDAL EFFECT OF MICROALGAE ON LEGUMES

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ABSTRACT

Currently, there is a growing awareness of the harmful consequences of the massive use of chemicals in conventional agriculture. The aim of organic farming is to reduce the use of chemical pesticides, herbicides and fertilizers and at the same time while increasing the global food production in a sustainable way. It is therefore necessary to look for new sources of substances with biostimulating effects that can be used as biofertilisers, or substances with biopesticidal effects. These compounds can help to improve crop quality, increase nutrient use efficiency and increase the tolerance to biotic and abiotic stresses, such as patogens and drought. Unicellular microalgae and cyanobacteria contain a wide range of substances with potential biostimulatory effects on higher plants, including phytohormones, vitamins, aminoacids, polyamines or polysaccharides. The ongoing project ALGASET aims to develop a biostimulating preparation containing microalgal biomass or its components. Three strains of unicellular microalgae (*Dictyospharium* sp., *Chlorella vulgaris, Trachydiscus minutus*) and four strains of filamentous cyanobacteria (*Trichormus variabilis*, *Tolypothrix tenuis*, *Arthrospira maxima* and *Nostoc entophytum*) have been tested for their potential effect on legumes (faba bean, soybean and pea). Firstly, a preparation for foliar application containing a wetting agent applicable in organic farming (potassium soap) and freeze-dried disintegrated microalgal biomass was developed. The effect of composition on the wetting properties of the formulation was investigated to make it suitable for foliar application. Then, the preparations were used in various activity tests, including the effect on seed germination, relative water content in plant leaves, biomass productivity, length of roots, photosynthetic activity and leaf senescence. Some microalgae species showed positive effect on most of the measured parameters (especially *T. variabilis* and *T. tenuis*). Then, the effect of microalgal biomass on the growth performance of *Pseudomonas savastonoi glycinea*, a phytopathogenic bacteria causing the blight of soybean, was tested. A significant inhibitory effect of *T. variabilis* was observed at a concentration of 0.5 % w/v, suggesting the potential use of this cyanobacteria as a biopesticide. Our results suggest that microalgae-based products can be an effective tool for enhancing legume growth in organic agriculture.

Keywords
Microalgae, biostimulation, biopesticide, legume, soybean blight

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Simona Lucakova is a graduate of biotechnology at University of Chemistry and Technology in Prague. After her graduation she started to work as a research assistant at the Institute of Chemical Process Fundamentals of the Czech Academy of Sciences and she continued in PhD. course at the Department of Biotechnology of University of Chemistry and Technology. Her research is mainly focused on microalgal cultivation, harvesting and valuable compounds production. The topic of her dissertation is the application of electrocoagulation method to biotechnology processes.

COMPANY PROFILE

The Institute of Chemical Process Fundamentals (ICPF) is one of the six institutes constituting the Section of Chemical Sciences of the Czech Academy of Sciences. The Institute functions as a center for advanced research in chemical, biochemical, catalytic and environmental engineering, biotechnology, physical chemistry and industrial chemistry. Except of systematic basic research, the Institute is also focused on applied research.

ARTHROSPIRA MAXIMA EXTRACT AS A HIGH VALUE BIOFERTILIZER IN BASIL *(OCIMUM BASILICUM)* SEEDLINGS

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ABSTRACT

Continued increase of human populations and use of chemical fertilizers remain a threat to the health and stability of human-ecological systems worldwide. To ameliorate this problem and achieve sustainable agriculture, a variety of ecofriendly technologies have been developed, including the production of cyanobacteria-based biofertilizers. This technology can be optimized through experiments that assess how plant growth is enhanced under different biofertilizer concentrations (g/L). Here we assess the biofertilizer potential of hydrolysates of the cyanobacteria *Arthospira maxima* on basil (*Ocinum basicullum*) under six different concentrations (0, 2.5, 5, 10, 20, and 40 g/L), and an additional treatment where we applied the registered product Optimar ® (4 mL/L). Wet mass (g), dry mass (g), leaf area (cm^2), and stem length (cm) increased with increasing cyanobacteria concentrations. Leaf count and root mass (g) also increased, but there were no differences among the 2.5, 5, 10, and 20 g/L concentration groups. Similarly, node count increased but did not differ between 5, 10, and 20 g/L. For all response variables except stem length, a concentration of 40 g/L yielded significantly higher biomass production. The beneficial effects of the application of Optimar ® were often surpassed when plants received cyanobacteria concentrations above 10 g/L. A chemical composition analysis of *A. maxima* revealed high concentrations of the phytohormones gibberellic acid, 6-Benzylaminopurine, and 1-Naphthaleneacetic acid that are likely responsible for the cyanobacteria's biostimulant activity. Further research is required to determine how other crop plants respond to different concentrations of *A. maxima* and the viability of creating an economically accessible product with a higher concentration of *A. maxima* using circular economy and biorefinery concepts to obtain high value-added by-products.

Keywords
Growth-promoter, cyanobacteria, sustainable agriculture.

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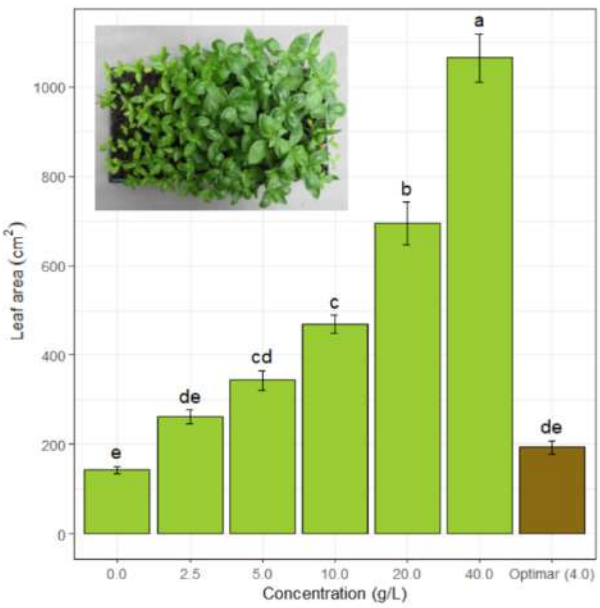

Figure 1. Leaf area of basil plants under different concentrations of *A. maxima* hydrolysate.

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Camila Andrea Marín Marín is a chemical engineer graduated from the Universidad of Antioquia (UdeA) in Medellín, Colombia in the year 2019. She has worked in different areas of engineering and education, as she has always considered herself to be curious by nature. For this reason, she has been interested in seeking alternatives towards the solution of different problematics that the world and her country, Colombia, face nowadays. During her undergraduate studies, she worked in the Bioprocesos (UdeA) research group where she participated in a project for the production of biopolymers (PHB) from bacteria, taking the first steps in research and biotechnology. Currently, Camila is a master's student in biological sciences at Universidad CES, and she works on a project that seeks to value the biomass of cyanobacteria to develop products for the agricultural industry, especially for strategic crops in Colombian agriculture. Additionally, she is recently a professor of Mathematics and Biostatistics at the Department of Science and Biotechnology of the Universidad CES.

INVESTIGATING ALGAE-BACTERIA INTERACTIONS AS BIOCONTROL FOR MARINE PATHOGENIC BACTERIA VIBRIO PARAHAEMOLYTICUS

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ABSTRACT

The interactions between bacteria and microalgae are complex and can range from detrimental to mutually beneficial. The focus of this study is to investigate the effectiveness of different combinations of algae and bacteria to biocontrol *Vibrio parahaemolyticus*, one of the leading cause of infectious diseases in aquatic organisms. It has been shown that microalgae are in fact capable of inhibiting some species of *Vibrio*, but the level of inhibition is dependent on the compounds excreted by specific microalgae species. Here, the growth of several *V. parahaemolyticus* strains was measured in the presence of different combinations of 5 phytoplankton species and up to 25 other bacterial species during 7-days experiments. Among the bacteria inhibiting the growth of V*. parahaemolyticus, Pseudovibrio sp., Pseudoalterromonas sp., Micrococcus yunnanensis* and *Pseudoalterromonas ECSM84* had the most significant effect, potentially coinciding with a release of antimicrobial compounds. These Vibrio inhibitions were further amplified by the presence of the following microalgae: *Chaetoceros mullerii, Pavlova lutherii* and *Tetraselmis suecica*. By screening the probiotic effect of different combinations of 5 microalgae strains and 25 bacterial strains, individually and in consortia, this study aims to provide better probiotic solutions to pathogenic *Vibrio* outbreaks, mostly related to aquaculture industry, through administering these consortia or individual strains as biocontrol.

Keywords
Vibrio, Alga-Bacteria interaction, Biocontrol, Coculture, Probiotic, Aquaculture.

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Mathieu completed his PhD in Biology at the University Pierre and Marie Curie in Paris (2006). In 2008, the European Commission awarded him with a prestigious Marie Curie outgoing postdoctoral fellowship to move to Australia (University of Queensland, Brisbane) and study the influence of environmental stresses in corals and their algae symbionts. Since January 2014, he is based within the Climate Change Cluster (C3) at the University of Technology Sydney, where he is currently Deputy Director and Associate Professor, co-leading a team on Algal Biosystems and Biotechnology research with Professor Peter Ralph. In this role, he is mentoring students and developing early career researchers doing world leading research on algal biotechnology.

COMPANY PROFILE

The University of Technology Sydney aims to be a world-leading university of technology. UTS is globally recognized as a leader in innovative research, engaged teaching and industry collaboration across key focus areas such as health, data science, sustainability and social futures. It is Australia's highest ranked young university in both the Times Higher Education and the QS Top 50 under 50 year old rankings. It is committed to high-quality research that is industry-aligned and focused on making a meaningful impact on the lives of Australians. The Climate Change Cluster (C3) is a prioritised strategic 'Research Strength' at UTS and is housed in a world-class, AUD $165 million building that contains state-of-the-art equipment worth >$30 million, including the world-first fully automated robotic algal phenomics facility but also microsensing equipment, molecular extraction and sequencing equipment, imaging facilities (e.g. electron microscopy) and high performance computing.

VARIATIONS IN BIOSTIMULANT RESPONSE ACCORDING TO PLANT SPECIES: THE CASE OF *ARTHROSPIRA* AND *NOSTOC*-BASED FORMULATIONS

Santini G.

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ABSTRACT

Cyanobacteria are receiving increasing interest in the scientific community and the agrochemical industry as a new biological source of plant biostimulants capable of improving yields and quality of agricultural and ornamental crops. However, the biostimulant effects may vary according to plant species as different plants may have different sensitivity and/or different abilities to absorb and transport the bioactive molecules contained in the product. In addition, the cultivation conditions can vary the responses of the plant to a biostimulant product.
In this work, data obtained in several trials performed over three years of experimentation are presented and differences in plant responses to treatments investigated. In particular, extracts and hydrolysates obtained from the same *Arthrospira* sp. and *Nostoc* sp. biomasses were applied by foliar spraying on different plant species of great agronomic interest in Italy.
Growth trials show that the effectiveness of the same microalgae-based biostimulant varies in relation to the plant species and can be enhanced in plants subjected to abiotic stress. In fact, the *Nostoc*-based formulation significantly increased the yield and root development in basil plants, but had no effects on tomato plants, while the *Arthrospira*-based formulations were more effective when applied on stressed tomato plants than on basil.
Our results highlight that the study on the application of a biostimulant on different plant species and conditions is crucial to ensure the credibility of a new product on the market.

Keywords
Biostimulant, *Arthrospira*, *Nostoc*

PRESENTER INFORMATION

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BIOGRAPHY

BSc degree in 2013 in Agricultural Sciences and Technologies at the University of Florence. MSc degree in 2017 in Plant and Microbial Biotechnology at the University of Pisa. Collaboration with F&M (Fotosintetica & Microbiologica S.r.l.) on isolation and characterization of microalgal and cyanobacterial strains and in the maintenance of the F&M algae culture collection. Currently Post-Doc Researcher at the Department of Agriculture, Food, Environment and Forestry (DAGRI) of the University of Florence, in the group led by Prof. Liliana Rodolfi, working on the use of microalgae and cyanobacterial strains for the development of new biostimulants.

RAISING METABOLIC PRODUCT EXPRESSION IN GREEN ALGAE

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ABSTRACT

In biotechnology the improvement of the production of bioactive compounds is a continuous challenge. However, in the case of microalgae the applied stress conditions can hinder the biomass production. In the photosynthetic organisms salt stress can interfere their metabolism, can trigger different physiological and biochemical mechanisms. We have investigated the effect of moderate salt stress on biotechnologically important microalgal strains. The microalgal strains can react differently to the applied salt concentrations. The mixotrophic, unicellular, flagellate *Euglena gracilis* showed only slight decrease in cell growth accompanied by a decrease in chlorophyll content under moderate salt stress, at the same time increase in the carotenoid content was detected. Furthermore, salt stress caused changes in the macro-organization of pigment-protein complexes and in the thylakoid stacking, however, we did not detect significant change in the composition and functioning of the photosynthetic complexes. Salt stress did not substantially affect the photosynthetic activity. Most importantly in the salt treated cells accumulation of paramylon was observed (Kanna S.D et al. 2021), that is a potent immunostimulatory and anti-inflammatory agent.
Part of this project was funded by the Hungarian National Research, Development and Innovation Office grant GINOP-2.3.2-15-2016-00058 and the European Union's Horizon 2020 Research and Innovation Programme under grant agreement N° 101000501.

Keywords
Microalgae, Euglena, Paramylon, Salt stress, Carotenoid

References
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BIOGRAPHY

Bettina Ughy obtained her PhD in a co-tutelle program from the University of Szeged and the University of Paris-Sud XI in 2006. During her PhD studies she used cyanobacteria as model organisms of her research. She has expertise in molecular biology, biochemistry and in the investigations of biological membranes. She studies the growth of microalgae populations and the individual cell divisions, as well. Her aim is to increase the yield of biomass and/ or the bioactive metabolites. To reach these goals, she investigates the effects of different stress conditions on the metabolism of microalgae.

COMPANY PROFILE

The Biological Research Centre (BRC), Szeged (ELKH) is an outstanding institution of the internationally acknowledged Hungarian biological research. The Biological Research Centre was founded in 1971. The 4 institutes of BRC - the Institutes of Biophysics, Biochemistry, Genetics and Plant Biology - employ about 260 scientists whose work is hallmarked by highly appreciated international scientific publications and patents. The research topics include several fields of molecular and cell biology from the industrial utilization of bacteria through controlled improvement of cultivated plants to the problems of human health and environmental protection. BRC is mainly a scientific basic research centre, but scientists of BRC play an initiative role in the foundation and promotion of biotechnological companies, as well as in educational duties. The successful activity and high-level scientific research pursued in BRC were also acknowledged by the European Molecular Biological Organization (EMBO) and in 2000 the European Union awarded the title of “Centre of Excellence“ to BRC. BRC became a member of EABA in 2022.

Cosmetics

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

MICROALGAE EXTRACTS FOR COSMETIC APPLICATIONS: INFLUENCE OF ABIOTIC STRESS ON THEIR ANTIOXIDANT POTENTIAL

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ABSTRACT

As cosmetic industries seek innovative solutions to keep up with societal changes, the increasingly stringent health, sustainability, and safety requirements have driven the demand for natural and bioactive ingredients to replace synthetic ones in their formulations [1]. In this regard, microalgae biomass is a rich source of biomolecules (e.g. pigments, lipids, secondary metabolites, among others) to be used for cosmetics and cosmeceuticals purposes. Imposition of abiotic stress (i.e., medium composition, temperature, light irradiation, etc.) can modulate the profile and quantity of the components produced, resulting in extracts with different properties [2,3]. As for the composition of the culture medium, several compounds have been highlighted for playing an important metabolic role in biomass accumulation and composition, such as nitrate, phosphate, ferrous sulphate, and sodium chloride. *Chromochloris zofingiensis*, for example, is known as a potential source of a powerful antioxidant, astaxanthin (approximately 50% of total carotenoid content) [4]. Ethanolic extracts of *C. zofingiensis* grown in nitrogen-limited medium showed enhanced ferrous-ion chelating ability (FICA, 59.9 %) and radical scavenging ability (RSA, 41.4 %) compared to the control, 49.9 and 5.8 %, respectively. Regarding pigments content, the control reached higher contents of astaxanthin (0.20 $mg \cdot g^{-1}$), total carotenoids (0.55 $mg \cdot g^{-1}$), and chlorophyll (1.46 $mg \cdot g^{-1}$) in contrast to the N-limited culture, 0.11, 0.29 and 0.33 $mg \cdot g^{-1}$, respectively. However, the N-limited culture achieved higher phenolics content (34.5 mg $GAE \cdot g^{-1}$) than the control (21.5 mg $GAE \cdot g^{-1}$). These results suggest that phenolic compounds have more influence on antioxidant activity than carotenoids and chlorophyll. When cultivated outdoors (i.e. without temperature and light irradiance control) also in N-limited medium, *C. zofingiensis* produced less phenolic content than under controlled conditions (12.3 mg $GAE \cdot g^{-1}$), however, FICA and RSA remained higher than control, 52.9 and 21.4 %, respectively. These results show that the continuous variation of temperature and light irradiation did not drastically impair the antioxidant potential of the microalgal extract.

Keywords
Abiotic stress; Antioxidants; Cosmetics; Microalgae; Natural products.

Acknowledgements
The authors would like to acknowledge the financial support of Fundação para a Ciência e Tecnologia (FCT), through grants UIDB/04730/2020, UI/BD/151529/2021, LA/P/0045/2020 (ALiCE) and UIDB/00511/2020 - UIDP/00511/2020 (LEPABE).

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BIOGRAPHY

Bioprocess Engineer (Federal University of Rio de Janeiro, 2015), Master of Science by the Postgraduate Program in Chemical and Biochemical Process Technology (Federal University of Rio de Janeiro, 2017) and currently PhD student at University of Porto (Doctoral Program in Chemical and Biological Engineering). Topics of expertise include polymer synthesis and characterization, enzyme immobilization, cultivation of microorganisms (microalgae and yeast) in bioreactors, and processing and characterization of microbial biomass.

COMPANY PROFILE

CIETI is a multidisciplinary group that supports scientific research seeking innovation, sustainability and excellence in products, processes, and systems. Topics of interest include biomaterials, nanotechnologies, engineering, processes, energy, environment, remote laboratories and test and debug system.

ARTHROSPIRA PLATENSIS AND *CHLORELLA VULGARIS* AS HIGHLY SUSTAINABLE INGREDIENTS FOR ECO-FRIENDLY COSMETICS

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ABSTRACT

Arthrospira platensis and *Chlorella vulgaris* are sustainable and promising microalgal species characterized by the possibility to utilize whole biomass without waste, easy growth without the need for pesticide or herbicide usage, and reduction of harmful greenhouse gas emissions. Both species represent valuable sources of bioactive compounds with different health-promoting properties, which makes them suitable for implementation in the cosmetic industry [1]. This research aimed to investigate the influence of different drying temperatures on the chemical composition of *A. platensis and C. vulgaris* since dehydration is one of the crucial production steps in the cosmetic, food and pharmaceutic industries. The influence of drying temperature on total protein, carbohydrate, pigment, and lipid content was evaluated, while gas chromatography was carried out to determine fatty acid composition. Antioxidant activity of methanolic: dichloromethane (1:1) extracts from differently dried (40, 60, and 100°C) samples were analyzed using reduction of the radical cation (ABTS), 2,2-diphenyl-1-picryl-hydrazyl (DPPH), oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays [2].

The highest protein content of *A. platensis* was observed for samples dried at 60°C followed by 40 and 100°C (59,59%±0,69%, 38,39%±4,64%, 32,51%±2,93%, respectively). *C. vulgaris* sample dried at 100°C has the highest protein content (44,77%±0,00%). The total lipid content of *A. platensis* was around 2% for samples dried at 40 and 100°C and around 5% for *C. vulgaris* samples dried at 60 and 100°C. Saturated fatty acids were the most represented in all *A. platensis* samples, followed by monounsaturated and polyunsaturated fatty acids while in *C. vulgaris* the most represented fatty acids were monounsaturated and polyunsaturated, among which essential omega-6 (linoleic acid) and omega-3 (docosahexaenoic acid) fatty acids were found. By implementing ABTS and DPPH methods, the highest antioxidant activity was observed for the *A. platensis* sample dried at 40°C. ABTS, DPPH, ORAC, and FRAP results showed the highest activity for *C. vulgaris* samples dried at 60°C.

Overall results point out that both *A. platensis* and *C. vulgaris* may be utilized by different industries in an economically and eco-friendly manner by using intermediate temperatures of drying while preserving the beneficial properties of both microalgae.

Keywords
Arthrospira platensis, Chlorella vulgaris, sustainability, cosmetic industry

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BIOGRAPHY

My research interests are focused on chemical characterization (HPLC, GC) and evaluation of the antioxidant potential of microalgae and macroalgae extracts. The main goal is the identification of novel and natural compounds for further applications in cosmetic and pharmaceutical industries and functional food development.

Biomaterial

ALGÆUROPE2022
13 - 15·DECEMBER·ROME

BIOMINERALIZATION BY MICROALGAE AS A TOOL TO VALORIZE STONE EXTRACTION LEFTOVERS

Biondi N.

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ABSTRACT

Biomineralization is a process performed in nature by several microorganisms through different metabolic pathways, like urea hydrolysis, photosynthesis, denitrification and sulphate reduction. Among the minerals produced by this process, those based on calcium carbonate are the most common (Zhu & Dittrich, 2016). Although urea hydrolysis is the most widely used in several technical applications (Dhami et al., 2013), photosynthesis by oxygenic microorganisms represents an interesting alternative for microbially induced calcium carbonate precipitation (MICP). In this respect, many studies focus on cyanobacteria, like *Synechococcus*.
This work was conducted with a multidisciplinary approach. Its aim was to identify microalgae able to perform biomineralization to produce new materials from stone extraction leftovers. A screening of several microalgal strains was performed and the selected ones were tested on inert materials to evaluate their growth capacity. Preliminary production trials of new materials from stone leftovers treated with live cyanobacteria were also performed.
The screening, including nine microalgae (mostly cyanobacteria), was performed in 100 ml flasks kept in an orbital shaker under continuous light. A culture medium enriched with Ca^{2+} and $NaHCO_3$ was used compared to a standard medium as control. Microalgal growth on inert materials was tested on three different stone leftovers (marble, travertine and pietra serena from Tuscany caves), in transparent vessels under continuous light, by mixing the inert material with a microalgal inoculum suspended in the enriched medium. New materials were prepared using stone leftovers of different granulometries mixed with microalgal inoculum and a binding substance. In both experiments, controls without microalgal addition were prepared.
All the tested microalgae were able to grow in the enriched medium, in some cases even more than in standard medium. In the culture grown in the enriched medium mineral crystals were visible under the microscope. The selected strains were able to persist or even grow on stone leftovers. The trials on new material production were only preliminary and further work is needed to scale-up and optimize the process, so as to reach a quality sufficient for a usable material.
Acknowledgments. Project INERTIAL is funded by EU-NextGenerationEU and Fondazione Cassa di Risparmio di Firenze.

Keywords
Biomineralization, photosynthesis, cyanobacteria, stone leftover, biodesign

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BIOGRAPHY

Natascia Biondi, PhD in Microbial Biotechnology, is a researcher at the Department of Agriculture, Food, Environment and Forestry (DAGRI) of the University of Florence. She has more than 20 years of experience in the microalgae field and has participated to several EC funded projects (FP4, FP7, H2020). She is secretary of the Italian Association for the Study and Application of Microalgae (AISAM). Main topics of research: microalgae cultivation and application in the food, nutraceutical and agricultural fields.

TRANSITION TO ALGAE-BASED PLASTICS

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ABSTRACT

There is an urgent need for an alternative to oil-based plastic. The increasing pollution caused by plastic production and growing levels of plastic waste creates a dangerous threat to all living beings on earth. Recycling of plastic is one of the options but will not be able to cover the total demand and quality. Alternative resources are needed in the direction of renewable resources. Among all biomass resources, algae has proven to be the most sustainable for production of petroleum replacements. Our goal is that 20% of the worldwide plastic production be algae based by the year 2040.

Algae can be harvested and naturally farmed; the process can be scaled and can be done everywhere around the world. Researchers have demonstrated proof of concept that algae can be an alternative resource to petroleum produced products, from fuels to plastics. The objective of new research should be to obtain a better understanding of challenges for large scale algae farming and what is necessary to turn algae into a significant resource for production of plastic. Who are the key stakeholders, and what are their interests and challenges? Why is algae not yet embraced by industry? Who are the stakeholders, and what do they need?

It can be assumed that all industries adopting algae-sourced materials will need guarantees that they are viable alternatives to petroleum products. Algae cultivators need some guarantees of market demand and fair price for their produce. Chemical manufacturers will require assurance that their materials will be purchased by product manufacturers. Product manufacturers will desire that the materials show the same or improved performance, offer drop-in solutions, competitive price points, and training for rapid adoption.

These goals can be reached when all stakeholders work together in a joint effort. Next steps will require the establishment of the collective vision and organization of an independent non-profit group focused on algae-based materials, in which the board members would represent key stakeholders of the processes. The collective can define a strategy, establish shared measurements and studies, reinforce activities, supports and initiate research. The objective would be to dramatically impact the plastic industry worldwide, functioning as a strong backbone, with a team dedicated to aligning and coordinating the efforts of all stakeholders. The collective would represent all stakeholders, from the cradle to grave, including not only business interests, but government, designers and consumers.

Keywords
Macroalgae, Microalgae, Algae Cultivation, Chemical Manufacturing, Bioplastic, Biodegradable plastic.

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BIOGRAPHY

My passion is to solve problems and play a role in making our world sustainable. I m a visionary entrepreneur. I love beautiful things and get inspired by nature. Born in the Netherlands with a German mother and Hungarian Father, married to a Swedish wife and 2 kids. Living in Amsterdam. The last 20 years I have run GHYCZY a family business in hand made high end furniture. Building a worldwide brand inspired by the 'club of Rome'. Last year I was introduced to algae and its potential made me change my mind to dedicate my life on the transition to algae-based plastics.

