ALGÆUROPE2021

07-10·DECEMBER·ONLINE

ABSTRACT BOOK

Organizers:

DLG Benelux

EABA EUROPEAN ALGAE BIOMASS ASSOCIATION

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SCHOTT glass made of ideas

European Union - Young Algaeneers Innovation Awards funded by:

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INDEX

WELCOME LETTER EABA

Dear participants

Welcome to this 2021 edition of the EABA: ALGAEUROPE Conference.

On behalf of the EABA Steering Committee, the Scientific Committee and the Industrial Committee, we are pleased to welcome you for this 2021 edition of the AlgaEurope conference.

This strange period will make, once again, that we will not be able to find ourselves physically in the same place. This configuration, as we all know, has consequences on several levels. A first consequence is a whole new logistics that we are still learning. And even if the EABA, by organizing several webinars during the year 2021 (7 technical, 3 flash and 3 conferences), has acquired some experience, the fluidity of the interventions, especially for a hundred, remains a challenge. Another consequence, which impacts all the participants, is the absence of “off” moments, which are so important, and which make the value of these conferences. Finally, a consequence on the participation rate, precisely because of the degraded configuration of the event.

EABA team continues and organize Algae Europe in partnership with DLG largely thanks to the quality of the speakers and the abstracts received. A hundred presentations are planned this year with a clear renewal of approaches in known themes. This edition will also be an opportunity to present a few round tables, around experts from different backgrounds. A little more business and market-oriented discussions in addition to the usual science sessions.

EABA intends to continue its close collaboration with the European Commission and the European strategy in general with two sessions dedicated to the DGs who support us but also through a session orchestrated by Blue Invest. Finally, we would like to apologize for the summaries left pending and would like to stress that the choices made are only a mirror of the growing success of the AlgaEurope conference.

After Florence (2014), Lisbon (2015), Madrid (2016), Berlin (2017), Amsterdam (2018) and Paris (2019) and 2020 web-based by videoconference. The 2021 edition will again be in digital form but will nevertheless reflect the dynamism of the algae ecosystem.

Wish you all an intense 2021 Algae Europe Conference as the a unique opportunity for learning and sharing!

Jean-Paul Cadoret

President

Vitor Verdelho

General Manager

WELCOME LETTER DLG

Dear Algae professionals,

Hereby we warmly welcome you to the AlgaEurope online 2021 Conference! Due to the current uncertainties with regards to Covid-19 this edition takes place in an online format. We are optimistic that AlgaEurope 2022 will take place physically in Rome.

Since years AlgaEurope is one of the most global comprehensive conferences about science, technology and business in the Algae Biomass sector organized by industry professionals.

One of the key success factors of AlgaEurope is the close cooperation between EABA - European Algae Biomass Association and DLG Benelux.

This years' program of AlgaEurope is once again very comprehensive and more than 70 speakers will share their knowledge and expertise within the field of Algae. Some 11 sessions spread out over 4 days will give the participants a full update on the Algae industry.

This years' edition also includes the nomination for the European Union - Young Algaeneers Innovation Award, funded by the European Union. This contest is open to young people from companies, research institutes and other organizations involved in the Algae Biomass sector in Europe (EU and EEA countries).

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 the European Commission for funding the Innovation Awards, a sustainable blue economy is essential to achieving the objectives of the European Green Deal and ensuring a green and inclusive recovery from the pandemic, funding the Innovation Awards underlines this objective.

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 2021 and we hope to see all of you live again next year!

Best regards,
DLG Benelux B.V.

Kuno Jacobs
Managing Director

WELCOME LETTER EUROPEAN COMMISSION

Dear AlgaEurope participants,

Algae in all their forms represent a largely untapped resource in Europe. They can be used to produce food, feed, pharmaceuticals, bioplastics, fertilisers, biofuels and more. Algae do not only provide healthy and low-carbon alternatives to the existing products. While growing, algae consume carbon, nitrogen and phosphorus – thus regenerating and decarbonizing ocean ecosystems.

The potential of algae is recognised in various EU initiatives such as the Sustainable Blue Economy strategy, Farm to Fork, or Strategic Aquaculture Guidelines. In order to increase the sustainable production, consumption and use of algae and algae-based products in the European Union, the European Commission is preparing a comprehensive cross-sectoral EU algae initiative. Because of their small carbon and environmental footprint, increasing the production and use of algae will help achieve the objectives of the European Green Deal, the transition to a green, circular and carbon-neutral EU.

To develop a thriving regenerative algae industry requires developing and investing in innovative technologies. Whether this is in algae cultivation, production of new products or restoration of marine ecosystems, the objective is to boost algae production, create new green jobs and businesses in the blue economy.

By funding the ***European Union - Young Algaeneers Innovation Award***, the European Commission wishes to highlight its commitment to the sector and offer entrepreneurs and academia the possibility to contribute to this transformation by showcasing their innovative services and products that could make a positive impact on ocean health and the new blue economy.

Felix Leinemann

Head of Unit Blue Economy Sectors, Aquaculture and Maritime Spatial Planning, European Commission, DG Maritime Affairs and Fisheries

Putting the Blue into the Green

The European Commission is working for healthy seas and a sustainable use of the oceans and of marine resources in the EU and worldwide.

The EU's new approach to a sustainable blue economy will help to deliver both the European recovery and the European Green Deal. The objective is a strong, sustainable, resilient and climate neutral model for the blue economy.

The Commission has adopted a new vision for EU aquaculture – Blue Farming – and is preparing an EU algae initiative to unlock algae potential in Europe and to enable the development of a regenerative EU algae sector.

Targeted actions, like the #TasteTheOcean campaign with top chefs across Europe are encouraging consumers to buy and enjoy sustainable fish and seafood.

Follow us:

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#BeGreenGoBlue #EUGreenDeal #TasteTheOcean

European Commission

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BIOASIS GRAN CANARIA

The Blue biotechnology and Aquaculture Platform (BIOASIS Gran Canaria), combines the R&D capabilities of the local research centres with the support of the public bodies involved in the development of the business and innovation activities related to the sector

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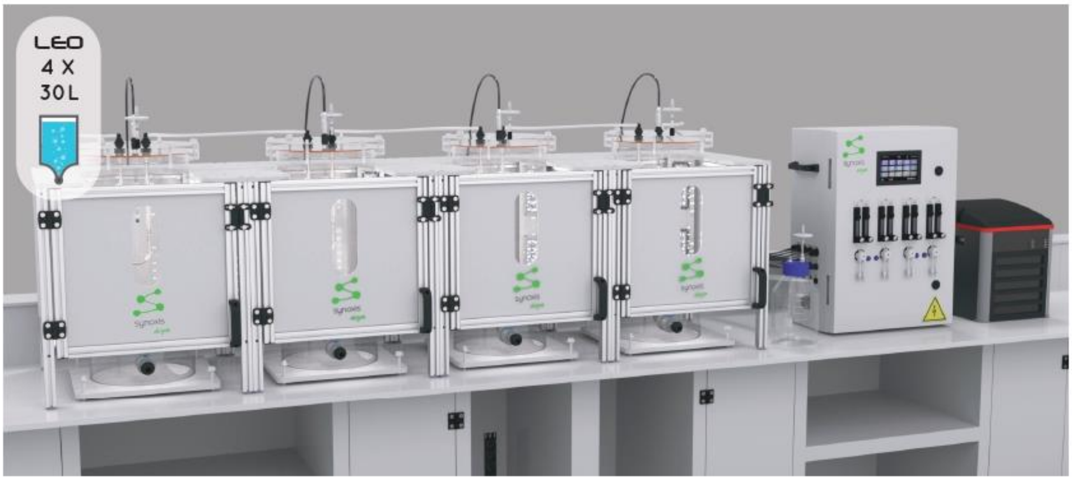

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AlgaEurope 2021 Conference Program

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

All times are mentioned in Central European Summer Time (CEST)

Conference Day 1 - Tuesday 7 December 2021

09:30 am - 10:00 am: Conference Opening

Jean-Paul Cadoret - EABA President
Vitor Verdelho - EABA General Manager

Morning Chairman: Sammy Boussiba

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

Mario Tredici (University of Florence, Italy): Microbes: Food for the Future

10:30 am - 12:10 pm: Scientific Session 1: Algae Biology and Production

10:30 am - 10:45 am: Matteo Ballottari: CO2 supply modulates lipid remodeling, photosynthetic and respiratory activities in chlorella species

10:45 am - 11:00 am: Jiri Masojídek: Physiological changes in the outdoor culture of chlorella g120 during trophic conversion from heterotrophic to phototrophic growth regime

11:00 am - 11:15 am: Javier Dávila: Increasing vertical mixing in HRAP to enhance photosynthetic light integration

11:15 am - 11:30 am: Gergely Ernő Lakatos: Psychrophilic Monoraphidium sp. B cultivation in outdoor thin-layer raceway pond for high-value products

11:30 am - 11:40 am: COFFEE BREAK

11:40 am - 11:55 am: Giovanni Rusconi Clerici: e-Pond®. Bioelectroactive filter helps growing food-grade microalgae on recycled streams rich in organic forms of nutrients

11:55 am - 12:10 pm: Fritz Wintersteller: Why tubular glass photobioreactors?

All imes are mentioned in Central European Summer Time (CEST)

Conference Day 1 - Tuesday 7 December 2021

12:10 pm - 12:40 pm: Scientific Session 2: Biomaterials from Algae

12:10 pm - 12:25 pm: Pablo Alvarez: Chlorella vulgaris high-glucose syrup as carbon feedstock for pha-producing bacteria

12:25 pm - 12:40 pm: Gordon Brinitzer: Fatty acid based biopolymers from Chlorella vulgaris biomass for its application in sporting goods

12:40 pm - 02:00 pm: Lunch Break

Afternoon Chairman: Luis Costa

02:00 pm - 03:00 pm: Scientific Session 3: Technology and Processing

02:00 pm - 02:15 pm: Eleonora Sforza: LED technology development to maximize energy conversion efficiency: application of flashing blue-red light in continuous photobioreactors

02:15 pm - 02:30 pm: Fernando Pagels: Continuous pressurized solvent extraction in cyanobium sp. Pigments bioprocessing

02:30 pm - 02:45 pm: Francesca Casagli: Optimizing algal productivity and nitrogen recovery from digestates by adapting solid retention time and alkalinity

02:45 pm - 03:00 pm: Daniel Figueiredo: Electrocoagulation to harvest and enrich Nannochloropsis oceanica biomass for agricultural applications

03:00 pm - 03:15 pm: Virtual Tour 1: DUPLACO

03:15 pm - 03:45 pm: Thematic Session 1: Training

03:15 pm - 03:30 pm: René Wijffels: A green generation with energy for the future

03:30 pm - 03:45 pm: Ira Levine: The Algae Foundation's workforce development, education and training platforms

03:45 pm - 03:55 pm: COFFEE BREAK

All times are mentioned in Central European Summer Time (CEST)

Conference Day 1 - Tuesday 7 December 2021

03:55 pm - 04:25 pm: Thematic Session 2: Algae Initiatives in the EU

03:55 pm - 04:10 pm: Vincent Doumeizel: The need for global regulations in the seaweed industry

04:10 pm - 04:25 pm: Adrien Vincent: The case for Seaweed investment in Europe

04:25 pm - 04:40 pm: Virtual Tour 2: REBECA Project

04:40 pm - 06:10 pm: Scientific Session 4: Bioremediation with Algae

04:40 pm - 04:55 pm: Marta Sá: Capturing and re-using CO2 using Deep Eutectic Solvents and Microalgae

04:55 pm - 05:10 pm: Alice Ferreira: Combination of photo-Fenton oxidation with Microalgae Cultivation for Improved Bioremediation of Piggery Wastewater

05:10 pm - 05:25 pm: Valeria Mezzanotte: Treatment and valorizaton of a dairy by-product by euglena gracilis

05:25 pm - 05:40 pm: Supattra Maneein: Compositional changes in spring- and summer-collected Sargassum muticum after methanol extraction or water washing

05:40 pm - 05:55 pm: Bruno Assis Pessi: Is microalgae production under greenhouse beneficial?

05:55 pm - 06:10 pm: John Benemann: Microalgae Production and Utilization in the USA - Recent Developments

06:10 pm - 06:25 pm: Virtual Tour 3: SCHOTT AG

06:25 pm - 06:40 pm: Closure of the Conference Day

All times are mentioned in Central European Summer Time (CEST)

Conference Day 2 - Wednesday 8 December 2021

Morning Chairman: Hywel Griffiths

09:30 am - 10:00 am: Keynote Speaker Day 2

Céline Rebours (Morforsking SA, Norway): Seaweed in Salmon-Driven Integrated Multi-Trophic Aquaculture (IMT)

10:00 am - 12:40 pm: Scientific Session 5: Algae for Food and Health

10:00 am - 10:15 am: Thomas Lafarga: Pilot-scale production of A. platensis as a source of functional and bioactive proteins

10:15 am - 10:30 am: Stig A. Borgvang: From fundamental algae research to applied industrial practice - algae to future project

10:30 am - 10:45 am: Fabian Abiusi: Algae proteins: Is Galdieria the new Spirulina?

10:45 am - 11:00 am: Anabela Raymundo: Strategies to engage the consumers improving the sensory profile of microalgae biomass

11:00 am - 11:15 am: Gabriel Bombo: Isolation of novel microalgae mutants with improved protein contents and pigments for food applications

11:15 am - 11:25 am: COFFEE BREAK

11:25 am - 11:40 am: Lena Stiefvatter: The microalgae Phaeodactylum tricornutum as an omega-3 fatty acid and carotenoid source- High bioavailability of nutrients in healthy volunteers

11:40 am - 11:55 am: Olivier Lépine: Phycocare to decrease oncology side-effects: a first clinical study

11:55 am - 12:10 pm: Dorit Avni: The anti-inflammatory properties of marine microalgae in LPS-induced macrophages

12:10 am - 12:25 pm: Ana Teresa Gonçalves: Algae biomass as potential additives for fish gut health modulation

12:25 am - 12:40 pm: Ana Morao: Algae omega-3s as a sustainable alternative to fish oil

All times are mentioned in Central European Summer Time (CEST)

Conference Day 2 - Wednesday 8 December 2021

12:40 pm - 02:00 pm: Lunch Break

Afternoon Chairman: Gabriel Acien

02:00 pm - 03:00 pm: Thematic Session 3: Industrial Committee Session

A4F - Round Table: Carbon Capture and Utilization for Climate Change Mitigation

03:00 pm - 04:00 pm: Scientific Session 6: Algae Genetics and Improvement

03:00 pm - 03:15 pm: Fengzheng Gao: Improved industrial tisochrysis lutea strains for fucoxanthin and docosahexaenoic acid production

03:15 pm - 03:30 pm: Frederico Perozeni: Metabolic engineering for the production of geraniol in Chlamydomonas reinhardtii

03:30 pm - 03:45 pm: Aneika Leney: Native Mass Spectrometry; the key to unlocking the secrets behind phycobiliprotein production and quality

03:45 pm - 04:00 pm: Mafalda Trovão dos Santos: A non-GMO approach to isolate novel Chlorella vulgaris mutants with higher biomass productivities for food and feed application

04:00 pm - 04:10 pm: COFFEE BREAK

04:10 pm - 05:40 pm: Thematic Session 4: Blue Invest

04:10 pm - 04:25 pm: Panel Discussions, Moderator: Catherine Frideres (Blue Invest)
- Magdalena Andreea Strachinescu - Head of Unit, Maritime Innovation, Marine Knowledge and Investment, DG MARE, European Commission
- Felix Leinemann - Head of Unit, Blue Economy Sectors, Aquaculture and Maritime Spatial Planning, DG MARE, European Commission
- Jean-Paul Cadoret - President, European Algae Biomass Association and Chief Scientific Offier, ALGAMA
- Chris Coyle - Founder, CEO and Futurist, Tethra Advisors and Officers

04:40 pm - 05:40 pm: Pitch Session
Investor Panel: AquaSpark, Astanor Ventures, Faber Blue Pioneers, Blue Horizon Vetures

06:00 pm - 06:15 pm: Closure of the Conference Day

All times are mentioned in Central European Summer Time (CEST)

Conference Day 3 - Thursday 9 December 2021

Morning Chairwoman: Alexandra Mosch

09:30 am - 10:00 am: Keynote Speaker Day 3

Patrick Sorgeloos: Underestimated role of (micro and macro) algae in microbial disease prevention in intensive aquaculture systems

10:00 am - 12:25 pm: Scientific Session 7: Algae Biorefinery and multi-use approaches

10:00 am - 10:15 am: Gabriel Acien: Industrial scale production of microalgae biomass for agriculture and aquaculture related applications

10:15 am - 10:30 am: Daniela Bárcenas Pérez: Sequential and efficient production of fucoxanthin from the diatom Phaeodactylum tricornutum using high performance countercurrent chromatography

10:30 am - 10:45 am: Calvin Lo: Eutectic solvents with tuneable hydrophobicity for lipid extraction from microalgae

10:45 am - 11:00 am: Apurav Koyande: Potential biorefinery approach of chlorella sp. By liquid triphasic flotation system as sustainable downstream process

11:00 am - 11:10 am: COFFEE BREAK

11:10 am - 11:25 am: Christian Kleinert: In situ extraction (milking) of Botryococcus braunii on a semi-technical scale

11:25 am - 11:40 am: Evelyn Ruales: Bioproducts and bioenergy recovery from microalgae biomass treating wastewater: a biorefinery approach

11:40 am - 11:55 am: Caroline Autréau: Lactobacillaceae for seaweed fermentation

11:55 am - 12:10 pm: Marta Bellver Catalá: Cultivation of cyanobacteria in wastewater for multi-resource recovery

12:10 pm - 12:25 pm: Virtual Tour 4: ALGOLINER

12:25 pm - 02:00 pm: Lunch Break

All times are mentioned in Central European Summer Time (CEST)

Conference Day 3 - Thursday 9 December 2021

Afternoon Chairwoman: Liliana Rodolfi

02:00 pm - 03:00 pm: Thematic Session 5: Young Algaeneer's Corner

Chairman: Jean-Paul Cadoret

02:00 pm - 02:05 pm: Agnieszka Paulina Lipinska

02:05 pm - 02:10 pm: Peter Schmedes

02:10 pm - 02:15 pm: Mette Møller Nielsen

02:15 pm - 02:20 pm: Sophie Steinhagen

02:20 pm - 02:25 pm: Pedro Moñino Fernández

02:25 pm - 02:30 pm: Rita Mota

02:30 pm - 02:35 pm: Koen Mulder

02:35 pm - 02:40 pm: Serena Lima

02:40 pm - 02:45 pm: Barbara Guimarães

02:45 pm - 02:50 pm: Julie Billy

02:50 pm - 02:55 pm: Arriana Rizzo

02:55 pm - 03:20 pm: European Union - Young Algaeneers Innovation Award: Prize distribution

03:20 pm - 03:50 pm: Thematic Session 6: Funding and Investors

03:20 pm - 03:35 pm: Information will follow shortly.

03:35 pm - 03:50 pm: Olivier Raybaud (SWEN Capital Partners): Funding innovation to regenerate our ocean

03:50 pm - 04:00 pm: COFFEE BREAK

All times are mentioned in Central European Summer Time (CEST)

Conference Day 3 - Thursday 9 December 2021

04:00 pm - 04:40 pm: Thematic Session 7: Expert Round Table

Chairman: Avigad Vonshak

Speaker: John Benemann, Amha Belay, Michael Borowitzka, Gabriel Acién

Topic: Use of algal biomass: Is there a conflict between the options ? (Food, Feed or environment)

04:40 pm - 06:10 pm: Scientific Session 8: Algae Monitoring, from Nature to Industry

04:40 pm - 04:55 pm: Marta Coelho: Impact of macroalgae harvesting on bioactive compounds

04:55 pm - 05:10 pm: Dorinde Kleinegris: A closer look into the microbiome of microalgae cultures

05:10 pm - 05:25 pm: Aurelie Couzinet-Mossion: Benthic diatoms: How to grow to obtain bioactive lipids?

05:25 pm - 05:40 pm: Nádia Correia: Bioprospection, growth optimization and industrial cultivation of novel microalgae strains obtained from local ponds

05:40 pm - 05:55 pm: Aurélien Parsy.: Growth of marine microalgae and cyanobacteria in artificial industrial effluents supplemented with nutrient rich wastewaters

05:55 pm - 06:10 pm: Bert Groenendaal: AlgaeDemo: Demonstration of large scale seaweed cultivation

06:10 pm - 06:25 pm: Virtual Tour 5: LLDC

06:25 pm - 06:30 pm: Closure of the Conference Day

All times are mentioned in Central European Summer Time (CEST)

Conference Day 4 - Friday 10 December 2021

Morning Chairman: Pi Nyvall

09:30 am - 10:00 am: Keynote Speaker Day 4

Keynote speaker

10:00 am - 11:15 am: Scientific Session 9: Environmental Impact

10:00 am - 10:15 am: Olivier Lépine: CO2 emissions: Paris we have a problem!

10:15 am - 10:30 am: Lea Braud: Unraveling modelling strategies of algae systems: a first step towards improved transparency

10:30 am - 10:45 am: Joseph Pechsiri: Comparative LCA of Novel and Conventional microalgae Reactors

10:45 am - 11:55 am: Scientific Session 10: Algae for Plants

10:45 am - 11:00 am: Flavio Martini: The Potential Use of C. reinhardtii and C. sorokiniana as biostimulants on maize plants

11:00 am - 11:15 am: Gaia Santini: Arthrospira-based biostimulants and their effects on different plants

11:15 am - 11:25 am: COFFEE BREAK

11:25 am - 11:40 am: Ana Álvarez González: Production and assessment of a microalgae biofertilizer from wastewater treatment system

11:55 am - 12:55 pm: Scientific Session 11: Algae Biofilms and EPS Production

11:55 am - 12:10 pm: Andrea Fanesi: Bubbling for light: microalgae biofilms control in PBRs

12:10 pm - 12:25 pm: Yolanda Soriano-Jérez: A coupled cfd-dpm approach to predict microalgae cell adhesion on different materials using a xdlvo-temporal model

12:25 pm - 12:40 pm: Aurélie Pham.: Adaptive Laboratory Evolution for enhanced EPS production in biofilm forming microalgae

12:40 pm - 12:55 pm: Pauline Delran: Extraction and separation of exopolysaccharides from Tetraselmis suecica produced as biofilm

12:55 pm - 01:05 pm: End of the Conference

CONFERENCE DAY 1

ALGÆUROPE2021

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Scientific Session 1:
Algae Biology and Production

ALGÆUROPE2021
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CO2 SUPPLY MODULATES LIPID REMODELLING, PHOTOSYNTHETIC AND RESPIRATORY ACTIVITIES IN *CHLORELLA* SPECIES

Ballottari M[1].

Cecchin M[1], Paloschi M[1], Busnardo G[1], Cazzaniga S[1], Cuine S[2], Li-Beisson Y[2], Wobbe L[3]

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[3]*Bielefeld University, Center for Biotechnology (CeBiTec), Faculty of Biology, Universitätsstrasse 27, 33615, Bielefeld, Germany.*

ABSTRACT

Microalgae represent a potential solution to reduce CO_2 emission exploiting their photosynthetic activity. Here, the physiologic and metabolic responses at the base of CO_2 assimilation were investigated in conditions of high or low CO_2 availability in two of the most promising algae species for industrial cultivation, *Chlorella sorokiniana* and *Chlorella vulgaris*. In both species, high CO_2 availability increased biomass accumulation with specific increase of triacylglycerols in *C. vulgaris* and polar lipids and proteins in *C. sorokiniana*. Moreover, high CO_2 availability caused only in *C. vulgaris* a reduced NAD(P)H/$NADP^+$ ratio and reduced mitochondrial respiration, suggesting a CO_2 dependent increase of reducing power consumption in the chloroplast, which in turn influences the redox state of the mitochondria. Several rearrangements of the photosynthetic machinery were observed in both species, differing from those described for the model organism *Chlamydomonas reinhardtii,* where adaptation to carbon availability is mainly controlled by the translational repressor NAB1. NAB1 homologous protein could be identified only in *C. vulgaris* but lacked the regulation mechanisms previously described in *C. reinhardtii*. Acclimation strategies to cope with a fluctuating inorganic carbon supply are thus diverse among green microalgae, and these results suggest new biotechnological strategies to boost CO_2 fixation.

Keywords: Chlorella, CO2, photosynthesis, respiration, carbon fixation

BIOGRAPHY

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About the author:

Prof. Matteo Ballottari graduated in Biotechnology at the University of Verona in 2004. He is member of the several national and international scientific society, among which the Young Academy of Europe. Matteo Ballottari is involved in national and European projects financed by public and private funds about the exploitation of photosynthetic organisms to produce biofuels and high value products. Prof. Ballottari group (SOLE-LAB) studies plant metabolism in microalgae and higher plants, focusing on how it might be improved to produce biomass and high value products through an interdisciplinary approach ranging from omics to biophysics, biochemistry and genetic engineering.

PHYSIOLOGICAL CHANGES IN THE OUTDOOR CULTURE OF *CHLORELLA* g120 DURING TROPHIC CONVERSION FROM HETEROTROPHIC TO PHOTOTROPHIC GROWTH REGIME

Masojídek J.[1,2]
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ABSTRACT

Up to now, scarce information about major photochemical changes accompanying the metabolic shift from heterotrophy to photoautotrophy in microalgae cells has been available. In this work the trophic conversion from heterotrophic to photoautotrophic growth regime was studied in the microalga *Chlorella vulgaris* g120. The culture was first grown in the fermenter and then transferred into outdoor thin-layer cascades. Various photosynthesis monitoring techniques were used to correlate to the physiological response of the *Chlorella* g120 culture during photoacclimation. As for comparison the phototrophic strain *Chlorella vulgaris* R-117 was cultured in parallel. A build-up of dissolved oxygen concentration (DO) measured *in-situ* indicated increased photosynthetic activity. Other variables as photo-chemical yield and

electron transport rate (measured by Chl fluorescence) measured *in-situ* and *ex-situ* also represent reliable markers as they reflect the physiological status of outdoor microalgae cultures. Here, we show that *Chlorella* g120 can undergo the metabolic shift from heterotrophic to phototrophic growth regime, but its conversion (photoadaptation) is incomplete due to ineffective light-harvesting function. A rather high respiration rate is interpreted as a strategy to dissipate the unused light energy. *Chlorella* g120 was shown as the strain characterized by a small-antenna size strain (low chlorophyll/cell ratio <1%) which should be favourable for light utilization in dense cultures (Babaei et al. 2021, Masojídek et al. 2021). The high Car/Chl ratio (= 0.63) may be of interest from biotechnological point of view for carotenoid (namely lutein) production.

Keywords: Chlorella, chlorophyll fluorescence; oxygen evolution; trophic conversion

References:

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BIOGRAPHY

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Company profile:
Centre Algatech Třeboň is a part of the Institute of Microbiology of the Czech Academy of Science, a public research institution with the headquarters in Prague. The Centre consists of four laboratories: Laboratory of photosynthesis, Laboratory of algal biotechnology, Laboratory of algae cell cycles and Laboratory of anoxygenic phototrophs.

INCREASING VERTICAL MIXING IN HRAP TO ENHANCE PHOTOSYNTHETIC LIGHT INTEGRATION

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ABSTRACT

An abstract is a single paragraph, without indentation, compendious summary of a paper's substance including research question, background, purpose, methodology, results, and conclusion in 175 to 275 words.

Industrial production of microalgae is mainly carried out in High Rate Algae Ponds (HRAP) of large size, typically with more than 1000 m2 surface. In these situations, light absorption by microalgae is usually the bottleneck for culture growth (Torres-Franco et al. ,2021, y más referencias). In this work we have studied the influence of vertical mixing on the frequency of light/dark cycles and on the oxygen production. To increase vertical mixing, we submerge in the culture aerodynamic profiles of particular geometry and configuration to generate vortex tubes with horizontal axis parallel to the flow direction, a system patented by the University of Seville (PCT ES2019070842).

We have studied the generation of these vortices with Computational Fluid Dynamics (CFD), using Ansys-Fluent software. Obtaining the trajectories of tracer particles we have evaluated light absorption and oxygen production, using the photosynthesis model of Camacho-Rubio et al. (2003). Experimental work has also been carried out at the facilities of the University of Almería, in raceway ponds of 40 m length and 2 m wide to validate the results obtained with the numerical simulations.