COMPANY PROFILE

FG Foundation is a non-profit independent organization that leads the transition to algae-based plastic. The vision of the foundation is that algae will play an important role in solving plastic problems and contribute a better and sustainable environment. The goal is that by 2040 20% of the worldwide plastic production is algae based. The foundation connects stakeholders, initiates and fund research, create awareness, setup education and advise in governmental agreements.

ECOLOGICALLY INNOVATIVE PHB PRODUCTION TECHNOLOGY USING CYANOBACTERIA GROWING IN WASTEWATER

Grivalský T

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ABSTRACT

Polyhydroxybutyrate (PHB) is a polymer synthesized by microorganisms and is of interest as a biodegradable plastic alternative (e.g., in the packing industry) because of its ability to completely degrade into water and carbon dioxide under natural environmental conditions without using any industrial facilities.

This work aims to develop an eco-innovation process that addresses two essential areas of human activity: a new alternative way of producing bio-based degradable plastics by reusing waste. Nutrients recovered from wastewater were used to grow the cyanobacteria *Synechocystis* sp. PCC6714 Mt_a24 for the production of PHB. The cultivation conditions were optimized to grow the cyanobacteria *Synechocystis* sp. and produce PHB in a cultivation unit – a thin-layer raceway pond (TL-RWP) with a working volume of 100L, culture layer depth of 2 cm, and flow velocity of 0.2 m/s using urban wastewater.

One of the significant problems in mass cultures – the presence of contaminants, was solved by (i) partial decontamination of wastewater by UV and (ii) by culturing *Synechocystis* in a highly alkaline environment (pH≈10.5). It allowed us to control the bacteria and grazers (e.g., *Poterioochromas* sp.), which can destroy the cultures within a few hours. During these conditions, the culture reached stationary phase after two weeks of growth. The biomass concentration in the stationary phase varied between 2.7 to 3.4 g of dry weight per liter. Because PHB is synthesized only in response to conditions of physiological stress, mainly conditions in which nutrients are limited, we chose the strategy of keeping the culture in TL-RWP until the nutrients were consumed. A ripening process was observed in the late stationary phase, accompanied by chlorosis with specific color changes and an odor. The phenomena of PHB accumulation during nutrient stress was observed. Moreover, the photosynthetic performance changes measured by Fv/Fm were achieved. After maintaining the culture for two weeks in the stationary phase, the harvested culture had a PHB content of 25 % of dry biomass.

Keywords
Cyanobacteria; Polyhydroxybutyrate; Wastewater; Biodegradable plastics; Cultivation

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Since 2017 dr. Tomáš Grivalský is a postdoc at the Centre Algatech, Institute of Microbiology in Třeboň. He studied at Comenius University in Bratislava, Slovak Republic, and obtained BSc. degree in Biology in 2010, MSc degree in Molecular Biology in 2012. He defended his PhD thesis in the field of molecular microbiology at Comenius University in Bratislava in 2016 in collaboration with the Institute of Molecular Biology, Slovak Academy of Science in Bratislava. In 2016-2017 he worked as a research scientist at the Institute of Soil Biology, Biological Centre of the Czech Academy of Science in České Budějovice.
At the Centre Algatech, he is focused on genetic engineering and developing sustainable eco-innovation biotechnological production of valuable compounds from microalgae and cyanobacteria.

COMPANY PROFILE

ALGATECH Centre is an internationally recognized, largest institution for microalgae studies in the Czech Republic focused on basic and applied research of microalgae, cyanobacteria and photosynthetic bacteria in the field of photosynthesis, algae cell cycles and anoxygenic phototrophs as well as the R&D in microalgae biotechnology.

CONTINUOUS RECOVERY OF HYDROCARBONS FROM MICROALGAE BY MILKING

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ABSTRACT

Continuous oil recovery from microalgae using the *in situ*-extraction (milking) process (DE 10 2014 005 372 A1) is a promising approach to reduce the downstream costs. Depending on the process and the algal strain, these account for a major part of the total process costs and make an economic recovery of low-cost compounds from microalgal biomass difficult. The green alga *Botryococcus braunii* is particularly suitable for the process of continuous oil extraction due its ability to secrete large amounts of long-chain hydrocarbons (oils) into an extracellular matrix. The application of *in situ*-extraction eliminates the cost intensive steps of harvesting, dewatering and cell disruption [1].

Based on previous studies, various *Botryococcus braunii* strains were investigated with respect to growth, lipid accumulation and solvent compatibility. The strain Showa (B-Race) was identified as a suitable candidate for the process of *in situ*-extraction [2]. Further, the suitability of this strain for the *in situ*-extraction process was demonstrated using 3 L bubble column reactors [3], the optimal extraction time was determined, and essential cultivation parameters such as nutrient requirements, temperature, and the light/dark cycle were optimized.

The main objective of current investigations is the scale-up of the continuous extraction of extracellular oils from strain Showa. To evaluate the long-term effect of the *in situ*-extraction process on the culture characteristics, all performed optimization steps were combined and the longest possible continuous oil extraction was performed in 6 L flat-panel-airlift-reactors. In this poster, the results for continuous oil extraction over 80 days *in situ*-extraction of *Botryococcus braunii* strain Showa are presented and compared with previous extractions. Over 80 days of continuous oil extraction, 126 % of average biomass concentration in the 6 L Flat-Panel-Airlift-Reactor were recovered as oils during the *in situ*-extraction process. Therewith, a biomass associated oil productivity of 15.7 ± 2.2 mg g_{DW}^{-1} d^{-1} and volumetric oil productivity of 41 ± 6 mg L^{-1} d^{-1} was achieved. Compared to the 3 L bubble column reactors [3], the oil yield could be increased by 48 % using 6 L FPA-reactors and optimized culture conditions.

Keywords
Botryococcus braunii, Milking, *in situ*-extraction, oil

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Professional experience

Since Jan. 2012	Research Associate Anhalt University of Applied Sciences Prof. Carola Griehl, Competence Center Algal Biotechnology, Köthen
2011 - 2012	Student assistant Anhalt University of Applied Sciences Prof. Carola Griehl, Research Group Algae Biotechnology, Köthen
2011 - 2011	Professional internship Murdoch University Prof. Michael Borowitzka, Algae R&D Centre, Perth, Australia
2010 - 2011	Student assistant Anhalt University of Applied Sciences Prof. Carola Griehl, Algae Biotechnology Group, Köthen, Germany

Education

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USE OF MICROALGAE TO INCREASE THE DURABILITY OF OFFSHORE STRUCTURES IN SEAWATER

Marie RENAUDIE[a,b]

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ABSTRACT

Several early degradations of cementitious materials, such as offshore wind turbines, were reported in a marine environment [1]. Various protective strategies were developed and applied with varying degrees of success, requiring high investments. Besides, cement production represents about 6% of global greenhouse gas emission. Therefore, the durability of marine infrastructures must be urgently improved, in order to reduce both their economic cost and their environmental impact. Numerous studies focused on self-healing building materials, that is, the sealing of cracks by the material itself, designed to include microcapsules [2] or bacteria [3]. Those materials are now used extensively, but not suitable for marine conditions. On the other hand, microalgae, naturally present in sea water, are being used for a growing number of applications, such as biomineralization [4,5].
Our research project aims to provide the proof of concept of an original bioprocess, for the production of a microalgae-based mineral coating to protect building structures. To this end, we gathered a multidisciplinary team, combining skills in Bioprocess Engineering and Civil Engineering. We developed a 5L falling film photobioreactor, enabling the continuous recirculation of a microalgal culture on cementitious samples and the deposition of a mineral layer. For validation purposes, we operated a similar apparatus without microalgae. The produced biomaterial will be characterized by chemical, microscopic and micromechanical analysis. The impact of the produced solution on the durability of structures in a marine environment will also be quantified by chloride diffusion tests, permeability and mechanical tests on residual properties.
The ultimate purpose of the project is to provide recommendations and perspectives for an industrial development of the technology in the coming years.

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Marie RENAUDIE obtained her PhD Thesis in Bioprocess Engineering (biohydrogen production) in 2019 at the University of Strasbourg (Laboratory IPHC - UMR CNRS 7178). Then, she occupied several academic and industrial positions, working on topics ranging from Environmental Technology to Pharmaceutical Bioproduction. In April 2022, she joined Centrale Nantes as a Postdoctoral Fellow in the laboratory GeM, with a scientific project devoted to the development of bioprocess engineering solutions for civil engineering using microalgae biomineralization.
In September 2022, she was recruited as an Assistant Professor in the Department Process and Bioprocess Engineering of Polytech Nantes in the laboratory GEPEA.

FROM N_2 TO CYANOPHYCIN: HIGH-VALUE COMPOUND PRODUCTION THROUGH BIOLOGICAL NITROGEN FIXATION IN CONTINUOUS SYSTEMS

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ABSTRACT

Diazotrophic cyanobacteria have the ability to fix atmospheric nitrogen in their biomass during photosynthetic growth, so they could be a viable alternative to traditional and high energy demanding production processes, such as ammonia production [1]. Moreover, cyanobacteria produce many commercial relevant compounds, such as phycocyanin, zeaxanthin, β-carotene, polyhydroxyalkanoates, proteins and PUFAs [2], as well as cyanophycin (CGP), a non-ribosomal synthesized polyaminoacid compound, composed of equimolar amount of arginine and aspartic acid[3]. The industrial application of cyanophycin is not still consolidated, but it is a promising starting point for the synthesis of many important chemicals.
In this work, two diazotrophic cyanobacteria (*Anabaena cylindrica* PCC 7122 and *Nostoc* sp. PCC 7120) were phototrophycally cultivated in continuous system under N_2 fixing conditions to assess the possible stable production of cyanophycin. Indeed, *A. cylindrica* was already cultivated in a continuous cultivation system diazotrophically, obtaining remarkable biomass productivities[4]. The effect of the inlet phosphorus concentration on cyanophycin productivity was investigated with both the cyanobacterial species. The residence time and the incident light intensity were kept constant, according to previous literature on cyanophycin production in continuous system[5] and on continuous cultivation of diazotrophic cyanobacteria[4]. Then, to assess the effect of other operating variables a second set of experiments was carried out with *Nostoc* sp., because in the preliminary experiments was more efficient both in biomass, nitrogen and cyanophycin productivity. Overall, it was observed that the production of cyanophycin was strictly dependent on the concentration of phosphorus present in the culture medium: only decreasing the P quota, it was possible to measure a higher amount of cyanophycin in the biomass. It was achieved a stable and continuous production of cyanophycin under diazotrophic conditions, obtaining a maximum cyanophycin productivity of 15 mg_{CGP} L^{-1} d^{-1}.

The research is funded by the Israeli Ministry of Science grant 3-1736 and the Italian Ministry of Foreign Affairs and International Cooperation (MAECI) under award protocol number MAE01432042020-12-03, CUP C99C20001980005.

Keywords
Continuous system, polypeptide, *Nostoc* sp., arginine, aspartic acid

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BIOGRAPHY

Giulia earned her master's degree in Chemical and Process Engineering at the University of Padua in 2017. During the degree internship, she started working with algae, dealing with a microalgae-based wastewater treatment suitable for biopolymer production. Then, she collaborated within the project "Advanced systems for waste recovery". The following year, as a research fellow, she studied how to increase the productivity of Spirulina cultures in outdoor industrial photobioreactors. Currently, she is a PhD Student in Industrial Engineering, and her research topic is about a sustainable industry with low energetic and environmental impact, based on the industrial cultivation of cyanobacteria.

NOVEL TOOLS AND STRATEGIES FOR THE PRODUCTION OF BIOPLASTIC IN CYANOS

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ABSTRACT

Polyhydroxybutyrate (PHB) is a very promising alternative to most petroleum-based plastics with the huge advantage of biodegradability. Biotechnological production processes utilizing cyanobacteria as sustainable source of PHB require fast *in situ* process analytical technology (PAT) tools for sophisticated process monitoring as well as sustainable methods for PHB production and processing. In my talk, I will present a novel PAT tool for online monitoring, as well as innovative strategies for up- and downstream processing of this relevant biomaterial.

Keywords
Synechocystis sp., bioplastics, PAT, biomaterial, sustainability

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Oliver Spadiut completed his PhD in Biotechnology at BOKU University, Vienna, Austria, in 2008 before doing a 2-year PostDoc at KTH, Stockholm, Sweden. Since 2010, he has been employed as University Assistant in Biochemical Engineering at TU Wien, Vienna, Austria. In March 2015, he got his Habilitation in “Biotechnology” and is currently Associate Professor and PI of the research group “Integrated Bioprocess Development.” He has published more than 150 papers in reputed journals and is serving as a peer-reviewer for 80 scientific journals.

COMPANY PROFILE

The TU Wien is Austria's largest research and educational institution in the field of technology and natural sciences. More than 4,000 scientists are researching "technology for people" in five main research areas at eight faculties. The content of the studies offered is derived from the excellent research. More than 27,000 students in 55 degree programs benefit from this. As a driver of innovation, TU Wien strengthens the business location, facilitates cooperation and contributes to the prosperity of society.

Genetics - Synthetic Biology

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HIGH-LIGHT INDUCED LIPID REGULATORS IN MICROLAGAE

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ABSTRACT

Microalgae hyper accumulate lipids (mainly TAG or storage lipid) when subjected to stress such as: high salinity, high light intensity and nitrogen starvation. A Lipid regulator isolated under nitrogen depletion stress resulted in high lipid accumulation but failed in productivity that makes cultivation unsustainable for bioenergy production at large scale. Whereas our high-light induced two stage (48hrs + 48hrs) cultivation experiments revealed increased lipid yield and enhanced growth in Nannochloropsis gaditana when subjected to 1000uE light intensity as compared to standard condition, 100uE. Moreover, light intensity stress provided better growth than nitrogen depletion state. Therefore we aim to fill this trade off through our present work by identifying for first time lipid regulators under high-light intensity stress.

To reveal and isolate the early and late TF's (Trans factors/regulators), RNA-seq at several time points post stress was done. Among them 22-were down and 3-up regulated. When compared with nitrogen deprivation studies we observed only 2-down regulated TF's were in common (rest 18 were different and no TF was upregulated). It shows that may be for the similar effect of lipid induction the cause of abiotic stress may be perceived in different pathways, which can be the reason in their difference in lipid yield. It is relevant to note that key lipid synthesis gene-DGAT (kennedy pathway) was upregulated during light stress in our study but not in nitrogen deprivation. Further, by visualising the transcription regulatory networking hubs under light stress based on correlation (among transcript level of TFs and metabolic genes and metabolites), putative regulators were identified that regulates pathways like lipid metabolism, photosynthesis and carbon fixation.

As part of future plan (a) Quantitative proteomics of the same for stress and control is also being undergone. (b) Successful rational metabolic engineering would be attempted after identification of the regulators. The resulting mutant with the most lipid yield would be able to channelize the carbon flux towards storage lipid (TAG) keeping biomass production and carbon assimilation high. This algal platform would ideally balance the lipid-biomass trade-off that can make microalgae a sustainable solution for biofuel feedstock.

Keywords
Lipid regulator, Nannochloropsis, light stress, trans factor/regulator, RNA seq

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I am currently a post-doctoral scholar working with prof. Ondrej Prasil in laboratory of photosynthesis at Institute of Microbiology AS CR, Centrum Algatech, Třeboň, Czech Republic. My research focusses on the ''isolation of novel lipid (TAG) regulators in microalgae *Nannochloropsis* under high light stress'' that can lead to improved lipid yield and ultimately sustainable production of carbon neutral fuel. To help undertake the projects and achieve the goals I am generously funded by Czech academy of sciences post-doctoral fellowship and Marie curie International mobility of researchers - MSCA-IF IV (Institute of Microbiology of the CAS). Working on my present project enabled me to experience hands-on several interdisciplinary fields from biochemical engineering process optimisation, cloning, MS quantification, advanced genetic intervention to transcriptome data analysis. My endeavour provides me an opportunity to work in various labs, thus helping me to establish my network and build long-term collaborations that is not only beneficial in knowledge exchange but also opens up avenues to apply for future grants and grow as an independent scientist.

My Ph.D (Indian institute of technology, Dhanbad) was primarily based on (a) Formulation of medium for microalgae *Nannochloropsis* sp. cultivation that can enhance the biomass production and sequester carbon using flue gas and low cost nutrients (b) Optimisation of several independent environmental conditions and nutrients so that lipid, PUFAs are maximised by using statistical tools.

My ambition is to contribute in an original manner to the field of algal biology that can provide “Green solutions for tomorrow”.

HOW STABLE IS THE EXPRESSION OF TRANSGENES IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM*?

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ABSTRACT

In recent years, interest in microalgae as biotechnological hosts has increased rapidly. Compared to the classic model organisms for metabolic engineering, bacteria and yeast, the photosynthetic microalgae present a sustainable alternative for producing high-value compounds, such as diterpenoids. Microalgae have the additional advantage of producing the terpenoid precursor molecules, geranylgeranyl diphosphate (GGPP) and farnesyl pyrophosphate (FPP), as part of their native metabolism for photosynthetic pigments and membrane sterols, respectively. With potable water becoming a restricted resource, marine microalgae such as *Phaeodactylum tricornutum* are particularly attractive as they can grow on salt and wastewater. Engineering terpenoid production in *P. tricornutum* requires the introduction of terpene synthases and cytochrome P450 enzymes (**1**). For biotechnological applications stable expression of the transgenic sequences is key. So far, not much data has been published on the long-term stability of transgene expression in *P. tricornutum*, although some studies show a rapid loss of transgene expression upon selection pressure removal (**2**).

Using a range of novel and established DNA parts based on the MoClo syntax we generated a variety of strains producing the diterpene manoyl oxide (**3**). We will present data on the effect of promoters on transgene expression levels. Additionally, we assessed the long-term expression of exogenous DNA in a time-course experiment. Here we showcase potential gene silencing which is aggravated when strains are maintained in shorter subculturing periods and when the selection pressure is removed. In conclusion, we highlight that *P. tricornutum* has great potential for synthetic biology approaches and show areas in which further research may help to enhance the production of diterpenoids in this microorganism.

Keywords
Diatoms, high-value compounds, epigenetics, metabolic engineering, synthetic biology

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BIOGRAPHY

I completed an BSc in Biological Sciences at the University of Dundee before moving to Cambridge for my PhD in Plant Sciences. I love microalgae, epigenetics, and sustainable biotech. Outside of the lab I enjoy playing volleyball and practicing clicker training with my horse James.

A STRONG AND TUNEABLE PROMOTER FOR THE PRODUCTION OF DITERPENES IN THE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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ABSTRACT

Microalgae like the marine diatom *Phaeodactylum tricornutum* are emerging biotechnological chassis for the sustainable production of high-value products such as terpenoids. Microalgae are naturally optimised to produce precursor molecules for terpenoid production, such as geranylgeranyl diphosphate (GGPP) and farnesyl pyrophosphate (FPP), because these compounds are already produced in high amounts to make photosynthetic pigments and membrane sterols, respectively. An additional benefit over fermentative hosts is that algae exhibit rapid growth rates in simple mineral salt solutions using light as energy input to fix CO_2. Over the last years, we and others have domesticated a range of established and novel DNA parts (constitutive and inducible promoters, 5'UTRs, 3'UTRs, targeting peptides) based on the MoClo syntax that can be used for efficient and high throughput Golden Gate assembly for *P. tricornutum*.

Often it is desirable to regulate transgene expression using either tunable promoters or riboswitches. While a predicted thiamine riboswitch was found not to be functional in *P. tricornutum* (Llavero-Pasquina et al., 2022), we have demonstrated tuneable regulation of transgene expression using a vitamin-dependent promoter. We tested this promoter by driving the expression of fluorescent reporter proteins as well as a range of diterpenoid biosynthetic enzymes (e.g. diterpene synthases, GGPP synthases,). The generated strains showed an increase in protein abundance, as well as diterpenoid titer, compared to strains in which the transgenes were expressed under the control of standard promoters such as the fucoxanthin chlorophyll a/c binding protein (*FcpA*) promoter. We have engineered some of the diterpene-producing strains further by targeting rate-limiting enzymes involved in the biosynthesis of GGPP and could show a further increase in diterpene titer.

In summary, our work shows that the adoption of synthetic biology approaches in *P. tricornutum* to metabolic engineering can help with strain selection and the production of terpenoids in this microalga.

Keywords

Genetic parts, Tuneable promoter, Metabolic engineering, Terpenoid

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BIOGRAPHY

Katrin obtained a PhD from the University of Copenhagen, Denmark, while working with Prof Anne Osbourn at the John Innes Centre in Norwich, UK, studying the role of cytochrome P450 enzymes in the biosynthesis of triterpenes. In 2011 she joined the lab of Prof Joerg Bohlmann at the University of British Columbia, Canada, investigating cytochrome P450s in terpenoid pathways using genomic, metabolomic and biochemical approaches. In 2014 she joined Prof Alison Smith's lab at the University of Cambridge, UK, to study the diatom Phaeodactylum tricornutum as a chassis for the production of high-value compounds such as lipids and terpenoids. Her interests are the development of genetic parts to regulate transgene expression in microalgae such as *P. tricornutum* and the freshwater algae *Chlamydomonas reinhardtii*.
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ENGINEERING 5'UTRs TO ENHANCE REGULATED GENE EXPRESSION IN THE GREEN ALGA *CHLAMYDOMONAS*

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ABSTRACT

Substantial progress has been made in developing bioengineering tools for the model organism and green biotechnology host *Chlamydomonas reinhardtii* (Sproles et al., 2021). However, one outstanding issue is unpredictable and/or weak heterologous expression. Here we studied two B vitamin responsive elements in Chlamydomonas, a riboswitch and a promoter, that have been useful in regulating transgenes but often result in reduced overall expression. We asked whether we could address this issue through a 5'UTR engineering approach. We started by testing the effect of different individual and dual/chimeric 5'UTRs on reporter constructs under a non-regulatable but strong promoter, P_{PSAD}. From this we observed that a particular chimeric 5'UTR (PSAD fused to THI4 plus RBCS2 intron) led to a 2-fold increase in expression, compared to the control with promoter-cognate UTRs. We then tested these parts on constructs regulated by a thiamine riboswitch or the B12-repressible promoter, P_{METE} (Helliwell et al., 2014; Mehrshahi et al., 2020). Again, we observed an improvement in expression levels, of 2 to 5-fold compared to the control, which demonstrates that these otherwise poorly expressed systems can be optimized by modifying their 5'UTRs. Finally, we are testing the utility of these parts in a metabolic engineering strategy to produce a terpenoid of industrial interest. In conclusion, we demonstrate the efficacy of 5'UTR engineering in Chlamydomonas, suggesting the potential of a similar strategy to be applied in other eukaryotic organisms.

Keywords
Synthetic Biology, Riboswitches, Promoters, Metabolic Engineering

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BIOGRAPHY

Gonzalo obtained his PhD in Molecular and Cell Biology from the University of Edinburgh, where he studied pre-mRNA splicing in the group of Prof. Jean D Beggs. Since 2017, he has been working in the Plant Metabolism group of Prof. Alison G Smith to study mechanisms of gene regulation in algae, and to develop molecular tools to control transgene expression.

ENGINEERING LIPID PROFILES IN THRAUSTOCHYTRIDS

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ABSTRACT

Thraustochytrid T18 is a commercially relevant source of docosahexaenoic acid (DHA). The three main fatty-acid species produced by T18 are C16:0, docosapentaenoic acid (DPA)-n6 and DHA. This strain has also been developed in-house as a GM workhorse. The replacement of eicosapentaenoic acid (EPA) derived from fish oil is highly sought-after in the microbial oil industry. T18 possesses a PUFA synthase system which is responsible for the production of DHA and DPA-n6. One of our strategies to meet the need for a larger variety of high-value PUFAs includes engineering a classical pathway of elongases and desaturases for fatty-acid synthesis in T18 using C16:0 as substrate. Transcriptomics studies identified native regulatory elements. Endogenous T18 promoters are used in their native context to express transgenes while simultaneously knocking out the native coding sequence via homologous recombination. Co-expression of transgenes at a particular native site, using a 2A Picornaviral system, improves product yield over expression of transgenes at discrete sites. Transgenes including fatty-acid elongases and desaturases are expressed in T18. Elements of a vestigial native T18 classical pathway are used. Different permutations of genes introduced at the same native T18 sites result in strains producing different species of high-value PUFAs.

Keywords
Heterotroph, transcription, transgenes, 2A, EPA

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BIOGRAPHY

Holly Rasmussen has been a Research Scientist at Mara Renewables Corporation since 2013. Pathway engineering and molecular genetics experiments are her primary focus and obsession. She has a BSc (Double Major, Biochemistry/Molecular Biology and Religious Studies) from Dalhousie University and a can-do attitude.

COMPANY PROFILE

Mara Renewables Corporation is a marine microbial biotechnology company based in Nova Scotia, Canada. Our flagship strain, Thraustochytrid T18, was isolated from the Bay of Fundy, home of the world's highest tides. R&D at Mara encompasses a wide range of strains and methodologies. Many of the strains we work with come from our own collection of marine isolates. Using techniques from the tried-and-true to the cutting-edge, Mara works to supply diverse markets with marine microbial oil, protein, and co-products.

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SEPARATION OF VALUABLE PIGMENTS FROM *PHAEODACTYLUM TRICORNUTUM* VIA HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY

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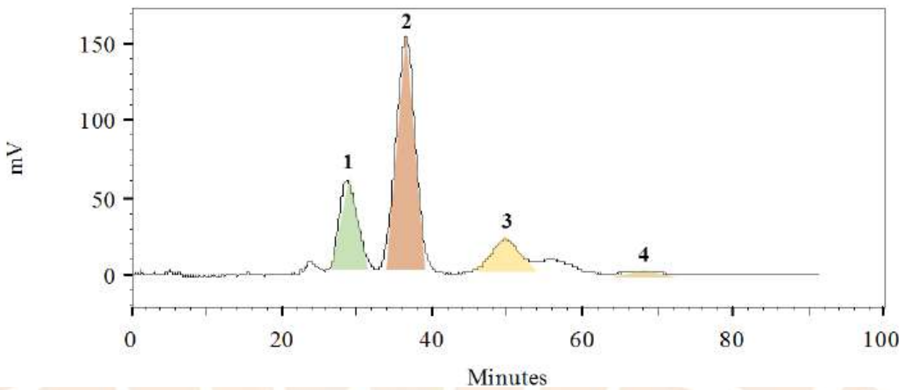
ABSTRACT

The marine diatom *Phaeodactylum tricornutum* is a recognized source of pigments such as fucoxanthin, diadinoxanthin, diatoxanthin and chlorophyll *c*, which are reported to have health-promoting properties for humans [**1**]. The present study reports the development and application of a high performance countercurrent chromatography (HPCCC) isolation method for the recovery of the pigments chlorophyll *c*, fucoxanthin, diadinoxanthin, and diatoxanthin from the diatom *P. tricornutum* in a one-step separation strategy. Biomass extraction and HPCCC isolation were unified using the same solvent system to improve the final recovery of the target pigments. The separation process was developed on an analytical HPCCC column (24 mL). The lower phase of the selected biphasic solvent system, consisting of *n*-heptane, ethanol, and water, was used as the mobile phase, while the upper phase was the stationary phase. The effects of flow rate and sample loading on resolution were studied in the ranges of 0.5-1 mL/min, and 20-40 mg, respectively. The rotational speed of the HPCCC column was set at 1600 rpm. The obtained optimized conditions were transferred to a semi-preparative HPCCC (134 mL - column) to process 120 mg of microalgal extract, resulting in the separation of chlorophyll *c* (**1**) (35.52 mg), fucoxanthin (**2**) (5.83 mg), diadinoxanthin (**3**) (1.28 mg), and diatoxanthin (**4**) (0.25 mg) with purities above 98% (**Fig.1**). The developed method did not need to be coupled with any other chromatographic technique to achieve the desired separation. Overall, the developed isolation method could provide a useful model for the recovery of valuable pigments from diatom biomass.

Figure 1.HPCCC separation of valuable pigments from *Phaeodactylum tricornutum*



Keywords
Microalgae; *Phaeodactylum tricornutum*; carotenoids, chlorophylls, high performance countercurrent chromatography (HPCCC).

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Acknowledgement
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PhD student at the Laboratory of Algal Biotechnology of the Centre Algatech (Czech Academy of Sciences) in association with the Faculty of Sciences of the University of South Bohemia, Czech Republic. Her PhD research focuses on the development of novel algal biorefinery systems for the production of valuable compounds by integrating optimised cultivation methods with high performance countercurrent chromatography (HPCCC). She is dedicated to creating new value chains by developing comprehensive and multi-target biorefinery processes using a combined approach of novel biotargets and in-demand active ingredients.

DEVELOPMENT OF ECONOMIC VIABLE ALGAE-BASED VALUE-CHAINS in NWEUROPE, WITHOUT AND WITH USE OF SIDE-STREAMS

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ABSTRACT

The Interreg NWE project IDEA envisions the development and enrolment of economic viable value chains based on micro-algae in NWEurope. More particularly, partners from Belgium, Germany, France, The Netherlands, Ireland and UK are involved which are countries exposed to a similar climate that is significantly different from the south-European climate. Although light intensities and temperatures are lower compared to south-Europe, there is a potential for algae growth. For some species, the NWEuropean climate may even have advantages, and can be economic viable when targeting higher value products (not bioenergy). Today algae based value chains are being established, comprising algae growth, algae harvest, algae processing and formulations with algae-based ingredients. IDEA aims to innovate and push the process development from individual solutions to a viable process chain targeting final compounds for food, feed and cosmetic applications.

In the first part of the IDEA project (2017-2021) IDEA addressed innovations related to: (1) algae species for NWEurope climate and cultivation efficiency for prolonged growth seasons in NWE, (2) development of an automated water recirculation, harvest unit & alternative CO2 resources, (3) storage concept, logistics and preservation of algae biomass, (4) regional activities for algae processing, potentially comparable to the milk industry, (5) >15 novel products formulations to targeting value generation in feed, food & cosmetics. Via an Interreg NWE capitalization initiative, the IDEA project was prolonged for another 2 year (till end 2023) during which special attention is given to circularity, being the use of different types of side-streams (water, CO_2, N/P, …) in phototrophic algae cultivation and the impact on the subsequent use of the algae biomass. More specifically, algae growth on low carbon process water and nutrients and CO_2 from digestors is being evaluated and demonstrated.

Some key findings of the IDEA project will be presented that are related to demonstrations of algae growth, algae harvest, medium re-use and the preparation of algae-based ingredients and application tests (including in vivo test trials). Findings have been translated into elements for a roadmap towards implementation of algae-based value chains.

Keywords

Algae value chains, side-streams, circularity, medium re-use, roadmap

References

This research was funded by NORTH-WEST EUROPE INTERREG, grant number NWE 639 as part of the IDEA project (Implementation and development of economic viable algae-based value chains In North-West Europe). www.nweurope.eu/idea

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Dr. Ir. Leen Bastiaens (Bio-engineering, PhD in Applied Biological Sciences) is project manager at VITO with 20 years of experience in applied scientific research (from TRL1 till TRL8) in the field of soil and groundwater remediation and biotechnology (lab to pilot scale). Since 2014 she became also involved in ‘renewable extracts and chemicals’ (lab & pilots) with projects related to algae harvesting and biorefinery of algae, insects and side-streams, and biosurfactants. She was active as coordinator/WP-leader/partner in multiple international research project and contract research directly for industrial partners.

COMPANY PROFILE

The Flemish Institute for Scientific Research (VITO NV) is a research Institute implementing client-driven research projects and developing innovative products and processes. The multidisciplinary skills and technological know-how of more than 900 researchers make this organization a crossroad of technology, where state-of-the-art technologies are successfully blended into practical applications.

SCREENING OF SEPARATION PROCESSES FOR THE RECOVERY AND RECYCLING OF DEEP EUTECTIC SOLVENTS FOR SEAWEED BIOREFINERS

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ABSTRACT

Deep eutectic solvents (DES) are novel green solvents which have been popularized recently not only due to their excellent solvent capabilities, but also to their characteristics such as: high thermal stability, low volatility, low vapor pressure, and tunable polarity (Hansen et al., 2021). Furthermore, DESs are environmentally benign which position themselves as sustainable alternatives to organic solvents (Khandelwal et al., 2016). Because DESs have been proven technically feasible and effective for biorefinery processes, they could be of great importance for the development of sustainable and circular biorefineries (Liang & Guo, 2022). These biorefineries maximize biomass utilization, minimize waste and chemical usage, and emphasize on the recovery and recycling of materials (Wang et al., 2021). Therefore, an efficient recovery and recycling of DESs are needed. However, these processes are challenging because DESs are often non-volatile and current separation technologies cannot separate efficiently the DES (Liang & Guo, 2022; Muilwijk, 2022). In this research bipolar membrane electrodialysis, nanofiltration and chromatography are carried out to recover and recycle deep eutectic solvents after alginate extraction from a model-seaweed mixture.

Keywords
Deep eutectic solvents, sustainable biorefinery, separation processes, membrane filtration, chromatography

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Oscar Elizondo Sada was born in Monterrey, Mexico. He obtained the title of Biotechnology Engineer with specialization in Bioprocess Engineering from the Monterrey Institute of Technology and Higher Education (ITESM). His Bachelor's thesis was focused on a biorefinery for the production of xylooligosaccharides from sugarcane bagasse for the development of prebiotics. He moved to The Netherlands to pursue his MSc. degree on Biotechnology specializing in Environmental and Biobased Technology at Wageningen University & Research (WUR). For his final course of the master program, he wrote a project about a biorefinery for single cell protein production from *Arthrospira platensis*. Currently he is doing his master thesis on the Biorefinery group from the Bioprocess Engineering department of Wageningen University. His topic is the screening of separation processes for the recovery and recycling of deep eutectic solvents for seaweed biorefineries.

CONVERSION OF MICROLAGAL-DERIVED POLYSACCHARIDES INTO BIOPOLYESTERS

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A.L. Carolas

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ABSTRACT

Plastics are an important part of the global economy: their production surged from 15 to 359 million tons between 1964 and 2018 and the plastics industry employs directly 1.5 million people in Europe, generating a turnover close to 350 billion euros in 2016. The plastic industry currently faces two major challenges: 1) 90% of global plastic produced is still derived from fossil feedstock; 2) the negative image linked to unsustainable end of life management practices.

Polyhydroxyalkanoates (PHAs) are biopolyesters that can substitute conventional plastics in numerous applications. They are renewable, biodegradable and bio-based. However, no sustainable value chain exists in Europe, and production developed elsewhere rely on plant-derived raw materials that and require arable land for their production and that compete with food and feed uses.

“NENU2PHAR - For a sustainable and European value chain of PHA-based materials for high-volume consumer products” is a project supported by a European grant of the private-public partnership “Bio-Based Industry Joint Undertaking”. It is developing an original route of production PHAs from sustainable and renewable resources: extraction of polysaccharides from microalgae, hydrolysis of polysaccharides into fermentable sugars and fermentation of the sugars by naturally occurring PHA-accumulating bacteria.

In NEU2PHAR, we have screened for different candidate bacterial strains able to convert the microalgal-derived sugar-rich hydrolysates into PHA. After removal of residual solids, hydrolysed algal biomass was shown to be a suitable carbon source for the fermentation process and two of the tested strains, *P. sacchari* and *C. necator*, accumulated significant amounts of PHA, 54% and 65% on dry weight respectively. A fermentation strategy was firstly developed using commercial raw-materials and validated pilot scale fermenters obtaining up to 85 g/L PHA within just 52 hours of fermentation. This process will be adapted to the use of the algal hydrolysates as main carbon source and scale-up performed to produce kg-scale samples of material for application testing, validating the feasibility of the CO2-to-biopolyester route developed in the project.

Keywords
Biorefinery, microalgae, biomass feedstock, fermentation, biopolyesters

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MSc in Chemical Engineering and PhD in Biotechnology with academic research experience in Portugal, Canada and Germany. Focused on the scientific and business aspects of industrial and marine biotechnology. Cofounder and CEO of Biotrend SA, board member of A4F SA and co-founder of Silicolife Lda. Member of the executive board of BlueBioAlliance. Very strong and broad bioengineering background in diversified biological systems for diverse applications. Advisor for private and public entities on industrial and marine biotechnology and the bioeconomy. Co-author of more than 40 contributions to international refereed papers and books and 4 patents.

COMPANY PROFILE

Biotrend develops fermentation-based biorefining processes. It provides advanced services covering process development aspects from strain screening to fermentation optimization, process integration, intensification, de-risking and scale-up from proof-of-concept to demo and commercial scale.

STARCH-RICH *CHLORELLA VULGARIS* PRODUCTION STRATEGIES AND COSTS AT SEMI-INDUSTRIAL SCALE IN DIFFERENT PHOTOBIOREACTORS

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ABSTRACT

The use of microalgae as a sustainable and renewable feedstock for biosourced plastic biorefineries is the core mission of the Nenu2Phar[1] European project, which the CEA is part of. Since 2021, the CEA team has been successfully triggering above-average starch content in *Chlorella vulgaris* CCALA924[2,3]. By exposing nitrogen-starved cells to high light, up to 70% of starch could be accumulated in the biomass. This growth strategy was validated extensively at laboratory scale under artificial light.

Further growth trials have since continued outdoors at pilot scale from November 2021 to November 2022 to take the proof of concept closer to industrialisation. 180 L flat panel airlift reactors with static mixers were used to establish the ease of culturing and the range of biomass and starch productivities attainable over winter and summer.

Starch production was affected due to seasonal changes as well as recurring contamination episodes which severely hampered production. Nevertheless, an average starch content of 38% dry weight was obtained during the culture period, and an overall increase in starch content could be observed in ageing, nitrogen-deprived cultures, with high starch content values ranging between 40% and 60% dry weight occurring in the second week of growth. Our results are in line with those obtained in other outdoor starch production pilot-scale studies from *Chlorella*[4,5].

Operational costs of microalgae production in four semi-industrial photobioreactors (1500L open raceway pond, 900L horizontal tubular photobioreactor, 180L flat panel with static mixers and a 140 L flat panel made on-site with plastic sleeves) harvested by centrifugation were also compared in order to select the most economically attractive strategy for an hectare-scale implementation. Based on the unit cost of biomass produced, the commercial flat panels with static mixers have the lowest operational costs as they present higher biomass concentrations than the other reactors, thus implying lower man work cost. However, these reactors were also more prone to contamination due to their geometry, which entails additional cleaning efforts and potentially higher operational costs. The selection criteria for the most economical reactor will be discussed.

Keywords
Chlorella vulgaris, outdoors, nitrogen-starved cultures, starch, operational costs.

References

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BIOGRAPHY

Sophie FON SING has a Bsc. (2006) and a PhD (2011) in Marine Science from Murdoch University, Perth Western Australia, Australia. She kickstarted her career in microalgal biotechnology by joining the university spin-off company originating from the microalgal biofuels project she worked on duringher PhD. Since then, she has been working in a variety of microalgae start-ups at pilot and demonstration scale and has assumed various roles such as research scientist, laboratory manager, R&D and operations manager. To date, her algae project portfolio includes bioplastics from microalgae, nutraceuticals, algal biofuels, aquaculture and environmental monitoring (toxic algae). Her broad research, field and industry experience has given her in-depth knowledge of the risks, challenges, opportunities, as well as the technical, regulatory and commercial aspects and requirements of the algae industry.

COMPANY PROFILE

CEA (France's Alternative Energies and Atomic Energy Commission) is a Research and Technology Organisation that produces, integrates and transfers science and technology to help resolve grand societal challenges and to exploit opportunities for new wealth creation, improved standards of living, preservation and build-up of economic competitiveness. CEA runs 10 research centers and 6 regional technological platforms located all over France, hiring over 16,000 people. Its core missions are to identify, develop and maturate Key Enabling Technologies from TRL 3 to TRLs 5-7 through public-private partnerships.

CEA operates the Microalgae Process Platform at CEA-Cadarache research center, which is a modular open platform devoted to designing, experimenting and validating advanced innovation technologies for cultivating and harvesting microalgae. It incorporates an analytical laboratory, a biology laboratory, pilot-plant facilities and demo-scale industrial systems.

TECHNO-ECONOMIC ASSESSMENT OF SUSTAINABLE AVIATION FUEL PRODUCED IN AN INTEGRATED ALGAL BIOREFINERY

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ABSTRACT

The need for sustainable energy sources for transportation sector is increasing every day. Today, civil aviation is responsible for 2% of the global carbon dioxide emissions and is expected to reach 3% by 2050. Sustainable aviation fuels from third-generation, non-food biomass feedstocks such as microalgae, stands out as a strong candidate to combat GHG emissions. The objective of this study was to assess the techno-economical viability of SAF production in an integrated biorefinery. Candidate microalgae species were selected as *Ettlia oleabundans, Nannochloropsis gaditana, Botryoccoccus braunii* and *Chlamydomonas nivalis.* Microalgae were cultivated in open raceway pond reactors, harvested and dewatered by centrifugation to be ready for extraction. Hydroprocessed esters and fatty acids (HEFA) were converted to SAF and blended with Jet A-1 to be used in civil aviation. The capital (CAPEX) and operational expenditures (OPEX) were calculated where downstream and upstream equipment costs were found to be 54% and 19% of the CAPEX while construction costs were accounting for 27% excluding land cost. The commercialization of SAF, like any other alternative bio-product, depends on the price competition with the conventional products. Although algal SAF is not viable to be used on its own, blending with Jet A-1 enables the blend to compete with fossil based jet fuels. Furthermore, the adoption of the integrated biorefinery concept, production of by-products and minimize the waste material from the production of bio-jet fuel is a promising step for commercialization.

Keywords
Techno-economic analysis, sustainable aviation fuel, integrated biorefinery

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BIOGRAPHY

Berat Z. Haznedaroglu is an assistant professor at The Institute of Environmental Sciences at Bogazici University (BU). He is currently director for İstanbul Microalgae Biotechnologies Research and Development Center and vice director for The Center for Life Sciences and Technologies. Berat's research group focuses on systems-level characterization of non-model microalgae and cyanobacteria for nutritional supplements, biofuels, value-added chemicals, and wastewater treatment applications. His group investigates the relationship between the expression and interaction of anabolic and catabolic reactions coupled to high-throughput systems biology tools including next-generation DNA sequencing, metabolomics, proteomics, and bioinformatics. Berat completed his postdoc at Yale University working on enhanced production of biodiesel from microalgae. He received his Ph.D. from the Chemical and Environmental Engineering Department of University of California, Riverside and his M.Sc. from the Civil and Environmental Engineering Department of Villanova University, PA. Berat holds a B.Sc. in Biology from the Middle East Technical University, Ankara, Turkey. Berat is Newton Advanced Fellow and associate editor for Frontiers in Microbiology Journal.

COMPANY PROFILE

Bogazici University (BU) is the highest ranking, non-profit, public university of Turkey, founded in 1863. With more than 150 years of research and teaching tradition, the administrative and academic quality is recognized and approved by the European University Association (EUA). BU is the only Turkish University among the first 150 universities based on Times Higher Education World University Rankings. Additionally, BU ranked 5th in the Times Higher Education BRICS & Emerging Economies Rankings. The official language of instruction is in English, and it maintains broad international educational exchange programs, with significant number of international students on campus. BU has over 600 faculty members and over 300 research staff and assistants. Among these, 70% of faculty members have received their Ph.D. degrees from internationally recognized prestigious academic institutions. The University offers PhD programs in 30, and MSc programs in 47 different fields. BU is affiliated with The Utrecht Network, The Mediterranean Universities Union (UNIMED), and The International Association of Universities (IAU).

A NOVEL EXTERNAL REFLECTING RACEWAY POND DESIGN FOR IMPROVED BIOMASS PRODUCTIVITY: MODELING AND EXPERIMENTAL RESULTS

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ABSTRACT

Decreasing the cost of microalgae cultivation is a vital step in unlocking its potential as a carbon sink and a renewable source of food, biomass, and other useful compounds. Currently, large-scale cultivation of microalgae typically occurs in outdoor raceway ponds where low biomass productivity and culture concentration can lead to high cultivation and downstream processing costs. This is due, in part, to the low solar incidence on the culture at various times throughout the year which reduces the illuminated culture volume and decreases the growth rate. This is particularly true in the winter months or at higher latitude locations. Thus, we propose two novel designs featuring vertical reflecting surfaces positioned on either the north side of an east-west oriented raceway pond or on the east and west sides of a north-south oriented raceway pond. These designs aim to increase the solar flux incident on the culture and improve the biomass productivity of outdoor raceway ponds. The resulting biomass productivity was predicted in our previous publication[1] by a coupled model of light transfer and microalgae growth kinetics. Overall, the modeling results predicted an increase in the biomass productivity for raceway ponds featuring a vertical mirror throughout the year. For example, the biomass productivity was predicted to improve by 36% in late September for a raceway pond featuring a south-facing mirror. To validate the predicted improvement in biomass productivity, proof-of-concept experiments are being performed using two 0.5 m^2 raceway ponds under solar conditions, with and without a vertical mirror, cultivating *Chlorella vulgaris* in a greenhouse at the AlgoSolis R&D facility located in Saint-Nazaire, France. Experimental results for a culture operated in batch mode show an increase in productivity of up to 15% for a north-south oriented raceway pond with east/west-facing mirrors during the month of August. Experiments are currently underway to assess the performance of an east-west oriented pond with a south-facing during the autumn months. This experimental campaign aims to quantify the actual increase in performance afforded by the mirror design and as well as any potential challenges associated with its implementation.

Keywords

Photobioreactor, solar energy, raceway pond, modeling, bioprocess engineering

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BIOGRAPHY

Jack Hoeniges received his PhD from the University of California, Los Angeles in mechanical engineering for his research on modeling light transfer phenomena related to outdoor cultivation of microalgae. His current research for his PhD at the University of Nantes focuses on novel methods for improving the biomass productivity of microalgae cultivation systems using experimental and modeling-based methods. He has applied and theoretical experience in heat transfer, light transfer, bio-based processes, and concentrated solar applications. With a passion for sustainability, space applications, and cross-cultural work environments, Jack will be open to employment opportunities in Europe following his defense in March of 2023.

IMPROVEMENT OF VERTICAL MIXING IN RACEWAY PHOTOBIOREACTOR

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ABSTRACT

The lack of adequate vertical mixing is one of the factors limiting the productivity of open raceway microalgae reactors. Thus, the existence of large gradients of light and the lack of vertical mixing does that the cells are mainly adapted to local irradiance instead of average irradiance. The adaptation to average irradiance allows maximizing the light utilization efficiency and then maximizing the biomass productivity of microalgae cultures. To overpass this problem different alternatives have been proposed, one of the more suitable being the utilization of blades to improve the vertical mixing. In this work, experimental studies were performed installing blades. The goal is to improve the photosynthetic efficiency, but also a better understanding of the light regime to which the microalgae cells are exposed in these systems and how to improve it.

Computational fluid dynamics (CFD) was used to optimize the design of blades. Moreover, a dynamic photosynthesis model (Rubio Camacho et al., 2003) allowing to estimate the photosynthesis rate as a function of the light regime to which the cells are exposed, including photo-adaptation and photo-inhibition phenomena was used. The combination of both tools allows for optimisation of the configuration of the system.

Results confirm that by using the blades the vertical mixing is improved also the photosynthesis rate improves, at the same time minimizing the energy consumption involved. The vertical mixing generated by the blades is dissipated after 4 m, but the photosynthetic benefits are observed including during 10 m in length of the channel. The frequency of exposition improves three times concerning conventional systems which allows an increase in photosynthesis rate of 25% and an increase of biomass productivity from 15.6 to 19 $g \cdot m^{-2} \cdot d^{-1}$. These results confirm the benefits of optimising the mixing in microalgae cultures, especially focusing on the movement of the cells between the different illuminated zones.

Additional work is underway to increase the photosynthesis rate and final biomass productivity, it is expected to increase the microalgae production capacity of actual photobioreactors up to 50%, regardless of location, maintaining low energy consumption and CAPEX.

Keywords
CFD, dynamic modelling, photosynthesis efficiency, vertical mixing, microalgae.

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Engineer, Master's in Computational Mechanics currently PhD student in Biotechnology and Industrial Bioprocesses dedicated to the development of industrial platforms for the production of microalgae. Young professional with more than 12 years of experience in the design and implementation of photobioreactors for the industrial of human food, animal feed, agriculture and environment. Experience in the private production industry and the research and development industry.

MICROWAVE ASSISTED EXTRACTION COMBINED WITH ENZYMATIC TREATMENT FOR CELL DISRIPTION OF *CHLORELLA VULGARIS*

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ABSTRACT

Microalgae are considered as a promising industrial source for the sustainable production of valuable compounds, such as high-quality protein, polyunsaturated fatty acids, dietary fiber minerals and vitamins useful in different industries (i.e., nutraceutical, food, animal feed and cosmetics). In addition, their significantly high growth rates and simple growth requirements make them attractive for the biorefinery industry (Canelli et al., 2021).

However, large amounts of reagents and high-energy inputs are needed for the release of valuable compounds due to the robustness, complexity, and chemical diversity of microalgae cell walls. Therefore, the disruption of cell walls plays a key role in the recovery of valuable compounds, so it is necessary to develop new specific disruptive processes for each species of microalgae that achieve a more efficient recovery of these compounds. This optimization will contribute to the reduction of energy, solvent and extraction time required and to limit the degradation of the target compounds, and thus, may considerably promote the implementation of the microalgae biorefinery concept (Karim et al., 2020).

The present work aims to evaluate the combination of different extractive methods applied to *Chlorella vulgaris* concentrated culture and biomass to enhance and optimize lipid and protein recovery. Several cell disruption methods have been tested including mechanical (microwave assisted extraction) and non-mechanical pre-treatments (enzymatic assisted extraction). Commercial enzymes including cellulases, lipases and proteases have been applied as algal cells are strong and stable due to the presence of polysaccharides such as cellulose and hemicellulose. Moreover, experimental designs were conducted through Design Expert software to optimize the operational parameters like extraction time, pH and type of enzyme. The cell wall disruption was evaluated by analysis of protein solubilization (Kjeldahl method).

Keywords
Cell-wall disruption, *Chlorella*, microwave, enzyme, protein solubilization

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Anna Margenat Mas holds a degree in Chemistry (2012) from the Chemical Institute of Sarrià (IQS), specialized in Organic Chemistry. She also studied a master's degree in Analytical Chemistry from the University of Barcelona (UB, 2015) and a PhD in Agrifood Technology and Biotechnology from the Polytechnic University of Catalonia (UPC, 2018).
Her research has focused on Environmental Chemistry in the evaluation of the incorporation of organic microcontaminants into plant matrices, their effects on crop productivity and human health. She has developed and optimized extractions of organic microcontaminant in plant matrices, multiresidue analytical methods with chromatography and preliminary metabolomics studies.
She is currently working as a researcher in the Circular Economy R&D business unit, in the R&D Bio-resources and Agrifood Technologies group of Leitat Technological Center, carrying out the scientific and technical development of R&D&I projects in the field of the use of by-products and the recovery of waste with different extractive and conversion technologies.

COMPANY PROFILE

Acondicionamiento Tarrasense (LEITAT Technological Center) is a non-profit association with its own legal and patrimonial personality, established in 1906. LEITAT is currently a Technological Center whose mission is to collaborate with companies and other entities to create economic, social and sustainable value, through R + D + 2i projects and technological processes based on innovation and creativity. LEITAT is a trademark of the private entity Acondicionamiento Tarrasense and is recognized by the Ministry of Science and Innovation. LEITAT has gained positioning and visibility in relevant research areas in the context of national and European public policies, such as biomedicine, nanomaterials, nanosafety, renewable energy and intelligent systems (IoT). LEITAT structures its R&D&i activities in 5 large areas of knowledge: (1) Health & Biomedicine; (2) Circular Economy; (3) Applied Chemistry & Materials; (4) Energy & Engineering and (5) Scientific & Technical Services.
The Biosources & Agrifood Technologies (BAT) division, belongs to the Circular Economy Department, and is composed of 9 researchers (3 doctors) in the fields of chemistry, food technology, environmental biology and biology. BAT division provides scientific and technological support to the agrifood industrial sector, with the aim to develop and provide technological solutions to the companies and improve their competitiveness.