The numerical results show that the frequency of the light/dark cycles can be increased by a factor larger than 10. Design and position of the aerodynamic profiles are the major factors determining the improvement of light/dark cycles. The oxygen production rate , comparing the flow without airfoils (with little mixing due to the turbulence generated near the walls of the pond) with some of the configurations with airfoils.

Keywords: Light integration, vertical mixing, HRAP, CFD

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PSYCHROPHILIC *MONORAPHIDIUM* SP. B CULTIVATION IN OUTDOOR THIN-LAYER RACEWAY POND FOR HIGH-VALUE PRODUCTS

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ABSTRACT

The vast majority of the commercial strains belong to the group of mesophilic or slightly thermophilic microorganisms that grow best in moderate temperatures with an optimum growth range from 20 to 45°C. This characteristic restricts their cultivation to tropical and subtropical zones. A possible solution to extend the cultivation area and the season is therefore to look for microalgae strains from cold habitats such as polar or glacier regions that grow under 20°C (1, 2).

The temperature requirements of *Monoraphidium* sp. B for growth was specified between 6 to 20 °C, while its favoured irradiance intensity was under 100 μmol m−2 s−1 during a sunny day (3). Psychrophilic microalgae can generate high-value compounds like polyunsaturated fatty acids and pigments. These valuable properties made *Monoraphidium* sp. B to a promising candidate for our outdoor trials. Three cultivation conditions were tested: cultivation in natural illumination; natural and artificial illumination; and nitrogen deprivation with natural and artificial illumination. Photosynthetic oxygen evolution, as well as two chlorophyll fluorescence techniques, were used for monitoring the activity of the culture *in-situ* and *off-situ*. Temperature, irradiance, pH, dissolved oxygen and carbon dioxide concentration was monitored and recorded to describe the environment of the culture. Samples were collected for further analysis of fatty acid and pigment profiles.

Saturated palmitic acid (C16:0), stearic acid (C18:0) and monosaturated oleic acid (C18:1) desirable for high-quality biodiesel production was found. Besides, gamma-linolenic acid (18:3n-6) possessing anti-inflammatory and anti-cancer actions were detected as well. Lutein, which might help to prevent or ameliorate the effects of degenerative human diseases, such as age-related macular degeneration or cataract, was extracted from biomass.

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Keywords: psychrophilic, microalgae, thin-layer raceway pond, biodiesel, food supplement, lutein
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Company profile:

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.

E-POND®. BIOELECTROACTIVE FILTER HELPS GROWING FOOD-GRADE MICROALGAE ON RECYCLED STREAMS RICH IN ORGANIC FORMS OF NUTRIENTS.

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ABSTRACT

Organic-rich **secondary products** of food industry and **wastewaters** are important sources of nutrients. In the **e-Pond® technology** microalgae cultivations are assisted by the introduction of an innovative microbial electrochemical technology that provides **recycled nutrients** in mineral forms from organic-rich streams. **Bio-electrochemical oxidation** of organic loads is performed by electroactive microbial communities, coupled to a special electroactive material (based on biochar), which enhances the exchange of electrons in biogeochemical reactions, towards the terminal electron acceptor [1-6]. In bench-scale tests, the e-Pond was fed with lactic-acid fermentation effluents at organic loading rates of 600 mg COD/L/day. Bioelectrochemical oxidation rates of about 200 mg COD/L/h were achieved. The obtained mineral medium, presented residual organic concentrations of around 90 mg COD/L but insufficient nitrate concentrations were recorded. In fact, results showed lower Spirulina growth performance. However, after enriching the mineral medium with NO_3 (at the level of Zarrouk medium at 50%), Spirulina growth rate achieved 130 mg DM /L /d, i.e. ~20% lower than obtained with the optimal growth solution (Zarrouk's 100% medium). As control, the e-Pond was run without electroactive materials: the obtained medium (enriched with NO_3) permitted to grow Spirulina at a rate of 46 mg DM/L/d, i.e. ~73% lower than using the standard medium. Higher residual organic concentrations (500 mg COD/L) in the medium were probably the main cause for partial inhibition of growth.

Our study demonstrates the important role played by the conductive material (i.e., biochar) in the operation of e-Pond. Future work will be focused to improve nitrogen recovery in the form of nitrates, trying to avoid possible losses by ammonia evaporation or denitrification processes.

Keywords: nutrients recovery; microalgae; wastewater; Spirulina; microbial electrochemical technologies; organic agriculture.

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WHY TUBULAR GLASS PHOTOBIOREACTORS?

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ABSTRACT

Large tubular glass photobioreactors operate profitably since more than 20 years and new systems are under construction today. This presentation highlights the underlying reasons for the growing adoption of tubular glass systems by the algae industry. Tubular glass systems enable cultivations at high concentrations with high volumetric productivities. Production in closed systems is predictable and sustainable because of high bio-security and no evaporation of water. Glass maintains a high light transmission over many years of UV exposure. Glass has a GRAS (generally recognized as safe) certificate by the FDA. Its smooth and hard surface almost prevents biofilm formation and allows for easy, scratch free cleaning without causing production downtime. The high price of glass is a myth and the lifetime of over 50 years results in much lower total cost

of ownership than systems made of disposable polymer materials. Finally, the presentation provides insights on the crystal-clear benefits of tubular glass photobioreactors.

Keywords:algae cultivation, tubular glass, photobioreactor

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Algalif Iceland ehf, Algatechnologies Ltd., Astabio Co. Ltd. Astaxa GmbH, A4F SA, Carlsberg A/S, Clearas Water Recovery Inc., ecoduna AG, Fukuoka Riken Kogyo Co. Ltd., Green Tech s.r.l., IGV GmbH, Instituto de Technologia, LGem b.v., microphyt SAS, Mitsubishi Kakoki Kaisha Ltd., PT Evergen Resources, Synoxis SAS, Varicon Aqua Solutions Ltd.

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SCHOTT is a leading international technology group in the areas of specialty glass and glass-ceramics. The company has more than 130 years of outstanding development, materials and technology expertise and offers a broad portfolio of high-quality products and intelligent solutions. SCHOTT is an innovative enabler for many industries, including the home appliance, pharma, electronics, optics, life sciences, automotive and aviation industries. SCHOTT strives to play an important part of everyone's life and is committed to innovation and sustainable success. The parent company, SCHOTT AG, has its headquarters in Mainz (Germany) and is solely owned by the Carl Zeiss Foundation. As a foundation company, SCHOTT assumes special responsibility for its employees, society and the environment.

Scientific Session 2: Biomaterials from Algae

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

CHLORELLA VULGARIS HIGH-GLUCOSE SYRUP AS CARBON FEEDSTOCK FOR PHA-PRODUCING BACTERIA

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ABSTRACT

Bio-based biodegradable plastics offer a valuable alternative to non-biodegradable, petroleum-based plastics. Polyhydroxyalkanoates (PHA) are natural polymers used in the production of bioplastics, have been reported to degrade in oceanic conditions [1] and can be obtained by a purely biotechnological route starting from a variety of carbon-rich biomass feedstocks. *Chlorella* sp is one of the most extended microalgae produced worldwide [2], it is a robust and easy growing species. In this work, a starch-producing *Chlorella* strain has been used as carbohydrate feedstock for fermentation of PHA-producing bacteria. Raw *Chlorella* biomass has to be processed prior to bacterial fermentation, in this sense high-starch *Chlorella* was subjected to high pressure homogenization in order to break cell wall and let starch available for enzymatic hydrolysis into simple sugars. Starch was then hydrolyzed into glucose by means of amylase and amyloglucosidase enzymes. The hydrolysate supernatant was successfully used as carbon feedstock for bacterial growth and PHA accumulation.

Keywords: bioplastic, polyhydroxyalkanoates, *Chlorella*, starch, fermentation

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Company profile:

CEA is a leading Research and Technology Organization in the field of energy. **M**icro**A**lgae **P**rocesses **P**latform (MAPP) 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. MAPP is involved in collaborative R&D projects with public and private stakeholders.

FATTY ACID BASED BIOPOLYMERS FROM *CHLORELLA VULGARIS* BIOMASS FOR ITS APPLICATION IN SPORTING GOODS

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ABSTRACT

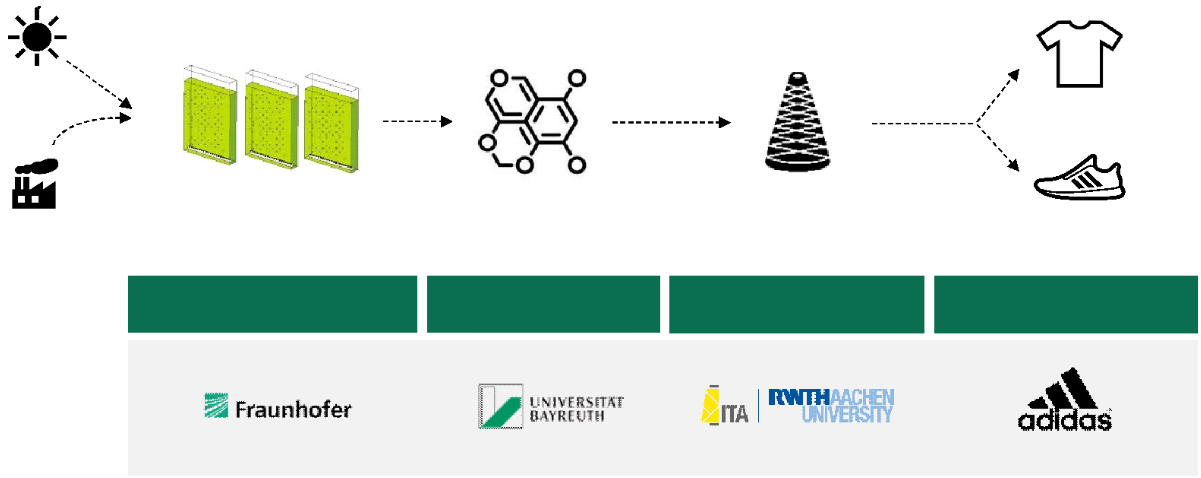
The innovation space BIOTEXFUTURE aims to promote the transformation of the textile industry from petroleum-based to biobased. In the AlgaeTex sub-project, the development of microalgae as a raw material basis for plastic filaments for the production of sustainable textile products are investigated.

Fraunhofer is developing a three-stage process to determine an economically best-case scenario with the fastest possible way for scaling, production, harvest and extraction of fatty acids (FA) from *Chlorella vulgaris*.

In the first stage, a heterotrophic pre-culture with an organic carbon source is intended. In the second stage a short, phototrophically growth phase in flat panel airlift reactors (FPA) with double-sided artificial illumination will be applied. In the final stage, lipid enrichment will take place under either phototrophic or mixotrophic conditions. The control system will manage different nutrient demands, productivities and shuttle the volumes between each stage to get a fast, robust and automated process with a maximum of suitable FA content in the biomass.

The fatty acids are further processed by transesterification to fatty acid methyl esters (FAME),purified and converted to dicarbonic acids which are then polymerized into spinnable polymers. This is the task of the Department of Macromolecular Chemistry at the University of Bayreuth with the final goal to develop melt-spinnable polyesters and polyamides.

Melt spinning, and the further processing of the polymers, is being investigated by Institut für Textiltechnik of RWTH Aachen University. The spinnability of the algae-produced yarns is being investigated and developed in direct interaction with their processability into knitted textiles. For validation purposes and to ensure the industrial relevance, adidas AG will be responsible for the final processing of the algae-based polymer yarns into high-quality textile demonstrators.



Keywords: Biotexfuture

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Company profile:

The Fraunhofer IGB has many years of extensive experience with many different algae species in the field of process development for microalgae cultivation, reactor development, and the processing of algae biomass. Processes and interesting biomass compositions are investigated and optimized for industrial applications in the framework of a future bio-economy.

Scientific Session 3: Technology and Processing

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

LED TECHNOLOGY DEVELOPMENT TO MAXIMIZE ENERGY CONVERSION EFFICIENCY: APPLICATION OF FLASHING BLUE-RED LIGHT IN CONTINUOUS PHOTOBIOREACTORS

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ABSTRACT

Flashing light is considered a promising strategy to increase light penetration in microalgal cultures, reducing self-shading effect [1]. In fact, it allows using high light intensity pulses, though avoiding culture photoinhibition. The real advantage of flashing light is a matter of discussion [2], because many variables can play a role, such as average light intensity, frequency, acclimation and cultivation mode. Several authors showed that frequency pulses below 100 Hz are able to increase biomass growth and photosynthetic rate in batch cultures [3]–[8], thus enhancing light utilization, but to match with the reaction kinetics of the photosynthetic process, a technological limit is also present. In addition, batch cultures are prone to acclimation phenomena, possibly covering the pulsed light effect.

In this work, *Arthrospira maxima* was cultivated in steady state continuous photobioreactors under red and blue LED lamps. Culture performance was compared under continuous and pulsed light, with frequencies in the range of 10-1000 µsec and pulses up to 70.000 µmol m^{-2} s^{-1} at different integral light intensities and residence times. It was found that frequencies between 100 – 200 µsec increase biomass concentration with respect to control for integral light intensities up to 300 µmol $^{-2}$ s^{-1}. Furthermore, pulse intensity affects the productivity curve by shifting the maximum and the wash-out condition at higher residence times. Interestingly, it was found that biomass concentration can be up to three times higher than the control under high intensity pulses for long residence times. To evaluate process efficiency, photosynthetic and LED efficiency were taken into account. Two experimental lamps were developed, with the purpose to improve photoconversion of power energy into light. It was found that the cultivation in continuous photobioreactors, allowing proper culture acclimation, makes flashing light advantageous from the energetic point of view, increasing the overall process efficiency.

Keywords: *Arthrospira; Spirulina; frequency; light pulse; multi-wavelengths spectrum; duty cycle*

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CONTINUOUS PRESSURIZED SOLVENT EXTRACTION IN CYANOBIUM SP. PIGMENTS BIOPROCESSING

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ABSTRACT

Phycobiliproteins and carotenoids are cyanobacterial pigments with huge economic significance owing to their bioactive capacity (e.g., antioxidant). In the industry, pigments are typically used as raw extracts, but no particular method of extraction has yet been defined due to the difficulties raised by the specificity of morphological characteristics of species [1]. In this study, a continuous pressurized solvent extraction (CPSE) method was optimized for the extraction of carotenoids and phycobiliprotein from *Cyanobium* sp., a marine cyanobacterium [2]. The optimization was performed using a Central Composite Design factorial model with three factors: time (t, 5 – 20 min), temperature (T, 30 – 70 °C) and flow (f, 1 – 4 mL min^{-1}); and two successive extracts were obtained: an ethanolic extract (carotenoids-targeted) followed by a water extract (phycobiliproteins-targeted). The content of pigments and the antioxidant capacity of extracts were evaluated in all tested conditions. The optimization provided a significant quadratic model for all the studied parameters. All the factors evaluated tend to influence pigment extraction in various ways, such as: the increase in temperature and time is more effective in terms of extraction rate, while the lower flow (translated to the device pressure) of extraction decreases the deterioration of the pigment content. Optimal conditions for co-extraction of carotenoids and phycobiliproteins in CPSE were T = 70 °C, t = 20 min and f = 1.5 mL min^{-1}, leading to a content of 31.6 ± 1.8 mg g_{DW}^{-1} for carotenoids and 133.4 ± 7.5 mg g_{DW}^{-1} for phycobiliproteins. The optimal condition was performed experimentally as a validation of the model.

Keywords: carotenoids, phycobiliproteins, antioxidant capacity, ethanolic extract, aqueous extract, successive extraction.

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CIIMAR - Interdisciplinary Centre of Marine and Environmental Research - is a leading research and advanced training institution of the University of Porto, working at the frontier of Ocean Knowledge and Innovation. CIIMAR uses knowledge-based approaches to promote the natural capital and the sustained management of marine resources through monitoring of ecosystems health, optimization of aquaculture, and biotechnological exploitation of the resources for environmental and human health applications.

OPTIMIZING ALGAL PRODUCTIVITY AND NITROGEN RECOVERY FROM DIGESTATES BY ADAPTING SOLID RETENTION TIME AND ALKALINITY

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ABSTRACT

Combining algae and bacteria in a High Rate Algal/Bacterial Ponds (HRABP) is an emerging technology for wastewater remediation which do not need an external oxygen supply and the nitrogen and phosphorus can be recovered in the algal biomass. However, HRABPs are complex ecosystems involving a wide biodiversity and being highly climate dependent, therefore still requiring optimization studies. In addition, the biomass harvesting is still a major challenge and algae-bacteria grown in outdoor reactors are generally operated with a Hydraulic Retention Time (HRT) equal to Solid Retention Time (SRT).

Decoupling HRT and SRT using membrane modules is a technological breakthrough that was shown to considerably enhance the process efficiency, even if these advantages have been assessed mostly at lab-scale. The benefits in decoupling HRT and SRT is probably even more suitable for specific media, such as digestates.

The objective of this work was to develop a computer assisted optimization strategy to optimize the use of a separation system. We used a model that was validated under realistic conditions, with two outdoor case studies, and for more than 600 days. The ALBA model has been shown to accurately predict the four tested seasons in two different climatic conditions. The model also highlighted a risk of N_2O emission resulting from a strong competition for inorganic carbon following a shortage in alkalinity, due to a strong nitrification activity, as experimentally observed when the growth medium was the digestate.

This model was therefore an opportunity to carry out an extensive optimization study for better understanding: i) the trade-off between algal productivity and nitrogen recycling, only considering operating conditions not susceptible of leading to N_2O emissions; ii) the complex interplay between SRT, HRT, liquid depth and alkalinity.

Keywords: algae/bacteria mathematical modelling, raceway optimization, biomass productivity, membranes, nitrogen recovery, nitrous oxide emissions.

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From November 2017, she was a PhD 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 discussing her Ph.D thesis in March 2021, she got a Postdoc position in the INRIA-BIOCORE team, where she works on the optimization of algae-bacteria systems.

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ELECTROCOAGULATION TO HARVEST AND ENRICH *NANNOCHLOROPSIS OCEANICA* BIOMASS FOR AGRICULTURAL APPLICATIONS

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ABSTRACT

Electrocoagulation is a promising low-cost technology to efficiently concentrate and harvest microalgae biomass before secondary dewatering steps [1]. However, electrochemical reactions and sacrificial electrodes lead to physical changes in media and release undesired salts that affect the harvested biomass [2]. In this study, effects of Fe, Zn, and Mg electrodes were evaluated on *Nannochloropsis oceanica* harvesting performance and on chemical elementary composition of electrocoagulation biomass and supernatants. All electrodes recovered *N. oceanica* biomass at efficiencies above 95%, concentrating it 3-7 times. Electrocoagulation affected biomass and supernatant chemical elementary concentrations depending on the electrode material. While Zn electrodes increase Cu in the biomass, Fe and Mg electrodes enhance Mn. Moreover, co-precipitation and accumulation of Mg and Ca happened in all electrodes, leading to more enriched biomasses. A consequent experiment tested the bioavailability of electrode's biomass-metals (Fe, Zn, and Mg) on *Solanum lycopersicum* seedlings. Metal enriched biomass from each electrocoagulation was added to nutrient solutions lacking the respective metal, which were used to grow *S. lycopersicum*. After 13 days, plants grown in nutrient solutions with Fe and Mg enriched biomasses had no visible symptoms of chlorosis and had no differences in chlorophyl and root and shoot weights, compared to plants grown in non-limiting nutrient solutions (Fig. 1). The proven bioavailable Fe and Mg from electrocoagulation electrodes make the harvested biomass a promising resource for agricultural applications.

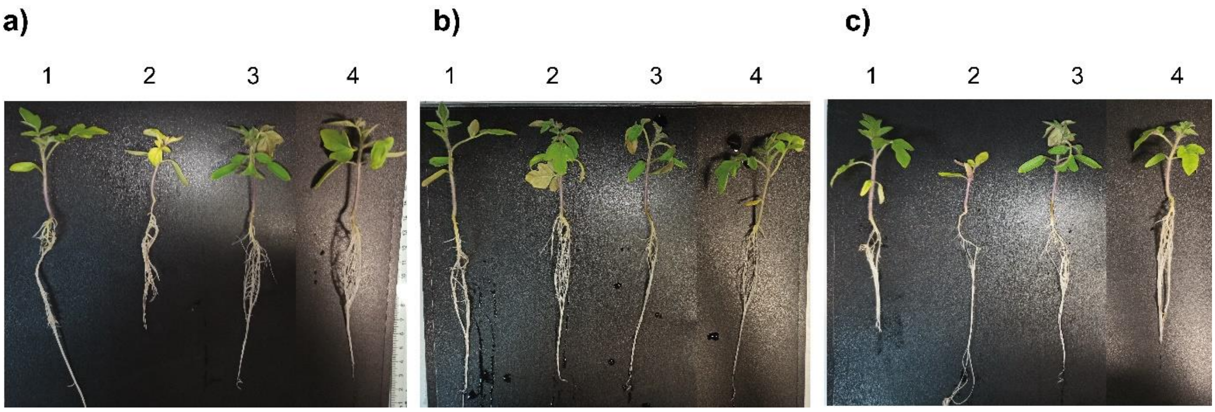

Figure 1. Bioavailability of electrocoagulation iron (a), zinc (b), and magnesium (c) metals to *Solanum lycopersicum* seedlings grown during 13 days in nutrient solutions: 1 – metal deficient solution supplemented with biomass harvested by electrocoagulation using respective deficient metal electrodes; 2 – metal deficient solution; 3 – complete nutrient solution; 4 – complete nutrient solution supplemented with biomass harvested by centrifugation using equal biomass concentrations of solution 1.

Keywords: Microalgae, Electrocoagulation, Alternative electrodes, Harvesting, Plant nutrition, Metal bioavailability

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Company profile:

The GreenCoLAB is a collaborative platform between research and industry, that aims to develop in short turn, new technologies, products and services in order to optimize the current industrial production of algae-based compounds for cosmetics, pharmaceuticals, food and animal feed, ensuring that these products respond to market needs.

Thematic Session 1: Training

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07-10·DECEMBER·ONLINE

CAPTURING AND RE-USING CO2 USING DEEP EUTECTIC SOLVENTS AND MICROALGAE

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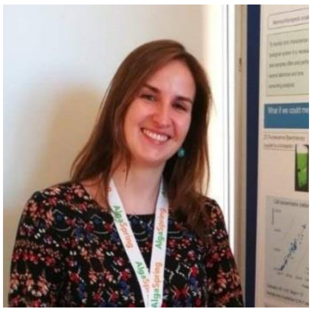
ABSTRACT

Microalgae are photosynthetic microorganisms naturally adapted to capture CO2 from atmosphere due to the presence of a specific enzyme, carbonic anhydrase (CA). The aim of this work was to extract this enzyme from fresh microalgal biomass using a low-cost green solvent, deep eutectic solvent (DES). This combination of DES with CA, when placed in a gas-membrane contactor, will capture and purify CO2 simultaneously, which can be subsequently used to cultivate microalgae biomass. This process results in a closed-loop, where CO2 is fixed to generate more CO2 fixation-capacity, and can potentially be coupled to the biorefinery of high-value compounds for a growing market. The combination of these two readily available technologies as one engineered solution may lead to a breakthrough in the Carbon Capture and Utilization (CCU). Three distinct microalgae were processed – Tisochrysis lutea, Chlorella sp., and Spirulina sp. – with three DES – Choline chloride-Urea (ChCl-U), Choline chloride-Poly(ethylene glycol) (ChCl-PEG), and Poly(ethylene glycol)-Urea (PEG-U). To evaluate the most promising microalgae-DES pair, CA activity was evaluated with a specific enzymatic activity kit and through CO2 absorption (solubility and diffusivity). Preliminary results indicate that: DES is a suitable solvent to extract CA from microalgal biomass, preserving its activity; CA extraction efficiency differs between DES and microalgal species, indicating the potential for further biorefinery processes; from the tested DES, the ones containing PEG were favorable to maintain CA activity. This work paves the way towards a disruptive CCU approach.

Keywords: Tisochrysis lutea; Chlorella sp.; Spirulina sp.; Carbonic Anhydrase, gas- membrane contactor; CO2 fixation.

BIOGRAPHY



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Company profile:

OnePlanet Research Center is a multidisciplinary collaboration between Wageningen University & Research (WUR), Radboud University, Radboudumc, and the nano- technology world-player imec. OnePlanet uses the latest chip and digital technologies to contribute to a healthier and sustainable society, though the development of game changing innovations.The Bioprocess Engineering group focuses on development of novel biotechnological processes for production of pharmaceuticals, healthy food ingredients, bulk chemicals, and biofuels. Our challenge is to produce high quality bio-based products in a sustainable and economical way to prevent depletion of natural resources and to increase development of a (bio)technological industry.

COMBINATION OF PHOTO-FENTON OXIDATION WITH MICROALGAE CULTIVATION FOR IMPROVED BIOREMEDIATION OF PIGGERY WASTEWATER

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ABSTRACT

Livestock systems have expanded and intensified to fulfill the increasing food demand related to population growth. This has been accompanied by the generation of large amounts of wastewater (WW), leading to the excessive discharge of nutrients into aquatic environments, resulting in eutrophication problems and reduced water quality [1]. Microalgae can assimilate these nutrients (N and P) cleaning the WW and producing valuable biomass. However, livestock WW have very high ammonia levels, high pH, and dark color that inhibits microalgae growth. To overcome these hurdles, dilution is usually first strategy, but freshwater is a scarce resource [2–4]. Thus, the implementation of a pre-treatment step to minimize the WW toxicity and improve pollutant biodegradability, can enhance microalgae cultivation. Photo-Fenton (PF) oxidation was applied to piggery effluent at several Fe^{2+}/H_2O_2 ratios, proving to be very efficient in removing chemical oxygen demand (92.6 ± 0.4 %), total suspended solids (98.5 ± 0.1 %), and color (99.7 ± 0.2 %), but not NH_4^+ (19.4 ± 0.1 %), using 1 g Fe^{2+}/L and 10.5 g H_2O_2/L. Nonetheless, the microalga *Tetradesmus obliquus* was able to grow in undiluted effluent, even at high levels of NH_4^+ (~1200 mg/L), achieving an average volumetric productivity of 107 mg/L/d, compared to 154 mg/L/d in Bristol medium, after 15 days of cultivation (Fig. 1). When grown in PF-WW, pH tends to decrease until 3-4, minimizing contamination, especially cyanobacteria, and maintaining a monoculture of *T. obliquus* in piggery effluent. Furthermore, PF has a disinfecting potential (due to H_2O_2 and UV radiation) for decreasing pathogens, minimising the limitation of WW-grown microalgal biomass for more high-value applications.

Keywords: Piggery wastewater, Microalgae, Pre-treatment, Photo-Fenton, *Tetradesmus obliquus*

A- Bristol medium **B- Photo-Fenton wastewater**

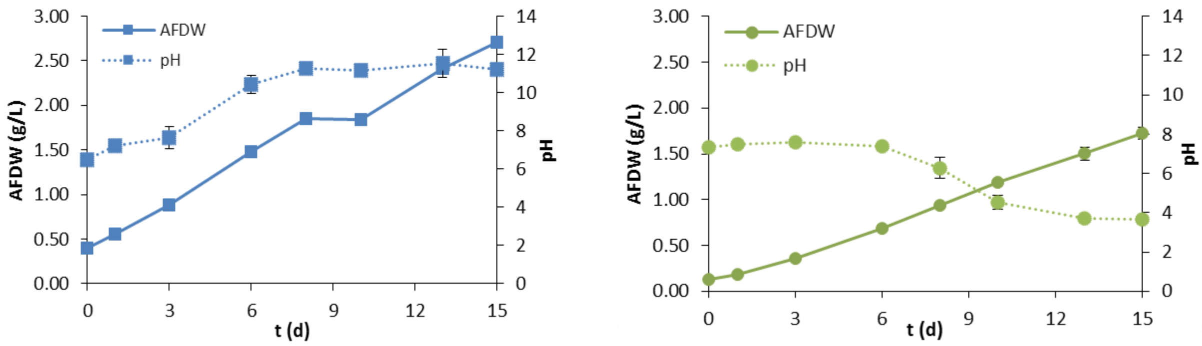

Figure 1. a) *Tetradesmus obliquus* growth and pH evolution in undiluted piggery effluent pre-treated with photo-Fenton (B), compared with synthetic (Bristol) medium (A), for 15 days of cultivation. Error bars indicate standard deviation (n=2).

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TREATMENT AND VALORIZATON OF A DAIRY BY-PRODUCT BY *EUGLENA GRACILIS*

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ABSTRACT

Dairy factories produce large amounts of liquid by-products which cannot properly be defined as wastewaters because they just contain milk residues, whose disposal poses anyway serious problems related to their high organic and nitrogen content. To valorize them, in a circular economy perspective, an experimental work is being carried out, based on microalgae-bacteria systems. Considering the high COD of such substrate, *Euglena gracilis* has been chosen as inoculum, as it needs organic matter and low pH and its biomass is rich in proteins, lipids and Paramylon, a valuable β-glucan. Lab-scale (column photobioreactors, 3.5 L working volume) and pilot scale (flat panels, 55 L working volume) tests have been conducted in duplicate at different dilutions of a dairy by-product and the pilot tests have confirmed the good results of the lab-scale ones. 10-days batch cultivation and 10% dilution have been proven as the best culturing conditions. CO_2 supply was not needed: working in non sterile conditions, a rich bacterial population developed and the CO_2 derived from bacterial degradation of COD produces was enough to keep pH between 4.1 and 4.3 (optimal for *Euglena*). Removal efficiency was 99-100% for NH_4-N both in lab-scale and in pilot scale tests, and 83% and 57% in lab-scale and pilot-scale tests, respectively. Standard deviations among replicates approached to 0. Microalgal cell counts were always in the order of 10^6 cells/mL..

Keywords: Dairy by-product, Euglena, COD, NH_4-N

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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.

COMPOSITIONAL CHANGES IN SPRING- AND SUMMER-COLLECTED *SARGASSUM MUTICUM* AFTER METHANOL EXTRACTION OR WATER WASHING

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ABSTRACT

Biomethane production using *Sargassum muticum* as a source of biomass during anaerobic digestion is partly limited by its low methane yields. The effects of extensive washing or aqueous methanol extraction were investigated on spring and summer-collected *S. muticum*. For the summer-collected samples, the biochemical methane potential (measured over 28 days) of the extensively washed and methanol-treated residues were statistically higher than the untreated biomass ($p < 0.05$) by 15.56% and 26.60%, respectively. The methane production profiles were fitted using the modified Gompertz equation. Additionally, the changes in the chemical and physical characteristics of the seaweed due to the pre-treatments were investigated. This study highlights the changes in the surface areas, polyphenolic (-65 to -83%), lipid (-21.7 to +12.2%), protein (+20.3 to +48.6%), total dietary fibre (+9.1 to +38.1%), and carbohydrate contents (>-22.2%). Correlations between these contents and the parameters of the modified Gompertz model will be further discussed. The effects of these treatments were dependent on the season or month that the seaweed was collected. The characterisation was completed to understand the underlying reasons for the increase in methane yield and identify any potential methane production inhibitors. Ultimately, informing on methods to increase methane yield during the anaerobic digestion of *S. muticum*, whilst potentially also obtaining high-value products.

Keywords: biofuel; macroalgae; anaerobic digestion; pre-treatment

BIOGRAPHY

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About the author:

I am at the end of my third-year as a PhD student at the University of Greenwich. I started my education in biomedical science and has since focused on biofuels for energy production, completing my Masters in Environmental and Energy Engineering at the University of Sheffield. I am currently researching the composition and use of *Sargassum muticum* and holopelagic Sargassum for energy production via anaerobic digestion. My primary focus is the role of polyphenolics from *S. muticum* in anaerobic digestion and its effects on methane production. I hope to further explore the uses of seaweed in my future career.

IS MICROALGAE PRODUCTION UNDER GREENHOUSE BENEFICIAL?

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ABSTRACT

In this work we analyze how greenhouses may affect microalgae production on a year scale, by modelling temperature and light intensity effects on growth rate. We then determine the dependency of greenhouse efficiency on climate and microalgae characteristics.

Outdoor production is susceptible to external weather changes, especially temperature and solar radiation fluctuations, leading to conditions significantly different from the theoretical optimal ones. Greenhouses may provide an interesting tradeoff to reduce contamination while modulating climate. To define the conditions for which greenhouses are economically profitable we propose a model for the marine green algae Tetraselmis suecica and adapt it to four other species (*Spirulina platensis, Dunaliella salina, Phaeodactylum tricornutum and Chlorella vulgaris*).

Experiments under a greenhouse were carried out for the marine green algae *Tetraselmis suecica*, shifting the temperature of two raceways compared to a reference raceway with free evolving temperature. A productivity model and a model for water temperature were then parametrized and validated accounting for the recorded evolution of temperature and light. We use real data of solar radiation and air temperature to simulate productivity in different regions of France.

Simulation of yearly cultivation demonstrates that greenhouse usage is beneficial in limited circumstances. At year scale, greenhouse efficiency is notable only for few species, e.g. *Spirulina platensis*, where productivity can be increased by 20 % compared to outdoor production without greenhouse.

Based on these results, cultivation under greenhouse is beneficial mainly to protect the culture against contamination and to increase productivity in cold regions for species susceptible to photoinhibition with optimal growth in high temperatures. Rotation of the cultivated species is also a good strategy to improve annual productivity.

Keywords: Greenhouse, raceway, modelling, temperature

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Graduated in chemical engineering in 2019 at Federal University of Rio Grande do Sul, Brazil, with a double degree at Ecole CentraleSupélec, France. Before graduating in 2017/18, I worked as an intern at INRIA and at the Oceanographic Observatory of Villefranche-sur-mer, working with cultivation of microalgae. I started my PhD at the end of 2019 with the topic of optimization of microalgae production via reduced metabolic models.

Company profile:

The goal of the INRIA Biocore team is to contribute to environment preservation, develop new renewable energy sources and avoid water pollution or the use of chemicals for crops. In this context, Biocore designs artificial microbial ecosystems, and develops mathematical models to analyse, control and optimize these processes.

MICROALGAE PRODUCTION AND UTILIZATION IN THE USA - RECENT DEVELOPMENTS

John R. Benemann

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ABSTRACT

There are many research, development and commercialization activities in the microalgae space ongoing in the USA. The recently concluded Algae Biomass Organization (ABO) Summit, held as virtual event from September 28 to October 27, provided a broad overview of these N. American activities (1). Many of the presentations at the ABO Summit were of collaborations between universities, private companies and US Department of Energy (DOE) National Laboratories. The US DOE has been a major source of support for this research, with a detailed semi-annual project review available online (2). Privately funded R&D and commercialization activities are also ongoing, with a large project funded by ExxonMobil being carried out by Viridos, Inc. (formerly Synthetic Genomics) on algal biofuels production. *Nannochloropsis* production is ongoing jointly by NBA Technologies, for animal feeds, and Qualitas Health, for nutritional products, with the latter successfully marketing an EPA supplement under their iWi brand. Heliae Development LLC is a leading company in the development and marketing of microalgae products for agriculture. Earthrise Nutritionals LLC is the major US producer of both Spirulina and phycocyanin, under their Linablue brand. Cyanotech Corp. produces both Spirulina and astaxanthin from *Haematococcus pluvialis.* Microalgae for wastewater treatment and nutrients recovery, with co-production of biofuels, are another focus of research and commercial activities. Thousands of wastewater treatment ponds operating in the US provide potential sources of low-cost algal biomass for conversion to biofuels using hydrothermal liquefaction. Many other commercial developments and research projects are ongoing, from basic biology and genomics to applied engineering and techno-economic analyses / life cycle assessments (TEAs/LCAs). The ultimate goal is to advance microalgae technologies from currently small niche to larger commodity markets.

Keywords: Microalgae, Biofuels, Animal Feeds, Nutritional products

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(1) www.algaebiomasssummit.org/

(2) www.energy.gov/eere/bioenergy/2021-project-peer-review-advanced-algal-systems-program

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CEO and co-founder (2009) of MicroBio Engineering Inc.; a founding director at the Algae Biomass Organization (USA) and on the Steering Board of the European Algae Biomass Association (EABA). Consultant and advisor to US and international government agencies and companies; principal investigator on many US Department of Energy funded projects; frequent conference speaker and author of many publications. Academic history: B.S. Chemistry and Ph.D. Biochemistry, both from the University of California Berkeley. Researcher, Chemistry Department, U.C. San Diego and Departments of Civil Engineering and Plant and Microbial Biology, U.C. Berkeley. Associate Professor, Department of Applied Biology, Georgia Institute of Technology.

Company Profile:

MicroBio Engineering Inc. (MBE), located in San Luis Obispo, California, is a research, consulting and engineering firm specializing in the research, development, design, construction and operation of algae cultivation processes for CO_2 capture and utilization, wastewater recycling, biofuels production, animal feeds and specialty bioproducts.

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Scientific Session 5: Algae for Food and Health

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PILOT-SCALE PRODUCTION OF *A. PLATENSIS* AS A SOURCE OF FUNCTIONAL AND BIOACTIVE PROTEINS

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ABSTRACT

The production of the microalga *Arthrospira platensis* was optimised to increase the protein content of the biomass while simultaneously minimising production costs. Overall, a biomass productivity of 0.61 $g \cdot L^{-1} \cdot day^{-1}$ was achieved when using a low-cost medium formulated using agricultural fertilisers. The process was further up-scaled using 80 m^2 raceway reactors achieving productivities within 20-40 $g \cdot m^{-2} \cdot day^{-1}$. The protein content of the produced biomass was around 60% on a dry weight basis. The produced biomass was used as a feedstock for the isolation of proteins rich in essential amino acids and potential applications in the development of novel foods suitable for vegan consumers. The isolated proteins had a protein content of approximately 90% and were assessed for their techno-functional properties. The protein isolate demonstrated high emulsifying, foaming, and gelling properties that were comparable to those of other commercial ingredients such as soy proteins. Moreover, the water and oil holding capacities were calculated as 2.2 and 2.9 $g \cdot g^{-1}$ respectively, suggested that the proteins could be also used in baked products or meat analogues. Finally, an *in silico* study was conducted to predict the potential utilisation of the isolated proteins as novel sources for bioactive peptides with applications in functional foods, cosmetics, and pharmaceuticals. A preliminary study using 27 protein sequences available at https://www.uniprot.org/ demonstrated that *A. platensis* is rich in previously identified peptides with varied bioactivities, especially in inhibitors of the enzymes ACE-I (EC 3.4.15.1) and DPP-IV (EC 3.4.14.5) that have implications in hypertension and diabetes. Moreover, the *in silico* analysis led to the identification of papain (EC 3.4.22.2) as the most suitable enzyme for the generation of bioactive peptide with biological activity.

Keywords: Vegan foods, Spirulina, Technofunctional properties, bioactive peptides, pilot-scale

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MSc in Industrial Computer Sciences and MSc in Chemical Engineering. Tomás completed his PhD in Agriculture and Food Science at University College Dublin in 2016. His doctoral studies led to a Wang Shang Fellowship, conferred by the International Society for Nutraceuticals and Functional Foods. In 2017, he was awarded a *Juan de la Cierva Formación* scholarship at IRTA. He is since 2020 working under the *Juan de la Cierva Incorporación* Programme at the University of Almería. He is a member of the Editorial Board of the International Journal of Food Science and Technology and Frontiers in Food Science and Technology.

FROM FUNDAMENTAL ALGAE RESEARCH TO APPLIED INDUSTRIAL PRACTICE - ALGAE TO FUTURE PROJECT

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ABSTRACT

The knowledge- and technology platform developed within the ALGAE TO FUTURE project aims to lay a foundation for an industrial microalgae production in Norway. In the project ALGAE TO FUTURE, funded by the Norwegian Research Council 2017-2021, with a consortium of 20 national and international research and industry partners, research and product development of microalgae biomass have been approached from multiple angles merging multiple research fields. The focus of the research has been bioprocess developments linked to lipids, carbohydrates and proteins, where species selection and cultivation conditions are used to obtain microalgae biomass with specific nutrient composition targeting specific products. We have chosen to target the development of three example products, namely 1) bread using algae biomass with high protein content, 2) beer using algae biomass with high content of starch and starch-degrading enzymes, and 3) fish feed using algae biomass with high PUFA content. These case studies have been chosen in order to demonstrate the use of algal biomass from various algae species with highly different nutrient composition suitable for different products. We have in this project studied the whole process line from small scale microalgae cultivation technology, upscaling cultivation, processing of algae biomass, shelf life, food/ feed product development, food safety and consumers attitudes. Some highlights from the four-year project period will be presented. Results from these activities may contribute towards the use of microalgae as part of the future Norwegian bioeconomy.

Keywords: Bioprocess, food, feed, nutrient composition, national microalgae network

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About the author:

Stig A. Borgvang has background as scientific advisor in WRM at National Authority and research institute levels, mostly related to eutrophication of freshwater systems. He has spent 6 years as deputy scientific secretary at the London based Paris Commission Secretariat. For the last 13 years, he has been actively involved in national and international co-operation related to the cultivation, processing and use of microalgae biomass. Currently, he is co-ordinating the ALGAE TO FUTURE project, funded by the RCN, with the *goal* to promote research that increases the level, profitability and sustainability of microalgae production in the bio-based industries in Norway.

Company profile:

NIBIO 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.

ALGAE PROTEINS: IS GALDIERIA THE NEW SPIRULINA?

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ABSTRACT

Galdieria sulphuraria is a polyextremophilic microalgae able to tolerate low pH (even pH 0.2), high temperatures (up to 57 °C) and high osmotic pressure (up to 400 $g \cdot L^{-1}$ of sugar and 2-3 M of salt). Due to these exceptional traits, *G. sulphuraria* can be cultivated using an organic carbon source without microbial contamination. In this work we optimized the light regime of *G. sulphuraria* grown autotrophically and under "oxygen balanced" mixotrophy, where intracellular recirculation of O_2 and CO_2 take place. Under oxygen balanced mixotrophic conditions, a closed reactor can be operated without any gas exchange.

Mixotrophically grown biomass contained 10% w/w of the blue pigment C-phycocyanin which, combined with its high areal biomass productivity (49 $g \cdot m^{-2} \cdot day^{-1}$), sums as one of the highest C-PC areal productivities ever reported (5 $g \cdot m^{-2} \cdot day^{-1}$) under constant illumination. C-PC extracted from *G. sulphuraria* was more acid- and thermostable than the currently used C-PC from Spirulina as the blue color was retained down to a pH 3 and up to 55 °C. *G. sulphuraria* had a protein content of 64% w/w and compared favorably with FAO's dietary recommendations of adults regarding amino acid composition. *G. sulphuraria* contains a high proportion of essential amino acids and has an amino acid profile superior to *Chlorella*, *Spirulina* and soybean protein. *G. sulphuraria* is especially rich in sulfur-containing methionine (1.5% w/w), and cysteine (1.1% w/w) and contains taurine (0.4% w/w). To conclude, aside from being a source of stable bleu pigments, *G. sulphuraria* also has potential in food and feed applications to overcome amino acid deficiencies.

Keywords: Amino acid profile, protein production, oxygen balance, acid stable phycocyanin, thermo stable phycocyanin, phycocyanin productivity

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F Abiusi, R.H. Wijffels, M. Janssen, Doubling of Microalgae Productivity by Oxygen Balanced Mixotrophy, ACS Sustain. Chem. Eng. 8 (2020) 6065–6074. https://doi.org/10.1021/acssuschemeng.0c00990.

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About the author:

Dr Fabian Abiusi has been working with microalgae for the last 11 years. His research has covered various applications of microalgae: fuels, pigments, waste water treatment and food.Dr Abiusi obtained his PhD degree from Wageningen University & Research in March 2021, with a thesis titled: ‘Oxygen Balanced Mixotrophy in Microalgae’. Dr Abiusi joined Algreen as Chief Scientific Officer last February with the goal of commercializing mixotrophic cultivation of microalgae.

Company profile:

Algreen B.V. is a start-up based in The Netherlands at the campus of Wageningen.Algreen is working as technical provider for microalgae sector. Algreen’s expertise lies in the cultivation and downstream processing of *Spirulina* and *Galdieria*, including mixotrophic cultivation.

STRATEGIES TO ENGAGE THE CONSUMERS IMPROVING THE SENSORY PROFILE OF MICROALGAE BIOMASS

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ABSTRACT

The use of microalgae as food ingredient has deserved special attention in recent times, considering its nutritional richness, particularly in terms of proteins, minerals, vitamins, lipids, antioxidants, and dietary fibres[1-4].

Despite several studies revealing a high potential of microalgae to enhance the nutritional profile of foods, their use still has a long way to go. One of the main problems is related to the consumer acceptance of new tastes, colors, and flavors that are present in microalgae-based-products, generally characterized by low sensory scores. Several strategies to engage the consumers can be used, namely: inclusion of low amounts of microalgae, biomass encapsulation, innovative gastronomy techniques or food education.

In the present work, two strategies were followed: i) microalgae was subjected to ethanol extraction to obtain less pronounced colors and flavors. The objective of this study was to compare the impact of adding 4% of raw and ethanol treated *Tetraselmis chuii*, *Nannochloropsis gaditana* and *Chlorella vulgaris*, produced by Algae2Future[5] partners, in technological aptitude, nutritional composition, and bioactivity of breads. This approach showed promising results during sensory evaluations and an improvement of dough rheology and baking performance; ii) another research line conducted by LEAF results from a collaboration with Allmicroalgae company. Different strains of *Chlorella vulgaris* were incorporated in several foods, like cheese, bread and vegan bars. These strains are produced by heterotrophic mode and random mutagenesis[6-7], that induces a deficiency in the production of chlorophyll. Heterotrophic *C. vulgaris* was found to be a versatile source of nutrients that can be used as a functional and sustainable ingredient to nutritionally enrich food reaching higher sensory scores, since it has appealing colors and a smooth aroma and flavor.

Keywords: ethanol treatment; heterotrophic microalgae, nutritional composition, sensory properties, food structure.

Acknowledgements: The work was supported by the Norwegian Research Council project Algae to Future, A2F (NFR 267872) and Portuguese Foundation for Science and Technology (FCT), through LEAF Research Center UIDB/04129/2020.

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Anabela Raymundo is Chemical engineer, MSc in Food Science and Technology and a PhD in Food Engineering. Assistant Professor with Habilitation, integrating the LEAF (Linking Landscape, Environment, Agriculture and Food) – coordination of the group Food and Feed.

Main research areas of interest: functional properties of macromolecules; development of new food products; evaluation of the rheological behavior of different food matrices and relations with the structural composition. Main work focused on the use of poorly exploited food sources (e.g., microalgae biomass and food industry by-products) for the development of high added value products. Participates in several national and international research. https://orcid.org/0000-0001-5266-1685.

Company profile:

LEAF (https://www.isa.ulisboa.pt/en/leaf/anuncios) is a research unit of ISA working on the whole agro-food chain, on a variety of scales, from cells and microorganisms trough sustainable and healthy food up to landscape design, by using a balanced combination of basic research with applied sciences and engineering to develop knowledge.

ISOLATION OF NOVEL *CHLORELLA VULGARIS* MUTANTS WITH IMPROVED PROTEIN CONTENTS AND PIGMENTS FOR FOOD APPLICATIONS

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ABSTRACT

Microalgae are unicellular organisms capable of living in the most diverse environments, growing faster than other photoautotrophs in non-conventional growth media that do not require potable water or arable land. Organisms with such characteristics can become an alternative food source for the future since the world population is increasing. Microalgae are already produced and consumed as a high-quality food. However, there is an urgent need to improve existing microalgae-based products since their organoleptic properties, such as their "grassy" taste, are not consensually accepted by the consumer. The present work aims to create new strains from microalgal species already registered as novel food, namely *Chlorella vulgaris*, to improve the quality of the produced biomass and the overall consumer acceptance. For this purpose, random chemical mutagenesis using the alkylating agent ethyl methane sulphonate (EMS) was carried out to generate mutants with higher protein contents and lower amounts of chlorophyll. Afterwards, the best performing strains were selected using visual scoring regarding pigmentation and flow cytometry techniques. Upon implementing this selection pipeline, two *C. vulgaris* strains were isolated, namely the C3 strain, a non-mutagenized isolate able to grow significantly faster on solid medium as compared to the wildtype (WT) culture and a second mutant (GL3) obtained from the C3 strain. Interestingly, although the C3 strain presented higher protein contents, the GL3 displayed vestigial chlorophyll contents, lower carotenoid levels, and higher protein content than the WT. The GL3 strain grown under heterotrophic conditions reached higher cell concentrations than the WT, strongly suggesting that the mutant strain GL3 might become a relevant source of protein, being suitable to be produced on a larger scale to generate food products with enhanced organoleptic properties.

Keywords: Random mutagenesis, microalgae, protein, food.

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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 on the project ProFuture at GreenCoLab where he carries out bioprospection, identification and isolation of novel species of microalgae, control of culture properties and taking part in experiments for selection and improvement of already established strains for consumption. He has also experience in HABs analysis, identification of microalgae and toxin monitoring

THE MICROALGAE *PHAEODACTYLUM TRICORNUTUM* AS AN OMEGA-3 FATTY ACID AND CAROTENOID SOURCE-HIGH BIOAVAILABILITY OF NUTRIENTS IN HEALTHY VOLUNTEERS

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ABSTRACT

In order to overcome food shortages due to the growing world population, microalgae could be a possible solution, as they contain large amounts of proteins and fatty acids (Ryckebosch *et al.*, 2012). A healthy diet requires omega-3 fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and it is recommended to consume fish once or twice per week for an adequate intake. If every individual would follow this instruction, our oceans would already be fished dry. Only 15% of this amount can be met by fisheries and other aquatic sources. Since microalgae are the primary producers of EPA and DHA, they could be used as an additional supplier. *Phaeodactylum tricornutum* (PT), a microalgae high in EPA, is already being used as feed for salmon and could potentially be used for human consumption, too (Sørensen *et al.*, 2016). In a clinical study (n= 22, age~ 26), the nutrient bioavailability, effects on blood levels, and intestinal safety were investigated after a two-week intake of PT (305 mg omega-3 fatty acids/ day) and compared to fish oil and fish intake in a crossover study. It was shown that PT increases plasma levels of both omega-3 and EPA, demonstrating equal bioavailability to fish oil. Additionally, the omega-6/3 ratio was reduced and the absorption of carotenoid fucoxanthin from PT was proven, which can prevent obesity (Gille *et al.*, 2019). The intestinal barrier and microbiome were not affected by PT, however short-chain fatty acid-producing bacteria, such as *Oscillospiracea* and *Akkermansia,* increased. As PT can be utilized for human nutrition, the present study is an important step towards the approval as a novel food.

Keywords: microalgae *Phaeodactylum tricornutum*, human, nutrition

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As a PHD student at the University of Hohenheim Stuttgart, I am working on microalgae as a new alternative for human nutrition. I am investigating the safety and bioavailability of nutrients and their health benefits, such as anti-inflammatory properties and improvement on gut health.

Company profile:
University of Hohenheim, Department for clinical nutrition, Stuttgart. Professor and head of the department Dr. med. S.C. Bischoff. The research interests of the department include all issues related to nutritional medicine, prevention and clinical research.

PHYCOCARE TO DECREASE ONCOLOGY SIDE-EFFECTS: A FIRST CLINICAL STUDY

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ABSTRACT

The use of existing effective oncology treatments is limited by their severe side effects. While many vitamin-based food, supplements are being used to alleviate minor side effects of oncology treatment, there is currently no solution for the major ones that have an impact on the nerves and the brains (peripheral neuropathy), present in more than 50% of the cases. Those limit the use of such oncology treatments or are so aggressive that the treatments are stopped in the middle when the patients can no longer bear them. Decreasing those side effects allows a better quality of life for the patient, and a better compliance with the prescribed oncology treatment. ALGOSOURCE's has developed a spirulina extract from his patented extraction technology to tackle this problem. A first clinical study (double blind versus placebo, Biomerieux) 2016-2019, has demonstrated the antioxidant effect of the product. A second clinical study with 110 patients has now started in 7 hospitals in France. It is the first time that Spirulina is being used within a clinical protocol in Europe, and there is currently no medical drug extracted from microalgae worldwide. In addition to the pure medical data and protocol, we review the industrial and regulatory developments associated with this new development for microalgae. We look at the future development of microalgae-based drugs and the necessary steps to reach this objective.

Keywords: Spirulina, oncology, clinical study, Phycocyanin

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Olivier Lépine is one of the founders and the Managing Director of AlgoSource, a group of companies dedicated to microalgae. Since 1993 AlgoSource has embarked on the development of Phycocyanin based products. Olivier has a master degree in Physics and chemistry and a master degree from the National School of Petroleum in France. He has served in the energy industry during 7 years abroad before joining the microalgae sector.

Company info:

AlgoSource vision is simple: microalgae for health. AlgoSource mission is to develop a microalgae industry that will help fulfilling the needs of our planet for us and the coming generation. The first company to commercially produce Spirulina in Europe, AlgoSource develops original efficient nutraceuticals to both industries and final consumers

THE ANTI-INFLAMMATORY PROPERTIES OF MARINE MICROALGAE IN LPS-INDUCED MACROPHAGES

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ABSTRACT

Inflammatory diseases such as inflammatory bowel disease (IBD) have become one of the leading causes of health issue throughout the world, having a considerable influence on healthcare costs. Existing treatments for IBD are not effective and emphasize the need for new biologic approach to improve IBD symptoms. Inflammation is mediated by cytokines produced by stimulated immune cells such as macrophages. Targeting pro-inflammatory cytokines such as Tumor necrosis factor-□ (TNF□) often reduce 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, anti-inflammatory potential of microalga was assessed in lipopolysaccharide (LPS)-stimulated murine RAW264.7 macrophages. Extraction were prepared using 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 the crude extract from micro algae significantly inhibited the secretion of TNF□ by 50- 60% ($p < 0.001$). Furthermore, the fractions effectively inhibited the LPS-induced TNF□ similar to the effect obtained by the crude extract. Interestedly, some fraction contained omega 3 and omega 7 that 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: microalgae, Inflammation, IBD anti-TNF cytokines

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Dr Dorit Avni heads the Sphingolipids and Natural Bioactive Compounds as Immune Modulators Laboratory. She specializes in in-vitro, ex-vivo and pre-clinical models, and practices a transdisciplinary approach in her research, employing genomic, immunologic, and metabolomic techniques. Her laboratory applies novel platforms and bio-active metabolites as immune system regulators to prevent and treat unmet diseases, such as COVID-19 and IBD.Her applied research approach is to introduce novel compounds based on algae as a functional food or drug.

PhD (Tel-Aviv University, IL) focused on novel pathways for the regulation of cytokines release. Post-doctoral fellowship (Massey cancer canter-VCU, USA) focused on sphingolipids in inflammation and cancer.

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

ALGAE BIOMASS AS POTENTIAL ADDITIVES FOR FISH GUT HEALTH MODULATION

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ABSTRACT

Fish gut stability and homeostasis is key for fish health, and new nutritional sustainable strategies to promote gut health are needed to develop new functional feeds. Algae functional properties (e.g. antioxidant, immunostimulant, prebiotic, wound healing) highlight their potential as gut health modulators to be included in diets as functional additives. The aim of this study was to assess the effects of algae biomass on seabream intestinal response and stability. For that gilthead seabream (*Sparus aurata*) intestinal explants were obtained from healthy fish and incubated with algae suspensions. Commercial dry biomass of three macroalgae (*Gracilaria gracilis*, *Fucus* sp. *Ulva* sp) and five microalgae (*Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Skeletonema* sp., *Tisochrysis lutea* and *Tetraselmis* sp.) were used, and the anterior and posterior intestine explants response was evaluated by the transcriptional modulation of a panel of genes. The expression of *glutathione peroxidase* was upregulated by microalgae in both intestinal sections, and *cyclooxygenase 2* expression was also upregulated by these biomasses suggesting immunoestimulation and higher antioxidant response. When evaluating genes coding for tight junction's proteins such as *claudin 12*, *occludin* and *zonula occludens 2* an increase in the last two was evident with microalgae in anterior intestine and with macroalgae in the posterior section. These results indicated a high potential of these biomasses to promote stabilization of the gut integrity, a golden feature for a functional ingredient targeting gut health. A validation trial was performed where intestine explants were incubated with pure saponins and the different algae, and the gut epithelium stabilization capacity of algae biomasses was confirmed to some extent, highlighting their potential as gut health and performance promoters.

Keywords: gut performance; functional algae; ex vivo; microalgae; macroalgae; gene expression

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Marine Biologist from University of Algarve, Master in Marine Biosciences and PhD in Marine Science from Tokyo University of Marine Science and Technology with specialization in Fish Health Management. Over the last years Dr. Gonçalves has been working in functional nutrition in aquaculture focusing on the health improvement of gut modulators such as probiotics, using nutrigenomics as a diagnostic, predication and monitoring tool. Presently, as a researcher in fish nutrition and immunology at GreenCoLAB, Dr. Gonçalves is dedicated to the investigation of the potential of algae as an ingredient for fish health promotion and improving the gut homeostasis in aquaculture.