MULTI-STR3AM: A SUSTAINABLE MULTI-STRAIN, MULTI-METHOD, MULTI-PRODUCT MICROALGAE BIOREFINERY INTEGRATING INDUSTRIAL SIDE STREAMS TO CREATE HIGH VALUE PRODUCTS

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ABSTRACT

There is a critical need to shift to more sustainable forms of food and feed production. Microalgae are increasingly recognised as a valuable source of raw materials for these markets. Large-scale production is held back by barriers of scale and price. MULTI-STR3AM project will respond to the growing interest in demand for sustainable products from microalgae by providing Europe with the first dedicated multi-strain, multi-process and multi-product biorefinery. This biorefinery will be able to produce a wide array of structurally diverse molecules, including proteins, lipids including fatty acids, polysaccharides and photosynthetic pigments such as carotenoids. The project integrates a large-scale production of microalgae, biorefinery and final product validation by end-users in the food, feed and fragrances markets. Large scale autotrophic microalgae production was accomplished in unilayer horizontal tubular photobioreactors with 1560 m^2/ 35 m^3 and cascade raceways with 2500 m^2 / 80-120 m^3 for *Nannochloropsis* sp., *Dunaliella Salina* and *Spirulina*. Fractions of disrupted biomass, carotenoid extracts and fragrances were evaluated as final products for food, feed and fragrances applications with promising results. The outcomes allowed for validation of the MULTI-STR3AM biorefinery with valorisation of all fractions of biomass in a zero waste approach.

The biorefinery was designed with all the necessary unit operations, and the mass balances were calculated (**Figure 1**). To evaluate the profitability of the proposed biorefinery, a detailed techno-economic evaluation was performed for the *Nannochloropsis* case study. Early in this study it was concluded that the biomass price was found to have a great impact on the economic profitability of the biorefinery, and the comparative analysis conducted revealed that a reduction of 40% in the biomass price, from 5.0 €.kg_{SFDW}^{-1} to 3.0 €.kg_{SFDW}^{-1}, increases the project IRR from 20.3% to 43.1%. Finally, the ultimate goal of the study was to evaluate if the proposed multi-product biorefinery is economically more attractive than a single-product value chain biorefinery. The multi-product biorefinery was found to reach higher profitability levels, with an IRR of 20.3%, which stands clearly above the single-product biorefinery, for which a negative IRR was obtained.

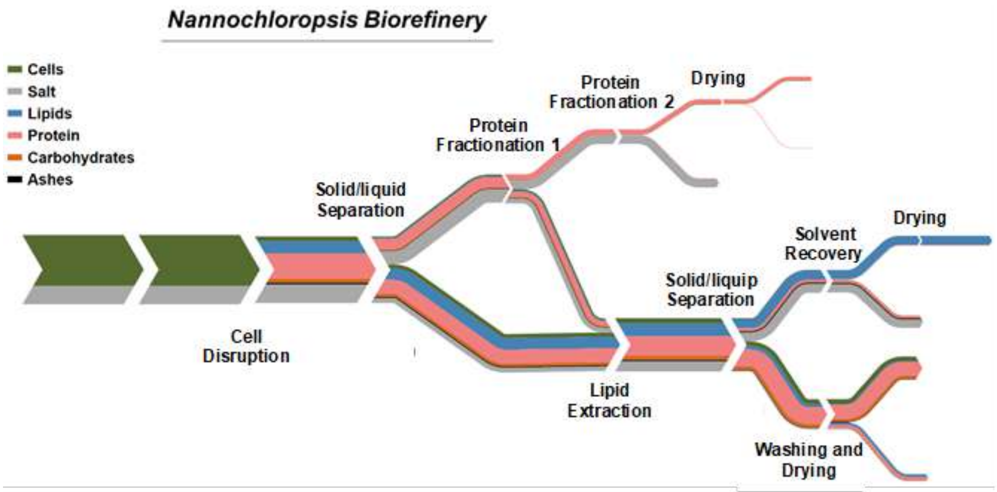

Figure 1. Sankey diagram of the proposed Nannochloropsis Biorefinery

Keywords
Microalgae biorefinery; multi-product biorefinery; circular economy; large scale production; techno-economic assessment

Acknowledgements
This research was supported by the Project MULTI-STR3AM which received funding from the Bio-based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No 887227. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio-based Industries Consortium.

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COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions, circular economy opportunities and Customer goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

VALORIZATION POTATO STARCH WASTEWATER BY SCREENING AND CULTIVATION OF VALUABLE MICROALGAE

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ABSTRACT

High-strength organic wastewater e.g., potato starch wastewater leads to high stress on the environment. This study aims to investigate the system for simultaneous potato starch wastewater treatment and nutrients upcycling for further biocompounds production through microalgae cultivation. Microalgae are capable to simultaneous removal of COD and recovery of N and P within the production of various valuable bio-compounds during wastewater treatment e.g. protein, lipid, pigments, etc [1]. The limited types of carbon sources that can be metabolized by the microalgae causes low removal efficiency of COD. Thus, high concentration of organic substances in the potato starch wastewater, including protein, starch, etc. which can not be directly consumed by microalgae, may significantly influence on the removal of COD and even inhibit the growth of microalgae [2, 3]. Therefore, having an optimal pretreatment of the potato starch wastewater before microalgae cultivation is necessary to conversion of the low bioavailable organic compounds. Hence, in the present study four different microalgae species were cultivated and screened in the effluent of the pre-treated potato starch wastewater by anaerobic acidification (AA). The microalgae cultivated in the effluent of AA process achieved higher growth compared to cultivation in synthetic media (control) in all cases. Among four microalgae (*Chlorella vulgaris, Chlorella sorkiniana, Scenedesmus obliquus, Haematococcus pluvialis*) cultivated in the effluent from AA at different dilution times (0, 10, 20, 30, 40) *Chlorella vulgaris* reached the highest optical density (≈1.4) in 10 times diluted effluent during 6 days. Furthermore, two stages system (AA and microalgae) contributed to a final removal efficiency of 63.4–95.0%, 87.44–98.99%, and 69.4–99.08% for COD, phosphorus, and ammonia, respectively, in a shorter period. This study presents a promising simultaneous efficient high strength organic wastewater treatment and biocompounds production by the microalgae.

Keywords
Anaerobic processes, Microalgae, High-strength organic wastewater, *Chlorella vulgaris*.

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Sonia currently is a Postdoc in the Biocon group under supervision of Prof. Irini Angelidaki at the Department of Chemical and Biochemical Engineering, Technical University of Denmark, DTU. She is working in BlueBioChain (EU project) with the aim of novel biorefinery supply chains for wastewater valorization and production of high market value bio products using microalgae.

LIPIDOMIC ANALYSES OF *PARACHLORELLA KESSLERI* DEPENDING ON PHOTOBIOREACTOR USED

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ABSTRACT

Microalgae are studied as interesting renewable sources of a huge diversity of molecules with applications in industrial fields such as energy, health and feed for example *(1)*. Among these molecules, lipids can be valorized as biofuel, bioactive molecules or in aquaculture*(2)*.
Parachlorella kessleri, a chlorophyta microalgae known as a lipid producer under nitrogen limitation *(3)* was cultivated in a photobioreactor of 10 L at laboratory scale until 69 days. Lipids were analyzed at different scale. Total lipid composition has been investigated using gravimetry, fatty acid composition was determined by GC-MS and lipidomic was realized using HPLC-MS. Total biomass obtained was 1,74 g at 69 days. Lipid class has been followed and triglycerides accumulation has begun at day 3 with a huge variety described by HPLC-MS. The main fatty acids are 16:0, 18:2 and 18:3. Glycolipids and phospholipids composition has also been studied.
The cultivation of *P. kessleri* in a raceway photobioreactor of 1 000 L is undergoing to evaluate the extrapolation of lipid composition when up-scaling.

Keywords
Parachlorella kessleri, lipids, total lipid, GC-MS, HPLC-MS

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Aurelie Mossion obtained her PHD in 2007 in Toulouse (France) dealing with the analysis of tea composition depending on the water used. Her skills in analytical chemistry allow her to obtain a post-doc in GEPEA at St-Nazaire to develop the analyses of lipids from microalgae. Then she has been recruited in the Mer Molecules Santé laboratory to continue in this field of research. She began to work on macroalgae, mollusks and, since few years, her field of interest is the lipids of benthic diatoms.

INNOVATIVE SOLVENT-BASED PHYCOCYANIN EXTRACTION FROM ARTHROSPIRA PLATENSIS (SPIRULINA)

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ABSTRACT

Arthrospira platensis is a cyanobacterium interesting for its high phycocyanin (C-PC; 10-20% w/w) and protein content (60-70% w/w) [1]. These components can be used as food/feed ingredients and additionally C-PC can be used for pharmaceutical applications due to its antioxidative, hepatoprotective and anti-carcinogenic properties [2]. Current methods for C-PC extraction from A. platensis involve cell membrane lysis to release pigments in an aqueous extraction medium. Conventional cell lysis techniques, such as freeze-thawing and bead milling require long processing time and high energy inputs and result in release of other cell components, jeopardizing the C-PC extract purity [3]. Natural Deep Eutectic Solvents (NADES) represent an emerging metabolite extraction technology which offers bio-safety, high extraction efficiency, improved stability and specificity towards the desired target compound.

The present work focused on C-PC extraction by emerging cell lysis (ultrasonication) and metabolite extraction with NaDES. Four NaDES were prepared by mixing the appropriate ratio of hydrogen bond acceptors (HBA), such as choline chloride, betaine, lactic acid and glycerol, with hydrogen bond donors (HBD), including malic acid and glucose. Extraction efficiency of the solvents was evaluated after 2 min of ultrasonic pretreatment of biomass. The highest extraction yield was reached by using glycerol:glucose-based solvent with 20% w/w water (2.85%). After selection of solvent combination, extraction efficiency was maximal at increasing the water ratio up to 45% w/w. The extraction yield was 13.3%, which is higher than values with pure water at p=0.05. Extract purity in C-PC by the tested NaDES, however, was higher when using pure water as the solvent. Extraction of proteins, alongside with cell lysis and fractionation of carbohydrates and lipids by Natural Switchable Hydrophilicity Solvents (NSHSs) was previously shown on Scenedesmus and Chlorella by Circular Extraction (CE) [4]. Future work should therefore investigate the possibility of obtaining proteins, specific fractions or molecules by NSHS in CE, preserving protein integrity and reducing the cost of recovery and procurement of the solvent.

Acknowledgements
This work was supported by the project PON “Ricerca e Innovazione” 2014-2020 – Action IV.5 “Dottorati su tematiche green”.

Keywords
Phycocyanin, spirulina, NaDES, SHS, circular extraction

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In January 2022 started the PhD studies at Department of Chemical Engineering Materials & Environment of Sapienza University of Rome. Is a member of a research group of Food and Biochemical Engineering Laboratory under supervision of prof. Marco Bravi.
Her research interests include downstream processing of raw materials in the most sustainable way, in full compliance with the principles of Green Chemistry and Green Engineering.
Special emphasis of her research is laid on employing switchable-hydrophilicity solvents for targeting bioactive compounds from the microalgal biomasses.

SCREENING EXTRACELLULAR LYTIC ENZYMATIC ACTIVITIES ON MICROALGAE-RECRUITED BACTERIA ISOLATES FOR DOWNSTREAM PROCESSING APPLICATIONS IN MICROALGAE BIOREFINERY

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ABSTRACT

The power of microalgae microbiome has been gaining increased attention by industrial sector to find sustainable innovative ways to improve microalgae culture growth speed, biomass and high value compound productivity. Additionally, microalgae biorefinery downstream processes have been explored and developed to not only increase microalgae biomass flocculation, enhance oil extraction efficiency and carbohydrate solubilization, but also to decrease the overall processes' expenses. [1–4] In a current study about the microbiome recruitment of two industrially relevant marine microalgae (i.e., *Phaeodactylum tricornutum* and *Nannochloropsis oceanica*) using natural waters from Sado and Tagus rivers in Portugal, 148 marine bacteria were isolated and identified. Besides their proposed potential to act as probiotic, these bacteria were characterized regarding their ability to produce extracellular lytic enzymes capable of catalyzing various compounds including lipids, proteins, and polysaccharides. When tested for extracellular lytic enzymatic activities, 84% and 90% of bacterial isolates from *N. oceanica* and *P. tricornutum,* presented at least one of the lytic enzymatic activities tested, respectively. The strength of the enzymatic activity was measured, and many bacterial isolates were had high levels of extracellular lytic enzymatic activities detected. Thus, suggesting them as good sources of powerful enzymes (e.g., chitinases, cellulases and amylases), useful for microalgae downstream processing. The genomes of bacteria holding high extracellular lytic enzymatic activities were fully sequenced and their genomes mined for identification of putative lytic enzymes and their overall pathways.

Figure 1 – Screening and measuring extracellular lytic enzymatic activities of isolates SADO10 and SADO49 (left and right halfs of the petry dish, respectively) in solid media using alginate(a), starch (b) and cellulose (c) as substrates. SADO10 isolate presented extracellular lytic enzymatic activities for all three substrates, while SADO49 presented no activity for the same substrates.

Keywords
Microbiome; Enzymes; Downstream processing; Genome data mining.

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Francisco Quintas Nunes has a master's degree in Applied Microbiology by the Faculty of Sciences of Lisbon University. He is a quality oriented, deeply curiosity driven, easily adaptable, with excellent attention to detail and critical thinking. Francisco has been involved in many research projects related to microalgae biotechnology and bacteria water bioremediation, along with his master's thesis in industrial enzyme characterization and evolution. He has worked for two years as a Quality Control laboratory technician at Sterifluids, a medical devices company. Recently he has won a full scholarship grant to start his PhD studies in a Molecular Biosciences program at Instituto de Tecnologia Química e Biológica (ITQB - NOVA University of Lisbon). His thesis work will be developed at Instituto de Biologia Experimental e Tecnológica (iBET) and at the Catholic University of Leuven, Belgium (KU Leuven). The thesis will be focused on the unravel and modulation of 1-aminocyclopropane-1-carboxylate and ethylene production pathways of microalgae to stimulate biomass and high value compound production.

COMPANY PROFILE

iBET (www.ibet.pt) is a private non-profit institution, a Biotechnology Research Organization acting as an interface between academic and private institutions while also creating and organizing autonomous knowledge and expertise. Target areas are biopharmaceuticals and novel therapies (ii) food and health with the development of new bioactive food supplement, new solutions for the detection of food frauds, food and water-born pathogens (iii) environment, with the development of new sustainable, energy saving, processes for both food and water industries.
iBET's infrastructure comprises 16 cutting edge laboratories, a GMP certified Analytical Services Unit and a Mass Spectrometry Unit, a Late Stage R&D and Bioproduction Unit (2000 m2) and access to the GMP manufacturers which, together, allows iBET to develop integrated solutions from R&D to phase I/II clinical trials.
As an R&D institution iBET has coordinated over 30 international projects and participated as work-package leader in more than 100 projects supported by the European Commission. iBET has several well-established collaborations in Networks of Excellence and long-term partnerships with companies from the Agro-food industry.

BIOCHEMICAL CO2 CONVERSION INTO VALUE-ADDED PRODUCTS USING MICROALGAE AND ACETOGENS: A TWO-STEP PROCESS

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ABSTRACT

Microalgal photoautotrophic cultivation present limits related to the high operative and investment costs required to maintain adequate light supply rate (reactors with high S/V ratio) to ensure satisfactory productivity [1]. Heterotrophy represents a promising solution: some microalgal strains can grow without light, metabolizing organic compounds as carbon and energy source [1,2]. Moreover, the integration of heterotrophic microalgal production with wastewater or effluents treatment can increase economic sustainability of the microalgal biomass production, eliminating the cost of carbon feedstock required in heterotrophy [3].
Through gas fermentation, some acetogens bacteria convert CO_2 and H_2 into acetic acid, using the Wood–Ljungdahl pathway [4]. However, acetic acid produced through this way presents a low economic value. Using acetate as substrate to produce compounds with higher economic value could be an efficient solution [5].

We propose here an innovative approach to overcome both the limitations associated with photoautotrophic growth of microalgae and the economic sustainability of microbial acetate production by gas fermentation. This approach is based on a two-stage fermentation process: the first step consists in the conversion of CO_2H_2-based feedstock into acetate through gas fermentation, using the acetogenic strain *Thermoanaerobacter kivui* in a stirred tank reactor (STR); the fermentation effluent, acetate rich, is used in the second fermentation step as cultivation medium for *Chlorella sorokiniana* grown in heterotrophy using acetate as organic source.

Chlorella growth was assessed starting from different acetate concentration - 1.1 g L^{-1}, 2.2 g L^{-1}, 3.3 g L^{-1} - in the medium, obtained by diluting and sterilizing (by microfiltration) the fermentation effluent of *T. kivui*. Biomass production and acetate removal were analyzed; the growth kinetics were modelled to determine the specific growth rate (μ_{MAX}), which was 0.075 h^{-1}. Good values of biomass productivity were obtained; no growth inhibition was observed until 3.3 g L^{-1} acetate concentration. As next goals, microalgal proteins accumulation and quality will be analyzed to prove their economic value for food and feed. Finally, *Chlorella* growth will be assayed without any medium sterilization procedure, with a perspective of increasing the energy and economic sustainability of the whole process, considering oxygen exposure as lethal to acetogens [6].

Keywords
Biochemical CO_2 conversion, Microalgal heterotrophy and effluents treatment, Acetogens, Two-step process, *Chlorella sorokiniana*].

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BIOGRAPHY

I'm Giacomo Proietti Tocca, I graduated in Biology at Roma Tor Vergata University. Then, I got a Master degree (summa cum laude) in industrial and Environmental Biotechnology at Sapienza, University of Rome. My research thesis concerned the development of bacterial contamination control strategy in microalgae cultures with wastewater treatment.
Currently, I'm a Ph.D student in Chemical Engineering at Polytechnic of Turin and Instituto Italiano di Tecnologia. Here, I'm researching for efficient strategies to valorize the CO_2-rich gaseous waste streams through biotechnological process. In particular, my work is based on CO_2 conversion into value-added products using acetogens bacteria and microalgae. My main interests see the fusion between microbiology, chemical engineering and biotechnology, using microorganisms as biocatalysts for the conversion of solid, liquid and gaseous waste into products which have an high economic value.

THE USE OF MEMBRANE ULTRAFILTRATION/DIAFILTRATION IN THE RECOVERY OF SOLUBLE PROTEIN OF *NANNOCHLOROPSIS SP.*

Ribeiro C.[a,b]

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ABSTRACT

The protein fraction produced from microalgae is now considered an alternative source of high-quality protein not only for animal feed [1] and human food applications [2] but also in some specific cases, for cosmetic and pharmaceutical applications [3]. The incorporation of microalgae protein in such application faces several challenges, mostly those associated with their organoleptic characteristics, such as odour, taste and colour [4]. To use these microalgae proteins in high-value applications it is important to find ways to reduce or eliminate the presence of chlorophyll. Several species of microalgae are known for their high protein content [5]. *Nannochloropsis* sp. is a small marine microalga [6], commonly known for the production of omega-3 fatty acids. Nevertheless, it has also an important content in total protein [7], both soluble and insoluble.

The research work developed within this context proposes a way to take full advantage of a *Nannochloropsis* sp. biorefinery, through membrane technology. The main focus of the work was to produce an extract enriched in soluble proteins, free from the insoluble compounds, and eliminate chlorophyll. The process proposed aims at minimizing membrane fouling, enabling the achievement of high permeate fluxes and reducing the frequency of membrane cleaning, contributing to an extended membrane lifetime. The supernatant obtained after centrifugation of ruptured *Nannochloropsis* sp. cells was processed by ultrafiltration using a membrane with a molecular weight cut-off of 100 kDa.

Two different operating approaches were evaluated: controlled transmembrane pressure and controlled permeate flux, under concentration and diafiltration modes. The characterization of the different protein factions was achieved not only by protein quantification through the BCA® method but also by the analysis of protein molecular weight distribution through SDS-PAGE. SDS-PAGE was used as a technique to monitor the performance of the system allowing to fine-tune the extracting conditions and assisting in the selection of the best membrane cut-off. Ultrafiltration operated in a diafiltration mode, and under controlled permeate flux conditions, led to the highest soluble protein recovery (78%) with the highest constant permeate flux (12 $L.m^{-2}.h^{-1}$) and the lowest membrane fouling.

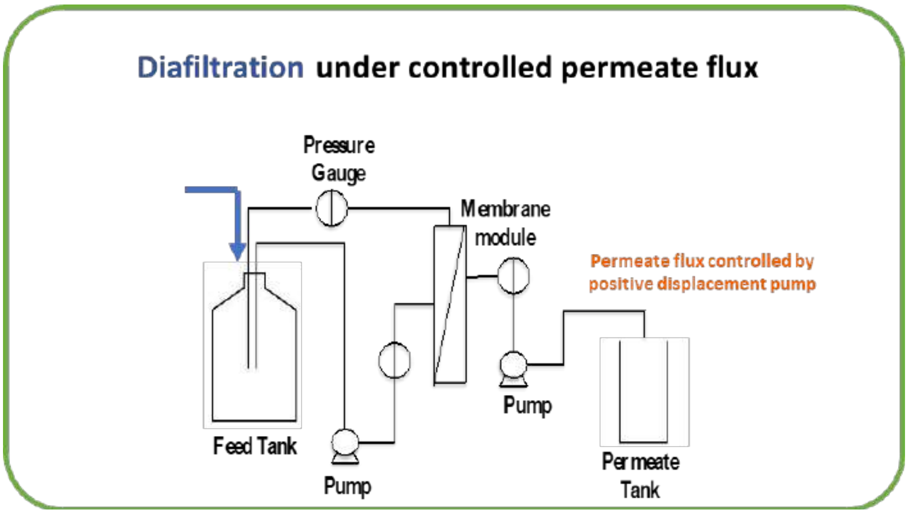

Figure 1. Representation of the diafiltration unit during the experiments performed under controlled permeate flux. Adapted from [8].

Keywords
Nannochloropsis sp.; protein recovery; ultrafiltration; controlled transmembrane pressure; controlled permeate flux

Acknowledgements
This research was supported by the Project Multistr3am which received funding from the Bio-based Industries Joint Undertaking (JU) under the European Union's Horizon 2020 research and innovation programme under grant agreement No 887227. The JU receives support from the European Union's Horizon 2020 research and innovation programme and the Bio-based Industries Consortium. The work received also funding from the project Algaref project (n. 45375), cofinanced by Programa Operacional Regional de Lisboa, Portugal 2020 and the European Union, through the European Regional Development Fund (ERDF).

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Chemical Engineer with a Master's degree in Chemical and Biochemical Engineering (NOVA University of Lisbon 2013). PhD candidate in Refining, Petrochemical and Chemical Engineering (NOVA University of Lisbon 2022) specialising in microalgae biorefineries, and with almost 5 years of accumulated experience in the fractionation of microalgae from laboratory scale to industrial scale.

COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions, circular economy opportunities and Customer goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

Transversal - LCA

ALGÆUROPE2022
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ENVIRONMENTAL EVALUATION OF A MICROALGAL-BASED FUNGICIDE

López-Herrada, E*.

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ABSTRACT

A life cycle analysis of the production process of a fungicide based on amphidinols has been carried out. Amphidinols are bioactives produced by the photosynthetic dinoflagellate microalga *Amphidinium carterae* (De La Crouee & Thomas, 2017). The production of 1 L of fungicide (concentrated product) was taken as functional unit. Two scenarios were evaluated: (A) biorefinery, considering two high value co-products (carotenoids and fatty acids); and (B) biofungicide as only product. Inventory data were scaled-up from previous work on pilot-scale reactors (Molina-Miras, 2018), as well as, lab-scale downstream equipments (López-Rodríguez, 2019, 2020, 2021). A yearly production of 1,940 grams of amphidinols, equivalent to 22,000 L of fungicide, was selected as the production objective. The required industrial equipments and the input and output flows were obtained with the SuperPro Designer software v7. Once the inventory was obtained, a dedicated LCA software was used (Air.e LCA v3.12.0.10 with the EcoInvent v.3.7.1 database). This software allowed calculating the carbon footprint, as well as other key environmental indicators, such as Ecotoxicity and Eutrophication of seawater and freshwater or Human Toxicity. A first conclusion is that incorporating a biorefinery approach, the environmental impacts per functional unit are much lower since they are distributed among several co-products. For instance, in the scenario A, 56.7 t of $CO2_e$ would be produced in 15 years linked to 330,000 L of fungicide, while for the scenario B, the $CO2_e$ produced would be 330.92 t. Despite photosynthetic biomass is a sink of anthropogenic CO_2, harvesting and downprocessing have large carbon footprints that exceed the biomass fixed carbon. In addition, different commercial agricultural fungicides were compared under the same LCA methodology. The results obtained showed a lower impact of the microalgal product in most of the indicators, including carbon footprint. Due to their composition, most of the commercial fungicides present, important toxicity impacts (on marine and human health) compared to the microalgal fungicide. In conclusion, our study allowed demonstrating that from an environmental perspective, microalgal bioprocess can be more attractive than the current chemical processes in use, with benefits that go beyond the reduction of the greenhouse gases.

Acknowledgements

This work was funded by the State Research Agency (grant PID2019-109476RB-C22) of the Spanish Ministry of Science, Innovation and Universities and the General Secretariat of Universities, Research and Technology of the Andalusian Government (grant: P18-RT-2477).

Keywords:

LCA; microalgae; bioprocess; antifungal

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BIOGRAPHY

After completing a bachelor's degree in Industrial Chemical Engineering and a Master's degree in Chemical Engineering, my research career begins in the bioprocessing of microalgae. Specifically, I have worked on the design, characterization, optimization and scaling of photosynthetic microorganism culture systems, as well as their environmental impact through the implementation of LCA's. My main research interest is the reduction of the environmental impacts produced in the processing of microalgae. In addition, I have experience in other research fields such as reduction of CO2 emissions and development and optimization of fertilizer analysis. Since 2022 I have been developing my research as a Predoctoral Researcher in the Department of Chemical Engineering of the University of Almería.