Company profile:

The **GreenCoLAB** is a non-profit private organization a collaborative platform between research and industry, whose research & innovation agenda is based on the exploration of microalgae as an essential component for the food, feed, nutraceutical, bioenergy, wastewater and cosmetic industries.

ALGAE OMEGA-3S AS A SUSTAINABLE ALTERNATIVE TO FISH OIL

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ABSTRACT

Fish oil has traditionally been the primary source of long chain omega-3 fatty acids, which are essential nutrients for human diets as well as many aquaculture and animal feeds. The demand for fish oil is growing rapidly, due to an expanding aquaculture sector as well as rising demand in pet and livestock feeds, while the availability of fish oil from wild caught fish has levelled off over the past decade. Fish oil is not easily replaced and alternative sources are required to meet the growing global demand.

One of the most promising alternatives is microalgae - the original source of long chain omega-3s. Corbion is a leading producer of algae omega-3 products (AlgaPrime™ DHA), using Brazilian sugarcane as feedstock and a renewable energy source. To understand the environmental impacts of Omega-3s produced by heterotrophic algae, a comprehensive Life Cycle Assessment (LCA) was conducted for AlgaPrime powder and liquid products for six impact categories. A comparison with fish oil, using data publicly available in LCA databases, indicated that both AlgaPrime product formats offer a lower impact on climate change than fish oil.

A paper summarizing the results of the LCA has been accepted for publication at Algal Research and the results will be shared at the Algae Europe.

Keywords: Omega-3 DHA, Life cycle assessment (LCA), Heterotrophic algae, sustainability

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Thematic Session 3: Industrial Committee Session

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

Scientific Session 6: Algae Genetics and Improvement

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

IMPROVED INDUSTRIAL *TISOCHRYSIS LUTEA* STRAINS FOR FUCOXANTHIN AND DOCOSAHEXAENOIC ACID PRODUCTION

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ABSTRACT

Tisochrysis lutea is an emerging industrial microalgal species that can produce high-value compounds, i.e., fucoxanthin (40 000-80 000 USD/kg) and docosahexaenoic acid (DHA, 25 000-75 000 USD/ton). The current production yields of fucoxanthin and DHA are still too low to make *T. lutea* a competitive feedstock for these products. In addition, biomass production is limited at low temperatures during winter (< 20 °C) in temperate regions, making *T. lutea* a seasonal species with a high biomass selling price (400-800 €/kg).

In the EU project MAGNIFICENT, we focus on improving the content and productivity of fucoxanthin and DHA in *T. lutea* by process optimization and developing improved strains. The optimal growth and operation conditions (temperature, light intensity, dilution rate and biomass concentration) were determined for fucoxanthin and DHA productivities, at different scales (0.4-190 L), indoors and outdoors at AlgaePARC (www.algaeparc.com).[1, 2] In addition, *T. lutea* was cultivated under low light (50 µmol m^{-2} s^{-1}) to induce fucoxanthin production and low temperature (15 °C) for all year-round production; followed by high-throughput cell selection using fluorescence-activated cell sorting. We obtained two improved strains (flagella-less and winter strains) and achieved the highest ever reported fucoxanthin (9.8 and 2.1 mg g^{-1} d^{-1}) and DHA (12.6 and 4.8 mg g^{-1} d^{-1}) productivities, from indoors to outdoors, using the optimal cultivation parameters obtained in this study.[3-6] The production strategies and obtained robust strains have been applied at commercial scale by our partner NECTON, S.A. (Olhão, Portugal) to address the present challenges of low productivity and seasonality. This project opened new possibilities for fucoxanthin and DHA cost-effective production, and supports the planning of future R&D strategies and industrial applications.

Keywords: *Tisochrysis lutea*, fucoxanthin, docosahexaenoic acid, fluorescence-activated cell sorting, improved strains

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6 **Gao, F.**, Teles, I. D., Wijffels, R. H., & Barbosa, M. J. (**2022**). Fucoxanthin and lipid production by cold-adapted *Tisochrysis lutea*. *New Biotechnology*, 66: 16-24.
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About the author:

Dr. Gao is a Postdoctoral Researcher in Bioprocess Engineering group at Wageningen University. He is involved in the European project MAGNIFICENT working on *Tisochrysis lutea* strain improvement for fucoxanthin and DHA production.He holds a PhD in Aquatic Products Processing and Storage Engineering obtained at Ocean University of China (2019) and a PhD in Bioprocess Engineering obtained at Wageningen University (2021). He obtained an EMBO Short-Term Fellowship after obtaining his PhD. During the past three years, he gave >10 talks on Microalgal Biotechnology topics during several large international conferences. He won the silver medal of the Best Microalgae Awards 2020.

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 world’s best in the field of Agriculture & Forestry.

METABOLIC ENGINEERING FOR THE PRODUCTION OF GERANIOL IN *CHLAMYDOMONAS REINHARDTII*

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ABSTRACT

Geraniol is a molecule that has interesting organoleptic and aromatic properties. It is also widely used for its repellent properties against many insects and as an antibacterial and antifungal agent. Finally, it is used in various drug therapies thanks to its ability to fight many types of tumors. All the reasons mentioned above make it a molecule of industrial and commercial interest. With the aim of providing an alternative to complicated chemical synthesis, here we report a synthetic biology approach to engineer *Chlamydomonas reinhardtii*, a model organism for green algae, to make this species become a sustainable bio-factory for geraniol production. In particular, we introduced in the *C. reinhardtii* nuclear genome the two key enzymes geraniol synthase (GES) and geranyl pyrophosphate synthase (GPPS) necessary for the biosynthesis of this monoterpene. A set of engineered strains was thus generated expressing only GES or only GPPS, differing in number of gene copies introduced or in heterologous proteins localization. The production of geraniol was verified in these strains by GC-MS while no evident growth phenotype was observed in engineered strains. Geraniol production was thus investigated in different growth conditions (autotrophy / mixotrophy and high light / low light) and different growth stage. The results reported show that it is possible to obtain a production of geraniol at high levels using *Chlamydomonas rienhardtii*, making this organism an excellent alternative for the production of this compound.

Keywords: Chlamydomonas, terpenes, synthetic biology, metabolic engineering

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Federico Perozeni holds a PhD in Biotechnology obtained at the University of Verona in 2020. He is now a post doc researcher at the SOLE-LAB group coordinated by Prof. Matteo Ballottari at the University of Verona. Federico Perozeni is involved in several national and international research projects focused on the genetic engeenring of microalgae to produce high value products. He published since 2017 13 papers in peer-reviewed international journal.

NATIVE MASS SPECTROMETRY; THE KEY TO UNLOCKING THE SECRETS BEHIND PHYCOBILIPROTEIN PRODUCTION AND QUALITY

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ABSTRACT

Mass spectrometry is a rapidly emerging technology that can tell us exactly what is happening to a product in space and time. Moreover, the information mass spectrometry can now provide about proteins could provide a breakthrough for algae biotechnology. Here, we demonstrate how state-of-the-art analytical mass spectrometry tools have been developed to monitor high value phycobiliprotein products extracted from algae. Mass spectrometry analysis of phycobiliproteins revealed unique structural details that are invisible to other biophysical techniques yet their presence correlates with its attractive blue colour. Moreover, each spectrum is unique to a certain algae species meaning that we can rapidly identify the source of algae used by another manufacturer through analysis of their phycobiliprotein containing product. Overall the data shows the power of mass spectrometry has to transform the phycocyanin production industry whilst simultaneously providing direct information on the quality of these high-value phycobiliprotein products.

Keywords: mass spectrometry, phycobiliproteins, phycocyanin, algae species identification

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About the author:

Aneika C. Leney's research group is developing protein mass spectrometry tools to monitor phycobilisomes from algae. Dr Leney is interested in how these complexes respond to light and how this information can be used to bio-engineer novel, natural, coloured protein complexes for food, cosmetic, therapeutics or biochemical applications. Her recent work has further expanded into using mass spectrometry as a diagnostic tool to identify algae from algae blooms and to also identify algae species retrospectively from supermarket products containing algae extracts.

A NON-GMO APPROACH TO ISOLATE NOVEL *CHLORELLA VULGARIS* MUTANTS WITH HIGHER BIOMASS PRODUCTIVITIES FOR FOOD AND FEED APPLICATIONS

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Associação Oceano Verde – Green CoLAB, Portugal

ABSTRACT

World's population is estimated to reach 9 billion people in 2050 [1], which raises significant concerns about the future food and feed demand [2,3]. In this context, microalgae are widely recognized as promising alternatives to conventional feedstocks, due to their interesting nutritional profiles, rich in protein and bioactive metabolites [4,5]. However, only a limited number of microalgal strains, including *Chlorella vulgaris*, are currently approved for human consumption (EU2017/2470). Despite all the investment in R&D, microalgal products struggle to reach competitive market prices, mainly due to low productivities obtained in industrial production [6]. Accordingly, non-GMO approaches (DIRECTIVE2001/18/EC) [7,8] for strain improvement, such as random mutagenesis, might be key to increase overall biomass productivities [9–11]. This work aimed to develop novel food-grade non-GMO *Chlorella vulgaris*' mutants with improved growth performance. Accordingly, chemically induced random mutagenesis was used to generate 11 new mutants. Two mutants, 200A and 200D, displayed 16% and 7% higher biomass productivities (2.21 ± 0.03 and 2.00 ± 0.05 g/L/day, respectively) than the wildtype (1.86 ± 0.09 g/L/day). On the other hand, the protein and chlorophyll contents in mutants 200A (23.78% ± 1.17 and 1.71 ± 0.07 g/100g, respectively) and 200D (27.94% ± 1.23 and 1.74 ± 0.08 g/100g DW, respectively) were slightly inferior to the wildtype (29.66% ± 2.41 and 2.00 ± 0.16 DW). Further work is required to validate these results on a larger scale; however, these novel mutants are very promising in what concerns a more productive industrial cultivation of *Chlorella vulgaris*.

Keywords: Strain improvement; Random mutagenesis; Microalgae; *Chlorella vulgaris*;

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Thematic Session 4: Blue Invest

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CONFERENCE DAY 3

ALGÆUROPE2021

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Scientific Session 7:
Algae Biorefinery and Multi-Use Approaches

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INDUSTRIAL SCALE PRODUCTION OF MICROALGAE BIOMASS FOR AGRICULTURE AND AQUACULTURE RELATED APPLICATIONS

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ABSTRACT

After five years the SABANA project conclude with the demonstration of industrial scale facilities producing microalgae biomass for agriculture and aquaculture related applications. Three industrial facilities of 1.7, 2.0 and 3.0 ha were finally build and operated, including harvesting and processing of the biomass. The project, supported by the EU Blue-Growth program, demonstrate that microalgae production can be sustainable when integrating the recycling of waste streams such as wastewater and manure. In spite of using waste streams the microalgae biomass has enough quality to be applied on both agriculture and aquaculture. Processing of the wet biomass was performed by using high-pressure homogenizers and enzymatic hydrolysis, avoiding the use of organic solvents or drying steps. On this way, the bio-stimulants and bio-pesticides already obtained demonstrates to improve the production of largely different crops, up to 15% while reducing the requirement fertilizers up to 10%. Positive effects were related with both the enhancement of root systems and inhibition of pathogens, especially fungi. Concerning aquaculture, results confirm that including up to 4% of microalgae biomass into the aquafeed provide pro-biotic effects increasing the health of fish and improving the quality of fillets. In addition to these products, SABANA project also contribute to the microalgae field by providing valuable knowledge about monitoring methods, advanced control systems, temperature control strategies, improvement of mixing and light utilization in open reactors, etc.. Models and tools already developed are also a valuable result because they can contribute to the further development of new microalgae related industrial processes.

Keywords: Industrial facilities, biorefinery, agriculture, aquaculture, wastewater

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SEQUENTIAL AND EFFICIENT PRODUCTION OF FUCOXANTHIN FROM THE DIATOM *PHAEODACTYLUM TRICORNUTUM* USING HIGH PERFORMANCE COUNTERCURRENT CHROMATOGRAPHY

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ABSTRACT

Phaeodactylum tricornutum is a rich source of fucoxanthin, an orange-brown carotenoid with several health benefits [1,2]. The present study reports, for the first time, the development and application of an efficient, and scalable high performance countercurrent chromatography (HPCCC) isolation method to obtain fucoxanthin from the diatom *P. tricornutum* using a multiple sequential-injection separation strategy. The biomass extraction and HPCCC isolation processes were unified using the same solvent system to improve the fucoxanthin final recovery. A multiple-sequential injection HPCCC method was developed combining two elution modes (reverse phase and extrusion). The lower phase of the selected biphasic solvent system (*n*-heptane, ethyl acetate, ethanol and water, ratio 5/5/6/3, *v*/*v*/*v*/*v*) was used as the mobile phase, while the upper phase was the stationary phase. Ten consecutive sample injections (240 mg of extract, each) were performed leading to the separation of 38 mg fucoxanthin with purity of 97% and a recovery of 98%. The process throughput was 0.189 g/h while the efficiency per gram of fucoxanthin was 0.003 g/h. Environmental risk and general process evaluation factors were used for assessment of the developed separation method, which was compared with existing fucoxanthin liquid-liquid isolation methods. The developed method does not need the use of another chromatographic technique to achieve the desired purity of fucoxanthin. Overall, the developed isolation method may represent a useful model to produce fucoxanthin from diatom biomass on a large scale.

Acknowledgement: TAČR-NCK grant (TN010000048/03, J.C, D.B-P, P.H. and A.S.). National Programme of Sustainability I, MŠMT, Czech Republic (ID: LO1416, J.C., J.K. and P.H.) and the Grant GAJU 017/2019/P.

Keywords: Fucoxanthin; *Phaeodactylum tricornutum*; high performance countercurrent chromatography (HPCCC); countercurrent chromatography (CCC); centrifugal partition chromatography (CPC).

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EUTECTIC SOLVENTS WITH TUNEABLE HYDROPHOBICITY FOR LIPID EXTRACTION FROM MICROALGAE

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ABSTRACT

Eutectic solvents (ES), a new class of eco-friendly designer solvents, hold the potential to integrate the algal biomass pretreatment and lipid extraction within a single step. However, most of the reported ES are hydrophilic, unsuitable for lipid extraction. Furthermore, the lipid recovery from ES is challenging due to the low volatility of ES. Therefore, in this study, we developed ES with tuneable hydrophobicity to enable both extraction and recovery of lipids. The combinations of various compounds (e.g., carboxylic acids and quaternary ammonium salts) were screened based on their ability to dissolve water and model oils. The ES dipolarity were also quantified through solvatochromic analysis. Made of imidazole and hexanoic acid, the hydrophobicity of this combination shifted depending on the compositional ratio. The ES dissolved model oils at low imidazole content, whereas at higher imidazole content, the ES became miscible with water. Thus, by adding imidazole to the ES-oil solution, the oils could be efficiently recovered (~75%) from the ES with high purities (> 85%). Furthermore, we also used the imidazole/hexanoic acid ES to extract lipids from intact microalga *Nannochloropsis oceanica*. A preliminary result showed that the extraction on wet biomass reached higher lipid yield than on dry biomass, indicating the further simplification for the algal lipid extraction process.

Keywords: eutectic solvents (ES), tuneable hydrophobicity, lipid extraction, imidazole, hexanoic acid, *Nannochloropsis oceanica*

References: Lo et al. (2021). RSC Advances, 11, 8142-8149. DOI: 10.1039/d1ra00306b

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Calvin completed his MSc in Biotechnology at Wageningen University & Research. In his MSc thesis, he studied the mechanical cell disruption for microalgae. He recently finished his PhD at the same university on the topic of biorefinery, where he developed semi-hydrophobic eutectic solvents for lipid extraction from microalgae. Currently, he is working as a postdoctoral researcher to further develop novel solvents for microalgae biorefinery.

Company profile:

Wageningen University & Research is a public university in Wageningen, Netherlands, specializing in technical and engineering subjects and an important center for life sciences and agricultural research.

POTENTIAL BIOREFINERY APPROACH OF *CHLORELLA* SP. BY LIQUID TRIPHASIC FLOTATION SYSTEM AS SUSTAINABLE DOWNSTREAM PROCESS

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ABSTRACT

Microalgae are potential sustainable renewable sources of energy but are highly underutilized due to the expensive and time-consuming downstream processing. This study focused on optimisation of different parameters of ultrasonication-assisted LTF system (U-LTF) to extract proteins, lipids and carbohydrates from *Chlorella sorokiniana* CY-1. The parameters involved were optimized and the final recovery efficiency of proteins, lipids, and carbohydrates was determined. A control run involving conventional three-phase partitioning and a 15-fold scale-up system with the recycling of phase components were also performed. Gas Chromatograph (GC-FID) and Fourier Transform Infrared (FTIR) spectroscopy were used to examine the potential of extracted products as a source of biofuel. The optimised parameters were ammonium concentration of 40 w/v% with volume ratio of 1:1 for $(NH_4)_2SO_4$:t-butanol; flotation air flowrate of 100 mL/min for 5 min; ultrasound pulse settings of 20 s ON/ 30s OFF at pH of 6.5. The resultant protein separation efficiency, protein recovery, lipids recovery and carbohydrates recovery were 26.81±5.93 %, 96.59±8.15 %, 61.02±0.91 % and 52.69±1.90 % respectively. The comparison study showed that U-LTF system gave higher yields than the control runs. A large-scale system (20 ×) observed similar yields. The recycling of phases was successful and extraction for multiple cycles is possible. This biorefinery approach is crucial in commercializing microalgae for biodiesel and bioethanol generation with a side product of purified proteins as feed.

Keywords: biorefinery; downstream processing; liquid triphasic flotation; microalgae.

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IN SITU EXTRACTION (MILKING) OF *BOTRYOCOCCUS BRAUNII* ON A SEMI-TECHNICAL SCALE

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ABSTRACT

The costs of recovering value substances out of microalgae biomass are a major bottleneck of microalgae biotechnology. Based on the common process chain, the steps of harvesting, dewatering and cell disruption for obtaining intracellular products mount up 50 to 80 % of the total process costs [1]. An opportunity for reducing these costs is the process of *in situ* extraction (milking) of microalgae, in which the product, secreted from the cells, is extracted repetitively during the cultivation from the culture suspension without destroying the cells. A suitable microalga for this process is *Botryococcus braunii*, which actively secretes long chain hydrocarbons (C18 to C37), amounting to up to 85 % of the biomass in an extracellular matrix around the cells. For the extraction of this hydrocarbons during the cultivation without cell disruption, an *in situ* extraction process was developed, patented at Anhalt University of Applied Sciences (DE102014005372A1) and approved at laboratory scale (3 L cultivation volume) [2]. The aim of current investigations is the scale up of the laboratory scale extraction process to technical scale for evaluation of the economic and ecologic feasibility. Therefore, two *B. braunii* strains (Showa and Bot22), which were identified previously as potential candidates [3], were optimized in terms of extraction time and particle diameter of the culture suspension flowing through the solvent. The cultivation and *in situ* extraction of these two strains was transferred from 3 L bubble column to 6 L FPA-reactors (semi-technical scale). The data presented show results for the optimization of the strain dependent optimal extraction time, the optimization of particle diameter and the *in situ* extraction over 45 days at semi-technical scale.

Keywords: *Botryococcus braunii*, *in situ* extraction, milking, hydrocarbons

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BIOPRODUCTS AND BIOENERGY RECOVERY FROM MICROALGAE BIOMASS TREATING WASTEWATER: A BIOREFINERY APPROACH

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ABSTRACT

In the microalgae biorefinery approach, the extraction of value-added bioproducts (like natural pigments, biopesticides and biostimulants) from microalgae would produce residual biomass that could still be valorized to obtain bioenergy and enhance the feasibility of the process (Arashiro et al. 2020). In addition, the use of wastewater as growth medium would improve the sustainability of the process, while also providing sanitation (SDG nº 6). The aim of this study is to assess the potential microalgae biomass downstream processes for value-added products (biopesticides and biostimulants) extraction followed by biogas production from residual biomass. Additionally, the co-digestion with primary sludge to increase the biogas production was also assessed. The biomass was harvested in a pilot raceway pond treating wastewater in Almería (Spain). The influence of the downstream processes applied on physicochemical properties and anaerobic biodegradability of microalgae biomass was studied in mesophilic biochemical methane potential (BMP) tests. The results evidenced the high methane yield of mono-digestion trials of microalgae biomass after the extraction of biopesticides (518 mL CH_4/g VS) as compared to raw microalgae (303 mL CH_4/g VS) and primary sludge (317 ml CH_4/g VS). Upon co-digestion, the methane yield was increased by 22% after the extraction of biopesticides, and the process kinetics by up to 14% after the extraction of biostimulants. According to the results, by combining the extraction of biopesticides and biostimulants, and the production of biogas from residual biomass, we would not only obtain value-added compounds but also more energy (around 5−10% higher), as compared to the single recovery of biogas, in agreement with previous studies (Arashiro et al. 2020). The proposed process appears as a promising strategy for resource recovery from wastewater, moving towards a circular bioeconomy.

Keywords: Anaerobic digestion, biogas, bioproduct, biorefinery, circular economy, microalgae, wastewater, resources recovery

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Arashiro, L.T., Ferrer, I., Pániker, C.C., Gómez-Pinchetti, J.L., Rousseau, D.P.L., Van Hulle, S.W.H., Garfí, M. (2020) Natural pigments and biogas recovery from microalgae grown in wastewater. ACS Sustainable Chemistry & Engineering, 8, 29, 10691–10701.

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Evelyn Ruales is currently a first year PhD candidate at the Universitat Politècnica de Catalunya·BarcelonaTech. Her research is focused on anaerobic digestion technology from microalgae biomass treating wastewaters. The recovery of renewable energy and useful bio-products from microalgae biomass is a key aspect in the microalgae biorefinery approach and the bio-based circular economy. Despite being a full-scale mature technology, aspects in microalgae such as community resilience, production rates, etc. can still be improved. Her research undertakes to develop novel methods and approaches to improve the microalgae anaerobic digestion for integrated energy and resource recovery.

Company profile:

The Group of Environmental Engineering and Microbiology (GEMMA) is located at 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 biotechnology, water supply, wastewater and solid waste treatment, and bioenergy generation.

LACTOBACILLACEAE FOR SEAWEED FERMENTATION

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ABSTRACT

Seaweeds are recognized as natural and abundant resources with great potential for transformation by many industries. However, one bottleneck during processing is that they rapidly decompose once harvested due to their high water content. In this context, seaweed preservation by fermentation, and not only by ensilage methods used in agriculture, are promising techniques. Strains from the *Lactobacillaceae* family are good candidates for this work. The aims of this study were to isolate strains of *Lactobacillaceae* from fresh seaweeds, to identify them and to select the best ones for fermentation using different screening methods. Strains of *Lactobacillaceae* were isolated from fresh seaweeds directly after harvest or cultured in MRS broth for 48 hours before isolation. Presumptive strains of *Lactobacillaceae* were confirmed by PCR and identified by gene sequencing. Proteolytic activity was determined by diffusion method on skim milk agar. Bacterial growth and NaCl tolerance were evaluated by monitoring the optical density at 600 nm in 96-well plates. Carbohydrate metabolism was studied using GEN_{III} microplates. 17 isolates of *Lactobacillaceae* were identified from fresh seaweeds and 35 others isolated from food matrices were added for screening. Most strains were found to be weakly proteolytic. Isolates from seaweeds showed higher growth rates and were more tolerant to 3.5% NaCl. Carbohydrate metabolic profiles on GEN_{III} microplates showed a large variety of profiles depending on the origin of the bacterial isolates. This work will be pursued by the development of mini models for solid state fermentation. Metagenetic and peptidomic approaches will be used to assess the fermentation potential of a seaweed-based substrate by the most promising strains or cocktails of strains.

Keywords: Seaweed, fermentation, *Lactobacillaceae,* screening

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After a master's degree in marine biology and human health, I worked in OLMIX S.A. as a project manager before starting my PhD. I am now in my last year of PhD on preservation of seaweeds by fermentation and promotion of fermented seaweed-based products. My PhD is financed by a CIFRE grant on a collaborative project between OLMIX and the MALIM research team of the ABTE laboratory at the University of Caen Normandie. After my PhD, I would continue to be involved in marine biotechnologies.

Company profile:
OLMIX S.A. is a company specialized in marine biotechnologies and green chemistry. It uses seaweeds to develop innovative products which will be used for animal care and plant care. The main goals of this company are to reduce the use of antibiotics and phytosanitary products in farming thanks to seaweeds.

CULTIVATION OF CYANOBACTERIA IN WASTEWATER FOR MULTI-RESOURCE RECOVERY

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ABSTRACT

Cyanobacteria have been cultivated as promising platforms to produce many valuable compounds such as lipids, proteins, carbohydrates, polyhydroxyalkanoates (PHAs) and natural pigments, among others. The use of wastewater as an alternative to commonly used nutrient sources has been pinpointed as an opportunity to reduce the production costs of cyanobacteria and related bioproducts. The aim of the present study was to evaluate the cultivation of cyanobacteria in treated wastewater in a pilot scale photobioreactor for the production of lipids, polyhydroxybutyrate (PHB) and phycobiliproteins (PBPs). A 30 L photobioreactor, fed with secondary effluent from a wastewater treatment plant, was used to grow *Synechocystis* sp. Three different hydraulic residence times (HRT) (6, 8 and 10 days) were tested to determine the optimal cultivation conditions. Harvested biomass was either used to extract and quantify PBPs, or transferred into 3 L vertical column photobioreactors to stimulate PHB and lipid accumulation under nutrients (N and P) starvation. A maximum productivity of 0.177 g L^{-1} d^{-1} and a biomass concentration of 1.413 g L^{-1} was reached with an HRT of 8 days. A maximum PBP productivity of 7 mg L^{-1} d^{-1} was obtained by using this HRT. However, a slightly higher content of PBP (7%$_{dw}$) was obtained under 6 days of HRT. The maximum accumulation of PHB was obtained with the biomass harvested during the period with an HRT of 6 days. A 4.8%$_{dw}$ of PHB was reached after 7 days of nutrients starvation. Finally, a high content of lipids (aprox. 40%) in the cells under P limitation was attained. In conclusion, this work demonstrates the feasibility of cyanobacteria cultivation in wastewater to produce bio-based valuable compounds within a circular economy approach.

Keywords: cyanobacteria, photobioreactor, wastewater, PHB, phycobiliproteins, lipids

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I am a PhD student at the Universitat Politècnica de Catalunya (UPC). I hold a BSc in Biotechnology (Universitat Politécnica de Valencia) and a MSc in Applied Blue Biotechnology (La Rochelle Université). Since then, I have been working in the cultivation of cyanobacteria for the screening of bioactivities and recovery of bioproducts. My current interest focuses in the recovery of natural pigments (mainly phycobiliproteins) from cyanobacteria grown in wastewater under a circular economy framework.

Company profile:

The Group of Environmental Engineering and Microbiology (GEMMA) carries out interdisciplinary research in the fields of environmental biotechnology, wastewater treatment and water supply, solid waste treatment and bioenergy generation. GEMMA aims at contributing to sustainable development by using ecoinnovaition and biotechnology as main tools.

Thematic Session 5: Young Algaeneer's Corner

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SEX CHROMOSOMES EVOLUTION: NEW INSIGHTS FROM BROWN ALGAE

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ABSTRACT

Brown algae (Phaeophyceae) represent a unique group to study aspects of sexual reproduction, due to the rich variation of life cycles, fertilization modes and sex determination systems found in this lineage. Particularly interesting are the aspects of the evolution of sex determination during the haploid life stage (UV sex chromosomes) and the various degrees of sexual dimorphism between gametes of closely related species, ranging from isogamy to oogamy. In this study, we used high-quality genomic, transcriptomic and genetic marker data to investigate the genomic architecture and evolutionary history of the sex chromosomes across two major brown algal orders (the Ectocarpales and the Laminariales) and correlate it with the different life history traits. Comparative genomic analysis with the model brown alga *Ectocarpus sp*. revealed that the sex chromosomes are derived from a common ancestral sex-determination system and have been conserved across at least 180 MY of evolution. Despite the conservation of a number of core genes, the sex-chromosome regions are, however, highly dynamic and could be related to the contrasting levels of sexual dimorphism and life cycle strategies.

Keywords: UV sex chromosomes, brown algae

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PALMARIA PALMATA: UPDATED HATCHERY AND CULTIVATION METHODS TO IMPROVE BIOMASS YIELD

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ABSTRACT

The red macroalgae *Palmaria palmata* is a highly demanded biomass; however, current supply from wild harvest is unsustainable and cannot meet the increasing demands [1]. This paper summarizes recent updates regarding domesticating *P. palmata* for its cultivation: 1) factors involved in stable tetraspore production outside the natural peak season of fertility, 2) efficient spore use and seeding of cultivation substrates and 3) optimal substrate configuration to maximize yield using longline cultivation. Induction of spore formation in vegetative sporophytes exposed to different treatment levels outside the natural season of fertility was evaluated. In pursuit of optimizing the area-specific production, we compared biomass yields from longline cultivation trials around Denmark using different types and deployment configurations of seeded growth substrates. Analysis of selected biogenic polyamines revealed elevated concentration in ripe fertile sporophytes compared to non-fertile individuals, indicating their potential involvement in sporogenesis. Day length and nutrient concentration showed sequentially importance in inducing spore formation however; we did not obtain a spore release during off-season. A vertical flow-through spore-seeding method showed high efficiency in seeding nets. Adding male gametes to enable fertilization of female gametophytes showed to increase the number of seedlings on substrates. Detained and germinated tetraspores showed the ability to re-attach to substrates up to 40 days post release using the GMA-seeding method, hence germinated spores constitute a secondary seeding material to prolong the seeding season and further improve spore use efficiency. Seeded net substrate with a cultivation depth of 1.4 m resulted in highest biomass harvest per meter longline. In conclusion, we report 5-17 times higher efficiency in the spore seeding 1 km of growth substrate with *P. palmata* tetraspores.

Keywords: Dulse; hatchery seeding; spore use efficiency; propagules; re-attachment

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About the author:

My current research focus is to improve hatchery protocols and cultivation methods for macroalgae. Furthermore, I am assessing the effect of harvesting wild Fucus sp. in Danish waters to provide updated knowledge for management.

NEW FACILITIES FOR LARGE SCALE HATCHERY PRODUCTION OF SEAWEED IN DENMARK

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ABSTRACT

With 8,750 km of coast line and coastal waters of high hygienic standards, Denmark has an obvious potential for high quality seaweed production. At DTU Aqua, we conduct research within commercial utilization of seaweed by developing stable, cost-efficient methods for aquaculture production of various species of commercial interest, and by providing management input on commercial harvest of wild stocks to authorities.

Recently, we established a new state-of-the-art commercial-sized hatchery (900 m^2) that allow us to conduct high quality research within various commercially relevant aspects of seaweed production. We focus on supporting the seaweed industry by providing the scientific knowledge for seed production of a wide range of seaweed species and by developing protocols for large scale production. At the moment, our main focus evolves around red seaweed species, as there is a high demand for farming protocols within this group and diversification is key to develop the seaweed industry. Our facility has strict biosecurity measures and a specialized separate quarantine area that allow us to work with non-native species in a safe way with no risk for the surrounding environment.

Keywords: hatchery, biosecurity, species diversification, industry support

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About the author:

I do research on utilization of marine resources within: 1) seaweed cultivation and; 2) management of marine vegetation. I focus on optimization of culture techniques of different seaweed species spanning from greens, to browns, to reds with hatchery protocols for enhanced seed production, eco-type selection, site-selection for grow-out, bioremediation potential, and biomass quality as essential lines of topics. In addition, I work with management of marine vegetation, e.g. in relation to environmental impact assessments of fisheries and commercial harvest of wild seaweed. This includes marine habitat mapping of macroalgae and eelgrass and development of new techniques for management use.

UPSCALING SEA LETTUCE AQUACULTURE: HOW CULTIVATION CONDITIONS AFFECT THE INDUSTRIAL-SCALE PRODUCTION OF *ULVA FENESTRATA* IN AN OFF-SHORE SEAFARM

Sophie Steinhagen[1]

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ABSTRACT

Compared to Asia, seaweed aquaculture is in its infancy in Western Europe. However, due to the multi-applicable use of this renewable resource there is a rapidly growing interest in efficient production technologies to foster a large-scale industrial production of seaweed biomass. Especially the sustainable oceanic cultivation of seaweeds is attractive as it does not compete with terrestrial crops for space and freshwater, and as extractive organisms seaweeds do not afford fertilization whilst providing high biomass yields.

This study monitored the effect of seasonality on the overall biomass performance (growth, biomass yield), chemical composition (fatty acid, protein, carbohydrate, pigment, phenolic, biochar, ash, and element composition), fertility (total amount of fertile thallus tissue), and biofouling (total coverage) of Swedish off-shore cultivated *Ulva fenestrata*, in order to find suitable harvest times for biorefinery purposes. Specimens of *U. fenestrata* were cultivated in an off-shore seafarm in the Kosterfjord, Sweden from October to five different harvesting points in April, May and June. Statistical analyses confirmed that there was a significant difference in overall biomass performance and biochemical composition among the time points. Our study confirmed the large scale off-shore cultivation potential of northern hemisphere *U. fenestrata* and underpins suitable harvest time points to facilitate industrial valorization processes of the off-shore cultivated biomass. Together, these results indicate that seasonality and the selection of harvest periods are crucial factors to consider in order to facilitate high yields, respective quality as well as desired biochemical traits in future oceanic *Ulva* farms.

Keywords: Aquaculture, Biomass valorisation, Ulva, biochemical compounds, protein, carbohydrates

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I am a researcher at the University of Gothenburg (Sweden) dedicated to investigating the marine biodiversity of macrophytes and supporting a sustainable seaweed aquaculture in the Northern Hemisphere. During my PhD at the GEOMAR Helmholtz Centre for Ocean Research (Germany), I investigated the taxonomy and systematics of different genera of green algae and the impact of environmental factors on their distribution, invasiveness, and blooming potential. I am having a passion for teaching phycological and molecular related topics, combined with an interest for communicating marine biology to a broader audience.

SCALE-UP OF NON-AERATED MIXOTROPHY WITH *GALDIERIA SULPHURARIA*

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ABSTRACT

Oxygen-balanced mixotrophy is a promising cultivation method that increases autotrophic productivity and biomass concentration by adding an organic substrate while removing any kind of external aeration. Substrate supply is carefully adjusted so that photosynthetic oxygen production is balanced with respiratory oxygen consumption. In turn, carbon dioxide released by substrate oxidation is recycled by the photosynthetic machinery, resulting in only a small loss of the organic carbon provided.

So far, this process has been developed in a laboratory setting [1, 2]. The next step involves moving from benchtop reactors to large-scale tubular photobioreactors, which requires addressing several challenges. For example, under outdoor conditions cells face light and temperature changes that are absent in controlled lab environments. In addition, substrate feeding needs to be re-designed to take into account the different fluid behavior in large volumes and maintain the oxygen balance. On top of these, there is an increased risk of bacterial and fungal contamination due to the supply of an organic carbon substrate to the system.

Our approach to achieve a successful scale-up consists of two different parts: (1) scale-down of the process to study large scale conditions in the lab before going outdoors and (2) using the acidophilic microalga *Galdieria sulphuraria* as our model strain to reduce the chance of contamination while exploiting its metabolic versatility.

Keywords: oxygen balanced mixotrophy, *Galdieria sulphuraria*, acidophile, scale up, tubular photobioreactor, scale down

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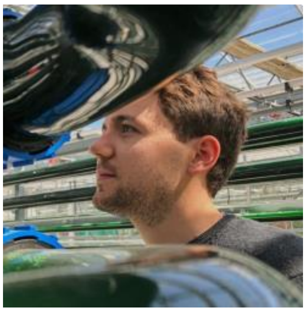

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About the author:
Pedro Moñino Fernández was born in Spain in 1995. In 2013, he started his scientific path with a BSc degree in Biotechnology in the Technical University of Madrid. Later he moved to The Netherlands to continue his studies with a MSc 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.

CYANOFLAN: A VERSATILE CYANOBACTERIAL POLYMER FOR THE COSMETIC INDUSTRY

Rita Mota

Jorge Cardoso

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ABSTRACT

With a more environmentally conscious society, the demand for natural alternatives that can replace harmful chemical/synthetic origin compounds is rapidly increasing worldwide. In particular, the cosmetics and personal care industry has been actively seeking for natural ingredients that can contribute to the quality, performance, value, and lifespan of formulations, while promoting a sustainable and eco-friendly economy. In this context, cyanobacteria-derived products are still an unexploited resource, offering an opportunity to address key challenges in the cosmetics and personal care industry. The CyanoCare project aims to develop innovative and added-value products from cyanobacteria, starting by Cyanoflan: a unique extracellular polymer naturally produced by a marine unicellular cyanobacterium [1]. Cyanoflan is a complex and versatile macromolecule that can be applied in cosmetic and personal care formulations as a rheology modifier, showing a viscosity about 1.5 times higher than xanthan gum (a natural ingredient commonly used as thickener in cosmetic formulations) [2]. Furthermore, *in vitro* and *in vivo* results demonstrated Cyanoflan biocompatibility with human cells and bioactivity, since it has antioxidant and anti-inflammatory properties, providing protection to the skin and promoting its regeneration [3]. In addition, the biomass surplus can also be used to generate value based on a circular economy model, having the potential to be commercialized as a dietary supplement, for example. The incorporation of natural, renewable and sustainable raw materials such as Cyanoflan into commodities and premium products from the cosmetics and personal care industry will allow reaching the expanding market of environmentally conscious consumers following nature's lead.

Keywords: biopolymer, cyanobacteria, Cyanoflan, cosmetics, sustainability

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Rita Mota is Consultant and Researcher at i3S-Institute for Research and Innovation in Health, Univ. Porto (Portugal), with a PhD in biotechnology field. RM is specialized in cyanobacterial extracellular polymers, studying its characteristics, biosynthesis, production optimization and novel applications. RM is also Invited Professor at School of Engineering, Polytechnic of Porto. RM started the CyanoCare in 2017 aiming to develop innovative and value-added products from cyanobacteria. RM has attended entrepreneurship and acceleration programs (e.g. ClimateLaunchpad2018, EIT-Climate), wining several innovation awards (e.g. Born From Knowledge Award, National Innovation Agency-ANI).

CYANOBACTERIAL CELL FACTORIES: CO2-BASED CHEMICALS

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ABSTRACT

At Photanol, we have developed cyanobacterial cell factories for a large number of compounds, such as biofuels, sweeteners and organic acids. The company was founded in 2008 and since then Photanol has been expanding its technology platform by continuously developing and adding more products to its portfolio. The ability of cyanobacteria to capture (sun)light and CO_2 to grow can be redirected towards production of chemical commodities by genetic engineering. The resulting biosolar cell factories present an opportunity for global concerns such as rising atmospheric CO_2 levels and (future) uncertain/fluctuating availability of sugar and oil as raw material. Moreover, direct conversion of CO_2 into chemical commodities is more efficient than first fixing CO_2 by plants into sugar as feedstock for such commodities.

As an example, Photanol's proprietary technology has resulted in efficient organic acids production using fully optimized strains and pathways. Next to metabolic engineering of cyanobacteria, we also develop an upscaling process to assess industrial feasibility. We are running developed strains in photobioreactors at different scales, including pilot- and demonstration-scale.

Keywords: cyanobacteria, cell factories, CO2-based chemicals, genetic engineering,

References: https://photanol.com/

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About the author:

As an alumnus of Future Planet Studies at the university of Amsterdam, I have always been driven by urge that we need to exchange our current oil-based economy for a circular and sustainable alternative. During my studies I have deepened my knowledge in microbiology and chemistry, firmly believing that these disciplines can make a crucial impact in establishing this alternative economy. As a strain engineer at Photanol, we create cyanobacterial production strains with the aim of capturing CO2 and using this as a feedstock to create biochemicals directly in the presence of sunlight.

Company profile:

Joining us as junior researcher, Koen has been with Photanol since 2017. He is now the unflappable, results-driven lead our engineers need to guide them through the cultivation of our latest and greatest strains.

AUTOCHTONOUS MICROALGAE EXPLOITATION FOR WASTEWATER REMEDIATION AND CONVERSION IN 5-HMF

Lima S.

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ABSTRACT

Over the last decades the seek for renewable feedstocks have increased the interest in microalgae, a fast growing biomass able to efficiently fix atmospheric CO_2 that contain high-value biomolecules and source of a suitable biofuel. Today, microalgal cultivation is still too expensive to provide a market competitive biodiesel product, and therefore it results advantageous to couple microalgal growth with processes such as wastewater treatment. Autotrophic microalgae, in fact, are promising in treating wastewaters thanks to their ability in employing inorganic compounds (nitrates and phosphates) as nutrient for their growth. Furthermore, the produced biomass may be employed for conversion in 5-HMF, a platform chemical compound for the synthesis of valuable chemical building blocks and biofuels [1].In our work, we isolated from the environment microalgal strains, characterized the biomass content, and employed them for treating urban wastewaters [2,3]. We aimed at evaluating their ability in decreasing COD, nitrogen and phosphorous. In a first step we employed four strains of microalgae in a batch procedure [4]. As subsequent step, we employed two microalgal strains of *Chlorella* in different ratios with activated sludge, with the result that all the analysed parameters decreased more efficiently than before. In parallel, we developed a procedure for the conversion of microalgal biomass in HMF. Future perspectives are to growth microalgae with activated sludge in a lab-scale raceway in continuous to assess the best operational condition in terms of nutrient removal, and to better understand the relationship between algae and bacteria. We also aim at optimizing the procedure for the conversion in HMF. The results of this work may have a role in developing new procedures and strategies for the industrial exploitation of microalgal biomass.

Keywords: autochtonous microalgae, wastewater treatment, HMF, biofuel

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2015, Master degree in Industrial Biotechnology at the University of Palermo, Italy, with the thesis “Chlorella zofingiensis, a microalgae as feedstock for biodiesel” hold at the institute “Hes So Valais-Wallis”, Sion, Switzerland.2020, PhD in Chemical Engineering at the University of Palermo, Italy, with the thesis: Technologies for cultivation and exploitation of microalgae in industrial applications.2016-2018 Visiting scientist at the University of Kent, UK and at the Nord University, NO Currently post doc researcher at the University of Palermo

Company profile:
The Engineering department of the University of Palermo comprises more than 30 laboratories, 5 library and 20 didactic classrooms. It includes 5 different engineering degree courses, including Chemical and Biochemical Engineering.

SELENIUM ACCUMULATION IN NANNOCHLOROPSIS OCEANICA

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ABSTRACT

Selenium (Se) deficiency in fish can interfere with growth, cause greater fish mortality and hinder the fish immune response [1]. This study aims to assess which inorganic selenium (Se) species is suitable to produce Se-enriched *Nannochloropsis oceanica* (*N. oceanica*) biomass for aquafeed applications. The toxicity of Selenium was assessed by investigating the effective concentration for 50% growth inhibition (EC_{50}) and Se bioaccumulation of the two inorganic forms of Se, sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4). Toxicity results showed that selenate had a greater negative effect on cell growth than selenite. Total intracellular Se was analysed by inductively coupled plasma - optical emission spectrometry (ICP-OES) and high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS), which revealed that selenite was better taken up by *N. oceanica*. Following the toxicity trials, a concentration of 30 µM of selenite in the growth medium resulted in Se bioaccumulation with a minor effect on cell growth. Thus, 30 µM of selenite was selected for batch pilot-scale cultivation in a 1500 L tubular photobioreactor, resulting in Se-enriched biomass with a intracellular content of (0.104 - 0.159 $g_{Se}/kg_{biomass}$). The results from this pilot-scale study are fundamental for a proof of concept laboratory to pilot-scale production and are a critical bridging step for the potential use of Se-enriched *N. oceanica* for aquafeed [2].

Keywords: *Nannochloropsis oceanica*, selenate, selenite, toxicity, accumulation

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About the author:

Bárbara Guimaraes is doing a PhD at Wageningen University and Research in Bioprocess Engineering Department. She obtained her BSc in General Biology (University of Aveiro, Portugal). She undertook an MSc in Industrial Biotechnology (University of Strathclyde) and completed her dissertation at Xanthella, evaluating microalgae-cyanobacteria co-cultures in response to narrow light spectrum exposure. Bárbara then accepted a position at Xanthella Ltd working as an Algal Technologist and within Product and Technical Support. In her current research she is looking into the use of mineral-enriched algal biomass for aquaculture feeds, specifically selenium and its bioavailability.

Company profile:

The Bioprocess Engineering chairgroup, research focuses on novel biotechnological processes for production of pharmaceuticals, healthy food ingredients, bulk chemicals and biofuels. Our challenge is to produce high quality bio-based products in a sustainable and economical way to prevent depletion of natural resources and increase development of a (bio)technological industry.

LIGHT SENSITIVITY CHARACTERIZATION OF A CYANOBACTERIA STRAIN FOR INDUSTRIAL APPLICATION

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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.

The aim of our study is to cultivate a promising cyanobacteria strain at industrial scale and produce natural extracts. According to previous studies, the sensitivity of our strain to high irradiance is a constraint for the robustness of its mass cultivation. As a consequence, understanding the strain light response is the first step to manage its production at industrial scale. This work is thus focusing on the evaluation of light that the strain can support and also on the evaluation of growth kinetic performances in order to be able to predict de productivities of our strain.

In order to define the light transfer in the photobioreactor, a parameter is commonly used: the mean rate of photon absorption by the biomass or MRPA. This parameter depends on the incident photon flux density, the specific illuminated surface ratio of the photobioreactor and the cell concentration in the PBR. The goal of the study is to work with increasing MRPA values and to study the impact on the kinetic and photosynthetic parameters of the strain.

The first results show that this strain seems to be less sensitive to light than the strains used in previous studies. Growth kinetic parameters remain stable at high illuminations and photosynthetic parameters such as pigment content fluctuate as expected.

Keywords: Light transfer, Cyanobacteria, Photobioreactor, Mean Rate of Photon Absorption

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About the author:

She is a PhD student in bioprocess engineering working on microalgae 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.

DIATOMS BIOPHOTONICS IN HYDROGELS AND MICROPHYTOBENTHIC COMMUNITIES

Arianna Rizzo

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ABSTRACT

The project is part of an European consortium called BEEP which stands for “Bioinspired and bionic materials for enhanced photosynthesis”, which studies the light management in the marine organisms, such as macroalgae, corals, marine bacteria and microalgae, to develop new materials through a biomimetic approach. The final objective is to improve the natural light exploitation for biotechnology applications (e.g., novel photobioreactors).

My project explores the light management in a group of microalgae called diatoms inhabiting the upper mm of the sediment in the intertidal mudflats. Here, they form an algae community called microphytobenthos[1]. This biofilm is mainly dominated by benthic diatoms that are highly efficient at photo-regulating, being exposed to extreme light variations during tidal cycles[2]. They use a complex mixture of mechanisms to photo-regulate, including endogenic vertical movements within the sediment matrix and species cycling/stratification at the sediment surface. These movements modulate the light environment inside the sediment but are extremely difficult to investigate under natural conditions [3]. Thus, the first objective is to imitate the natural conditions building an artificial biofilm (i.e. a hydrogel) and study the diatoms response to a single stimulus, e.g., the light exposure, and their consequent orientaton inside the gel.

Study the diatoms biophotonics properties will help to inspire new materials and devices like transducers, fiber optics and photonic crystals [4].

Keywords: diatoms, light, biophotonics, hydrogels

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BIOGRAPHY

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Arianna Rizzo is a Marine Biologist interested in the sustainable use of marine natural resources. In 2019, she obtained her MSc in Marine Biology at the University of Ancona (Italy) after an internship at SAMS, in Scotland, where she studied the production of astaxanthin from Haematococcus pluvialis. After working in Norway for 7 months at the NIBIO Research Institute, on the industrial production of microalgae, she joined the RSBE (Remote Sensing & Benthic Ecology) group at the University of Nantes. Here, under the supervision of Dr Bruno Jesus, she works on the diatom biophotonics as part of the BEEP project.

Company profile:
MMS (Mer Molécules Santé). RSBE team has several projects on the structure and functioning of intertidal ecosystems: e.g., benthic ecology, biodiversity and ecophysiology of benthic microalgae, hyperspectral and satellite remote sensing.

Thematic Session 6: Funding and Investors

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Thematic Session 7: Expert Round Table

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Scientific Session 8:
Algae Monitoring, from Nature to Industry

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07-10·DECEMBER·ONLINE

IMPACT OF MACROALGAE HARVESTING ON BIOACTIVE COMPOUNDS

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ABSTRACT

Bioactive compounds from macroalgae have been increasingly explored in recent years, due to their health benefits. The aim of the present work was to evaluate the association between bioactivity expression and the harvesting location and period of Portuguese red and green macroalgae. *Codium* spp. and *Osmundea pinnatifida* were harvested, every two months, between January and September 2020 at different rocky shores, duly washed and dried, and characterized in terms of bioactives, namely fatty acids profile, phenolic compounds, carotenoids and chlorophylls. *Codium* spp. (0.093 ± 0.0012 mg/100g_{DW}) contained higher amounts of polyunsaturated fatty acids than *Osmundea pinnatifida* (0.068 ± 0.0001mg/100g_{DW}). Fatty acid composition was affected by both location and period of harvesting. Seaweeds harvested in May had 35% higher total fatty acid content than seaweeds harvested in January). Similar trends were shown for phenolic compounds and carotenoids. *Osmundea pinnatifida* contained 3-fold higher content of polyphenols than the *Codium* spp., among which anthocyanins, in particular cyanidin predominated. Furthermore, antioxidant activity of *O. pinnatifida* was 1328.28 ± 75.32 µmol of Trolox Eq./g_{DW} in May while in January it was 20% lower (1071.16 ± 21.41 µmol of Trolox Eq./g_{DW}). *Codium* spp. presented higher amounts of the natural pigments, carotenoids (zeaxanthin, astaxanthin) and chlorophylls (a and b). In this case total carotenoids content varied between months of harvesting - January presented 184.55 ± 2.92 µg β-carotene eq./g_{DW}, while July presented 151.24 ± 3.54 µg β-carotene eq./g_{DW}. The location also had a significant impact ($p<0.05$), while seaweeds from Aguçadoura presented 123.34 ±13.65 µg β-carotene eq./g_{DW} those from Viana - presented 49.29 ± 12.06 µg β-carotene eq./g_{DW}. The results presented are very important to understand the required management of harvesting period and location in order to guarantee macroalgae with higher health-promoting properties.

Keywords: Macroalgae, *Codium* spp.; *Osmundea pinnatifida*; bioactive compounds, phenolic compounds, antioxidant capacity,

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About the author:
Marta Coelho (female) has acquired solid experience in the field during her Degree in Biosciences with specialization in Microbiology (ESB-UCP) and Master's degree in Biotechnology (IST-UTL), namely production and characterization of functional ingredients and food, by-products upgrading and genotoxic and cytotoxic effects of food products. Currently, she is a PhD student focused on the revalorizing of agro-industrial by-products through Ohmic heating as a new process for development of functional ingredients with biological bioactivities and their potential application. Also, she researches in the macroalgae field in ALGALUP project.

A CLOSER LOOK INTO THE MICROBIOME OF MICROALGAE CULTURES

DMM Kleinegris

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ABSTRACT

Although bacteria are commonly co-occurring in microalgae cultivation systems, little is known about their impact on microalgae production. Certain bacteria have been described to stimulate or promote microalgae growth [1-3], while others can compete with the microalgae for resources or produce toxic substances, and therefore decrease the production yields [4]. Moreover, specific bacteria have also been associated with culture crashes [5], and knowledge of bacterial composition is also important in the context of product quality when the algae biomass is to be used for future food and feed. A better understanding of how the bacterial community composition is related to specific taxonomic microalgae groups and growth conditions can help optimizing microalgae production. We investigated the microbial community of eleven candidate microalgae strains in stock culture, maintained in two different growth media, and followed the bacterial composition for three *Phaeodactylum tricornutum* strains during batch cultivation in bubble columns. Our results reveal that different microalgae genera in stock culture, although kept at the same cultivation conditions over several years, display separate and unique microbial communities. Further, different strains of the same genus had very similar bacterial community compositions, although originating from different habitats. However, when maintained in a different growth medium, the bacterial composition changed to different community structures for different strains of the same genus but remained relatively stable during batch cultivation for each *P. tricornutum* strain. This indicates that microalgae can modulate their microbial communities and that different microalgae genera could create distinct conditions that select for dominance of specific bacteria. However, other factors such as the composition of growth medium also affect the formation of the microbial community structure. Currently, work is ongoing to study the microbiome of *Microchloropsis gaditana* during the upscaling from lab to pilot-scale and during production in 750 tubular photobioreactors.

Keywords: microbiome, medium, strains, scale-up

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Company info:
NORCE is a new and forward-looking research institute, with expertise in a wide range of fields. We deliver research and innovation in energy, health care, climate, the environment, society and technology. Our solutions address key challenges for society and contribute to value creation on the local, national and global levels.

BENTHIC DIATOMS: HOW TO GROW TO OBTAIN BIOACTIVE LIPIDS?

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ABSTRACT

Marine benthic diatoms represent an under-exploited pool and therefore constitute a potential resource for the valorization of lipids of interest in health and nutrition. In this framework, a screening of more than one hundred strains from a rarely investigated source, namely intertidal mudflats stored in the NCC regional collection, was performed. Based on the lipid production and growth capacity evaluated by high-throughput Fourier Transform Infrared spectroscopy, six strains (*Amphora* sp. NCC169., *Entomoneis paludosa* NCC18.2, *Nitzschia* sp. NCC109, *Nitzschia alexandrina* NCC33, *Opephora* sp. NCC366 and *Staurosira* sp. NCC182) have been selected [1]. These six species were then produced in airlift photobioreactor (PBR) for the first time to establish their capacity for growth and lipid production. A thorough lipid study was also conducted by lipid class fractionation and fatty acid analyses by gas-chromatography coupled to mass-spectrometry [2]. Neutral lipids (TAG, sterols) and polar lipids (glycolipids, phospholipids) were evaluated for their potential for production of lipid of interest. Three species (*E. paludosa*, *N. alexandrina* and *Staurosira* sp.) have been selected to evaluate the bioactive potential of extracted lipid fractions. The glycolipids fractions shown antiproliferative activity on the MCF-7 (breast) and NSCLC-N6 (lung) cancer cell lines as an antibacterial activity on grampositive bacterial strains (*B. subtillus*). This study demonstrated also the impact of culture conditions (light and nitrogen) on the fatty acid quality [3]. *Amphora* sp. and *Opephora* sp. did not grow in PBR and so need to develop another PBR design such as biofilm one.

Keywords: benthic diatoms, screening procedure, fatty acid, bioactive lipids

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Aurelie Couzinet-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.

BIOPROSPECTION, GROWTH OPTIMIZATION AND INDUSTRIAL CULTIVATION OF NOVEL MICROALGAE STRAINS OBTAINED FROM LOCAL PONDS

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ABSTRACT

Bioprospection of novel strains adapted to local conditions is key to the successful industrial-scale production of microalgal biomass. A novel *Chlorococcum amblystomatis* strain was isolated from a freshwater pond at Allmicroalgae and high biomass productivities in large-scale industrial photobioreactors (PBR) were described[1]. Moreover, increasing the current biomass productivity while reducing the operational expenditure (OPEX) is essential for the future of industrial producers. Thus, the present work was focused on optimizing biomass productivity of autotrophic and heterotrophic growth of this strain. *C. amblystomatis* registered highly concentrated cultures (89.55 $g.L^{-1}$ in 5-L fermenters) upon heterotrophic growth being optimized by a Surface Response Methodology. Afterward, a two-stage growth approach was tested, where heterotrophically grown cells were used as inoculum for large-scale producing units operated under photoautotrophy. It has been previously shown that this approach reduces the scale-up time and overall costs[2,3]. As the producing unit type influences the biomass costs as well[4,5], growth in open raceway ponds was compared to PBRs. *C. amblystomatis* achieved significantly higher productivities in a 2.6-m^3 PBR (0.13 $g.L^{-1}.day^{-1}$) than those observed in 2.9-m^3 raceways (0.02 $g.L^{-1}.day^{-1}$). Moreover, regarding photoautotrophic growth optimization, urea appeared to be a promising nitrogen source (0.26 $g.L^{-1}.day^{-1}$) in comparison to the basal industrial media using sodium nitrate (0.18 $g.L^{-1}.day^{-1}$) or ammonium sulphate (0.11 $g.L^{-1}.day^{-1}$). *C. amblystomatis* was reported to contain high contents of protein (55.72%), omega-3 (67.81% of total fatty acid methyl esters), chlorophyll (40.24 $mg.g^{-1}$) and lutein (5.37 $mg.g^{-1}$)[1], when compared to *Chlorella vulgaris*[3]. Moreover, the lutein content was higher than the reported for the lutein-producer *Muriellopsis* sp. (4.3 $mg.g^{-1}$ at 0.055-m^3 PBR)[1]. Overall, this free-living *C. amblystomatis* strain displays a promising nutritional profile, comparable to conventional feedstuffs[6,7].