SUSTAINABLE SOLUTIONS FOR THE INDUSTRIAL PRODUCTION OF MICROALGAE BIOMASS

Rodrigues, A.[1]

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ABSTRACT

Microalgae are considered a sustainable source of proteins, lipids, carbohydrates, and other functional biomolecules. Nevertheless, microalgae cultivation and drying costs are still high, mainly due to the considerable energy consumption[1]. Therefore, as an effort to reduce biomass costs and improve sustainability, Necton implemented two innovations at industrial scale in the scope of H2020 ProFuture project.

The first was an indirect hybrid solar dryer, an equipment never used before to dry single cell microalgae at industrial scale. This drying method was compared to the standard freeze drying via assessment of biomass quality parameters, including biochemical profiles, functional properties, and microbial safety. No significant differences were found between the applied drying technologies for total proteins, carbohydrates, lipids, and fatty acid profiles. On the other hand, some pigments showed significant differences, displaying up to 44.5% lower contents in solar-dried samples. Finally, analyses of microbial safety and functional properties of the solar-dried biomass showed suitability for food and feed products.

The second innovation was an off-the-grid tubular photobioreactor (PBR). The 27 m^3 flow-through glass PBR, is able to work only with photovoltaics energy, thus greatly reducing operation costs. The pumps work at the maximum speed when enough sun incidence is available, but slow down when the photovoltaic production reduces, and turn off before sunset. This behaviour fits the mixing needs of the microalgae cultures, because it is during high sun incidence that the cultures need to have an efficient gas-exchange (CO_2 injection and O_2 removal), while during the night the mixing needs are expected to be much lower. To access the quality and behaviour of microalgae cultures in this PBR, one cultivation cycle of *Nannochloropsis oceanica* was followed. The cultivation regime was swapped every week between 100% off-the-grid to a regime with minimum 5 minutes of pumping per hour, to infer any possible detrimental effect of the off-the-grid regime. Growth, fluorimetry and microbiological analysis showed no detrimental effect of the off-the-grid regime. Nevertheless, the culture only lasted for 6 weeks, which can be considered short for *N. oceanica*, therefore, further studies are needed to access the quality of the cultures under an off-the-grid regime.

Keywords
Microalgae, sustainability, industrial production, off-the-grid, solar dryer

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BIOGRAPHY

Alexandre Rodrigues holds a licentiate degree in Microbiology and Genetics from the Faculty of Sciences of Lisbon University and a PhD degree awarded by the Faculty of Medicine of Lisbon University. His PhD studies about zebrafish and xenopus regeneration were developed at the Centre of Regenerative Medicine in Barcelona (CMRB). After his PhD, Alexandre worked as a research technician in histology and genetics laboratories in Barcelona, after which he moved back to Portugal to work as a post-doc at the University of Algarve.
In 2017, Alexandre started collaborating with Necton as a sales consultant. Given his scientific background, in 2019 he moved to the Research & Development Department as a Project Manager, contributing to projects management, project design and student coordination. In 2022 he was officially nominated Coordinator of the growing R&D Department at Necton.

COMPANY PROFILE

Established in 1997, Necton is the oldest company in Europe that specialises in the cultivation and commercialisation of microalgae. The company was set on an ideal location to grow these microorganisms in the natural park of Ria Formosa on the sunny south coast of Portugal. Throughout the years, the company acquired extensive knowledge in cultivating marine, freshwater, and hypersaline species. Necton's current portfolio includes over 30 species, such as *Nannochloropsis oceanica*, *Tisochrysis lutea*, *Phaeodactylum tricornutum*, *Tetraselmis chui*, *Porphyridium cruentum*, and *Skeletonema costatum*. To produce the microalgae biomass the facility relies on more than 100 m^3 of horizontal tubular photobioreactors and a 200-m3 raceway pond.
Necton's growth is supported by a continuous R&D effort that includes participating and managing several national and European-funded research projects. Through this effort, Necton was able to achieve scientific, technological and market knowledge of microalgae cultivation and applications for the aquaculture and cosmetic sectors, among others. The company's history, experience and constant collaboration with universities, research organisations and other companies have made Necton one of the leading European companies in microalgae biotechnology.

Transversal - Business

ALGÆUROPE2022
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ALGICEL, CARBON CAPTURE AS-A-SERVICE

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Jonathan Burns-Tang (JBT)

Denmark, Algiecel

ABSTRACT

ALGIECEL was founded to take part in solving the challenge of converting the CO2 emissions of today´s production methods into feasible business opportunities. The Company uses natural micro-algae organisms, a highly compact and high-yield photobioreactor technology fitted into standard shipping containers and a new revenue sharing business model to offer carbon capture as-a-service to industrial clients.

The Impact and Vision
ALGIECEL positions itself as the provider of a technology easy to scale. ALGIECEL provides a business model that transforms the cost-challenge of CO2 removal and emissions penalties, into a profitable business opportunity across the entire value chain through revenue sharing from derivative product sales. The company's vision is to make it easy for clients to remove millions of tons of CO2 annually from industrial production without hurting the clients' bottom line.

The Customer
ALGIECEL´s photobioreactors can go where large Power-to-X projects cannot go. Specific target industries are biogas and fermentation companies. Here, small numbers of mobile container units can solve the CO2 emission problems of entire production sites - plug and play – while requiring little space by stacking and packing the containers on demand.

The Organization
ALGIECEL was founded by Henrik Busch-Larsen, who also co-founded and scaled Unibio, a leading sustainable protein company. A team of highly skilled people has been established. The team holds experience from notable companies such as Maersk, Falck, Nestlé, COWI, Chr. Hansen and BioPorto among others. The company collaborates with skilled partners such as RobLight, AN Group and the Danish Technological Institute (TI).

Keywords
Carbon-capture and utilization (CCU), microalgae production, innovation, feed, food, food supplements, circular economy

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BIOGRAPHY

Jonathan Burns-Tang has a master's in marine biology (cand.scient) from Copenhagen University. He specializes in industrial algae biology working with cultivation, taxonomy, and ballast water treatment of algae. He has formerly worked in DHI Denmark as a taxonomist in the ballast water treatment laboratory and as a project manager working as head of the algae cultivation department at the facility. Jonathan finished his masters in the summer of 2021 and has since worked in Algiecel. Here Jonathan has worked with the design of the reactor, regulations of algae products and lately as one of the laboratory scientists setting up the inoculum production for the reactor.

CEO Henrik Busch-Larsen is a successful serial entrepreneur with several board positions. He recently left his CEO position at the Unibio Group which he co-founded and accompanied for the last decade. He took Unibio from a small start-up with 3 employees to a 50 FTE company with a proven, full-scale technology and a USD 300 million valuation.

COMPANY PROFILE

Algiecel has been founded to take part in the challenge of transforming today´s CO2 emissions into feasible business opportunities.
ALGIECEL is transforming CO2 emissions and LED light into microalgae biomass and Oxygen. The company has developed a plug & play photobioreactor (PBR) that can be stacked and packed as it fits into standard shipping containers. The reactor is easily installable and requires little technical know-how from small and mid-size industrial clients.
The company has designed a novel **C**arbon **C**apture and **U**sage business model that transforms the cost challenge of CO2 removal into a profitable business opportunity across the entire value chain through revenue sharing from the microalgae biomass and carbon credit sales.

VALSAR – VALORISATION OF “SARGAÇO”, STRANDED ASHORE SEAWEEDS

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ABSTRACT

“Sargaço” is a designation from the northern Portuguese coast assigned to a mixture of seaweeds that grows on rocky platforms and is loosen and run aground on the beach due to tidal movement. It mainly includes brown algae, but also red and green algae.

Traditionally, sargaço was collected by local farmers and fishermen, dried and applied in agriculture, or sold fresh for industry. Today, the traditional collection of sargaço is practically extinct.

However, these macroalgae continue to run aground on the beach. This accumulation on shore brings several constraints not only for vacationers, due to the algal accumulation in the sand and sea and bad odour from algal decomposition, but also ecologically, making the habitat of many species practically anoxic.

On the other hand, this natural biomass, rich in organic matter, minerals and various bioactive compounds, can be a valuable resource for Man. Nevertheless, it is currently being wasted and underexploited.

It was in this sense that VALSAR – Valorisation of Sargaço on the Portuguese north coast emerged. This project aims to investigate and explore the potential of these stranded macroalgae and enhance this natural biomass, contributing to sustainable development and diversifying the local economy of coastal communities.

To this end, a multidisciplinary team is currently working on:

• Production of a biostimulant based on marine algae.

• Production of a biofertilizer based on a mixture of marine algae and organic waste;

• Identification of pharmaceutical and cosmetic properties of seaweed-based extracts, using cell lines that represent the dermis and epidermis.

Experimental trials are being conducted and interesting results have already been obtained. We look forward to sharing more information about our recent discoveries and the value of this underexploited biomass.

“ValSar: Valorization of Sargasso da Costa Litoral Norte” (MAR-04.03.01-FEAMP-0502) is financed by MAR2020, PT2020 through European funds (FEAMP) and supported by the LAG Coastal Litoral Norte.

Keywords

Macroalgae; Blue economy; Biostimulant; Biofertilizer; Sustainable Agriculture

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BIOGRAPHY

Master in Environmental Engineering from the University of Coimbra.
Her work has, since then, been focused on environmental assessment with a large component of spatial analysis (Geographic Information Systems). She has strongly contributed to the current available spatial registry of aquaculture and saltpan establishments and to the current application of the Salt of Figueira da Foz to the UNESCO World Intangible Heritage, under the project Integrated, Ecological and Sociocultural Evaluation of the Salgado da Figueira da Foz, funded by the Municipality of Figueira da Foz. As a researcher at the SUSpENsE project, she supported the development of strategic guidelines for Ecotourism based on an Ecosystem Services approach.
Currently is PhD Student Fellow- Living Labs for Sustainable Development: Unveiling the value of the Ecosystem Services concept. The main aim of project is to explore the value of the Ecosystem Services (ES) concept for creating Living Labs for sustainable development, using coastal saltwork-related systems as case study. It will be determined the baseline conditions for stakeholders to join and actively participate in an Ecosystem Services Living Labs by confronting stakeholders, in a participative approach. Her scientific path has been enhanced by her proven experience in science communication to a wide variety of audiences. This is the main task carried out in the VALSAR project: the communication of science, whether for peers, the school community and the local community.

Most Originals

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DYNAMIC CULTIVATION PLANNING OF MICROALGAE USING A PARAMETRIC LOGISTIC EQUATION WITH KALMAN FILTER

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ABSTRACT

Microalgae are promising producers of bioactive chemicals and feeds from carbon dioxide by photosynthesis. Planning of cultivation conditions for harvesting target biomass at target time is important in practical cultivation of microalgae. However, the cultivation planning is often a challenging task due to the complex interplay of growth conditions such as light flux, medium concentration, and temperature [1]. To overcome this challenge, the authors have recently proposed a parametric logistic equation capable of predicting the dynamic effect of light flux into culture and medium concentration on the growth profiles of microalgae [2]. The predictive ability of the proposed model for various light flux and medium concentration was demonstrated using the experimentally measured growth profiles of *Monoraphidium* sp., a microalgal strain isolated by the authors. Finally, model-based exploration of cultivation conditions was performed for the planning of cultivation cycles.

In this presentation, we first show our recent results on the parametric logistic equation and its experimental verification [2] (see Figure 1 (a) and (b)). Then, an extension of the proposed model is discussed to enable dynamic cultivation planning that changes the cultivation conditions in a dynamic manner based on the real-time experimental data. For this purpose, we introduce Kalman filter [3] that estimates uncertain parameters in the model based on the real-time experimental data. The parametric logistic equation combined with the Kalman filter enabled real-time compensation for the prediction error by updating the misidentified uncertain parameters, resulting in highly accurate prediction of harvesting time with the target biomass (see Figure 1 (b) and (c)).

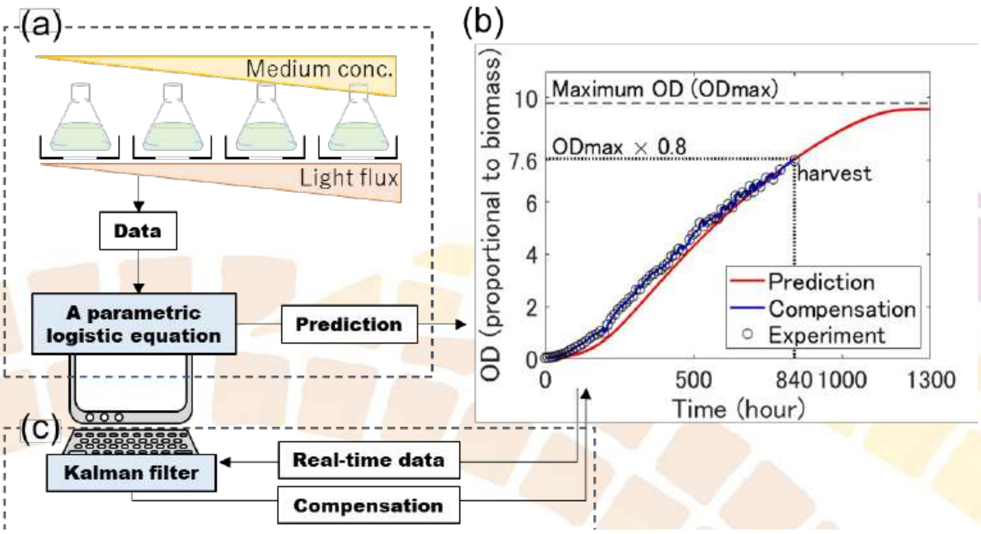

Figure 1 (a) Cultivation planning by parametric logistic equation. (b) Time series data of biomass. Circle shows experimental data. Red and blue solid lines show predicted and compensated results, respectively. (c) Compensation for the prediction error with Kalman filter.

Keywords
Microalgae, Kalman filter, Compensation, Logistic equation, Cultivation planning

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BIOGRAPHY

Kazuki Kambe received the B.S. degree in engineering from Keio University in 2021. In 2021, he joined Keio University, where he is currently a M.S. student. His research interests lie in mathematical model for growth prediction of microalgae and its applications to experimental operations. With one original paper in his curriculum, this is the first participation in a microalgae international meeting.

COMPANY PROFILE

Keio University founded in 1858 by Yukichi Fukuzawa has a history as Japan's very first private institution of higher learning. As of May 1, 2022, there are 33437 students of which 4796 enrolled in graduate schools in 10 undergraduate faculties and 14 graduate schools. Keio University leverages its strength as a comprehensive university to enhance cross-disciplinary activities for contributing to society through education, research, and medical care.

CHARACTERISATION AND DEVELOPMENT OF VARICON AQUA'S CELL-HI ALL-IN ONE NUTRIENT PRODUCT LINE

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ABSTRACT

The Cell-Hi All-In-One product range is the only complete, free flowing, dry and fully soluble algae nutrient blend available on the market. The products incorporate a balance of all essential, macro and micro nutrients coupled with vitamins. Each of the Cell-Hi products is based on industry standard formulations from literature and culture collections. The product is widely used in academic settings, as well as aquaculture and production facilities worldwide. There are over 24 peer reviewed academic journal articles in which the Cell-Hi product range is referenced [1]. We estimate between 2-5% [2] of OPEX costs associated with microalgal production at typical hatcheries as being attributable to the costs associated with making media for algae cultivation. To prove the efficacy of the Cell-Hi range we benchmarked the biological and economic performance of the product line against commercially available alternatives. Experiments were undertaken in a Varicon Aqua (CTC) controlled temperature chamber using 250 ml conical flasks aerated with 1% CO_2, and surface illumination of 150 μmol m^{-2} s^{-1} achieved with red/blue/white (400-760 nm) LEDs. The temperature conditions were fixed at specific temperatures depending on the trial strain 25 °C ± 1 °C for *Nannochloropsis spp*. and 20 °C ± 1 °C, for *Tetraselmis suecica*. Our results showed that *Nannochloropsis spp*. grown on Cell-Hi F2P had identical performance to laboratory made or commercially supplied nutrient (no significant statistical difference, average productivity of 0.15 g L^{-1} d^{-1} and final yield of 1.1 g L^{-1} over a 7-day batch). Similarly, no significant differences were observed in growth rates between T. suecica grown on Cell-Hi-WP and laboratory made equivalents (average productivity 0.25 g L^{-1} d^{-1} and I final yield of 1.7 g L^{-1} over a 7-day batch). Furthermore, techno-economic evaluation demonstrated 60% cost savings per kg of nutrient used within a hatchery when substituting conventional media protocols with Cell-Hi products. We have shown that our Cell-Hi range is comparable in biological performance, and superior in economic performance to many conventional alternatives (both pre-formulated and formulated from analytical grade chemicals). These findings provide a compelling argument for making the switch to Cell-Hi All-In-One within commercial hatchery operations.

Keywords
Cell-Hi nutrient range, all-in one, Varicon Aqua, algae fertiliser, fully soluble

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BIOGRAPHY

Marco Lizzul has an interdisciplinary background grounded within the life sciences having undertaken a BSc in Biochemistry at the University of York. This was followed by an MRes in Molecular Plant Biology and Biotechnology at Imperial College London. He completed an EngD at University College London, where his research project 'Integrated Production of Algal Biomass' centred on engineering approaches to the investigation and development of lower cost algal production strategies, including usage of waste streams as feedstock. During his doctorate he designed, constructed and commercialised an airlift photobioreactor system (the Phyco-Lift) with Varicon Aqua. Marco is a an active research scientist and has published within fields allied to algal biotechnology and biology; including biofuels, waste treatment & bioremediation, biochemical engineering, bioprocessing and process modelling. His research and development work has been supported by both the EPSRC and Innovate UK, and has previously been recognised for its potential importance to future UK economic activity with an Industrial Fellowship from the Royal Commission for the Exhibition of 1851. In his current role Marco is involved in the management of Varicon Aqua's R&D programme, innovation pipeline and provision of professional services, as well as certain aspects of communication and technical sales.

COMPANY PROFILE

Varicon Aqua is the manufacturer of the Phyco-™ range of photobioreactors and the Cell-Hi line of algal nutrients. To date we have deployed over 350 photobioreactor systems across the globe, ranging in scale from 5 L to 400,000 L. We are based in the UK and our team has more than 30 years' experience designing, constructing and deploying algal photobioreactors, high-rate algal ponds and aquaculture production systems worldwide. All Varicon Aqua products are built to the highest specifications using high throughput precision manufacturing processes. We combine this manufacturing experience with strong supply chain relationships, giving a systems wide understanding of both the equipment and production processes. This unique experience gives us a competitive advantage during client engagement and project specification, allowing us to provide bespoke photobioreactor and cultivation systems for a wide range of applications. We pride ourselves on our ability to support the client from conception through to commissioning and exploitation, an ability founded on a clear understanding of the interplay between the needs of the organism and our engineered solutions.

CYANOBACTERIA IN HOT PURSUIT: FULL GENOME ANALYSIS OF INDIGENOUS *EUHALOTHECE SP.* AND EVALUATION OF HIGH VALUE PIGMENTS (MYCOSPORINE-LIKE AMINO ACIDS AND PHYCOCYANIN)

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ABSTRACT

Cyanobacteria are oxygenic photoautotrophs that grow in diverse habitats and able to adapt to a wide range of environemnetal conditions. They synthesize a vast array of biologically active compounds and metabolites that have potential biotechnological applications. An indigenous halophilic cyanobacterium, representing a novel species of Genus *Cyanothece*, Cluster *Euhalothece* was isolated from a hypersaline environment in South Africa. The strain was found to have several interesting traits such as its ability to grow at a high salinity (120 g/L), survive in varying nutrient and light conditions and naturally produce relatively high quantities of pigments such as mycosporine-like amino acids (MAAs) and C-Phycocyanin (C-PC). C-Phycocyanin is a blue pigment applied in cosmetics and food as a natural dye, antioxidant and anti-ageing agent. Additionally, C-PC has application as a fluorophore in diagnostics therapeutics. Mycosporine-like amino acids are multifunctional molecules acting as antioxidants, photo-protective agents and natural UV sunscreen products. Thus, these natural pigments from marine cyanobacteria have great biotechnological applications and commercial value. Initially a comprehensive polyphasic approach *viz*., cell morphology, pigment composition and complete genome sequence analysis was conducted to identify and elucidate the taxonomic position of the strain. Full genome sequencing has further allowed us to understand the key genes and proteins responsible for its interesting properties including its adaptation to environmental conditions (stress acclimation) and pigment synthesis. High salinity concentrations significantly enhanced the production of MAAs as well as C-PC in *Euhalothece* sp. *In silico* analysis of the physicochemical properties of the C-PC amino acids were correlated to the thermostability and antioxidant activity of the purified protein. C-PC was found to be relatively thermostable (up to 45 °C) at a pH range from 5.0–8.0 with high antioxidant activity due to high content of non-polar, hydrophobic and aromatic amino acids. Bioinformatics analysis of MAAs data revealed a distinctive mys gene cluster that contained six genes (mysA to mysE), compared to four genes commonly found in MAAs-producing cyanobacteria. This research contributed to our understanding of some of the molecular, cellular and biochemical mechanisms of this cyanobacteria as well as identification of valuable metabolites.

Keywords
Cyanobacteria, hypersaline, genome analysis, mycosporine-like amino acids, c-phycocyanin

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BIOGRAPHY

Trisha Mogany is a researcher at Institute for Water and Wastewater Technology. She holds a PhD in Biotechnology from Durban University of Technology, South Africa. Her research focus over the past 10 years has been the algal biotechnology with a focus on the application of microalgae/ cyanobacteria for cosmoaceuticals, feed and food, bioremediation and aquaculture. She has experience in genomic analysis, *in silico* protein analysis and eludicating the metabolic pathways. Her expertise includes project design and management, data analysis and interpretation. Trisha is actively involved in a number of research projects funded by JICA-Science and Technology Research Partnership for Sustainable Development, Technology Innovation Agency and Water Research Commission. She has published six first-author articles and contributed to six peer reviewed conference proceedings. Being an emergering reseacher she focuses on mentoring and inspiring students. Trisha has an extensive supervision record of BTech/honors and mentored Inservice trainees/Interns and currently serves as co-supervisor for two master's students, one PhD. She is a recognized reviewer for several Elsevier journals and has acted as reviewer for a national and funding agencies. She is also engaged in several active collaborations with national and international institutions including Agricultural Research Council (ARC); Shiraz University of Medical Sciences, (Iran); Tokyo University of Agriculture and Technology, Nagoya University, Aichi Shukutoku University (Japan); Alexandria University (Egypt).

COMPANY PROFILE

The Institute for Water and Wastewater Technology is based at the Durban University of Technology. The IWWT has over the last 25 years developed into a "Centre of Excellence". Core research areas at the Institute are wastewater treatment technology, algal biotechnology and environmental health. The Institute hosts postgraduate students in a wide spectrum of disciplines in Science and Engineering. The Institute hosts one of two National Research Foundation South African Research Chair Initiative (SARChI), chairs in wastewater treatment in S.A. The strength of the Institute lies in its commitment to development of essential skills through student training. The Institute comprises of well-equipped laboratories with state of art high-end equipment. We conduct short courses and training on a routine basis for staff from municipalities, water utilities and industry to enhance their skills and keep abreast on the latest technology developments in the water and energy sector. Our approach is multi-disciplinary, combining the skills of biotechnology, chemistry, environmental health and various engineering disciplines.

BIOLOGICAL CONTAMINANTS DETECTION FROM NON-ASEPTIC MICROALGAE CULTURES OF *TISOCHRYSIS LUTEA* AND *PHAEODACTYLUM TRICORNUTUM*

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ABSTRACT

Biotic contaminations are a major economic hurdle in industrial-scale cultivation of photoautotrophic microalgae. In fact, different contaminants (e.g., amoebae, ciliates and fungi) are known to collapse microalgae cultures within a short time period (24-78 hours) if not detected and controlled on time. In this context, the present work focused on applying Next-Generation Sequencing (NGS) technologies to study contaminated cultures of *Tysochrysis lutea* and *Phaeodactylum tricornutum.* The aim was to detect and identify the microorganisms causing biomass loss and culture collapse. At this stage, an optimized PCR protocol was established with different primer pairs, some already published and others developed *in-house* to detect the presence of contaminants in the early stages – when microscopic detection can be misleading - during production scale-up. Molecular and phylogenetic analyses were performed in parallel with persistent microscopic observation of contaminated cultures to clearly identify harmful contaminants and evaluate their life cycle under controlled conditions. Data obtained so far strongly suggest that the Chrysophyte *Paraphysomonas* sp. and a Heterolobosea protist were the responsible agents for the collapse of *T. lutea* and *P. tricornutum* cultures, respectively, due to grazing. Upon their identification, Fluorescence Activated Cell Sorting (FACS) and micromanipulation enabled the isolation of both grazers. Several mitigation strategies are currently being tested (physical, chemical and environmental procedures) to find the most efficient and cost-effective solution to control or eliminate these grazers at lab scale without losing microalgae biomass or applying stress to the cultures. In conclusion, further research on these controlled ecosystems and microorganisms at industrial scale is necessary to avoid economic losses of microalgae ventures. Improvements in early detection/mitigation strategies are crucial to minimize biological contamination and enhance biomass productivity, ensuring the future sustainability of the algae production sector.

Keywords
Biological contaminants, microalgae cultures, grazers, NGS sequencing, Paraphysomonas, Heterolobosea

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BIOGRAPHY

Cristina Paulino holds a BSc and a MSc degree in Marine Biology with specialization in marine ecology and conservation. Currently lab manager at GreenCoLab with more than 12 years of experience and a wide range of knowledge in diverse fields: molecular biology, ecology, conservation, environmental DNA (eDNA) and biological contaminants detection in algae cultures. At the time of september 2022 with 12 publications in international scientific journals, participation in several conferences and collaborations in more than 10 national and international projects. A growing curiosity and scientific interest of exploring interdisciplinary areas, entangle the needs of contaminant microorganism's studies to solve problems at industrial scale production facilities, protocols development, ecosystems functioning, knowledge and network in multiple fields related to marine algae are some of the several qualifications acquired in the academic and professional career.

COMPANY PROFILE

GreenCoLab – Associação Oceano Verde is a non-profit private organization and a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of micro- and macroalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries. GreenCoLab was granted the title of «Collaborative Laboratory» by the Portuguese Science and Technology Foundation. It is therefore formally recognized as an R&D institution and is part of the national science and technology system, in accordance with the Portuguese Science Law.

GreenCoLab is composed of one research centre (Centre of Marine Sciences – CCMAR), one state laboratory (National Laboratory of Energy and Geology – LNEG), one university (University of Aveiro) and four companies, namely Allmicroalgae, Necton, Algaplus and Sparos, with the common goal of advancing the R&D on algae biotechnology. The GreenCoLab's multidisciplinary team covers expertise across the whole algae value chain; from the lab to large scale production, strain optimisation, scale-up, harvesting, biorefining to commercialization.

SCALING-UP C. MEROLAE CULTURES UNDER EXTREME ENVIRONMENTAL CONDITIONS ON THE MID RED SEA COAST OF SAUDI ARABIA

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ABSTRACT

The unicellular red alga *Cyanidioschyzon merolae* is a poly-extremophilic microalga that thrives in low pH and high temperatures. Its biomass contains interesting amounts of thermostable phycocyanin (PC) in addition to starches and beta-carotene and zeaxanthin carotenoid pigments. *C. merolae* strain 10D (*Cm*10D) was used for this work and kept in lab conditions (42 °C, 115 $\mu E \cdot m^{-2} \cdot s^{-1}$, pH 2.5). At the Algae Pilot Plant (KAUST, Thuwal, Saudi Arabia), inoculum was used to scale *Cm*10D in 8.0 L glass columns at outdoor conditions (39±4.0°C, average irradiation 1,503 $\mu E \cdot m^{-2} s^{-1}$, pH 2.5) for two weeks, where analytical-grade salts of macro nutrients of MA2 media [$(NH_4)_2SO_4$, KH_2PO_4 and $MgSO_4$] were replaced by industrial/agricultural grade salts. The inoculum was then transferred to a 60 L methacrylate column reactor (Varicon Aqua, UK) with constant CO_2 sparging at a rate of 0.2 $L \cdot min^{-1}$ at outdoors conditions (32.4±2.0°C, 1,351 $\mu E \cdot m^{-2} \cdot s^{-1}$, pH 2.5). The adapted culture was used as inoculum for a 1,000 L tubular photobioreactor (PBR, Varicon Aqua). Biomass from the first PBR was used as inoculum for two other 1000 L PBRs. Three different sources of CO_2 were tested across the three PBRs: 1) atmospheric CO_2 levels from pressurized air 2) "green" CO_2 from flue gas (Gulf Cryo®) and 3) commercial CO_2 (Alpha Gaz®). In all treatments gassing was supplied constantly (0.2 $L \cdot min^{-1}$) from 8 am to 5 pm. Average atmospheric temperature 42±2.0°C; culture temperatures 39±1.56 °C; and irradiance of 1,265 $\mu E \cdot m^{-2} \cdot s^{-1}$ were recorded during the experiment. Biomass reached up to 0.92, 1.26 and 1.45 $g \cdot L^{-1}$, after 7 days in these reactors, respectively. The low pH tolerance of *C. merolae* allows cultivation with continuous high injection rates of CO_2 at high temperatures. Promising biomass production and CO_2 injection results allow us to propose this species as highly adaptative to summertime dessert conditions as an excellent candidate for CO_2 uptake while producing valuable biomass which contains biomolecules such as starch and thermostable PC.

Keywords
CO_2, extremophile, high temperature, phycocyanin.

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BIOGRAPHY

Biotechnological Engineer by profession, Scholarship by the National Council of Science and Technology to obtain a PhD. in Industrial Biotechnology at the University of Almeria. 10 years of experience in the cultivation of microalgae outdoors. I have been participated in microalgae-based bioprocess development projects on a pilot and demonstration scale. With a great desire to expand the applications of microalgae on a commercial scale and expand the field of its applications. I am currently working on the project: “Algae Biotechnology Development in the Kingdom of Saudi Arabia”.

COMPANY PROFILE

King Abdullah University of Science and Technology (KAUST) has stablished the project *“Development of Algal Biotechnology in the Kingdom of Saudi Arabia”*, which has been funded by the Ministry of Environment Water and Agriculture (MEWA, project number: 52000003916), with the main objective of assess the potential of algae biomass production for animal feed using different side-stream effluents from industries. The project is under the direction of Dr. Claudio Fuentes-Grünewald.

CHARACTERIZATION OF THE MICROBIOME ASSOCIATED WITH *TISOCHRYSIS LUTEA* INDUSTRIAL PRODUCTION

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ABSTRACT

Microalgae-associated microbiomes have recently gained scientific interest since bacteria associated with microalgal cultures are known to exert a significant effect on their growth and biochemical composition. The increasing interest and market demand for microalgal biomass for different biotechnological applications (e.g., human and animal nutrition and nutraceuticals) led to the intensification of industrial-scale production. In this context, the main goal of the present work is to characterize and identify the microbiome associated with the industrially produced photoautotrophic microalga *Tisochrysis lutea* and evaluate its influence on the growth performance and nutritional profile of the cultures. Throughout the project, biomass production of *T. lutea* was conducted in 15- and 19-m^3 tubular photobioreactors at Necton S.A. facilities (Olhão, Portugal) during the summer/autumn seasons. Cultures were followed throughout six complete production cycles from inoculation until harvesting. The bacterial composition was evaluated through 16S rRNA gene amplicon sequencing. Determination of relative bacterial abundance and alpha and beta diversity were performed using the QIIME2 (Quantitative Insights into Microbial Ecology) pipeline. Parameters regarding production days, growth indexes, biochemical biomass profile (ash, protein, mineral, and fatty acids profile), abiotic factors, culture viability, and microbiome composition were assessed to find significant correlations. Across all the samples, the estimation of relative abundance revealed that the most dominant bacterial population belonged to the order Chitinophagales (24%), followed by Rhodobacterales (13%), Enterobacterales (12%), and Pseudomonadales (11%). When comparing best-performing algal cultures against those in collapse, the alpha diversity metrics showed no differences between culture viability parameters in abundance. However, there were significant differences in bacterial dominance and sample richness (Simpson diversity). These observations were confirmed by significant differences among communities in terms of beta diversity as determined by permutational multivariate analysis of variance (PERMANOVA) for unweighted UniFrac (q-values: 0.04) and weighted UniFrac Distances (q-values: 0.005). Collected information regarding the degree of differentiation between samples is key for the future establishment of a minimal tailored microbiome to be co-inoculated with the microalga to mediate and enhance the growth performance and quality of the produced biomass.

Keywords
Microalgae; Holobiont; Microbiome; Bacteria; Symbiosis; Biotechnology.

Funding
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BIOGRAPHY

About the author: Tamára Santos graduated in Marine Biology at the University of Algarve, where she obtained her MSc degree in Aquaculture. Since 2017, she has been involved in the bioprospection of novel microalgae strains as a researcher at CCMAR/University of Algarve - MarBiotech Research group. She was awarded a Ph.D. fellowship by the Foundation for Science & Technology (FCT). In her Ph.D. project, Tamára is researching innovative ways to enhance industrial microalgal biomass production using metagenomics. She has co-authored 23 papers in international peer-reviewed journals and presented ten poster presentations at international meetings.

COMPANY PROFILE

The Centre for Marine Sciences (CCMAR) is a multidisciplinary, non-profit research organization promoting research and education on the marine environment. Emphasis is placed on biological interactions and the sustainable use of resources. CCMAR publishes 150-200 articles annually and, in the last five years, had 15 patents approved and established two spin-off companies.
Necton is the oldest company in Europe, producing and selling microalgae. Serving the sectors of aquaculture and cosmetics since 1997, Necton has also gained scientific, technological, and market knowledge in the feed, food, nutraceutical, pharmaceutical, and bioenergy sectors. The company has a unique and distinctive experience in designing and operating different microalgae cultivation technologies, ranging from open to closed systems, the proprietary technology of the company makes it possible to cultivate freshwater, marine, and hypersaline microalgae of many genera.

A NEW LAB PROTOCOL TO IMPROVE PROTEIN ACCESSIBILITY OF *ARTHROSPIRA PLATENSIS*

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ABSTRACT

Microalgae have emerged as a good alternative feedstuff for monogastric diets, concerning their richness in many nutrients, especially proteins [1]. Particularly, *Arthrospira platensis* has high levels of proteins and several bioactive compounds, but its recalcitrant peptidoglycan cell wall affects accessibility of nutrients, mainly in monogastric animals because they cannot digest properly the cell wall [2, 3]. Therefore, it is important to develop some strategies to disrupt cell wall and improve nutrients accessibility. The use of exogenous enzymes, like peptidases, is well-accepted in diet formulation for monogastric animals [4, 5]. So, our research team implemented an *in vitro* lab protocol to find out a novel combination of peptidases active on *A. platensis* proteins, after microalgae incubation with enzymes, mainly from marine origin and produced by high-throughput technology, following these steps: 1) overnight incubation, in an orbital shaker (37 ºC and 160 rpm), of microalga (20 mg/mL suspension in PBS buffer), after its sonication (15 minutes in 7 cycles 70%), in a 24-well plate, with the previously produced enzymes at 20 µg/mL (*versus* a negative control with PBS and a positive one with trypsin); 2) plate centrifugation at 1500 x*g* (15 minutes, 4 ºC), followed by the recovery of 1 mL supernatant to a centrifuge tube; 3) Bradford assay to quantify the total protein solubilized; 4) 14% SDS-PAGE electrophoresis to determine protein degradation and solubilization; 5) and OPA assay to measure the peptides released from protein hydrolysis [6-9]. This lab protocol showed to be very sensitive to the *in vitro* assessment of protein accessibility and, therefore, it is a good screening methodology for the releasing of microalgae nutrients in the context of monogastric feeding.

Figure 1. Aspect of the *in vitro* incubation of *A. platensis* with peptidases in a 24-well plate

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Maria Spínola has 25 years old, and was born in Aveiro (Portugal). She got a Master in Veterinary Medicine (Abel Salazar Biomedical Sciences Institute, University of Porto, Portugal), and is currently a PhD student in Veterinary Sciences at Faculty of Veterinary Medicine of the University of Lisbon (Portugal). Research interests are related with the use of microalgae in Animal Nutrition.

Bioremediation

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PRELIMINARY SIMULATION OF A NEW FED-BATCH PROCESS, DESIGNED FOR CONTROLLING CONTAMINATION IN MICROALGAE CULTIVATION INTEGRATED WITH WASTEWATERS TREATMENT

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ABSTRACT

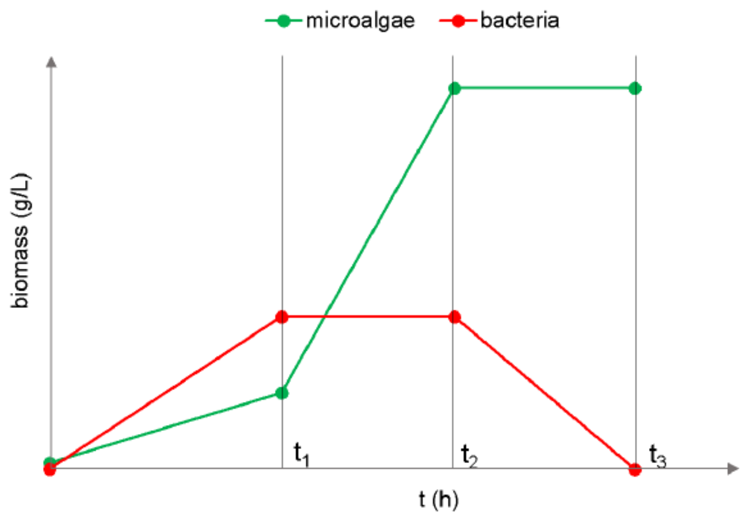
In recent years, the integration of microalgae cultivation with wastewaters treatment has received an increased interest and it has demonstrated to be a promising alternative to conventional biological processes.[1] It results in an increment of economic and environmental sustainability of the process, particularly in heterotrophic conditions, where synthetic substrates can be replaced by organics from wastes.[2,3] However, the presence of organic substrates increases the risk of contamination by microorganisms, like heterotrophic bacteria, which can compete with microalgae and compromise the quality of produced biomass.[4,5]

To face this problem, an alternative solution to sterilization is represented by strategies of nutrients feeding, that can generate a selective pressure for microalgae biomass production. With this aim, in our research group the uncoupled feeding strategy has been developed: carbon deplete/nitrogen replete and nitrogen deplete/carbon replete culture conditions are temporally alternated.[2] On one hand, with this strategy, contamination can be controlled, because microalgae are more competitive over bacteria when either nitrogen or organic carbon are not present in the culture medium.[4]

On the other hand, in real wastewaters carbon and nitrogen coexist, therefore we are currently developing a new fed-batch process (in figure) as described below:

at first, the culture is fed with carbon and nitrogen simultaneously and an exponential growth occurs until time t_1, when nitrogen is depleted, and a "fattening" phase starts until t_2. Here carbon is depleted too, and a "famine" phase starts, where microalgae can have a competitive advantage over bacteria (until t_3), because of their ability to accumulate energy reserve materials during the previous phase.

The aim of this work is the optimization of the parameters of this process, to control the contamination. Firstly, microalgal growth was tested, in batch, on glucose, galactose and sodium acetate and, μ_{max} and yields were estimated both for microalgae and bacteria. Subsequently, these values were used to simulate the process and, fixing an initial bacteria to microalgae ratio, the optimal values of t_1, initial carbon and nitrogen concentrations and microalgal and bacteria concentrations at t_1 and t_2 were estimated.



Keywords
Microalgae, Wastewaters, Bacteria contamination, Nutrient feeding strategies, Contamination control

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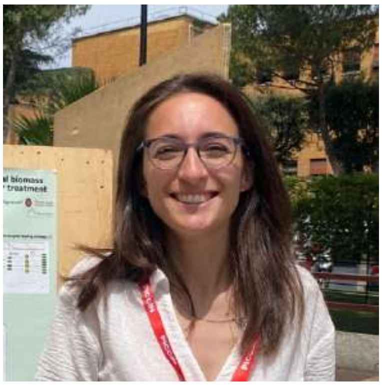

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BIOGRAPHY

Laura Capobianco was born in Rome on 11 May 1996, after the scientific and bilingual diploma at Liceo Scientifico “C. Cavour” in 2014, she studied Agro-Industrial Biotechnology and graduated with an experimental thesis entitled “The overexpression of gene ARF-5 in the model organism Arabidopsis Thaliana”. In 2019 Laura took the master’s degree in Industrial and Environmental Biotechnologies, with a thesis about the synthesis of bioadsorbents from olive pomace and the study of their use to remove arsenic from aqueous solutions. After that, Laura has been awarded for the ERASMUS+ traineeship, and she was near to go to Leipzig (Germany) to spend six months at the Helmholtz Centre for Environmental Research, but unfortunately the COVID pandemic aroses. During the pandemic period, Laura continued to cultivate her passion for the languages, studying German. Almost during the same period, in August 2020 she published her first paper on Journal of Environmental Management, entitled “Production of iron-coated adsorbent for arsenic removal by hydrothermal carbonization of olive pomace: effect of the feedwater pH”, together with Fabrizio Di Caprio, Pietro Altimari, Maria Luisa Astolfi e Francesca Pagnanelli. In October 2020, she won a scholarship in the PhD course of Chemical Processes for the Industry and the Environment conducted between the Department of Chemistry of Sapienza, University of Rome, and the company NextChem S.p.A.. During her PhD, Laura works on the integration between microalgal cultivation and wastewaters treatment; she is currently enrolled in the second year of PhD.

ORGANIC WASTE DARK FERMENTATION EFFLUENT AS CULTURE MEDIUM FOR CHLORELLA VULGARIS: PRELIMINARY ASSESSMENT OF THE PROCESS

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ABSTRACT

Microalgae growth on dark fermentation (DF) effluents is a promising secondary process, thanks to their ability of assimilating ammonium, phosphorus and certain VFAs and converting them into high-added value macromolecules [1, 2]. At present, microalgae have already been grown at pH 7-8 in synthetic or sterile medium, but knowledge about their cultivation on non-sterile DF effluents for lipids accumulation is still at a preliminary stage [3–5].
In this study, the growth of *C. vulgaris* NIES-227 was evaluated on the liquid fraction of a real DF effluent with a total VFAs concentration of 15.7 gCOD/L (3.0 gCOD/L acetic, 2.5 gCOD/L propionic, 3.0 gCOD/L butyric and iso-butyric, 2.8 gCOD/L valeric, and 4.4 gCOD/L hexanoic acids). The tests were conducted in 300 mL flasks on the DF effluent diluted 1:5, 1:2, and undiluted. Light intensity of 5.7 kLux was provided with LED strips, and pH was controlled at 8. The tests were inoculated with 9.5 mln cells/mL, i.e., 0.2 g/L of dry weight, of the preculture in exponential phase. Microalgae growth and VFAs were daily monitored.
C. vulgaris was able to grow on the non-sterile DF effluent at all concentrations, with a final microalgal cell count of 67-93 mln cells/mL and a final dry weight of the microalgae-bacteria consortium of 1.2-1.7 g/L for the DF effluent diluted 1:5 and 1:2, respectively. The test on undiluted DF effluent showed the highest final dry weight of 2.3 g/L but the lowest cell count of 38.0 mln cells/mL. VFAs were always depleted within 4 days, probably due to bacterial degradation of VFAs with longer carbon chains, which are hardly metabolized by microalgae [1]. Despite VFAs consumption, microalgae growth continued until day 9, probably due to the CO_2 resulting from bacterial VFAs degradation. A similar lipid content was obtained in the control (47%) and fermented 1:5 (48.8%), while it was higher for the fermented 1:2 (56.5%).
This work shows the potential of real DF effluents to grow lipid accumulators such as *C. vulgaris* without the need for sterilization, while contemporarily providing effluent depuration and higher COD recovery in the DF process [6].

Keywords
Microalgae, lipids, VFAs, dark fermentation, mixotrophy

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BIOGRAPHY

Cristina Cavinato is Associate Professor in Industrial Engineering at the University Ca’ Foscari of Venice, Department of Environmental Science, Informatics and Statistics.
Her research and development activity concern the chemical and process engineering for organic waste valorisation and wastewater treatment related to anaerobic digestion of municipal/agricultural organic waste (sludge, biowaste, manure, winery waste) for energy (hydrogen and methane production) and fermentation of solid organic wastes for chemicals production. Recently she is developing anaerobic digestion-microalgae coupled process for waste treatment and biomass/biobased compounds production.

She was involved in several International and National project and networks on organic waste valorization for energy and material recovery, she is co-author of n.3 patents, authored and co-authored more than 50 papers in international journals (corresponding H index of 24). She acts as external expert for Horizon 2020, Horizon Europe and BBIJU projects and of other international Research Institution. She is coordinator of the Research Institute of Green and Blue Growth at Ca’ Foscari University.

COMPANY PROFILE

Ca’ Foscari University attracts funding from regional, national and international programmes. It has a long-standing tradition in research and well established experience in EU funded projects (282 projects awarded since 2014, of which 187 within the Horizon 2020 Framework Programme. Furthermore, many grants have been obtained within REC-programme, EU DGs grants, Territorial Cooperation Programmes, Life, Creative Europe, EuropeAid, with Ca’ Foscari participating as Lead Partner or Partner) for a total of 75M€ awards (more than 60M€ in Horizon 2020 funding). It is a top Host Institution with more than 120 Marie Skłodowska Curie Individual Fellowship awarded, a Cofund Fellowship programme, 18 ERC grantees and an active and continuous participation in competitive research and collaborative programmes. The Department of Environmental Sciences, Informatics and Statistics (DAIS) of the Ca’Foscari University of Venice (UNIVE), was created in 1982 and is composed of 60 professors and researchers; DAIS has an interdisciplinary structure, combining knowledge in Engineering, Chemistry, Biology, Ecology, Economic, Earth Sciences, Informatics and Mathematics and is involved in several national and international projects, focused on environmental aspects.

RECYCLING NUTRIENTS FROM WASTEWATER TO GROW CYANOBACTERIA AND RECOVER PHYCOBILIPROTEINS

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ABSTRACT

Cyanobacteria are photosynthetic microorganisms able to synthesize a wide variety of molecules with commercial interest, such as the high-pigmented and water-soluble phycobiliproteins. The recovery of phycobiliproteins from biomass produced in standard media is still economically unfeasible, therefore, alternative nutrient sources such as wastewater are being investigated. Nevertheless, growing cyanobacteria in waste streams is usually related to poor culture stabilities and bioproduct social acceptance. In this study, a wastewater borne *Synechocystis* sp. strain was cultured in unsterile secondary effluent (SE) from an activated sludge treatment system, with and without supplementation of N and P (SE+nutrients) in a closed photobioreactor operated in batch mode. Results showed how the consortia cultured in SE removed inorganic nutrients more efficiently than the SE-nutrients one, reaching removal efficiencies up to 89% (ammonium), 54% (nitrate) and 86% (phosphate). However, the phycobiliprotein productivity was similar in both cases (8.2 and 9.6 mg L^{-1} d^{-1} in SE and SE+nutrients, respectively). Subsequently, cyanobacterial biomass and pigment production over time was studied by operating the photobioreactor in semi-continuous mode, with a hydraulic retention time (HRT) of 5 days. *Synechocystis* sp. remained the dominant species, showing a fairly stable total phycobiliprotein production, with contents up to 7.5$\%_{dcw}$ and purities up to 0.8 (corresponding to food grade). Similar results were obtained after scaling-up the process to a 30L photobioreactor, where a maximum 7.4$\%_{dcw}$ phycobiliprotein content was attained. In light of the results, the presence of contaminants of emerging concern (CECs) in SE, microalgal biomass and pigment-rich extract was also analyzed. Out of the 22 CECs found in SE, only 3 were detected in raw pigment samples. Prospective valorization of the produced pigments would require either the purification of pigment extracts or the regulation of their potential applications, for instance as natural dyes or paintings. In conclusion, *Synechocystis* sp. may be pinpointed as an interesting candidate to recover nutrients from wastewater and produce natural pigments.

Keywords
Cyanobacteria, bioproducts, natural pigments, wastewater, contaminants of emerging concern

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COMPANY PROFILE

The Group of Environmental Engineering and Microbiology (GEMMA) of the Universitat Politècnica de Catalunya·BarcelonaTech, is dedicated to interdisciplinary research, innovation, knowledge transfer and education in environmental engineering; particularly in the fields of environmental biotechnology, water treatment, biomass valorization and bioenergy generation. GEMMA was founded in 2005, and since then the group has played a remarkable role promoting ecoinnovation and biotechnologies for efficient wastewater treatment and reuse, along with biomass valorization.

GEMMA aims at contributing to sustainable development by means of fundamental and applied research, and through innovation in the water and energy fields. The goal of our research is to develop and promote sustainable, innovative water treatment and biomass valorization biotechnologies, capable of removing a wide range of pollutants, including contaminants of emerging concern.

During the last decade, we have been particularly focused on the recovery of resources from waste streams using microalgae and cyanobacteria, in order to obtain bioproducts (natural pigments, bioplastics, biofertilizers, alternative proteins) and bioenergy, in the framework of the circular bioeconomy.

WASTE MANAGEMENT FOR THE ISS USING SALTWATER MICROALGAE

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ABSTRACT

Discovering a way to safely convert urine to drinking water, through the use of algae, allows astronauts unique solutions to sustain life in space. On Earth microalgae can grow in wastewaters and in the process removes nitrogen, phosphorus, heavy metals, pesticides, pathogens, both organic and inorganic toxins. Microalgae is a self-sustainable sustainability concern, microalgae can be used for food, material, energy, clean air, and more. A microalgae-based bioregenerative life support system (BLSS) for space missions has been suggested for decades but it is now that major research is being done. One aspect of this research that needs improvement is CO2 fixation, test tolerance to urea, and adapting to the other numerous uses those algae have in space.

Through this research, I will determine the best microalgae for waste management applications. The algae must be high in proteins, lipids and ultimately survive in the harsh conditions of space.

The microalgae samples for this project consist of *Zooxanthellae* sp., *Chlorella* sp., and *Nannochloropsis* sp. and have all been cultivated in the lab. These samples were specifically selected as they all correlated between areas of high chlorophyll concentrations on NASA Geo Data and areas of known sewage water treatment from the Water and Sewer Authority. Both variables were input into a GIS (Geographic Information System) map to find the samples with the highest chance of success. All samples and microalgae were grown in separate BG-11 and urea mediums. Their morphology changes and the growth rate were determined using a spectrophotometer 600nm. It was determined that the microalgae of *Nannochloropsis* sp. and the sample of Isla Cabra that had the best growth in urea. These findings prove the potential that microalgae have for waste (urine) management applications on the ISS and opens the door for further research including chemical analysis of total proteins, carbohydrates, and lipids to determine other beneficial applications in space.

Keywords
Wastewater, Microalgae, Space, Nannochloropsis sp., Urine

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COMPANY PROFILE

The University of Puerto Rico, Río Piedras Campus is a public land-grant research university in San Juan, Puerto Rico. It is the largest campus in the University of Puerto Rico system in terms of student population and it was Puerto Rico's first public university campus.The university serves more than 18,000 students, 20% of whom are graduate students, and grants an average of over 3,000 degrees a year. Its academic offerings range from the bachelor to the doctoral level with 70 undergraduate programs and 19 graduate degrees including 71 specializations in the basic disciplines and professional fields. UPR-RP has consistently granted the largest number of doctorate degrees to Hispanic students under the United States jurisdiction.