Keywords: Bioprospection; *Chlorococcum amblystomatis*; Growth optimization; Microalgae cultivation; Industrial production, Microalgae-based feed supplement.

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Nádia Correia is currently in the 1st year of her PhD at University of Algarve in collaboration with Allmicroalgae Natural Products S.A., Centre of Marine Sciences (CCMAR) and National Laboratory of Energy and Geology (LNEG).

Previously, she completed her bachelor's degree in Biotechnology at Polytechnic Institute of Coimbra, and her master's degree in Biodiversity and Plant Biotechnology at University of Coimbra. Her master thesis was focused on characterization and analysis of biotechnological potential of a microalgae strain isolated at Allmicroalgae. Thereafter, she integrated a master grant into the COMPETE2020 project AlgaValor, related to microalgae integrated production and biomass prospecting.

Company info:

Allmicroalgae provides excellence in microalgae biomass production. Microalgae are cultivated in closed tubular systems or fermentation tanks. The company is very active in R&D, being involved in three H2020 and two P2020 projects and collaborating with more than 30 Portuguese and 50 international institutions, which allows accomplishing its high-quality standards.

GROWTH OF MARINE MICROALGAE AND CYANOBACTERIA IN ARTIFICIAL INDUSTRIAL EFFLUENTS SUPPLEMENTED WITH NUTRIENT RICH WASTEWATERS

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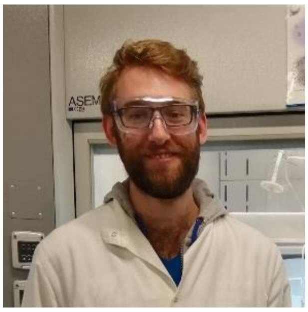
ABSTRACT

Recently, saline effluents such as produced waters from oil and gas production or waters from saline aquifers in sedimentary basins have gained interest in the field of microalgae. Studies have been conducted to determine the feasibility of using these streams as a culture medium to grow microalgae. Six marine photosynthetic microorganisms were studied (*Dunaliella salina*, *Nannochloropsis oceanica*, *Tetraselmis suecica*, *Picochlorum costavermella*, *Coccomyxa simplex* and *Synechococcus rubescens*). Experiments were performed to determine the extent of microalgal growth in artificial saline produced water and aquifer water supplemented with nutrient rich wastewaters, providing nutrients for the cell growth (*i.e.* liquid digestate and effluent of an urban wastewater treatment plant (WWTP)). Tests were performed in 96-wells microplates. Media were composed of approximately 7, 37 and 70 %v/v artificial produced water or aquifer water supplemented with 5 %v/v liquid digestate (final concentrations: 149-195 $mgN.L^{-1}$, 1.5-2.7 $mgP.L^{-1}$) or WWTP effluent (final concentrations: 3-39 $mgN.L^{-1}$, 0.2-1.3 $mgP.L^{-1}$). Media were completed to 100% with artificial seawater, corresponding to final salinities of 40, 70 and 110 $g.L^{-1}$. Only cultures supplemented with 5 %v/v liquid digestate showed significant growth, as 5 %v/v WWTP effluent did not provide enough nutrients for growth. Thus wastewaters can only be used to dilute the streams. With digestate, *D.salina*, *N.oceanica* and *T.suecica* showed the best growth rates and yields. They were selected to perform mixed cultures in 80 mL tubes in the same culture media as described previously. Population evolutions were followed. Depending on salinity and industrial effluent used, one species became predominant over the two others. It appears that mixed culture is a good solution to have a biomass production during a culture process where the culture media will evolve in terms of salinity and composition.

Keywords: aquifer water, liquid digestate, microalgae, produced water, WWTP effluent

BIOGRAPHY



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I'm a French PhD working in the field of microalgae in the TotalEnergies research center of Lacq (France) since April 2018 where I worked on the production and valorization processes for microalgae. Since February 2020, I have been working on my PhD thesis "Microalgae Culture for the Treatment of Salty Industrial Streams and Biofuels Production" in the University of Pau (France), in partnership with the company TotalEnergies (Parsy *et al*. 2020, Algal Research; Parsy *et al*. 2021, Algal Research).

CONFERENCE DAY 4

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Scientific Session 9: Environmental Impact

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CO2 EMISSIONS: PARIS WE HAVE A PROBLEM!

Olivier Lépine

Managing director, Algosource

ABSTRACT

The Paris agreement has set up an objective of 80% reduction of CO2 emissions in Europe, by 2050. An intermediate target is also set at 50% reduction in 2030. This applies to every sector and every human activity. The latest life cycle analysis (LCA) completed on the production of Spirulina, presented by Algosource in 2020 shows emissions in the order of 15 kg CO2, per kg of biomass produced dry weight. This is high in comparison to other agricultural vegetal productions: which are usually in between 1 and 5 kg CO2/kg dry. It is in fact similar to the emissions of meat production in Europe. At the same time the microalgae community communicates largely around the fact that microalgae "capture CO2" or are a solution for greenhouse gases emissions. The difference between the communication and the reality of the industry today is not sustainable and represent a challenge to EABA members. That means the industry has to shift towards cultivation systems that will significantly decrease microalgae emissions. In short it requires less water in the systems, higher productivity, renewable energy sources. After presenting a meta-analysis of published microalgae production LCA, that has never been done before, we will look into the technologies that will allow for the necessary CO2 emission decrease has required by the Paris Agreement.

Keywords: Climate change, LCA, cultivation technologies, CO2

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Journal of Cleaner Production, Volume 299, 25 May 2021, 126741

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Company info:
AlgoSource vision is simple: microalgae for health. AlgoSource mission is to develop a microalgae industry that will help fulfilling the needs of our planet for us and the coming generation. The first company to commercially produce Spirulina in Europe, AlgoSource develops original efficient nutraceuticals to both industries and final consumers

UNRAVELING MODELLING STRATEGIES OF ALGAE SYSTEMS: A FIRST STEP TOWARDS IMPROVED TRANSPARENCY

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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. The methodological choices made by LCA practitioners increase the variability of LCA results, therefore reducing their comparability. This study analyses the modelling approaches used to mitigate the environmental impacts of algae systems arising from the use of electricity, nutrients, and water. Based on a case study, guidelines to improve the transparency and reproducibility of LCA studies are then provided. An extensive literature review was conducted to evaluate the algae system modelling approaches in LCA studies published over the past 20 years. The outcomes of the review were used to conduct a LCA study of a novel biorefinery for phycocyanin production from *Arthrospira platensis*. Real pilot scale data were collected in the frame of the European SpiralG project (BBI-H2020). The literature review showed that three main strategies have been used to mitigate the environmental impacts of algae systems i.e. considering multiple products, using the bioremediation potential of algae, and designing closed-loop systems. Since solving the multifunctionality of algal biorefineries is one of the most controversial topics, different methods were compared in the case study. The results highlighted the strong link between the choice of functional unit, modelling perspective (attributional versus consequential), and method to solve multifunctionality. The understanding of methodological choices made by LCA practitioners is fundamental to facilitate the interpretation and comparison of LCA results. Therefore, the proposition of guidelines on algae system modelling approaches is a first step towards an improved transparency and reproducibility of LCA studies.

Keywords: LCA, system modelling, biorefinery, phycocyanin

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Léa Braud is a PhD Researcher in the UCD School of Biosystems and Food Engineering. 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.

COMPARATIVE LCA OF NOVEL AND CONVENTIONAL MICROALGAE REACTORS

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ABSTRACT

The versatility of microalgae biomass as candidates for various products and bioremediation needs is well documented. Conventional open-reactors are reliant on sunlight, take up a lot of space and yields can be constrained due to outdoor environment conditions. Conversely, closed-reactor systems like bubble columns occupy far less space, however they are subject to high energy demands, notably from lighting systems. The present study uses an attributional LCA approach to compare the environmental performance of such open/conventional reactors and an internally luminated novel closed reactor design, expressing impacts per kg biostimulant from *Scenedesmus almeriensis*. All performance data was collected from a pilot facility in Almeria, Spain. Urban-industrial symbiosis scenarios are also portrayed in the study, with the novel closed reactors being operated using wastewater and incinerator flue gas as sources of nutrients and carbon dioxide, respectively. Results show that under synthetic nutrient and carbon inputs in pilot operations in Spain, the cumulative energy demand for the novel photobioreactor system is similar to conventional vertically-stacked horizon bioreactors but are substantially more demanding than open raceways. However, when leveraging renewable energy sources and the photosynthesis process to consume wastestreams in urban-industrial symbiosis scenarios, the novel photobioreactor was able to achieve substantial improvements (up to 80%) in several impact categories e.g. eutrophication and climate change. This highlights that such closed and internally illuminated photobioreactors can be competitive with conventional open-reactors, and have potential to harness photosynthesis to reduce environmental burdens in urban-industrial symbiosis, while producing valuable biomass. Further research is needed on such reactors to determine possible economies of scale & associated gains in efficiencies, the potential of cultivating other species and developing other valuable products using such reactors.

Keywords: Microalgae, Scenedesmus, Biostimulant, Life Cycle Assessment, Urban Industrial Symbiosis, Climate Change, Eutrophication, bioreactor

BIOGRAPHY

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Scientific Session 10:
Algae for Plants

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

THE POTENTIAL USE OF *C. REINHARDTII* AND *C. SOROKINIANA* AS BIOSTIMULANTS ON MAIZE PLANTS.

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ABSTRACT

The use of plant biostimulants (PBs) on crops is a very promising application for the future agriculture to improve crop yield, but especially to prevent the effect of abiotic stresses. Micro-algae derived biostimulants represent an efficient tool to stimulate the root development, also under nutrient deficiency. The aim of this work is to test the stimulant ability of *Chlamydomonas reinhardtii* (CR) and *Chlorella sorokiniana* (CS) cells on maize roots. We tested two different extracts for both the algae species using un-treated and physically broken cells, to analyze if nutrients were more available after the disruption of cellular wall and membrane. Both CR and CS promoted the maize root system compared to the untreated negative control, but CS seemed to increase especially the number of secondary roots. The ICP-MS analysis showed that CR mostly affects the micro-nutrients accumulation on maize roots and shoots, while physiologic analyses showed that CS enhances the tolerance to abiotic stresses. Nitrogen (N) deficiency is one of the major problems in agriculture, affecting plant development and inducing visible chlorosis on shoots. Moreover, water deficiency is another negative abiotic stress for the crop yield, due to water deficiency itself or to the high soil salinity. Thus, the two CS extracts were tested under both N deficiency and drought stress showing an improved micro-nutrients accumulation and development of the root system compared to the control, respectively. Photosynthetic parameters were analyzed for all the experiments showing significant differences only for the CS untreated cells extract compared to the control under water stress condition.

Keywords: Micro-algae, Biostimulants, *Chlorella sorokiniana,* Maize, Root apparatus

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BIOGRAPHY

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I'm Flavio Martini, a PostDoc of the Department of Biotechnology at the University of Verona. My Ph.D. thesis was on functional analysis and expression of metal transporters in *A.thaliana* and poplar under the supervision of Prof. Antonella Furini. After my Ph.D. I joined the SOLE lab group of the Prof. Matteo Ballottari, focusing on the use of micro-algae as plant biostimulants and on the genome editing of several micro-algae species.

ARTHROSPIRA-BASED BIOSTIMULANTS AND THEIR EFFECTS ON DIFFERENT PLANTS

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ABSTRACT

In recent years, microalgae are emerging as excellent candidates for the production of biostimulants due to the high content of bioactives that positively affect plant growth and abiotic stress tolerance, and to the possibility of cultivating them in controlled systems, thus obtaining products with high and reliable quality compared to other feedstocks. However, a better standardization of the product does not necessarily imply a reproducibility of the effects on multiple plant species as, due to their metabolic diversity, different plants may have different sensitivity thresholds for the bioactive molecule(s). *Arthrospira* is one of the most studied microalgae for biostimulant production and several *Arthrospira*-based products are already on the market. In the present work, data obtained in several trials, in which extracts and hydrolysates prepared from the same *Arthrospira platensis* biomass have been applied to different plant species, are examined to detect differences in plant responses to treatments. The tested plants are among the most cultivated leafy (lettuce and basil) and fruit (tomato and vine) crops in Italy. Our results show that the effectiveness of the same *Arthrospira*-based biostimulant on plant yield varies in relation to the plant species and the doses applied and is enhanced in plants subjected to abiotic stress. This suggests that extensive agronomic studies are needed to deepen our basic knowledge of the effects of microalgae-based biostimulants on different plant species in order to enhance their biostimulant effects and develop tailored products adapted to specific crops and environments.

Keywords: Microalgae, biostimulants, *Arthrospira*, plant yield.

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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. Since 2013 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. Mario Tredici, working on the use of microalgae and cyanobacterial strains for the development of new biostimulants.

PRODUCTION AND ASSESSMENT OF A MICROALGAE BIOFERTILIZER FROM WASTEWATER TREATMENT SYSTEM

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ABSTRACT

Microalgae-based systems are an alternative to conventional wastewater treatment systems, reducing the cost and improving the sustainability of the process. The produced microalgal biomass could be used as a slow release biofertilizer, providing nutrients (mainly nitrogen and phosphorus) to the crops and encouraging circular economy. In this way, the need for inorganic fertilizers and the potential contamination of soil and groundwater would be reduced. In this context, the present study had two main objectives: to treat wastewater with microalgae and to assess the effect of microalgal biomass as biofertilizer on basil crops. Domestic wastewater was treated in two pilot scale high rate algal ponds with a total volume of 900 L, located outdoors in Barcelona (Spain), from February to June 2021. The biofertilizer was obtained by settling and centrifuging the microalgal biomass, which was then stored at 4ºC, without adding any chemical reagent or performing any kind pre-treatment. The biofertilizer, with a total solid content of 12% and nutrients concentration of 7.6%N, 1.6%P and 0.9%K, was tested in basil crops, using 1 L pots during five weeks inside a greenhouse. Three different treatments were applied in order to compare the microalgal biofertilizer with an inorganic fertilizer (control) and with a combination of both the biofertilizer and inorganic fertilizer. Plant biometrical parameters showed no statistical difference between the plants grown with the biofertilizer and the control with mineral fertilizer. Indeed, the plants grown with the biofertilizer presented higher leaf dry weight compared to the control. Thus, it can be concluded that the microalgae biofertilizer produced from wastewater is a promising alternative to inorganic fertilizers in basil crops.

Keywords: Wastewater, biofertilizer, crop, microalgae, circular economy

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Company profile:

The Group of Environmental Engineering and Microbiology (GEMMA-UPC) is dedicated to interdisciplinary research, innovation, knowledge transfer and education in environmental engineering; particularly in the fields of environmental biotechnology, water supply, wastewater and solid waste treatment, and resources recovery.

Scientific Session 11:
Algae Biofilms and EPS Production

ALGÆUROPE2021
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BUBBLING FOR LIGHT: MICROALGAE BIOFILMS CONTROL IN PBRS

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ABSTRACT

Biofouling in photobioreactors (PBRs) negatively impact the cultivation process by decreasing light penetration and therefore biomass productivity[1]. Biofouling results from the adhesion of single microalgal cells, and a later development of mature biofilms, on the surfaces of a reactor. The three-dimensional structure (i.e. spatial organization of cells) of such complex microbial communities strongly affects their cohesion and response to mechanical stresses and therefore may impact their resistance to cleaning procedures[2]. Despite the importance of understanding biofouling in PBRs, the 3D structure of microalgae biofilms has been seldom characterized. In this study, we aimed at understanding how microalgae (a diatom and a cyanobacterium) colonize the surfaces of PBRs by studying in the short-term (few hours) their adhesion dynamics and in the long-term (weeks) their development as a function of different hydrodynamic conditions aimed at reducing biofouling. The experiments were conducted in a flat-panel reactor and several hydrodynamic conditions were generated by air bubbling. Cell adhesion was monitored by time-lapse microscopy and video analysis and biofilm evolution by confocal laser scanning microscopy (CLSM) and optical coherence tomography (OCT). The results show that the adhesion dynamics are species-specific and that shear-stress generated by bubbles helps creating low-biofilm areas that improve light penetration. In these areas the biofilms appear to exhibit different 3D structures and a strong resilience to shear-stress is also identified. Overall, studying biofilm properties in PBRs allows a better comprehension of such complex microalgae lifestyle and helps improving bioprocesses stability and productivity.

Keywords: Microalgae biofilms, Biofouling, CLSM, OCT, PBRs

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A. FANESI is currently a Postdoc at the LGPM (Laboratoire Génie des Procédés et Matériaux, CentraleSupélec, France) and his research interest focuses on the physiological characterization of microalgae and bacteria (planktonic and biofilms) using microscopic (CLSM and OCT) and spectroscopic techniques (FTIR and Raman spectroscopy) in combination with advanced statistical analysis.

Company info:

The LGPM (Laboratory of Chemical Engineering and Materials Science) at CentraleSupélec is a multidisciplinary laboratory whose main areas of activity are the bioprocesses, chemical separative, downstream processes and (bio)materials engineering.

A COUPLED CFD-DPM APPROACH TO PREDICT MICROALGAE CELL ADHESION ON DIFFERENT MATERIALS USING A XDLVO-TEMPORAL MODEL

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ABSTRACT

Biofouling formation on photobioreactors walls causes negative consequences, culminating in significant economic losses [1]. Microalgal cell adhesion is influenced by the physicochemical surface and cells properties and the fluid dynamics present in the photobioreactor. This work aims to simulate and validate a temporal evolution of the cell adhesion on different materials in contact with a microalgae culture, using different flow rates to check the influence of shear stress on biofouling formation. To model the microalgal cell adhesion, a Computational Fluid Dynamic (CFD) simulation was carried out using an Eulerian-Lagrangian particle-tracking model (DPM) by the software Ansys Fluent®. The adhesion criterion was based on the forces and moments balance [3] including a XDLVO-temporal model. To validate the results, a commercial flow cell of the modified Robbins (MRD) device type coupled to a 35 L raceway photobioreactor, operated in continuous mode (0.17 day^{-1}), was fed with a marine microalga *Nannochloropsis gaditana* culture. The MRD was composed of transparent polymethyl methacrylate, polyethylene terephthalate glycol-modified, polycarbonate and polyvinyl chloride coupons for its validation. These coupons were evaluated over time measuring cell adhesion by Chlorophyll a fluorescence and characterized measuring the contact angle (with water, formamide and diiodomethane) by a goniometer using the sessile drop technique [4]. Two flow rates were tested: 7.5 L·h^{-1} and 30 L·h^{-1}, to know the shear stress influence. To our knowledge, this is the first work that includes a coupled CFD-DPM approach with time evolution of XDLVO forces as a tool to address the challenge to predict the microalgae in PBRs.

Keywords: Microalga cell adhesion, *Nannochloropsis gaditana*, Computational fluid dynamics, XDLVO

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BIOGRAPHY

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Graduated in Industrial Chemical Engineering (2018) and master's degree in Chemical Engineering (2020) from the University of Almería. Currently, I am performing my PhD studies in the Doctorate in Biotechnology and Industrial Bioprocesses Applied to Agri-Food and the Environment Program by the University of Almería, thanks to a predoctoral contract at the University of Almería associated to the project RTI2018-101891-B-100, in Biotechnology of Marine Microalgae Group (BIO-173). The objective of the project is the search for new transparent non-toxic coatings to build efficient photobioreactors, avoiding the biofouling formation to optimize biomass production and to reduce economic losses.

ADAPTIVE LABORATORY EVOLUTION FOR ENHANCED EPS PRODUCTION IN BIOFILM FORMING MICROALGAE

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ABSTRACT

Microalgae are widely used in different biotechnological fields as they can produce many interesting compounds (*e.g*. lipids or polysaccharides). Recently, the inalve start-up created a new technology for monospecific microalgae biofilm culture. It has numerous benefits including an easier way to harvest biomass that decreases energy and operating costs for dewatering. inalve has selected microalgal strains able to produce high value exopolysaccharides (EPS). The natural polymers excreted by the cells are composed of different carbohydrates. EPS have several functions, especially in the structural integrity of biofilms. Depending on physiological conditions, microalgae can produce EPS with various carbohydrate compositions leading to different bioactivities and industrial applications. Our objective was to select individuals with enhanced EPS productivity using the adaptative laboratory evolution method (ALE). ALE is based on selection principle, and selection is processed by imposing a specific stress on a population exhibiting genomic diversity. At the end, the selected microorganisms are non-genetically modified organisms. For this study, *P. purpureum* and *C. closterium* were chosen for their ability to secrete EPS under specific conditions. Therefore, these species were submitted during several months to several cycles of increasing stresses specifically chosen to promote the selection of biofilm-forming individuals which over-expressed the production of EPS to survive. Analyses including cell fatty acids and carbohydrates were performed to follow the adaptation processes. The productivity evolution was also followed. At the end of the ALE experiment, the wild type and the evolved strains will be compared in terms of productivity, composition and associated bacteria.

Keywords: Microalgae, Biofilm, Selection, Evolution, Exopolysaccharides

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About the author:
My college studies began with a BSc in Biology during which I discovered a passion for the marine environment. During my MSc in Marine Sciences, I develop a deep interest in the phytoplankton communities. Therefore, I chose to do an internship on these microorganisms for my Master's thesis at the Atelier de BioInformatique laboratory (National Museum of Natural History, France). Thanks to this experience, I learned different bioinformatics techniques that I wanted to use for my PhD. This is also the reason why I decided to focus my research on this thesis project combining experimentation and bioinformatics analyses at the Laboratoire d'Océonographie de Villefranche (Sorbonne Université, France).

EXTRACTION AND SEPARATION OF EXOPOLYSACCHARIDES FROM TETRASELMIS SUECICA PRODUCED AS BIOFILM

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ABSTRACT

Tetraselmis suecica is a green microalgae used in aquaculture. It is an important feedstock of valuable compounds such as proteins, lipids, polysaccharides, minerals and vitamins (Pereira et al., 2019). Commercialization of high added-value products from microalgae is mainly targeted to proteins and lipids, while polysaccharides and exopolysaccharides (EPS) are receiving limited attention due to high production costs. Moreover, EPS may be endowed with special physicochemical properties and important biological activities (Xiao et Zheng, 2016). Sulphated exopolysacharides produced by some microalgae have been studied and shown to have immunostimulatory activity, limiting the attachment of bacteria and interest in fighting infections in fish (Guzman-Murillo et Ascencio, 2000). When cultivated as biofilm, EPS secreted by T. suecica bind cells and allow them to adhere to solid materials. This work aims to study the valorization of EPS from T. suecica produced as a biofilm for industrial applications (e.g., health additives, animal food). Therefore, EPS extraction and separation from T. suecica exopolymeric substances was carried out by membrane separation. EPS extraction was operated by gentle mixing of the microalgae paste with salted water and purification tested with ultrafiltration membranes of molecular weight cut off ranging from 1 to 30 kDa. The 1 and 5 kDa membranes present the best properties for EPS concentration. EPS fraction was characterized in terms of molecular weight by SEC (Size exclusion chromatography) and biochemical content. The EPS exhibit a molecular weight of 5000 Da approximately. Determination of monosaccharides content by HPAEC (High Performance Anion Exchange Chromatography) after acidic hydrolysis showed that these EPS consisted of 9 different sugars: Kdo, galacturonic acid and glucuronic acid were the main constitutive monomers. In addition, the sulphate contents obtained by turbidimetric methods show that these EPS are sulphated, indicated possible bioactivities for Tetraselmis suecica EPS. Further research work is needed to gain a better knowledge of the physicochemical structure of these EPS and how they can exert bioactivities.

Keywords: Exopolysaccharides, membrane separation, HPAEC, SEC, aquaculture

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BIOGRAPHY

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Pauline DELRAN obtained a bachelor's degree in cell biology and a master's degree in plant resources development at the University of Strasbourg. Within the company Inalve (Nice - France) and in collaboration with the laboratory of chemical engineering (LGC) and the laboratory of agro-industrial chemistry (LCA) in Toulouse, Pauline is currently working on the development of sustainable biorefinery processes of microalgae for the production of protein meals and health ingredients intended for the field of aquaculture.

Company profile:

Inalve is a company based in Nice (France) which industrializes a patented process for the production of microalgae and markets microalgae-based products for the animal feed industry.

POSTER RESENTATIONS

Biomaterial

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

A NEW TECHNOLOGY TO PROTECT AGAINST BIOFILM FORMATION IN ALGAE CULTURES

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ABSTRACT

Microalgal cultures have grown to become a vital part in supplying a protein and vitamin rich source for fish and agricultural feed as well as nutritional supplements for us to use. With sustainability being a growing concern, the microalgal industry has expanded significantly in the last decades. Microalgal cultures today are primarily grown in closed photobioreactors to keep the culture in a controlled and defined environment with minimal risk of contamination. Closed photobioreactors also need a smaller growing area than open pond systems. However, algae can attach to the reactors' surface leading to biofilm formation which can occur in reactors of different material and dimensions. Preference in attachment of extracellular proteins, glycans and algae leading to biofilm on common reactor materials such as PVC, PE or Plexiglas depends also on reactor type and mixing system. Nevertheless, biofilm formation in algae culture processes impacts the productivity due to reduced light penetration and additionally required cleaning cycles. For this purpose, we developed a new coating solution to prevent biofilm formation on reactor surfaces during algae culture. Our solution is being tested on various potential photobioreactor surfaces with several different species Our coating solution adsorbs on commonly used reactor materials without chemical reactions within a short period of time. The polymer-based coating is completely inert and protects the surface from protein adsorption leading to biofilm prevention. Our new technology is easy-to-use, applicable in large systems and can impact biomass production and production costs by increasing light permeability, decreasing cost-intensive cleaning cycles due to decreased biofilm formation.

Keywords: Photobioreactor, biofilm, anti-fouling, microalgae

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About the author:

Tanja Schollmeier's background is in marine biology with a focus on the ecological importance of macro- and microalgae in various ecosystems. After completing a bachelor's degree at the University of Rhode Island, where she concentrated on the effects of harmful algal blooms, she completed her master's degree at the University of Alaska Fairbanks. During her master's she studied the importance of Arctic ice algae in the Arctic ecosystem, utilizing various biomarkers such as fatty acids and isotopes to achieve her goals. Today Tanja is part of a Start-up company developing a solution to prevent biofilm formation in commercial algal production.

Company profile:

faCellitate is a Venture Team of Chemovator GmbH. It was created from a BASF research project by an interdisciplinary team with long-term experience in material design. faCellitate specializes in the development of polymer surface coatings for laboratory consumables and biotechnological processes to create a fully synthetic, biologically relevant environment.

BIOFOULING FORMATION IN COMMERCIAL POLYMERIC SURFACES UNDER SALT STRESSFUL CONDITION OF DIFFERENT MICROALGAE

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ABSTRACT

One of the problems, which the culture of microalgae presents and that greatly increases production costs, is the biofouling formation on the inner walls of closed-photobioreactors (PBRs), which decreases the penetration of light into the PBR [1]. The aim of this work is the study of seawater concentration influence, both on the cell growth of flagellated and non-flagellated microalgae, and on cell and exopolymeric substances (EPS) adhesion in different polymeric materials (PVC, PC, PE, PETG, PMMA) and glass as well as a fouling release coating (Hempasil X3®) used as control. The adhesion of *Chlamydomonas reinhardti, Isochrysis galbana* and *Nannochloropsis gaditana* according to different seawater concentration in the medium (0%, 12.5%, 25%, 50%, 62%, 75% and 100%) were studied. Microalgae were grown in vessels of 0.2 L of culture in fed-batch mode with orbital shaking at 100 rpm and at 25 ºC. Measurements of growth, photosynthetic efficiency and carbohydrate and protein concentrations in the supernatant were performed during fed-batch cultures and, once the steady state was reached, the concentration of cells, carbohydrates and proteins adhered on each material were measured. Biomass concentration is lower with higher levels of salinity for the freshwater strain *C. reinhardii* and vice versa for seawater strains *N. gaditana* and *I. galbana*. For all of them, the more unfavorable tests in terms of cell growth the higher levels of adhesion were reached on the surfaces. The stress related to seawater percentage leads to increased excretion of EPS to the medium, which increases biofouling formation. The material with the lowest adhesion was Hempasil X3® and PE while those with the highest adhesion were PVC, PETG and PC depending on the growing conditions and species.

Keywords: Biofouling, salt stress, EPS adhesion, cell adhesion.

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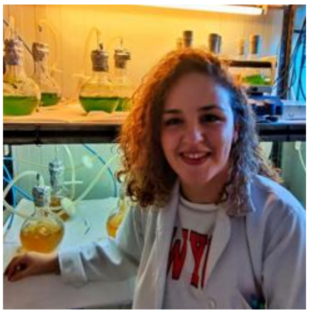

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CELLULOSE PRODUCTION OF DIFFERENT *SPIROGYRA* AND *NANNOCHLOROPSIS* STRAINS

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ABSTRACT

Current packaging material production should be adapted to the new environmental-friendly demands by using sustainable, natural feedstock such as biopolymers, which are commonly biodegradable, compostable, renewable, and recyclable. Cellulose is one of these biopolymers and so far it has been mostly obtained from vegetal sources. However, in the cell wall of plants, cellulose is highly protected by a matrix of lignin and an interfacial primer of hemicelluloses. Thanks to the absence of those biopolymers, algae contain more accessible cellulose. More specifically, microalgae, which are fast-growing organisms potentially obtainable on large scale, could eventually replace plants as cellulose sources. Recent studies have already assessed *Nannochloropsis sp.* for this purpose. In our study, several cellulose-containing microalgae are tested for cellulose production at laboratory scale: two *Spirogyra* species (SAG 170.80 and SAG 169.80) grown in modified Bold Basal medium and two *Nannochloropsis* species (*N. oceanica* and *N. gaditana*) grown in Marine F/2 medium. For the cellulose quantification we developed an analytical protocol that includes lipids extraction, alkali treatment, bleaching and acid treatment. Cellulose productivity of all the strains was calculated. Results showed that *Spirogyra sp.* productivities were much higher than *Nannochloropsis sp.* productivities. The best performing strain, *Spirogyra* SAG 170.80, was scaled up to a 25 L flat panel airlift (FPA) photobioreactor. An additional benefit of *Spirogyra sp.* is that this species can be easily harvested by means of mere filtration thanks to its filamentous morphology.

Keywords: Cellulose · Bioplastics · *Spirogyra sp.* · *Nannochloropsis sp.* ·

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Company profile

CEA is a leading Research and Technology Organization in the field of energy. **M**icro**A**lgae **P**rocesses **P**latform (MAPP) 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. MAPP is involved in collaborative R&D projects with public and private stakeholders.

COMPARATIVE STUDY OF PHYCOCYANIN PURIFICATION FROM BLUE GREEN ALGAE

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ABSTRACT

Phycocyanin, one of the major phycobiliproteins of blue green algae, has wide range of applications in food and pharmaceutical industry. But application of phycocyanin depends largely on its purity which in turn depends on the method of purification used. In the present study, we examined two different methods for extraction and purification of phycocyanin from six cyanobacteria viz. *Lyngbya* sp. (19R), *Phormidium* sp. (27R), *Phormidium* sp. (41R), *Nostoc* sp. (KS3A), *Nostoc commune* (CCC391) and *Anabaena variabilis* (CCC421). The purification method where acetate buffer was used for extraction was found more efficient than using phosphate buffer. In all the pre-chromatographic purification steps where acetate buffer was used, the purity and recovery of phycocyanin was higher except in *Lyngbya* sp. (19R) for which phycocyanin purity values were higher in case of phosphate buffer. When DEAE-cellulose 11 column was eluted with acetate buffer, highest purity of (4.68) was obtained in *Phormidium* sp. (41R) phycocyanin while maximum phycocyanin (46.90%) was recovered in *N. commune* (CCC391) while in case of elution with phosphate buffer, highest purity (2.37) was obtained in *Phormidium* sp. (41R) and maximum recovery (34.84%) in *A. variabilis* (CCC421).

Keywords: Phycocyanin, buffer, purification

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DEVELOPING BIOBASED AND BIODEGRADABLE ROPES FOR ALGAE AQUACULTURE

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ABSTRACT

Algae are key components of the marine environment and may play a significant role in addressing the EU strategical priorities; becoming climate neutral by 2050, the protection of biodiversity, the development of a circular economy and the contribution to the farm to fork strategy for sustainable food. The European algae industry is a promising emerging sector of the EU Blue Bioeconomy (Araújo et al., 2021). New applications of algae biomass are currently being explored for bioremediation and biomonitoring, biofuel production, biopolymers, cosmetics, and health products. However, the sector makes use of ropes that are petrol-based (non-biodegradable) and a growth in the industry will require more rope production which could significantly contribute to plastic waste and pollution in the marine environment. BIOGEARS, EU-funded EMFF project, addresses the challenge of minimizing the use of plastics in the sea by developing durable, fit-for-purpose and marketable, biobased ropes that will be tested at sea in Integrated Multi-Trophic Aquaculture (IMTA) systems integrating mussels and algae culture. The biobased ropes developed are biodegradable and can be sustainably managed in local composting facilities, when they reach their end of life, reducing carbon footprint along the whole value chain and hence contributing to waste reduction. Thus, contributing to sustainable algae production in Europe, as well as supporting strategies on bioplastics in the circular economy. BIOGEARS will conduct cultures of selected algae species at lab and pilot scale tests at different marine areas and sea conditions to assess technical, economic and environmental analysis of biobased ropes' performance (TRL5-7) and will use Blue Lab, which is an innovative laboratory to pilot new and economically viable solutions.

Keywords: algae, IMTA, bio-based, biodegradable, rope.

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MICROALGAE AND CELLULOSE-PRODUCING BACTERIA: A SYMBIOTIC ASSOCIATION FOR LIVING AND FUNCTIONAL MATERIALS

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ABSTRACT

The environmental pollution is a major problem that threatens the sustainability of our planet which has led scientific research to proliferate in several areas to obtain sustainable biotechnologies [1]. Microbial resources can provide sources of materials environmentally friendly and with suitable and desire properties that can reduce the use of petrochemical-derives. Microalgae, cyanobacteria, and cellulose-producing bacteria are some microorganisms that produce extracellular polymerics substances in response to environmental stress, protecting themselves. Some of this natural renewable exopolymers substances drew the attention of researchers due to their unique properties and potential such as bacterial cellulose and extracellular polymerics substances produced by microalgae and cyanobacteria [2]. Currently, the symbiosis between microalgae and bacteria has aroused interest for several applications [3], such as in the treatment of agricultural wastewater (removal of nutrients) and industrial wastewater (removal of organic and metallic pollutants) [4].

The present research aimed to develop the symbiotic association, namely: *(i)* microalga and cellulose-producing bacteria, to obtain living materials to be used in tissue regeneration; and *(ii)* cyanobacteria (that produce bioactive compounds) and cellulose-producing bacteria, to obtain bioactive materials with antimicrobial, anti- inflammatory, antioxidant, or anticancer properties (Fig.1). The results indicate that the incorporation of *Gloeocapsopsis c.f. dvorakii* and *Chlamydomonas debaryana* in the three-dimensional network of BC during biosynthesis increases cell growth and decreases BC production. FTIR spectrum confirmed the presence of microalgal cells in the BC network. Through cutting-edge methodologies, such as 3D printing and bioencapsulation, it is possible to obtain biomaterials with several functionalities: living materials and functional materials. It is expected that the new biomaterials will be used in the design of sustainable bio functional materials, capable of replacing current synthetic materials.

Keywords: Extracellular polymeric substances (EPS); Bacterial cellulose (BC); Microalga; Cyanobacteria

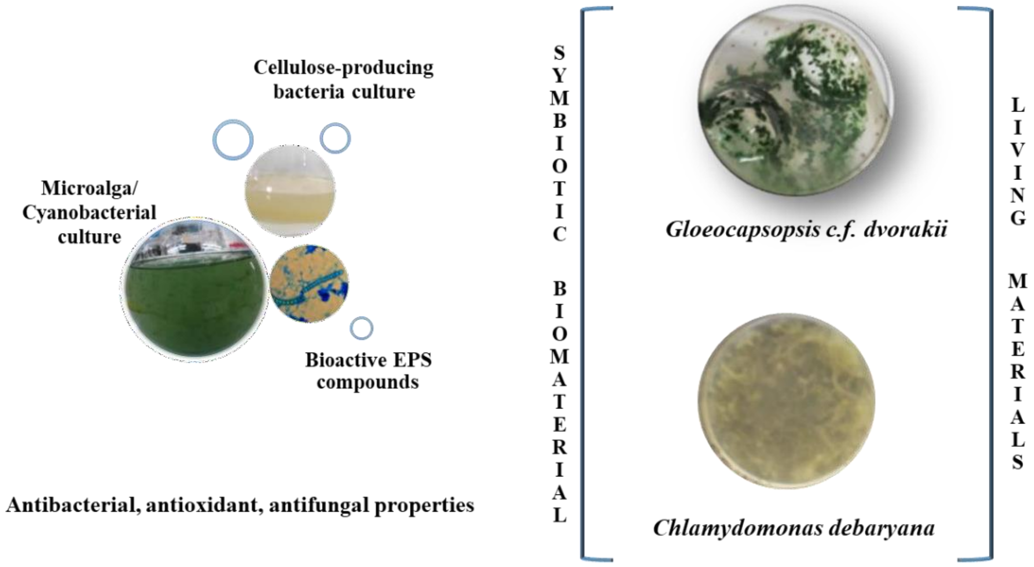

Fig. 1. Symbiotic association between microalga/cyanobacteria and cellulose-producing bacteria to obtain living materials.

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The financial support has been provided by the Foundation for Science and Technology (FCT) with a PhD grant (BD/6615/2020), through CIIMAR (UIDB/04423/2020 + UIDP/04423/2020) and by the European Territorial Cooperation Programme PCT-MAC 2014- 2020 through project REBECA-CCT (MAC/1.1.B/269).

THE POTENTIAL OF LAKE KINNERET ALGAE TO SERVE AS A NEW SOURCE FOR MICROBIAL PESTICIDE

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ABSTRACT

Algae are known as a "green" source for antimicrobial substances. Nonetheless, little effort has been invested in searching for an algal source for use against plant diseases. In the present study, we report on the first examination of micro-algal isolates collected from the Sea of Galilee (Lake Kinneret) for their potential to serve as a source for new plant pesticides for agricultural applications. Micro-algal isolates were cultivated under controlled conditions and the biomass was extracted using different organic solvents. The antimicrobial potential of the crude extracts was examined in vitro and on detached leaves against a wide variety of plant pathogens, including bacteria, fungi and oomycetes. Extracts from the P1 alga (code name because the species used is confidential) exhibited a minimal inhibitory concentration (MIC) of 2,500 ppm against the bacteria *Xanthomonas sp.*, *Erwinia amylovora* and *Clavibacter michigenensis*, and a relatively low MIC of 187 ppm against the oomycete *Plasmopara viticola*. Extracts from two other algal cultures, S1 and C1, exhibited a MIC of only ~150 ppm against *C. michigenensis*. Polarity-based fractionation (using a silica gel column) of the crude extract from the S1 algal culture showed at least two active metabolites: one relatively hydrophobic fraction (eluted with 10% methyl-tert-butyl ether in petroleum ether) and a second, relatively polar, fraction (eluted with acetone). These fractions demonstrated a MIC of 100 ppm against *C. michigenensis*. The latter fraction was further fractionated, and reached a MIC of 50 ppm. These results demonstrate the potential of algal isolate from Lake Kinneret as a source for new biopesticides.

Keywords: Antimicrobial, Pesticide, Algae, agriculture, extract, plant

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POSTER RESENTATIONS

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BIOTECHNOLOGICAL STRATEGIES FOR CULTURE OF THE FILAMENTOUS MICROALGA *KLEBSORMIDIUM NITENS* (BMCC149) RELEVANT FOR PRODUCTION OF FATTY ACIDS AND BIOACTIVES

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ABSTRACT

Recent studies have revealed the valuable biotechnological and nanotechnological potential of the green filamentous algal genus *Klebsormidium*. Species of this genus are producers of a wide variety of compounds that may potentially contribute to meet very specific needs of different industries (food, medicine, pharmaceutics, cosmetics, water treatment and remediation, bioindication, etc.).Additionally, *Klebsormidium* is particularly attractive for its strong stress-resistance and easy defragmentation, interesting qualities for its cultivation in photobioreactors [1-3].

In this study, a new strain of the filamentous microalga *Klebsormidium nitens* (BMCC149) is evaluated for biomass and fatty acids production from its cultivation in photobioreactors. In a first step, shear sensitivity of the microalga and culture medium were studied at a laboratory scale. In a second step, two types of closed photobioreactors, bubble column (CB-PBR) and tubular photobioreactor (T-PBR), were used to determine their influence on the growth rate, biomass yield and production of fatty acids and pigments. *K. nitens* could not be grown satisfactorily in the CB-PBR. The best biomass productivity results were obtained in the T-PBR, the maximum biomass productivity being 160 $mg \cdot L^{-1} \cdot d^{-1}$. The maximum values of 8 $mg \cdot L^{-1} \cdot d^{-1}$ and 0.3 $mg \cdot L^{-1} \cdot d^{-1}$ for linoleic acid (18:2ω6) and total carotenoids, respectively. Results from this study allow us to conclude that the *klebsormidium nitens* could be a candidate microalgae for commercial exploitation.

Keywords: *klebsormidium*, photobioreactor, fatty acids, bioactives

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CHARACTERIZATION OF AN INNOVATIVE FLOATING PILOT PLANT FOR THE PHOTOTROPHIC OUTDOOR CULTIVATION OF MICROALGAE

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ABSTRACT

Crucial challenges for the establishment of algae based biorefineries are the high demand of energy and land and the supply with nutrients for the cultivation of microalgae. Thus, novel and sustainable production systems are necessary to overcome these bottlenecks. At this point, a closed floating outdoor photobioreactor was developed and built at TUHH. The novel reactor system was placed and operated with a working volume of 160 liters on a public channel in an inland harbor of Hamburg-Harburg. It consists of transparent pipes, arranged horizontally, that can be immersed in the surface water of the channel for temperature control. The mixing is executed by a circulation pump and as a carbon source, air, enriched with CO_2, is injected in the pipes. For the characterization of the plant, three cultivation cycles were carried out with the local microalga *Tetradesmus obiquus* in repeated batch. For each single cycle, the pipes were immersed to 50 %, 25 % and 0 %, respectively, in the water of the channel. The cultivations were conducted until the cultures stationary phase and lasted 14 to 21 days. At high solar irradiances, maximum volumetric productivities of up to 0.24 $g \cdot L^{-1} \cdot d^{-1}$ were achieved whereat the average volumetric productivity was between 0.12 and 0.15 $g \cdot L^{-1} \cdot d^{-1}$. Cultivations were started with cell densities between 0.5 and 1 $g \cdot L^{-1}$ and maximum cell densities that were achieved until light limitation, were between 3.04 and 3.45 $g \cdot L^{-1}$. Last but not least, it was shown that the immersion of the photobioreactor in the surface water of the channel was beneficial for tempering in order to not approach critical conditions but maintain sufficiently high cultivation temperatures without active cooling or heating.

Keywords: microalgae, cultivation, offshore, floating, photobioreactor, temperature control

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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.

CHLORELLA: A POTENTIAL SOURCE FOR BIOFUEL APPLICATIONS

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ABSTRACT

Microalgae biofuels have been under the scope as potential alternatives to fossil fuels and energy sources [1]. However, the development of microalgae biofuels has been limited due to the economic and environmental cost of this type of processes [2]. For a better utilization of microalgae applications, it is essential to study the metabolic potential of the microalgae of interest. For this, it was the objective of our research to follow and compare the biomass production under compound accumulating conditions of different *Chlorella* stains with a specific interest on lipid production. The six species were grown at constant conditions for a period of three weeks with a limited amount of nutrients, aiming to induce lipid accumulation by N-starvation [3]. Biomass yield, number of cells and optical density were measured daily. The final biomass was characterized by its carbohydrate, lipid and protein content. The potential of the biomass as a biofuel was studied based on the fatty acid and elemental composition. *Chlorella vulgaris* NIES 227 was found to be the best strain for biofuel applications and as a feedstock for hydrothermal liquefaction. This strain was able to produce as much as 65% of lipid content in phototrophic conditions without a major reduction in biomass productivity. This strain also showed to have an ideal fatty acid profile for biodiesel, with the majority of the fatty acids being C16 and C18 chains. Its elemental composition revealed to have an interesting C/N, C/H and C/O ratio for hydrothermal liquefaction and the highest energy content (HHV= 30.3 MJ/kg) compared to the other strains (HHV= 26.1-23.4 MJ/kg).

Keywords: Biofuels, *Chlorella*, lipid production

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Adriana is an early stage researcher motivated by microalgae applications targeting societal and environmental needs.

Company profile:

CEA is a leading Research and Technology Organization in the field of energy. MicroAlgae Processes Platform (MAPP) 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. MAPP is involved in collaborative R&D projects with public and private stakeholders.

CHLORELLA VULGARIS, A PROMISING FEEDSTOCK FOR STARCH-BASED BIOPLASTICS

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ABSTRACT

SEALIVE [1] and Nenu2PHAr [2] European projects are both engaged in the development of new sustainable value chains, supporting the development of greener and cost-effective plastics. On the one hand, starch is well known for being used as a natural biopolymer base to integrate plastics blends [3], while on the other hand, it can be degraded into monomeric glucose to feed PHA producing bacteria [4]. *Chlorella vulgaris*, a robust microalga, recognized as a remarkable starch producer [5], is already widely cultivated at industrial scale [6]. Thus, in SEALIVE and Nenu2PHAr projects, different *C. vulgaris* biorefinery schemes are proposed, focusing respectively on the production of three fractions (starch, pigments and antioxidant molecules) or on the production of a fraction suitable to feed PHA producing bacteria. To optimize volumetric starch productivity, and thus decrease the production costs of the algal-starch, two culture schemes were developed. Firstly, the "2-reactor-strategy", where high biomass productivity is obtained in a first photobioreactor (PBR), while starch accumulation is quickly triggered in a second PBR, where culture is subject to nitrogen-deprived medium and high light intensities; and secondly, the "1-reactor strategy" where biomass production up to high-densities occurs in the same PBR in which starvation is induced. Total carbohydrate content raised up to a range of 50-70 %DW for both strategies, confirming that nutrient-deprivation plays a crucial role on starch accumulation. Results were validated at pilot scale (25L PBRs under artificial light) and semi-industrial scale (180L PBRs under natural light). Whereas production process under natural light is being optimized, different starch purification schemes are under development, in order to effectively include *C. vulgaris* feedstock into the production of biodegradable bio-based plastics.

Keywords: algae biorefineries, biomass production processes, starch, bioplastics

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[1] SEALIVE Project, https://sealive.eu/ This project has received funding from the European Union's Horizon 2020 Research and Innovation programme under grant agreement n° 862910.

[2] Nenu2PHAr project, https://nenu2phar.eu/ This project has received funding from the Bio Based Industries Joint Undertaking (BBI-JU) under grant agreement n° 887474. The BBI-JU receives support from

the European Union's Horizon 2020 research and innovation programme and the Bio Based Industries Consortium.
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About the author:
Ana Compadre is a research engineer in microalgae cultivation processes and technologies at CEA, France. Her current research topic is based on algal biorefineries, targeting the development of new bio-based formulations to provide viable alternatives to the current fossil-based plastics. Ana has been working for several years in the microalgae sector, having a combined experience in industry and research, acquired in three different countries (Portugal, France and the Netherlands).

Company profile:
CEA is a leading Research and Technology Organization in the field of energy. **M**icro**A**lgae **P**rocesses **P**latform (MAPP) 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. MAPP is involved in collaborative R&D projects with public and private stakeholders.

EFFECT OF THE OSMAC STRATEGY ON THE BIOACTIVE PRODUCTION IN *AMPHIDINIUM CARTERAE* USING EPIGENETIC MODIFIERS

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ABSTRACT

Dinoflagellate microalgae are associated to the production of high value products (e.g., carotenoids, fatty acids, bioactive compounds) with diverse biotechnological applications. For example, the marine dinoflagellate *Amphidinium carterae* is a source of amphidinols, DHA and peridinin [1]. Amphidinols are metabolites from the group of polyketides that show antibiotic and antifungal applications [2]. Peridinin, the major carotenoid and a pigment marker in most dinoflagellates, has medical applications [3]. The present study is aimed to further evaluate the bioactives' production of *A. carterae* (strain Dn241EHU [4]) by using the One Strain Many Compounds (OSMAC) strategy. Static-batch cultures were performed using a modified f/2 Guillard medium (N:P ratio of 5). Five chemical epigenetics (Jasplakiolide, suberoylanilide hydroxamic acids (SAHA), sodium butyrate, tricyclazole and 5-azacytidine) were evaluated at different concentrations. None of these compounds had influence on the expression routes related with amphidinols' production (evaluated as equivalent saponin mass in hemolytic assays). On the other hand, SAHA and tricyclazole were found promoters of peridinin synthesis. Thus, final concentration was three and two-fold compared to the control, respectively. Chlorophylls and total carotenoids increased by 175% with SAHA and 120% with trizyclazole, respectively. Despite these important results, SAHA and tricyclazole showed growth-inhibiting effects. Further assays using a two-phase culture, consisting in a first phase of optimum growth and a subsequent phase of epigenetic induction of peridinin synthesis, could prove the biotechnological potential of this approach. To our best knowledge, the treatment with epigenetic modifiers is tested for the first time in a marine dinoflagellate microalga. This work represents the first of further efforts aimed at including epigenetic modification in the OSMAC strategy to improve metabolites production of *A. carterae*.

Keywords: epigenetic modifiers, A. carterae, Peridinin, Bioactives, OSMAC

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ENHANCING THE SUGARS PRODUCTION FROM *DICTYOTA DICHOTOMA* BY ULTRASONIC PRETREATMENT

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ABSTRACT

Marine macroalgae show great potential as raw material to obtain bioproducts through the fermentation of the sugars generated from their polysaccharides hydrolysis. They offer the advantages of being accessible, renewable and not need land or freshwater for their cultivation. Also, algal biomass has an appreciable content of carbohydrates, lipids and proteins and low content of lignin which facilitates its hydrolysis. The purpose of this work was to study the effect of ultrasonic pretreatment on the enzymatic hydrolysis of the brown algae *Dictyota dichotoma*. For this, the seaweed was pretreated assaying various percentages of amplitude and pretreatment times in ultrasonic equipment and then hydrolysed with the enzymatic cocktail Cellic CTec 2 (117.53 units of cellulase per gram of dried solid). It was observed that the hydrolysis yield (percentage of reducing sugars obtained from hydrolyzable polysaccharides contained in algal biomass) increased as the amplitude and the pretreatment time rose as a consequence of the higher specific energy (MJ/kg) applied to the algae during the pretreatment. Thus, the seaweed hydrolysis yield improved from 9% (without pretreatment) to 24% when algal biomass was pretreated with an amplitude of 60% for 60 min (7.5 MJ/kg).

Keywords: *Dictyota dichotoma*; Pretreatment, Ultrasonic, Enzymatic hydrolysis

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I am Professor of Chemical Engineering in the Faculty of Sciences at the University of Cádiz. About ten years ago, I started working on a research line focused on obtaining different value-added products such as bioethanol, enzymes, lactic acid or bioplastic precursors (polyhydroxyalkanoates-PHAs) by fermentation several agroindustrial waste and subproducts previously pretreated and enzymatically hydrolysed. I am currently focused on the study of the production of PHAs from macroalgae. This work is being carried out within the framework of the project of the National Plan of R+D+i PID2019-104525RB-I00, of which I am co-principal investigator.

EXHAUSTED WHEY BY-PRODUCT AS ALTERNATIVE NUTRIENT SOURCE FOR *EUGLENA GRACILIS* CULTIVATION

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ABSTRACT

The dairy industry is one of the most productive sectors of Italian economy, but it produces an appreciable quantity of by-products, whose management/disposal often represents an environmental and economic issue.

Aim of this work was to valorize exhausted whey for growing *Euglena gracilis*, whose biomass is a source of proteins, lipids and carbohydrates, among which the paramylon, a β-glucan with interesting immunostimulatory properties. Exhausted whey (EW) is the final by-product of two consecutive cheese-making processes, with still a strong organic content (about 50.000 mgCOD/L).

EW was diluted 1:3 with distilled water and partly microfiltered to reduce bacterial load. Two different tests, TCW1 (with unfiltered medium) and TCW2 (with filtered medium) were conducted and compared with a control (SMG), in which *E. gracilis* was grown on standard medium, added with glucose as carbon source, until reaching the same COD value of the exhausted whey. All tests were conducted in 13-days batch cultivation mode, in duplicate, using 5-liter round flasks and in controlled conditions.

The best growth was observed in TCW2, with a maximum cell density of 1.28 * 10^6 cells/mL, but also TCW1 reached a maximum concentration higher than the control (SMG). In all tests, NH_4^+-N was completely removed and COD removal was above 70% both in TCW1 and TCW2 whereas in SMG the final reduction was only 52%. Overall, the results showed that the cultivation of *E. gracilis* on EW is not only possible but is even more efficient and sustainable than with the conventional protocol based on synthetic media.

Keywords: Dairy, Whey, By-product, *E. gracilis*, COD, NH_4^+-N

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Graduated in Science in Biology at the University of Milan, Italy (1998). Registered in the National Order of Biologists (2000). Currently Head of Aquaculture Division of the Spallanzani Institute. She coordinates a research group conducting various activities in the field of microalgae technology, with particular interest in the integrated microalgae culture system for nutrients recovery from wastewater and agricultural by-product and the extraction of bioactive molecules for cosmetics, nutraceuticals and feed purposes. Author or co-author of 19 scientific papers (Scopus) and over 60 presentations/posters to scientific congresses. H-index: 8

Company profile:

The Istituto Spallanzani is a non-profit research organization, stable member of The committee for Research and Experimentation in Agriculture. Its mission includes scientific research, experimentation and services in the areas of Aquaculture (Pisciculture and Microalgae), Quality and Safety of Products of Animal Origin, Reproduction.

IMPROVEMENT OF ARTHROSPIRA PLATENSIS DEWATERING BY ELECTRICALLY ASSISTED DEAD-END FILTRATION

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ABSTRACT

Microalgae have gained popularity in a wide range of industries food, pharmaceuticals, and biofuels. Before downstream processing, microalgae must be harvested which can be a constraint due to the associated costs, energy consumption and processing difficulties. Filtration has been proven to be a highly effective dewatering technique, however microalgae are difficult to filter at an acceptable rate, yield cakes with high specific resistances, and cause membrane biofouling.

This study explores the use of a constant electric field intensity with varying voltage to enhance the filtration kinetics of *Arthrospira platensis* (*A. platensis*). Two feed solutions with a concentration of 5% by weight are studied at first: dried and frozen *A. platensis*. This was done by dead-end filtration at a constant pressure (P) of 1 bar, for an electric field strength (I) of 0 and 60 A/m^2. Frozen *A. platensis* was then selected to study the effect of pressure and the electric field density on the filtration kinetics and cake dewaterability. The effect of combining the use of P (1, 2, 3, and 4 bar) with I (0, 30, 40, 60, and 80 A/m^2) on the overall filtration performance, energy consumption and product quality were assessed.

It was concluded that *A. platensis* cake is highly compressible and that the pressure does not have a strong influence on the filtration kinetics, but applying an electric field enhanced the dewaterability kinetics and cake dryness. For instance, at P= 1bar and 4 bar, applying an electric field strength of 60 A/m^2 decreased the filtration time respectively by 25% and 44% and increased the cake dryness respectively by 23% and 25%.

Keywords: A. platensis, Microalgae, Filtration, Dewatering, Electrodewatering, Extraction.