RECOVERY AND VALORIZATION OF GASEOUS AND LIQUID EFFLUENTS FROM THE WINE INDUSTRY FOR PRODUCTION OF *CHLORELLA* BIOMASS AND EXTRACTS

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ABSTRACT

REDWine is an international collaborative research project (https://redwineproject.eu) which aims to utilize carbon dioxide from wine fermentation off-gas and winery liquid effluent for *Chlorella* biomass and extracts production. The project comprises the implementation of a demonstration-scale *Chlorella* production unit designed by REDWine partners (A4F, COLDEP and LEITAT) at Adega Cooperativa de Palmela winery, comprising the following systems: water treatment, medium preparation, carbonation system with collection of fermentation off-gas, laboratory and inoculum preparation, pre-production system, demo-scale cultivation, thermoregulation, biomass harvesting, downstream processing, effluents treatment and data acquisition and control.
A4F installed in the winery a pre-production flat-panel photobioreactor to conduct preliminary trials and collect data from *Chlorella* cultivation on site along the year. A preliminary trial to compare the growth of *Chlorella* using pure CO_2 and fermentation off-gas showed that the use of off-gas didn't seem to have a negative effect in *Chlorella* cultivation. After the preliminary trial, A4F is successfully maintaining *Chlorella* production on site in a semi-continuous operation mode for over a year with an average biomass productivity of 0.07 g_{DW}/L/day. The biomass produced in this system is being sent to REDWine partners for end uses in food, cosmetics and agricultural sectors.
The characterization of winery liquid effluents and its incorporation in *Chlorella* cultivation was carried out at laboratory scale by IPS. The liquid effluents from the red wine manufacturing process in 2021 campaign were rich in carbon, moderate in nitrogen and low in phosphorous content, hence having a non-ideal nutritional composition for microalgal growth. Moreover, these effluents have a high variability of phenolic compounds that may negatively affect growth. The effluents have been tested for *Chlorella* growth at laboratory scale using two effluents with an increasing volume of incorporation in the culture medium (1%, 10% and 30%). *Chlorella* growth approximates the one of the control for 1% v/v. For 10% there was considerable microalgal and bacterial growth, and for 30% v/v there was no growth at all. These results indicate that, with 1% v/v of effluent in microalgal culture medium, *Chlorella* growth is viable and highlights the need for effluent pre-treatment to remove phenolic compounds.

Keywords
REDWine, *Chlorella*, fermentation off-gas, winery liquid effluents, circular economy

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BIOGRAPHY

Diana holds a master's degree in Biological Engineering (2017, Instituto Superior Técnico). At A4F, she works as a researcher in Lisbon Innovation Laboratory, where she collaborates in R&D projects and in microalgae production from laboratory to pilot scale.

COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions and customer characteristics and goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

EFFECT OF WATER TYPE ON THE COMPOSITION OF *TETRADESMUS ALMERIENSIS*

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ABSTRACT

The microalga *Tetradesmus almeriensis* was produced using 63 m^2 thin-layer cascade photobioreactors using either (i) freshwater supplemented with commercial fertilisers, (ii) primary-treated urban wastewater, (iii) secondary-treated urban wastewater, and (iv) pig slurry diluted with freshwater. The photobioreactors were operated in semi-continuous mode (0.3 day^{-1}) during 30 days. The goal of this work was to assess the effect of the water type on the microbial diversity of the biomass and on its composition, namely amino acid content, fatty acid profile, mineral content, volatile organic compounds content, among other characteristics.

Overall, the type of water used significantly affected the biomass productivity of the system, which was the highest when produced using freshwater supplemented with fertilisers (30.3 ± 2.5 $g \cdot m^{-2} \cdot day^{-1}$). The observed differences were attributed to the highest availability of nutrients in the different types of water. The nutrient recoveries in the wastewaters and the pig slurry were correlated with the biomass productivity, suggesting that most of the nutrients removed from the water were assimilated into biomass. The macromolecular composition of the biomasses produced using the different water types as well as the amino acid profiles and fatty acid profiles, which were significantly affected were attributed not only to the different nutrient composition and availability of the media but also to the microbial diversity of the biomass. Illumina sequencing revealed that the inoculated strain was only the most abundant in the cultures produced using freshwater supplemented with fertilisers (44.6%) and the diluted pig slurry (52.9%). When produced using primary or secondary wastewater, the genus *Tetradesmus* represented only 1.6 and 4.9% of the total eukaryotic populations, respectively. Several algal predators were identified in the cultures including parasites of the genus *Amoeboaphelidium* and rotifers from the genus *Adineta*.

The results of this work revealed that the type of water used has a striking effect on the composition and functionality (quality) of microalgal biomass. The results also highlighted the importance of characterizing microalgal cultures to identify potential pathogens and predators, which are not easily identified, to implement contingency actions and avoid the crash of the culture.

Keywords
Biomass production, waste management, biomass characterisation, pathogens, thin-layer reactors.

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BIOGRAPHY

MSc in Industrial Computer Sciences (University of Almeria, Spain), MSc in Chemical Engineering (University of Almeria, Spain) and PhD in Agriculture and Food Science (University College Dublin, Ireland). Tomás is currently a Senior Scientific Researcher at the Department of Chemical Engineering of the University of Almeria in Spain and at the Desalination and Photosynthesis Functional Unit of CIESOL Solar Energy Research Centre (Almeria, Spain). His research is related with the development and optimization of microalgae-based processes related with the capture of carbon dioxide, waste management, and food production with a special focus on the potential uses of microalgae in the functional foods industry.

COMPANY PROFILE

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DILUTION OF AGRO-INDUSTRIAL ANAEROBIC DIGESTATES WITH GEOTHERMAL WATER FOR THE LOW-COST PRODUCTION OF SPIRULINA IN A CIRCULAR ECONOMY APPROACH

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ABSTRACT

Anaerobic digestion (AD) is a natural biological process where organic matter is converted into biogas by a consortium of microorganisms working under anaerobic conditions. In addition to biogas, AD process generates a liquid/solid by-product, called digestate. The liquid fraction of this digestate is a nutrient-rich product mainly used for soil amendment. Face to the current increase of AD plants combined with the evolution of digestate management regulation, new digestate valorization strategies are currently been developed [1]. Microalgae cultivation is one of the promising approaches to manage liquid digestate while generating a valuable algal biomass. However, there are still some factors limiting a direct use of liquid digestate (strong turbidity, high ammonium concentration, etc.) for microalgae culture [2]**.**

In this context, the present study aims to investigate the feasibility of diluting liquid digestates with geothermal water for *Spirulina* cultivation. Thus, digestates coming from four different biogas plants were selected to perform this study. For each individual digestate, a screening experiment was first carried out in aerated 200 mL glass tubes to determine the optimal dilution rate generating a sufficient algal growth. Subsequently, a second culture stage was performed to evaluate if geothermal water could be used as substitute to demineralised water for digestate dilution in order to reduce the water footprint of the overall process.

Optimal dilution rates of 5x, 20x, 40x and 60x were obtained for dairy, agricultural, urban, and biowaste digestates, respectively. These results indicated the optimal ranges of values for ammonium concentration (60-121 mgNH_4^+/L) and turbidity (14-24 FNU), which allowed an appropriate *Spirulina* growth (μ=0.25-0.31 d^{-1}). Promising results were found with geothermal water, which generated similar growth responses than demineralised water, with all the digestate tested (μ=0.22-0.29 d^{-1}). These promising results highlight the potential of recycling nutrients from liquid digestate and valorising geothermal ground water to reduce the operational costs of microalgae cultivation by following a circular economy concept.

Keywords
Anaerobic digestion, Spirulina, digestates, circular economy, bioremediation

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BIOGRAPHY

In 2019, Marie-Ange received her chemical engineering degrees at the Toulouse INP-ENSIACET Graduate Engineering school in Toulouse (France). After her graduation, she worked at APESA (France) a French private technology center in environment on various microalgae projects. She has notably performed culture at different scales (laboratory to pilot scale), in various culture systems (vertical column, innovative PBR and raceway) and with several microalgae strains (*Spirulina*, *Nannochloropsis oculata*, *Tetraselmis suecica*, *Chlorella vulgaris*). In May 2021 she started a PhD with APESA company (France) and the University of Pau and Pays de l'Adour (UPPA - France). Her research project focused on the development of innovative photobioreactors for the intensive cultivation of microalgae applied at the bioremediation of agro-industrial effluents.

COMPANY PROFILE

SIAME (UPPA) - The SIAME laboratory is a team of the University of Pau and Pays de l'Adour whose research is carried out in the field of Engineering Sciences. The unit is structured in 4 teams: Complex flows and Energetics (EE), Geomaterials and structures of civil engineering (GS), Interaction Waves / Structures (IVS), High Voltage Processes (PHT). The research is based on experimental expertise, modeling and numerical simulations.

APESA - French private technology center in environment and risk control carries the values of sustainable development. APESA is organized in four areas: Management, Innovation, Animation and Valorization. The main activity sectors of the valorization group are composting, anaerobic digestion, the life end of bioplastics and effluents (including microalgae). APESA uses its experimental platform to carry out experiments aiming to validate the feasibility, to optimize the dimensioning and to help our customers decide of setting up projects and industrial solutions in the fields of energy recovery waste or biomass. From the deposit (waste, biomass, effluents), through the process (anaerobic digestion, composting, wastewater treatment) and up to the quality of the downstream product (digestate, compost, discharges) APESA can provide its expertise and knowledge.

SCENEDESMUS SP. AND *KOLIELLA ANTARCTICA* AND THEIR POTENTIAL IN FISH FEED REPLACEMENT UPON HPH-INDUCED CELL DISRUPTION

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ABSTRACT

Demand for food, feed, energy, and clean water will significantly increase over the next decades due to the foreseen human population increase. As agriculture is one of the sectors most impacted by this rising demand, decreasing the usage of clean water and the discharge of water containing excess nutrients is essential to protect the environment. Thus, efforts are being made to decrease the freshwater demand of the sector by using soilless plant production systems that offer increased productivities and lower water requirements as compared to those of traditional agriculture. However, discharge of nutrient-rich (drain)water still represents about 30% of the total water used. Therefore, the present work uses a circular economy approach that relies upon re-using drainwater as a culture medium for microalgae growth. Firstly, *Scenedesmus* sp. and *Koliella antarctica* strains were selected due to their different abilities for withstanding summer vs. winter outdoor conditions, respectively, and industrially grown in drainwater in a 19-m^3 industrial tubular photobioreactor. *Scenedesmus* sp. was in production for 8 days, while *K. antarctica* was produced for 18 days, reaching a dry weight of 1.8 and 1.6 g L^{-1}. Thereafter, a biorefinery approach was applied to generate 3 products: clean water, microalgal extracts and residual biomass (cell debris) for agriculture and aquaculture, respectively. Accordingly, after biomass harvesting, the concentrated culture with a dry weight of 120 g L^{-1} was disrupted using a high-pressure homogenizer (HPH) at 600 bars, 3 times, as previously optimized and defined as the ideal conditions for cell disruption. Processed biomass was then centrifuged to separate cell debris from the water extract. Cell debris of both species showed a promising biochemical composition, rich in proteins and carbohydrates, while *K. antarctica* displayed a high concentration of polyunsaturated fatty acids, accounting for about 78% of the total fatty acids. Cell debris were freeze-dried and later incorporated into feeds to assess their potential as a replacement of fish meal in aquaculture feeds. Water extracts will be later tested as a source of biostimulants in raspberry and avocado plants.

Keywords
Microalgae, biorefinery, bioremediation, high-pressure homogenization,

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BIOGRAPHY

Inês Maia is a student from the PhD programme Marine, Earth and Environmental Sciences, from the University of Algarve. Her thesis will focus on the bioremediation of drainwater from hydroponic productions, using a microalgae-based approach, for sustainable products. She finished her MSc programme on Molecular and Microbial Biology in 2019 with a thesis focused on the optimization of *Emiliania huxleyi*'s growth for production of novel compounds. Afterwards, she worked on a fellowship in the ALGAVALOR project, where she exploited the potential of isolated microalgae for agro-industrial wastewater treatment and of the produced biomass to be applied in agricultural production.

COMPANY PROFILE

University of Algarve is a higher education institution with 7,751 students, 1,604 of which following 56 postgraduate courses, including 45 and 11 MSc and doctoral programmes respectively. Teaching and research are focused on Life/Health Sciences and Technology, in particular Marine/Aquatic Sciences, often in collaboration with Centre of Marine Sciences (CCMAR).

Necton is the oldest company in Europe producing and selling microalgae. The company has a distinctive experience in designing and operating different cultivation technologies, with a cultivation facility of 4x19 m^3 photobioreactors and a raceway of 200 m^3, where it cultivates species including *Nannochloropsis, Tisochrysis*, *Phaeodactylum*, *Tetraselmis* and *Skeletonema*.

UTILIZATION OF POULTRY MANURE EXTRACT IN MIXOTROPHIC CULTIVATION OF *AUXENOCHLORELLA* PROTOTHECOIDES: EFFECT OF GLUCOSE ENRICHMENT ON BIOMASS GROWTH AND BIOCHEMICAL COMPOSITION

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ABSTRACT

Significant amounts of poultry manure (PM) are produced due to intensification of agricultural production systems. Nutrient recovery and reutilization from agro-industrial wastes in the framework of the circular economy has thus received increasing attention. PM is rich in nutrients and protein content which renders it ideal as a low-cost microalgae growth medium. Microalgae can provide a valuable source of biomass due to their rapid growth rates and ability to grow in various substrates under heterotrophic or mixotrophic conditions. Mixotrophic cultivation could enhance the productivity by utilizing both inorganic and organic carbon substrates. In this study, *Auxenochlorella protothecoides* was cultivated in a PM extract supplemented with glucose without any other added nutrients. The aim was therefore to evaluate the effect of different glucose concentrations (0-30g/L) in the medium on biomass growth and biochemical composition under mixotrophic conditions. The medium was diluted in order to achieve 1 g/L proteins and enriched with 0,10,20 or 30 g/L glucose. The experiment was carried out in 500 mL Duran flasks using 200 mL sterile PM medium and 20 mL inoculum under aseptic conditions, LED panel illumination (5000 lux), photoperiod of 16:8 h and temperature 26 ± 2 °C. The cultures were agitated with filtered sterilized air (0.2 L/min). Each culture was harvested after achieving glucose removal higher than 90%. *A. protothecoides* was capable to acclimatize and proliferate in the PM substrate, reached the maximum biomass concentration at 20 g/L glucose (2.92 g/L), while the lowest at the 0 g/L (0.34 g/L) and 30 g/L (1.43 g/L). Proteins were found to be the most abundant substance in dry biomass (37.4-43.1% w/w), followed by carbohydrates (15.8-24.4%) and lipids (7.1-12.8%). Protein, carbohydrate and lipid productivity was enhanced by the addition of glucose compared to the control (0 g/L), achieving the highest (43.1, 24.2,12.8 respectively) at 20 g/L glucose. Our results indicate that glucose-enriched PM extract can be used as a renewable nutrient source for microalgal cultivation. In addition, the algal biomass is a promising animal feed supplement due to its biochemical composition.

Keywords
Poultry manure, microalgae, glucose, mixotrophy, *Auxenochlorella protothecoides*

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Giorgos Markou works as a Researcher (grade C) at the Institute of Technology of Agricultural Products of HAO-DEMETER. He received his first degree (MSc equivalent) on Agricultural Engineering (Crop Science, Agricultural University of Athens-AUA) in 2003, and in 2010 received a second MSc degree on Energy Systems Management and Renewable Energy Sources (AUA). Since 2013, he holds a PhD degree (AUA) on the topic of production of microalgal biomass for biofuels generation and wastewater treatment. Since 2013, he completed 3 Post-doc projects (1) Department of Agricultural Engineering, HAO-Demeter, Greece (2014-2015) and (2) Pegasus Marie Curie, WFO KU Leuven, Belgium (2015-2016), (3) IKY-Siemens Scholarship grant for Excellence from Greek Scholarship Foundation (IKY), Greece (2016-2017). GM is the author of more than 60 peer reviewed articles (SCI system), 9 book chapters and over than 10 other publications (total references over than 3400; h index 28; Scopus database). So far, he has participated in 6 delivered research projects and is involved in 5 ongoing ones. He has a broad research interest, focusing on waste and wastewater valorization and nutrient (nitrogen, phosphorus etc.) recovery, biomass production (microalgae, cyanobacteria, higher plants), and biomass utilization (food, feed, biofuels, high-value products etc.). He is a member of the Editorial Advisory Board of the journal Algal Research (Elsevier), Bioengineering (MDPI), Biomass (MDPI) and Topic Editor-in-Chief in MDPI. GM is an active reviewer in peer-reviewed journals, and evaluator for funding research proposals.

COMPANY PROFILE

ITAP is the only public research Institute in the broader public sector that deals with the quality, safety, processing and preservation of foodstuff. Three of its laboratories are ISO 17025 accredited: Microbiological analysis laboratory, Physico-chemical analysis laboratory and Genetic Identification laboratory. A significant number of research programs have been funded by the European Union and by the GSRT and have been implemented at the Institute or are in progress. Through these programs, the Institute has established very good cooperation with many Universities and Research Centers in Greece and abroad. The results of the research have been published in international and domestic journals and have been announced in conference proceedings. Moreover, cooperation with the Greek food industry is constantly increasing in various forms (research projects, cooperation agreements and technical support services). In particular, support services contribute significantly to solve issues in the production process, food quality and hygiene assurance and development of new products by applying modern methods and technologies. The Institute is fully equipped and uses classical and modern techniques and equipment such as PCR, GelElectrophoreses, PFGE, DGGE, HPLC, GC, GC / MS, High Pressure System Unit (the unique laboratory-scale High Pressure system Unit established in Greek laboratories), microscopes and many more.

MICROALGAL BIOREMEDIATION OF AN N-RICH INDUSTRIAL EFFLUENT

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ABSTRACT

Agricultural and industrial activities require the use of large quantities of raw materials and generate large amounts of nutrient-rich solid, gaseous, and liquid waste streams. These have been linked to a widespread increase in the pollution of freshwater systems, leading to a drastic surge in the occurrence of eutrophication events.[1–3] The need to reduce nutrient run-offs and preserve the available water resources represents simultaneously a major concern and a great opportunity to develop nutrient removal and recovery processes for wastewater streams.

Microalgae have been exploited for the treatment of different industrial wastewaters, coupling microalgal biomass production with resource recovery and waste valorisation, demonstrating the potential of microalgal-based systems to integrate a circular economy model.[4]

Outdoor microalgae production systems are susceptible to seasonal variations in radiation intensity, photoperiod, and temperature, which impact the annual productivity and economic feasibility of large-scale operations.[4] As these parameters are unique to every location, it is important to study their patterns within the geographical area of interest before the implementation of any production unit. In this sense, this work aims to assess the feasibility of cultivating a naturally established endogenous microalgal assemblage for the bioremediation of a nitrogen-rich effluent derived from an industrial fertilizer production plant (ADP Fertilizantes, Portugal). The experiments were caried-out by uninterruptedly operating a 700 L pilot-scale raceway pond in semi-continuous mode from 2019 to 2022 to assess its long-term performance accounting for year-round variations. Nitrogen removal efficiency, biomass productivity, and the ecosystem's species evolution were followed.

Additionally, since microalgae represent a potentially sustainable alternative for the enhancement and protection of agricultural crops, several production cycles were performed under different pH values (6.5, 8.0 and 9.5), to promote alterations in the microalgal assemblage and, consequently, in the biomass batches obtained. The attained biomass was processed into three extracts tested as alternatives to chemical agronomical products. These extracts were applied as biostimulant priming treatments to rice (*Oryza sativa* L. Japonica) under optimal and salinity stress conditions (80 and 160 mM) to assess their impacts on the germination process.

Keywords
Microalgae, nutrient recycling, wastewater bioremediation, circular economy, biostimulants.

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BIOGRAPHY

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COMPANY PROFILE

A4F - Algae for Future, located in Portugal, is specialized in the microalgae, macroalgae, biorefinery and fermentation sectors. A4F has more than 20 years of accumulated experience in algae research & development and algae production (up to industrial scale). A4F provides services for the design, build, operation and transfer (DBOT) of commercial-scale algae production units, using different technologies that better adapt to our Customers' business. We work closely with our Customers to select the best technology and bioengineering solutions for the intended application, taking into account the specific site conditions and customer characteristics and goals. Additionally, A4F also develops standard operating procedures for optimized algae production, according to production goals and with industry best practices.

MICROALGAL BIOREMEDIATION OF ORGANIC WASTE: EXAMINING POTENTIAL FOR CIRCULAR WASTE MANAGEMENT

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ABSTRACT

The finite nature of the planet's resources, coupled with the ever-increasing world population, necessitate a departure from the linear 'take-make-dispose' food system and the adoption of circular bioeconomy to produce highly nutritious biomass. Anaerobic digestion, the leading organic waste disposal technology in the EU and the UK [1], is considered the hub of modern circular bioeconomy. In addition to bioenergy production, there are opportunities for nutrient recovery through valorisation of nutrient-rich anaerobic digestate effluent streams. Digestate, commonly used in conventional agriculture as fertilizer, could also serve as a nutrient source for microalgal cultivation, which offers significant advantages in terms of both wastewater treatment and production of high-value biomass [2]. However, this approach comes with challenges, such as consistency and scalability; the effectiveness of the process can significantly vary for different anaerobic digestion/algal cultivation setups [3], and careful design of 'waste-to-feed' supply chains tested in pilot scale is necessary.

In this study, we tested a circular fruit and vegetable waste treatment/utilization process. The process consists of anaerobic digestion of fruit and vegetable waste, and subsequent pilot-scale microalgae cultivation using the digestate produced as a nutrient medium. Supplementation of algal biomass to black soldier fly larvae (BSFL) is also investigated as an additional step towards high nutritional value feed production. Anaerobic digestion of fruit and vegetable waste was not only efficient in terms of biogas production (0.20-0.48 L CH_4 g^{-1} VS), but also generated digestate with a nutrient profile suitable for cultivation of protein-rich *Chlorella vulgaris*. Moreover, microalgae hyperaccumulated vitamin B_{12} that was biosynthesized during the anaerobic digestion step, exceeding the respective B_{12} content of *Chlorella vulgaris* grown in conventional algal growth media. These findings indicate that, in addition to macronutrients (C, N, P), digestate could serve as a source of high-value micronutrients which set microalgae apart from conventional dietary proteins, as they are known hyperaccumulators of vitamins and other components terrestrial plants lack or have only small amounts of.

Keywords
Anaerobic digestion, vitamin B_{12}, *Chlorella vulgaris*, BSFL.

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BIOGRAPHY

My interests are broadly at the intersection of biochemistry and biotechnology sectors. I have experience in characterisation of vitamin biosynthesis pathways and engineering metabolism of native and novel chemicals in photosynthetic organisms. As manager of the Algal Innovation Centre (AIC) at University of Cambridge, my remit is to facilitate R&D, educational and outreach projects, particularly between industry and academic collaborators.

COMPANY PROFILE

The Algal Innovation Centre (AIC) is a Centre for Excellence with test and scale-up facilities, connecting the entire pipeline of algal research from strain selection and improvement, through harvesting and processing, to development of underpinning technology and engineering solutions. The AIC's laboratory is a secure, research grade glasshouse on a sealed foundation with gross floor area of 164 square meters. It has laboratory grade benching, RO water supply environmental data collection, access to autoclaving, centrifugation and a wide range of algae reactors. The AIC also provides training courses for PhD students and postdoctoral researchers.

MICROALGAL-BASED CARBON ENCAPSULATED IRON NANOPARTICLES FOR THE REMOVAL OF MICROPOLLUTANTS FROM WASTEWATER

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ABSTRACT

Hydrothermal carbonization (HTC) is a low-impact thermochemical process[1] that can be used to produce microalgal-based carbon encapsulated zero-valent iron nanoparticles[2] (ME-nFe). Due to the high sorption capacity and the reducing power of this particular adsorbent Me-nFe can be used to remove micropollutants from wastewater, providing a new option for their removal that still need to be optimized in most wastewater treatment plants[3]. Here we present a laboratory-scale adsorption study focusing on the removal of specific organic pollutants: Amisulpride (AMS), Ofloxacin (OFX), Metoprolol (MET), Sulfamethoxazole (SMX), Clarithromycin (CLM), Gabapentin lactam (GBP), Propyphenazone (PHP), Carbamazepine (CBZ), Irbesartan (IRB) and Diclofenac (DCF) from aqueous solutions made with ultrapure water. The effect of different doses of ME-nFe (0.1, 1, and 3 g/L), and contact time (2 and 10 minutes) were evaluated to treat solutions containing all the compounds at 1.5 ug/L and 500 ng/L. Samples properly filtered at 0.2 µm, were stored in autosampler vials and were then analyzed by UPLC-MS. Considering all the compounds, the best results were obtained using a Me-nFe concentration of 1 g/L with two minutes of contact time regardless of the level of contaminants. In the 500 ng/L test, Diclofenac, Ofloxacin, Clarithromycin and Irbesartan were removed efficaciously (94 ± 1 %, 96 ± 1 %,83 ± 3 %,97 ± 1 %, respectively); Amisulpride, Metoprolol, Sulfamethoxazole, and Carbamazepine concentrations were halved (47 ± 4 %, 56 ± 3 %, 49 ± 3 %, 64 ± 1 % respectively). However, the removal was not adequate for Gabapentin lactam and Propifenazone (26 ± 6 %, 22 ± 5 %). Pearson correlation was performed on the 500 ng/L test to understand if a linear correlation could be detected between the experimental data (removal efficiency) and the main chemical-physical characteristics of the pharmaceutical compounds. The only significant correlation was found between the removal efficiency and the hydrophobicity of the compounds (r=0.73 pvalue=0.015).

Keywords
Microalgae; Micropollutants; Adsorption; Hydrothermal carbonization

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BIOGRAPHY

Since 2018 he works at Università degli studi di Milano Bicocca, focusing on alternative processes to treat urban wastewater using microalgae and microalgae derived nanoparticles. During the last years he was involved in the active management of a microalgal pilot plant (raceway), microalgal cultivation at pilot and lab-scale, lab-scale production and characterization of microalgal-derived nanoparticles through the Hydrothermal carbonization. Recently, he focused on the detection of micropollutants in wastewaters through UPLC-MS and the development of alterntaive treatments tecniques.

He was a research participant within the following competitive projects:

IMAP: “Integration of microalgal based processes in wastewater treatment”, funded by Cariplo Foundation, working in lab-scale testing and dissemination activities.

PerFORM WATER 2030,” Platform for Integrated Operation Research and Management of Public Water towards 2030”, funded within the framework of the European Regional Development Fund 2014-2020 and the Lombardy Region call "Accordi per la Ricerca e l'Innovazione", working in lab-scale testing as PhD student.

ACIDOPHILIC MICROALGAE FOR THE TREATMENT AND VALORIZATION OF BUTTERMILK

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ABSTRACT

Buttermilk is a dairy processing by-product with high organic content (COD 10,000-14,000 mg/L), chiefly made of lipids, lactose and proteins, high organic nitrogen concentration (125-200 mg Total N/L) and low pH, quite rich also in phosphorus. It seems thus a good culture medium for acidophilic, mixotrophic or heterotrophic organisms which should uptake nutrients and organic matter producing an effluent more suitable for a conventional wastewater treatment. *Euglena gracilis* is a protozoan, able to grow in autotrophy, mixotrophy and heterotrophy. It can produce and accumulate paramylon, a β-glucan valuable for many applications in the fields of nutrition and cosmetics. *Galdieria sulphuraria* is a thermoacidophilic red microalga whose optimal pH is below 2. Its biomass is particularly rich in proteins, carbohydrates, and pigments, including phycocyanin.
E.gracilis was cultivated in lab reactors (3.5 L) in batch for 24-72 h, at room temperature (22-26 °C) to maximize the production of biomass and paramylon and to limit contamination by bacteria, fungi and molds, which was too high in repeated batch mode. The optimal pH resulted to be 3.5 and the best dilution of buttermilk in tap water 1:5. The optimal duration of batch cultivation was 48 hours. The growth rate in mixotrophy (3.13 to 7.8 mg/L/day) was much higher than in heterotrophy (2.4 mg/L/day). The removal efficiencies of COD, Total N and Total P in mixotrophy were 32 ÷ 48%, 34 ÷ 45% and 11 ÷ 15%, respectively, and the maximum density, at 48 hours, was 0.6 ÷ 0.8 g TSS/L. The same dilution was optimal for *G.sulphuraria* which was grown in mixotrophy in flasks (100 mL, pH 1.8, 37 °C). The density of *G.sulphuraria* was 1.5 g TSS/L after 70 hours and 2 after 160 hours without any nutrient addition. The addition of nitrogen led the density to 2 and 3.5 g TSS/L after 70 and 160 hours, respectively. The removal efficiency of total carbon was 40% and raised to 70% after nitrogen addition. The mean phycocyanin content was 6.5% w/w.
The presented data indicate that *E.gracilis* and *G.sulphuraria* can be efficiently used for the valorisation of buttermilk.

Keywords
Euglena gracilis, Galdieria sulphuraria, buttermilk, biomass production, removal efficiency

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BIOGRAPHY

Master degree in Agricultural Sciences at Milan University, PhD in Sanitary Engineering at Politecnico di Milano, post-doc on treatment and reclamation of textile dyeing wastewaters at Politecnico di Milano. Currently Associate Professor at University of Milano Bicocca, charged for the courses of Environmental Impact Assessment (MD in Environmental Sciences and Technologies) and of Applied Ecology (BD in Biological Sciences). Member of IWA (International Water Association), ORGAGEC, International Scientific Board of IMU (Intelligences des Mondes Urbains) and of several editorial boards and scientific committees. Author or co-author of 74 scientific papers (Scopus) and over 100 presentations/posters to scientific congresses

COMPANY PROFILE

The Department of Earth and Environmental Sciences (DISAT) has been the first department of the Università degli Studi di Milano Bicocca, started in 1993 as part of Università degli Studi di Milano and become an autonomous institution in 1998. Research activities at the Department of Earth and Environmental Sciences (DISAT) focus on environmental evolution and on the dynamics of planet Earth. Researchers, teachers and technicians belong to diverse disciplines, including geology, chemistry, biology and physics.

REMEDIATION OF INDUSTRIAL CO2 EFFLUENT FROM A NICKEL REFINERY BY ALGAE

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ABSTRACT

In the race to net zero carbon emissions there is no single magic bullet, and a range of different technologies will be needed to achieve this challenging goal. The RICE project (Reducing Industrial Carbon Emissions) aims to take the leading cutting-edge technologies from the laboratory and demonstrate viability at industrial scale. This will provide valuable process data for scale-up, realistic estimates of CAPEX and OPEX, and de-risk the technologies ready for industry to adopt. One of the technologies being demonstrated for carbon capture and utilization is algae technology. A novel commercial scale algae photobioreactor has been deployed to the Vale Europe Ltd. Nickel refinery in Clydach (Wales, UK), see Figure 1. The reactor is fed using effluent CO2 from the nickel process and is being used to grow a variety of different algal species. Co-location on the industrial site brings inherent advantages. This paper will describe the reactor deployed, highlight the benefits of co-location, discuss the productivity of the reactor, and discuss the refinement of the algae into products using membrane based downstream processing. The algal facility can produce refined protein, lipids, and carbohydrates all capable of being transformed into animal feeds and bulk chemicals. In addition, a range of high value pigments have been isolated at both food grade and analytical grade from Spirulina and Porphyridium species. Thus, in our synergistic process, we are generating a circular economy that creates value by recycling waste materials to new products in a cost-effective sustainable process that helps mitigate climate change.

Keywords
Industrial, commercial, algae production, biorefinery

Figure 1: The algal photobioreactor deployed at Vale Europe Ltd.

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BIOGRAPHY

Professor Darren Oatley-Radcliffe is a chemical and bioprocess engineer at the Energy Safety Research Institute (ESRI) at Swansea University. He is a Chartered Chemical Engineer (C.Eng), elected fellow of the Institution of Chemical Engineers (FIChemE) and elected fellow of the Higher Education Academy (FHEA). He is highly experienced in the design, development and scale-up of chemical and biological processes and has extensive industrial experience gained in oil and gas, the pharmaceutical, and fine chemical industries. His research interests lie in the design and application of novel technologies and processes for water treatment (including desalination), water recycling and re-use, and algae processing. He is recognised internationally as an expert in membrane nanofiltration. He has published several book chapters, around 50 articles in refereed scientific literature and sits on the editorial boards of several international journals. He has chaired and delivered lectures at numerous international conferences and has received several significant awards: most notably he was awarded the title of ‘Vanguard’ at the GSK CEO Sustainability Awards 2009 for his contribution to a novel pharmaceutical production facility.

ALGAL INNOVATION CENTER AT UNIVERSITY OF CAMBRIDGE

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ABSTRACT

Scalability is one of the main bottlenecks that hinder the transition to the exciting future of algal biotechnology. The Algal Innovation Centre (AIC) is a Centre of Excellence with test and scale-up facilities for the algal sector, which connects the entire pipeline of algal research: from strain selection and improvement, to development of engineering solutions, training, and delivering advice to agencies and companies.

The AIC is a 164 m^2 secure research laboratory-grade glasshouse, licensed to grow non-native algal species and GM organisms. The AIC houses a wide range of algal growth and harvesting equipment. These include lab-scale setups (controlled temperature/light flask incubator and laminar flow hood), pilot-scale setups (Varicon Aqua Ltd 1L & Phyco-Lift 2 x 60L bioreactors, 10L vertical polycarbonate airlift bioreactors, 5-15L hanging-bag bioreactors, 2 x 150L horizontal rotary bioreactors) and harvesting equipment (flocculation, Membranology Ltd membrane harvester, 4x1L centrifugation) which can facilitate a wide range of research projects.

We can support projects with a variety of analytical methods including lipid profiling (using GC-MS), Coulter cell counting, flow cytometry, pigment profiling (HPLC-PDA), GC-MS Metabolomics, FT-IR fingerprinting, cell culture collection (temperate and polar species), nutrient analysis and metabarcoding and metagenomics sequencing.

Since its opening in 2016, the AIC has delivered several research and training outputs for UK (BBSRC, NERC, Leverhulme Trust, the Royal Society) and EU (Horizon2020, Marie Curie, European Space Agency and EU EIT-Food) funders. We also deliver training courses for both academic and industrial researchers at all levels, as well as wider outreach projects.

The AIC poster will highlight take home findings on challenges and solutions of growing algal culture outside of the lab environment.

Keywords
Algae, scale-up, bioremediation, photobioreactor, harvesting

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BIOGRAPHY

Kostas Papadopoulos is a researcher in the Algal Innovation Center (Department of Plant Sciences, University of Cambridge).

Kostas completed his Ph.D. and his undergraduate studies at the department of Chemical Engineering, at the University of Patras. His research interests lie in the area of circular economy and algal biotechnology. Much of his work has been on photosynthesis-based waste bioremediation and recovery of energy and high added value products. He has explored using modelling and other data analysis tools to develop and design scalable algal processes.

COMPANY PROFILE

The Algal Innovation Centre (AIC) is a Centre for Excellence with test and scale-up facilities, connecting the entire pipeline of algal research from strain selection and improvement, through harvesting and processing, to development of underpinning technology and engineering solutions. The AIC's laboratory is a secure, research grade glasshouse on a sealed foundation with gross floor area of 164 square meters. It has laboratory grade benching, RO water supply environmental data collection, access to autoclaving, centrifugation and a wide range of algae reactors. The AIC also provides training courses for PhD students and postdoctoral researchers.

IMPROVING MICROALGAE PRODUCTION AND CIRCULARITY IN RACEWAY PONDS

Pinto, B. [1,3]

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ABSTRACT

Facing the increment predicted for the upcoming years in both human population and, consequently, food requirements, there is an urgent need to improve the productivity and sustainability of food and feed sources. Aware of the current problematic, the project ALGAVALOR (MicroALGAe: integrated production and VALORization of biomass and their diverse applications) targets microalgae as an important and versatile resource. In this sense, the project aims to improve microalgae production and increase the valorisation of the biomass and its extracts in different applications, such as for the feed, food, cosmetic and agriculture sectors. Necton S.A., a partner in ALGAVALOR, is an experienced microalgae producer, that participated in two pertinent tasks of the project to achieve a more effective and sustainable microalgae production. The two tasks were focused on Technologic Revamping and Circular Economy, respectively.

In the first task, we tested the use of LEDs in raceway ponds to artificially extend the light period and prevent a bioenergetic shift of the cells from photosynthesis to a respiration-only stage. The use of LEDs resulted in higher nighttime growth which increased the productivities of target metabolites. During the day, the impact of the LEDs was higher under lower sunlight irradiances and was worthwhile only after the culture surpassed 0.5 g dry weight L^{-1} (0.1 m depth).

In the second task we focused on sustainability and tested two different strains isolated near a swine farm (*Chlorella* sp. and *Scenedesmus* sp.) for growth in swine wastewater, with the objective of lowering the freshwater footprint and uptake the excess nutrients present in the raw swine wastewater. A reduction of 72 % of ammonia (NH_4^+) and near 100 % reduction of phosphates (PO_3^{4-}) was achieved during the initial 10 days of operation in a 110 m^3-industrial raceway. After 27 days of cultivation, more than 70 kg of wet paste (dry weight around 20%) was harvested, frozen and delivered to another partner to test its usage as a biofertilizer.

Keywords
LED light, raceway, *Nannochloropsis*, Swine wastewater, bioremediation

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BIOGRAPHY

Bruno Pinto has a bachelor in Biology and a Master degree in Biotechnology. Since his master he developed an interest in microalgae and their commercial value and applications. Being the focus of his thesis, the treatment of swine wastewater using microalgae, with the results being presented in a poster intitled bioremediation of swine wastewater with locally isolated microalgal strains. After this first contact with the microalgae world, he has responsibilities in several P2020 projects such as ALGAVALOR, SizeMatters and MARemPÓ, all focused in three main hot topics: circular economy, increasing value in residues from other industries, and sustainability.
Counting with three years of experience in microalgae culture, up-scaling from balloons to industrial systems, inoculum maintenance, industrial microfiltration of cultures using membrane systems, extraction of polysaccharides from brine water, using ultrafiltration membranes, among other skills. Since the beginning Bruno is focused in his work, hardworking, and proactive.

COMPANY PROFILE

Necton is the oldest company in Europe specialised in the cultivation and commercialisation of microalgae. After several years studying microalgae up to pilot scale, a small group of young entrepreneurs established Necton in 1997. As microalgae need plenty sunlight and water, the company was set on an ideal location to grow these organisms – the natural park of Ria Formosa in the south coast of Portugal. During the years the company acquired an extensive knowledge in cultivating not only marine, but also freshwater and hypersaline species. Necton's current portfolio includes over 30 species, such as Nannochloropsis oceanica, Tisochrysis lutea (formerly Isochrysis galbana Tiso), Phaeodactylum tricornutum, Tetraselmis chui, Porphyridium cruentum, and Skeletonema costatum, which are sold to various markets, including Aquaculture and Cosmetics. Biomass is produced in ~100 m3 of horizontal tubular photobioreactors, which are planned to be expanded to ~150 m3 in the next year. Necton's growth is also supported by a continuous R&D effort, currently with 13 national and European-funded research projects, including the recently approved Horizon Europe project "REALM", which Necton coordinates. The company's history, experience and constant collaboration with universities, research organizations and other companies, have made Necton one of the leading European companies in Microalgae Biotechnology.

MICROALGAE AND THEIR POTENTIAL USE IN NITRATE REMOVAL

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ABSTRACT

Eutrophication is a major problem in water bodies all over the world in which nitrate plays a prominent role. Due to increased nitrate pollution, research is being carried out globally to utilize microalgae for bioremediation. Hence, our first study was conducted to investigate the effects of various concentrations of nitrate on two eukaryotic green microalgae, *Chlamydomonas* sp. MACC-216 and *Chlorella* sp. MACC-360. Both microalgae were cultivated in modified Tris-Acetate-Phosphate (TAP) media prepared with three different concentrations of sodium nitrate — 5 mM, 10 mM, and 15 mM. Both microalgae were capable of entirely eliminating nitrate when cultivated at 5 mM nitrate. It was also observed that high nitrate concentration (15 mM) led to an increase in lipids in *Chlamydomonas* sp. MACC-216, while *Chlorella* sp. MACC-360 did not show any lipid accumulation. Furthermore, *Chlamydomonas* sp. MACC-216 and *Chlorella* sp. MACC-360 were cultivated in synthetic wastewater, where both microalgae grew well and showed significant nitrate removal capacity. Moreover, *Chlamydomonas* sp. MACC-216 performed better than *Chlorella* sp. MACC-360 in nitrate removal from synthetic wastewater. We further investigated *Chlamydomonas* sp. MACC-216 in our second study to assess its total nitrate removal capacity under combinations of three light colours (referred to as blue, red, and white light) and three light intensities (50 μmol m^{-2} s^{-1}, 100 μmol m^{-2} s^{-1}, and 250 μmol m^{-2} s^{-1}), where both light colour and intensity were found to affect the performance of microalgae in nitrate elimination.

Keywords
Nitrate, *Chlamydomonas*, *Chlorella*, bioremediation, light

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BIOGRAPHY

Vaishali Rani is a PhD student at the Biological Research Centre in Szeged, Hungary. With no prior experience in the field of microalgae, she started her journey with microalgae when she started her PhD. For the past three years, she has been working on the relationship between microalgae and nitrate. According to her, it is just the beginning, and she wants to continue learning about microalgae in the future and then employ them for wastewater treatment.

BIOREMEDIATION OF HYDROPONIC GREENHOUSE EFFLUENT AND BIOAGRICHEMICAL APPLICATIONS WITH MICROALGAE: A CASE STUDY IN FINLAND

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ABSTRACT

Agriculture is the main cause of nutrient pollution in the Baltic Sea Region, as well in Finland, which causing severe impacts on the water quality, altering ecosystems and causing eutrophication[1]. Finland's long, cold winters mean that vegetable and plant production commonly occurs in greenhouses using hydroponic farming, which offers better water management and less use of mineral fertilizers. Although the farms operate a closed-loop hydroponic cultivation, there are still drawbacks on management and reuse of the nutrient solution. By all means, effluents from the hydroponic greenhouses are still overloaded with nutrients[2]. An action is vital to identify environmentally friendly and cost effective solutions towards reducing nitrogen and phosphorous loads originating from agricultural activities. A promising approach to deal with agricultural waste streams relies on the recirculation of these streams for the cultivation of photosynthetic organisms. From perspective of circular bioeconomy and growing trends in sustainable agriculture, the produced biomass can also be considered for bio-agrichemical applications in a biorefinery concept.

In this study, hydroponic greenhouse effluents were used for cultivation of the microalga *Tetradesmus obliquus* to demonstrate its bioremediation performance and highlight the potential of biomass transformed from the wastewater. Cultivation trials were performed in a tubular photobioreactor under natural light and supplemental red and blue LED lights in a fully controlled greenhouse environment. The culture achieved a maximum DW of 6.2 g L^{-1} and 100% removal efficiency of N and P. At the exponential phase of growth, the biomass had a protein content of 45-50% of DW and a carbohydrate fraction representing 20% of DW. At the stationary phase, the carbohydrates increased to 60% of DW. The PUFA content was higher during the exponential phase, representing more than 65% of total fatty acids. Throughout the cultivation, the predominant carotenoids were Lutein and β-carotene.

The microalga *Tetradesmus obliquus* was demonstrated to be a suitable candidate for an algal biorefinery designed for wastewater treatment and a multi-product pipeline. Ongoing and planned studies focus on multi-benefit valorization approaches for agricultural re-use of the biomass as bioagrichemicals e.g. biostimulants, biopesticides.

Keywords
Microalgae, Nordic region, nutrient uptake, hydroponic effluent, bioagrichemicals.

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BIOGRAPHY

Sema Sirin (Ph.D) is a researcher at University of Turku, Finland. She has a background in algal bio-refinery, especially in the downstream processes and green wastewater treatment technologies. She is also the project manager of "NordAqua" Nordic Center of Excellence funded by NordForsk (www.nordaqua.fi), which focuses on unlocking the value of Nordic micro and macroalgae for the benefit of society and blue bioeconomy.

COMPANY PROFILE

The University of Turku (UTU), Finland´s second largest multidisciplinary university, is an internationally competitive research-led university whose operation is based on high-level research. The UTU is recognized for the quality of research, teaching, and excellent support services.

COMPARATIVE GROWTH STUDY AND BIOMASS VALORIZATION OF ISOLATED MICROALGAE UPON COMMON MEDIA AND HYDROPONIC WASTEWATER CULTIVATION

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ABSTRACT

Several microalgal species can utilize nutrient elements of agricultural wastewater for their growth; thus having the potential to be a reliable and economically viable solution for bioremediation. Therefore, wastewater offers a promising option for producing microalgae biomass and the related high added value products[1]. In the present study, effluent water from hydroponic cultures was used for isolating naturally present microalgal species that could possibly be of biochemical interest and play a crucial role in wastewater treatment. This process was roughly divided in five successive steps: size separation, general enrichment and three separation stages. Briefly, a wide variety of methods were used, including serial dilution, agar plating, agar streaking and micropipette isolation. With the implementation of the above-mentioned methods, four different strains were isolated and characterized with morphological, biokinetic and molecular taxonomy methods. The microalgal strains were subsequently cultured under steady conditions in both commonly used media and in hydroponic effluent to estimate the potentiality of hydroponic wastewater as an alternative medium for microalgae as well as evaluate the bioactivity of the biomass obtained. Thus, extracts were produced by freeze-dried biomass of the selected microalgae strains cultured in both regimes with effective cell disruption protocols. Characterization of the produced extracts was carried out for evaluating their antioxidant capacity, total phenolic, flavonoid content, pigments as well as protein content. Finally, *in vitro* cytotoxicity assays were assessed on human cell line and cell viability was estimated. The results indicate that when the strains are cultivated using hydroponic effluent exhibit similar and sometimes even higher growth rates and cell densities compared to the control pinpointing their ability to grow effectively hydroponic effluent as a culture medium. Additionally, the study showed that extracts produced by species cultured in wastewater provide new insights into their beneficial role as sustainable feedstock of value-added extracts for many applications.

Keywords
Hydroponic effluents, wastewater, microalgae

References
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OPTIMIZING PROTEIN CONTENT IN MICROALGAE UNDER FED-BATCH CULTIVATION USING WASTEWATER

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ABSTRACT

Microalgae biomass produced using waste streams as feedstock has been proposed as a promising alternative source of protein. This study aimed to optimize protein content in microalgae biomass production using municipal wastewater as a growth medium. A fed-batch mode experiment under laboratory conditions was designed for the cultivation of the freshwater chlorophytes, *Lobochlamys segnis* F12, and *Klebsormidium flaccidum* NIVA-CHL80. The generated biomass harvest and medium supply rates were controlled by a daily biomass concentration adjustment using optical density. The fed-batch regime was repeated 6 times to monitor the biomass production, protein content and nutrient uptake. On average, the biomass production of *L. segnis* and *K. flaccidum* was 238 ± 17 and 278 ± 35 mg L^{-1} day^{-1}, where the protein content was 64 ± 10 and 44 ± 4%, respectively. For *L. segnis*, the average replacement of the medium was 21 ± 3% day^{-1}, and the daily loads of NH_4^+ (9.2 ± 0.9 mg L^{-1}) and PO_4^{3-} (5.2 ± 0.9 mg L^{-1}) were completely removed within 24 hours. Whereas for *K. flaccidum*, the average medium replacement was 24 ± 4% day^{-1} with complete removal of the NH_4^+ load (13.8 ± 3.2 mg L^{-1}). However, this strain showed a low and fluctuating removal (average 24 ± 14% day^{-1}) of PO_4^{3-} that partially accumulated in the medium over time. Our study demonstrated that the fed-batch cultivation with the appropriate initial biomass concentration and the medium replacement supported the microalgae production with high and stable protein content.

Keywords
Protein, wastewater, nutrient removal, cultivation, chlorophytes

PRESENTER INFORMATION

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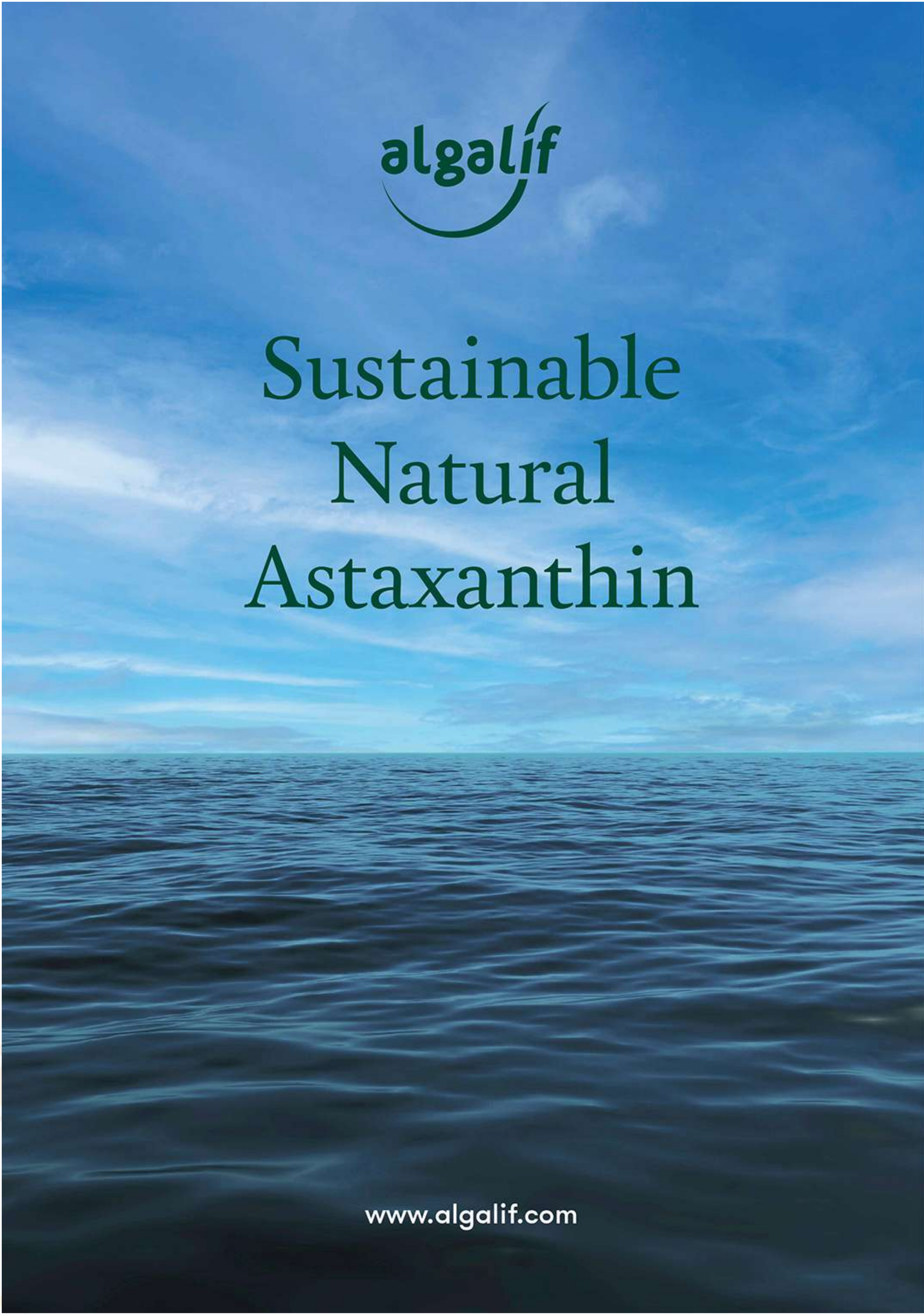
BIOGRAPHY

Research Scientist. Holds a PhD in Ecology from University of South-Eastern Norway. Has more than 10 years of experience with microalgae research, including the field of photosynthesis and fatty acid production.
Research interest is microalgae cultivation in the context of circular economy. Currently participating in the national and international projects related to the use of recycled nutrients for microalgae production.

COMPANY PROFILE

NIBIO – Norwegian Institute for Bioeconomy is one of Norway's largest institutes, which leads research on multifunctional agriculture, rural development, plant sciences, environmental protection, and natural resource management. NIBIO's Division of Environment and Natural Resources has a strong focus on resource management, bioprocesses, renewable energy, nutrient recirculation, and sustainable end products as part of the circular economy.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