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INTEGRATED ANALYSES OF PHOTOTROPHIC BIOMASS PRODUCTION AND PIGMENT PRODUCTION IN MICROALGAE AND CYANOBACTERIA

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ABSTRACT

Pigments are a group of compounds that are intensely coloured which are applied in various industries to colour other materials. The increase in environmental distress and sustainability issues with synthetic pigments have increased the demand for natural pigment production, transitioning towards green alternatives (circular bioeconomy approach) [1]. Natural pigments are produced by a number of organisms (e.g. bacteria, cyanobacteria, microalgae, macroalgae, plants). Cyanobacteria (prokaryotic) and microalgae (eukaryotic) are unicellular phototrophic microorganisms with the ability to capture solar energy and CO_2 and use it to synthesise a diverse range of sugars, lipids, and amino acids [2, 3]. This makes them attractive for the production of a wide range of high-value products including industrial chemicals, pharmaceuticals, nutraceuticals, and animal-feed supplements [1, 4, 5]. The advantages of microalgae as production platform are attributed to their high growth rates, their ability to grow in freshwater, seawater or brackish water, their comparatively low-cost cultivation using non-arable land. The pigments derived from cyanobacteria and microalgae including chlorophylls, carotenoids and phycobiliproteins have unique properties that can be translated into advanced technical and commercial products [6-8]. This study focuses on the development of strain-specific pigment profiles (qualitative and quantitative) and cultivation strategies to optimise natural pigment production through an automated high-throughput screening procedure to characterise nutrient and light. The combined identification of both biomass productivity and pigment concentration will enable the development of economically feasible pigment production scenarios with enhanced pigment yields and quality. The high-throughput screens enable the establishment of dose-response models of various strains and guide the understanding of differences in strain-specific and pigment-specific production scenarios.

Keywords: Pigments; Microalgae; Cyanobacteria; High-throughput; Biomass.

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Charu completed her BSc Hons of Technology – Biotechnology (Anna University, 2017) with first class Distinction. To advance her education to the next level she undertook her Master of Biotechnology (University of Queensland, 2019) successfully completed with a GPA of 6.5/7. Currently focused on the development of next generation algae systems to produce renewable fuels, foods, feeds, recombinant proteins and high value products. PhD is around the development of a design and validation of a high-throughput robotic nutrient screen and light screen for higher pigment yields, based on incomplete factorial analysis.

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MICROALGAE PULSED ELECTRIC FIELD EXTRACTION OF PROTEINS²

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ABSTRACT

Microalgae constitute an interesting source of proteins for the food industry[1]. Among the various cell disintegration technologies, PEF presents the advantages of being mild and selective, offering a potential easier way for the upstream separation and purification steps. In this study, batch PEF treatment has been applied to *Tetraselmis chuii* under a wide range of conditions: 50-600 pulses of 40µs duration, 2.5-28 kV/cm and 2.2-30 MJ/kg_{DW}. *Tetraselmis chuii,* a salted microalgae strain, has been provided as frozen paste. The cell disruption step is applied to thawed samples, and then centrifuged at 5,000g for cell debris removal and at 11,000g for retaining only “soluble” proteins. Protein yield and purity have been quantified in the supernatants and compared to the ones obtained after thawing of the original biomass. Results show that a very small portion of proteins is released after PEF treatment even at high energy input. Maceration of 6 hours after PEF shows an increased protein yield compared to control. Protein purity, being defined as the protein concentration divided by the dry mater of the 11,000g supernatant is observed to be slightly increased. PEF treatment of a salted microalgae strain presenting a high electrical conductivity has been demonstrated. However, PEF was not able to induce a sufficient electroporation of the cell wall to allow the release of large amount of intracellular protein as already observed on *Chlorella vulgaris* and *Neochloris oleoabundans* by Lam et al.[2]

Keywords: Pulsed electric field, protein extraction, Tetraselmis chuii

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I am a PhD student of Nantes university at the GEPEA laboratory and of Algosource. My thesis subject is on the extraction and purification of functional proteins from microalgae for food application.

Company profile:

Algosource is a small company that aims at extracting high-value bioactive ingredients from microalgae for nutraceutic, agrofood, cosmetic & healthcare.

MOZZARELLA BY-PRODUCT AS NEW CULTURE MEDIUM FOR THE PUFA PRODUCER *AURANTIOCHYTRIUM MANGROVEI*

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ABSTRACT

Dairy industry is one of the main food industries in Italy and Europe, with tons of cheese produced every year. After the production of "Mozzarella", tons of effluents are generated, in particular the stretching water (MSW). This effluent is rich in residual nutrients such as: reducing sugars, mineral salts and proteins. These compounds could be useful for formulation of more sustainable microbial media, in particular for heterotrophic microrganisms that requires organic carbon and other nutrients for their growth. The heterotrophic cultivation mode for microalgae is economically interesting because of the high cell density obtained. Moreover, heterotrophic cultivation is important for the obtainment of high added value compounds such as proteins and polyunsaturated fatty acids (omega-3 oil). *Aurantiochytrium* is a genus of heterotrophic microalgae promising for the production of docosahexaenoic acid (DHA, C22:6n-3). MSW has been used as culture medium for the production of DHA from *Aurantiochytrium mangrovei.* The biomass production from this food waste has been increased after an enzymatic hydrolysis of MSW. Without enzymatic hydrolysis of MCW, a lower biomass growth was obtained in terms of dry weight (up to 2.3 g L^{-1}) respect to the 5.33 g L^{-1} of the hydrolyzed samples. In combination with MSW, an alternative nitrogen source has been used (spent brewery yeast), and the optimal combination of these factors has been established using response surface methodologies. A biomass higher than 10 g L^{-1} was obtained with the optimized conditions.

Keywords: Sustainability, biorefinery, food waste, bioconversion

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Giovanni Luca was born in 1992 and graduated in 2018 in Food Science and Technology at the University of Naples “Federico II”. To date, he is a PhD student working on optimization and valorization of food waste and by-products through biotechnological approaches.

MULTIPRODUCT BIOREFINERY ON A CHIP: EXPLOITING ACOUSTIC WAVES TO HARVEST, EXTRACT AND SEPARATE MICROALGAL CELL COMPONENTS

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ABSTRACT

Microalgae are remarkable organisms which convert carbon dioxide into functional proteins, sugars, oils, pigments and platform chemicals. However, due to the high cultivation and processing costs, current single-product biorefinery approaches need to be disrupted if economic feasibility is to be achieved. Unique phenomena occur when combining microfluidic channels with external fields such as laser, electric, gravity and acoustic. These allow precise manipulation, extraction and even separation of cells and cell structures, otherwise not possible in conventional systems. For instance, low frequency acoustic pulsing waves can gently open the microalgal cells, releasing their components, while high frequency acoustic standing waves can separate them based on their acoustic properties. The former phenomena is based on cavitation and specific resonance effects, while the latter is driven by acoustophoresis forces. Advances in 3D printing technologies make it possible to mass-produce and stack together large numbers of these chips, effectively scaling-out and bringing the process throughput to specific required levels. Learning how to harness these forces could become a Promethean moment for many bioprocess applications spanning from development of drugs and novel foods, to space exploration.

Keywords: Algal multiproduct biorefinery; External fields; Acoustophoresis; Microfluidics

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After completing his PhD at the Politechnica University Timisoara, Romania, and the Biological Research Center Szeged, in Hungary, working on novel microbial – microalgal biohydrogen production approaches, Dr. Boboescu was offered a Mitacs Accelerate fellowship at the Sherbrooke University in QC Canada. Here, he developed and scaled-up novel second and third generation biorefinery technologies together with some of the biggest North American companies active in the fields of biofuels, biocommodities and specialty chemicals. Recently, Dr. Boboescu joined the Bioprocess Engineering group of Wageningen University and Research, The Netherlands, after securing a Marie Skłodowska-Curie Fellowship and an NWO Science XS grant to develop a novel acoustic biorefinery approach.

OPTIMIZATION OF INDUSTRIAL CULTIVATION IN THE NATIVE STRAIN *TETRASELMIS STRIATA* AND WASTEWATER REVALUATION: A CASE STUDY IN GRAN CANARIA

Bustamante, B., Aleman, M., Venuleo, M & Portillo, E.

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ABSTRACT

Tetraselmis is a microalgae with many nutritional properties and different bioactivities. This microalgae has a DW composition of approximately 15% lipids, 42% carbohydrates and 24% proteins. Due to its exceptional nutritional content, the biomass of the genus *Tetraselmis* is generally used in aquaculture feed. *Tetraselmis striata* is a species which has a rapid acclimatization to environmental changes, rapid growth and resistance to contaminations. This makes it an ideal candidate for industrial cultivation purposes. Surrounded by the sea, the Canary Islands (Spain) offer optimal conditions for industrial marine microalgae production due to their strategic economic location and stable year-round sunny weather. The objective of this study was to optimize water and nutrient efficiency for the cultivation of a native strain of *Tetraselmis striata* at a semi industrial scale scenario. These are key factors to obtain an economic and environmentally sustainable production. There was no significant difference in productivity between the cultivations using new media (0,096 g L^{-1} day^{-1} ± 0,04) and the productivity of the cultivations using recirculated supernatant (0,099 g L^{-1} day^{-1} cycles 1 to 5 and 0,072 g L^{-1} day^{-1} cycles 6 to 10 of recirculated supernatant). Nitrogen availability significantly affected the productivity at different stages of the cultivation being lower at nitrogen limitation conditions.

Keywords: *Tetraselmis striata*, recirculated supernatant, nutrient limitation, water efficiency, nutrient efficiency.

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After graduating at the University of Plymouth in Marine Biology and Coastal Ecology I undertook a master on Sustainable fisheries at the University of Gran Canaria, Spain. I then worked for six years in the private sector as the senior biologist cultivating *Haematococcus pluvialis* at an industrial scale in Tenerife, Spain. Finally I was offered the opportunity to work for the Canarian Institute of Technology to do research on two marine strains; *Tetraselmis striata* and *Dunaliella salina* and in order to optimize their cultivation in terms of profitability, quality and sustainability.

Company profile:

The Canarian Institute of Technology (ITC) promotes blue biotechnology through experimental development and applied research of new marine microalgae production and processing technologies, on pilot and industrial scale (demonstrative projects) with application in the Canary food, cosmetics, agriculture and environmental industries.

PHOTOSYNTHETIC PRODUCTION OF CYANOPHYCIN BY SYNECHOCYSTIS SP.: A DATA DRIVEN OPTIMIZATION

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ABSTRACT

Recently, cyanophycin has gained a lot of interest as a potential raw material for the industrial sector, where the applications focus on its chemical derivatives, such as poly(aspartic acid), PASP, and arginine. The main drawback of cyanophycin accumulation in autotrophic organisms is the high variability of the related synthesis and accumulation processes, as its intracellular content depends on the growth phase and on environmental conditions. Before investing on large-scale production systems, a reliable assessment of operating variables should be performed. According to standard chemical engineering procedures, this can be preliminarily done by using models calibrated on lab data and process simulations. However, the available knowledge on the complex interactions occurring in photosynthetic microorganisms cultivation, where light plays a major role and affects the metabolic pathways, is still insufficient to develop a detailed mechanistic model of the process. On the other hand, data-driven models based on response surface model (RSM) methodology have proven efficient in optimizing the performances of various chemical and biochemical processes, with only slight differences in optimum prediction with respect to knowledge-based ones[1]. In this work, the Design of Dynamic Experiment (DoDE) approach was applied with the aim of maximizing the cyanophycin production in *Synechocystis* sp. PCC 6803 batch experiments. The Design of Dynamic Experiments (DoDE) is a new data-driven approach that capture the dynamic aspects of a time-evolving system, in fact, it allows to take into account time-varying inputs. The factors considered are temperature, incident light intensity and phosphorus inflows, to estimate a two factors interaction model.

Keywords: cyanobacteria, polypeptide, Design of Experiment, model

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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.

POSTCOVID RECOVERY AND RESILIENCE PROGRAM HELPING BY BIOINDUSTRIAL MICROALGAE CULTIVATION COMPLEXES

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ABSTRACT

Microalgae play an important role in complex and successfully antiviral fight.as well as based on short retourning time of invested funds help the economic recovery.Developing our armament, tools and procedures our primary goal is to forgive in this mondial fight the immune system strenghtening. Corresponding of the actually challenges, the developed integrated cultivation complexes will produce a various immunological end products and raw microalgae serving to extraction of active substances.

The therapeutical products department will focus on bio disinfectants based on bioalcoolic substances and bio-ozone. The efficient supply of breathing apparatus will be resolved by high therapeutical biooxygen with oxygen isotopes content captured from photosynthetic algae growth process. The complex will be controlled by artificial intelligence.

The possibility of development to microalgae biorefinery with own bioenergy production is in conformity with POSTCOVID programs of UN-OECD-EU and several country-programs strongly affected by COVID 19 SARS 2 pandemic.This poster presents the steps of development of the integrated industrial complexes which can allows the necessary microalgae cultivation for the above purposes.

Keywords:
microalgae, immunology, COVID 19 SARS2,industrial complexes, biorefinery, bioozone

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2. Bela Ferenc Tozser, Bettina Szabo, Marianna Percsics: High efficiency, sustainable and safe bioindustrial microalgae cultivation complexes for circular economical sustainable development, AlgaEurope2019
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4. Bela Ferenc Tozser: Bioindustrial complexes for integrated bioenergy production, climate and environment protection in Southern Europe,Belgrad,2017

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Company profile:

Since 1996 the associated profile is integrated engineering on climate, environment, health protection, bioenergetics, bioindustrial products, equipments biorefinery complexes, circular economy and zero carbon emission. Since start of pandemic our activity has been focused to antiviral activity by microalgae and use of microalgae diversity for POSTCOVID recovery and resilience programs.

PRE-CONCENTRATION IN PILOT-SCALE CULTIVATION OF NATIVE CANARIAN MICROALGAE STRAINS BY THE APPLICATION OF SELECTED FLOCULANTS

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ABSTRACT

One of the main bottlenecks in the mass microalgae production is the high cost of the downstream processes that can be significantly reduced by the pre-concentration of the algal culture. In the present study, we investigated microalgae biomass flocculation as a pre-concentration step by the application of three different flocculants on laboratory and pilot scale cultures of three microalgae species native to the Canary Islands (Spain). Natural polymer chitosan and two chemical flocculants $FeCl_3$ and $AlCl_3$ were tested on freshwater *Chlorella sorokiniana* and two marine microalgae: *Dunaliella tertiolecta* and *Tetraselmis striata*. Biomass recovery of 85 % or more was accepted as sufficient for the flocculation to be considered successful. After a preliminary screening at laboratory scale to find the most promising doses of flocculants for each experimental system, pilot scale cultures were tested to confirm the effectiveness of flocculants in a more realistic production scenario. The results of this study shows that chitosan and $AlCl_3$ were efficient in doses of 0.1 g/L where $FeCl_3$ was efficient in doses of 0.2 g/L for *D. tertiolecta* and *C. sorokiniana*. In case of *T. striata,* flocculants were more effective in lower doses: chitosan at 0.04 g/L, $AlCl_3$ at 0.08 g/L and $FeCl_3$ at 0.1 g/L. This may be due to significant autoflocculation properties of *T. striata*, as reported by previous studies. Both chitosan and $FeCl_3$ can be successfully applied to pre-concentrate all three native strains at pilot scale with a reduction of the cost compared to conventional separation techniques. Moreover, the biomass obtained by harvesting the most concentrated fraction after flocculation was also analyzed for its possible application in food and feed industry.

Keywords: microalgae, downstreaming, pre-concentration processes, flocculation, chitosan

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Laura Figueira, Msc in Aquaculture specialized in biotechnology and BSc in Marine Science, grew up in the field of microalgae biotechnology during her academic career. Her final Msc project was about the study of the optimal growth parameters in the microalgae *Aureoumbra geitleri sp.nov*. in a cultivation chamber and its upscaling to an outdoor cultivation system. In 2019, she worked as full-time project technician at he Spanish Bank of Algae (BEA). Since 2020, she entered the Instituto Tecnológico de Canarias (ITC) as junior researcher where she is studying several biotechnological applications of native microalgae strains.

Company profile:

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

SALINITY AS AN ABIOTIC STRESS TO MODULATE BIOACTIVE COMPOUND PRODUCTION IN MARINE MICROALGAE

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ABSTRACT

There are evidences correlating the production of bioactive compounds with salinity modulation in the culture medium[1]. In this study, the effect of salinity on the growth and biochemical composition of biomass for three microalgae was assessed: *Chrysochromulina rotalis*, *Amphidinium carterae* and *Heterosigma sp*. Firstly, the growth of the microalgae at different salt concentrations was evaluated through an one-step process (**S1**). Once optimal salinities for each microalga were selected on the basis of the maximum growth rates reached, cultures were subjected to hypo and hyper saline shock by a two-step process (**S2**). In this procedure, cells cultured under optimal salinity were harvested by centrifugation and abruptly resuspended and exposure to different salinity during 48h. Assays were performed at a temperature of 18±1°C under 100 μmols^{-1}·m^{-2} irradiance supplied in 12:12 (light:dark) ON/OFF cycles using f/2[3] formulation as culture medium. The biochemical composition (carotenoids and fatty acids) and bioactivity (haemolytic response) of the biomasses obtained at **S1** and **S2** were analysed. *C. rotalis* reached biomass (Pb), fucoxanthin and PUFAs maximum productivities at **S1** for a salinity of 30 psu (14, 0.03 and 0.42 mg·L^{-1}·day^{-1}, respectively). *A. carterae* showed maximum growth at 35 psu (Pb=17.24 mg·L^{-1}·day^{-1}) while its haemolytic activity was maximal under hyposaline conditions for both **S1** (1.97±0.05 ESP at 15 psu) and **S2** (1.67±0.06 ESP at 20 psu). *Heterosigma sp.* showed no growth variation under hyposaline culture conditions (**S1**) reaching a maximum Pb of 12.33mg·L^{-1}·day^{-1} at 20 psu. While two maximum Pb plateau zones were observed in **S2**; one from 35-50psu (Pb=12.25mg·L^{-1}·day^{-1}) and another one from 5-20psu (9mg·L^{-1}·day^{-1}) with a maximum carotenoid and PUFAs content of 0.67% and 1.74% on biomass d.w. (fucoxanthin - EPA accounted for 28.04% - 59.32%, respectively).

Keywords: Microalgae, salinity, bioactive compounds, *C. rotalis*, *A. carterae*, *Heterosigma*

Acknowledgements:
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POSTER RESENTATIONS

Bioremediation

ALGÆUROPE2021
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ADAPTIVE LABORATORY EVOLUTION FOR IMPROVING MICROALGAE TOLERANCE TO A TOXIC EFFLUENT

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ABSTRACT

Hydrothermal liquefaction (HTL) is a process that converts wet biomass into a biocrude that needs to be further upgraded to obtain a biofuel [1]. HTL is a promising technology for producing biofuel from microalgae. However, HTL produces large volumes of an Aqueous Phase (AP) rich in various inorganic and organic compounds [2]. This AP needs to be treated before being released. Since it contains potential nutrients (such as ammonium and phosphate), its reuse in the microalgae culture is a promising way of recycling the AP. However, AP contains high level of organic compounds that are toxic to microalgae growth. For that reason, we used the adaptive laboratory evolutionary (ALE) technique [3] to acclimate *Chlorella vulgaris* NIES 227, an efficient lipid producing microalgae strain, and develop its resistance to the AP's toxicity. The microalgae were grown in a 1L photobioreactor with media containing an increasing concentration of AP. After a gradual acclimatization, it was possible to double the AP content in the culture medium from 0.167% to 0.67% without observed negative effects on the culture, while improving the growth rate from 0.6 d^{-1} to 1.7 d^{-1}. Finally, the growth of the adapted strain of *C. vulgaris* in a medium enriched with 0.5% AP was compared to the growth of the original non-adapted strain on the same medium. When compared to the non-adapted strain, the adapted one performed better for both the maximum growth rate (1.09 d^{-1} vs 0.89 d^{-1}) and the biomass dry weight after 4 days of culture (2.65 $g.L^{-1}$ vs 1.97 $g.L^{-1}$).The ALE proved to be an efficient and promising technique for adapting microalgae strains to environmental stresses.

Keywords: Adaptive Laboratory Evolution (ALE), Hydrothermal Liquefaction (HTL), Aqueous Phase, reuse

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Dr. Florian Delrue has participated in various collaborative and industrial projects (more than 25 projects so far including ANR DiesAlg 2011-2015, ALGUEX ADEME 2018-2020, ANR RafBioAlg 2018-2021) and he has acted as coordinator for 14 industrial projects (CEA/industry contracts with budgets ranging from 50 to 300k€). He has also managed the set-up of the lab and pilot components of the CEA Tech-GB3G algal test bed (1M€ budget in 2013 and 2M€ budget in 2020). He is the author of 13 papers (*h*-index 9, WOS) and 4 patents.

Company profile:

CEA is a leading Research and Technology Organization in the field of energy. MicroAlgae Processes Platform (MAPP) 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. MAPP is involved in collaborative R&D projects with public and private stakeholders.

ALGAE-BACTERIA CONSORTIA, A NEW AVENUE FOR BIOREMEDIATION AND HYDROGEN PRODUCTION

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ABSTRACT

Microalgae are microorganisms capable of surviving and adapting to many environmental conditions. Their complex metabolism allows them to activate or inhibit specific metabolic routes and produce metabolites in order to respond adequately to different stimuli. Thus, microalgae are powerful factories able to secrete many high-value end-products one being hydrogen (1). On the other hand, microalgae are also capable to grow in wastewater using pollutants as nutrients making themselves useful tool to bioremediate polluted water (2). Interestingly bacteria have similar abilities for metabolite production and bioremediation capacity. Therefore, combining algae and bacteria is a powerful avenue to benefit from and improve their hydrogen production and bioremediation skills (3). Here we report our investigation on consortia resulting from the co-cultivation of the freshwater green alga Chlamydomonas reinhardtii and different bacteria strains. Consortia of Chlamydomonas either with Methylobacterium sp, Escherichia coli or Rhizobium etli were able to improve and sustain hydrogen production depending on the light condition and the carbon sources used (4 and 5). Whereas other Chlamydomonas-bacteria consortium demonstrated bioremediation capabilities. A specific consortium was able to remove nitrate (NO3-), nitrite (NO2-) and ammonium (NH4+) from synthetic medium while generating a much larger amount of biomass compared with the single corresponding monocultures. Taken together our research indicate that the quest for finding suitable partners for algae can be a promising biotechnological approach to produce hydrogen and for bioremediation.

Keywords: Algae, consortia, hydrogen, remediation

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Dr Alexandra Dubini is a distinguished researcher at the University of Cordoba, UCO, Spain. She received her PhD from the University of East Anglia, UK and then worked 10 years as a permanent research scientist at the National Renewable Energy Laboratory in Colorado, USA. In 2015, she joined the UCO where she currently is the PI of national project “multivalga” and was the coordinator of European project called “WABA”, both using algae bacteria consortia properties for wastewater bioremediation and their biomass for agronomical valorization. She also specialized in biofuels production and more specifically hydrogen from green algae.

ALGACYCLE: VALORISATION OF GREENHOUSE EFFLUENTS WITH MICROALGAE: SUSTAINABLE AQUAFEEDS AND BIOSTIMULANTS FROM DRAIN WATER

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ABSTRACT

The human population is foreseen to significantly grow over the following decades and, with it, the demand for food, feed, energy, and clean water. Besides the decline in freshwater supply, one major concern is the discharge of agro-industrial water containing excess nutrients, which is known to have a negative impact on the environment. Soilless plant production systems have been gaining relevance in modern agriculture as a substitute or complement to traditional agriculture, having key advantages such as lower water requirements, increased productivities, improved nutrient usage and production on non-arable land. Nonetheless, the discharge of water rich in nutrients still represents about 30% of the total water used. To counteract these effects, the implementation of a circular economy approach seems to be a valid solution, upgrading otherwise waste streams into value, tackling environmental concerns while generating novel goods. The ALGACYCLE project focuses on the re-use of drainwater from soilless plant production units as a medium for microalgae growth. The envisioned downstream process with a biorefinery approach will allow for three different products to be obtained: clean water, microalgal extracts and biomass for agriculture and aquaculture, respectively. Two strains were selected for summer and winter outdoor conditions, *Scenedesmus* sp. and *Koliella antarctica*, and lab-scale experiments will access the impact of drainwater on growth performance and biochemical composition. Pilot-scale production of these strains will proceed under outdoor conditions for maximum removal of excess nutrients, and the microalgal biomass will be harvested and processed using a high-pressure homogenizer. After fractionation, two different products will be obtained: water extracts for biostimulants in agriculture and cellular debris for feed in aquaculture. Clean water will be a by-product of this downstream process.

Keywords: microalgae, biorefinery, biostimulants, feed, aquaculture, bioremediation

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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, where her thesis was 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*.

CO_2 BIO-FIXATION BY MICROALGAE AND OPTIMIZATION OF CARBON SUPPLY IN CULTURE SYSTEMS

PRUVOST J.[1]
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ABSTRACT

Microalgae or cyanobacteria can be used to fix CO_2 from industrial effluents. However, the gaseous CO_2 must be first transferred into the liquid phase in the form of dissolved inorganic carbon (DIC) to then be assimilated and thus biofixed by microalgae.

In practice, it is rather simple to maintain a sufficient DIC concentration by enriching culture medium with carbonates or the gas phase in CO_2. Determining the carbon fate in the process, such as the carbon absorption/desorption, reveals crucial when aiming at decreasing for example (i) the operating cost of a commercial application (ii) or CO_2 re-emitted in the outlet gaseous phase in the perspective to valorize flue gas through microalgal biomass various applications.

A model allowing predicting CO_2 mass fluxes including effects on growth has been developed and validated. Its use enabled to highlight several aspects, such as the compromise between carbon biofixation and CO_2 removal, or the effect of several typical operating conditions, such as carbon enrichment in the gas and liquid phases (pre-carbonation), the choice of mixing or cultivation technology (airlift, mechanically-stirred PBR, open or covered raceway), or culture systems arrangement (single, series, parallel).

As example of result, a high CO_2 content in the gas phase could lead to a very low CO_2 removal yield: for the airlift technology currently used, only 1% of efficiency could be obtained under normal conditions of use. The major role of photosynthetic growth as a carbon sink to develop processes for flue gas treatment was shown: a single or multi-stages system in parallel reveals interesting to make the best use of the available CO_2; for limited areas, the multi-stages in series proved more attractive.

Keywords: CO_2, photobioreactors, flue gas, optimization, modeling

BIOGRAPHY

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About the author:
Jeremy PRUVOST obtained his PhD Thesis on photobioreactor engineering in 2000 and is currently Full Professor at the University of Nantes. He is Director of the AlgoSolis R&D core-facility (www.algosolis.com) since 2015 (6-10 people), and Director of the GEPEA Laboratory (www.gepea.fr) since 2018 (225 people).
Jeremy PRUVOST is working in bioprocess engineering. His main research activities are related to the valorization of microalgal biomass, through the development and optimization of processes for microalgal culture (photobioreactor engineering). He also participated in more than 30 other projects on microalgae. More infos :

- Publication (open access – HAL database)
- Publications (Google Scholar)
- Research Gate profil

Company profile:
The GEPEA laboratory, with more than 220 people, constitutes one of the most important French research cluster in chemical Engineering. Its originality is to link fundamental and applied research to develop chemical engineering in Environment, Energy, Food industry and Valorization of microalgae and marine products.

CYANOBACTERIA-BASED EXOPOLYMERS: A PHYSIOLOGICAL RESPONSE TO WATER CONTAMINATION BY MICROPLASTICS

Gomes R.[a]

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ABSTRACT

Plastic was developed in the early 20th century and has been found in almost everything since then. In the environment, plastic undergoes various chemical, physical, and biological processes, which causes the release of small particles - microplastics (MPs). The lack of a selective removal method and its wide use makes its accumulation on land and sea inevitable, causing negative impact in the fauna and flora life [1]. Cyanobacteria exposure to stress conditions of growth or external substrates can induce the production of extracellular polymeric substances (EPS). The protective formed EPS can act also as a network that promote the adherence of the MPs to the microalga [2]. Focusing on the potential of cyanobacteria-based EPS to bioremediation [3], the goal of this study is, on one hand evaluating the physiological response of the cyanobacteria *Cyanocohniella calida* to MPs presence and, on the other hand, determinate the phytoremediation potential of this cyanobacteria to water contamination by MPs. For that *C. calida* was placed in water contaminated with MPs at low (0.05 mg/L) and high (5 mg/L) concentration, under static and aeration conditions, and in two distinct growth stages (exponential and stationary). Biochemical parameters such as biomass, pigments and EPS production were assessed. The MPs had impact on the biomass and pigment production in both growth stage. In the stationary growth phase, a higher EPS production, compared with the exponential phase, was observed. The higher concentrations of MPs generate higher EPS production (up to 28% under aeration conditions). The heteroaggregation of MPs to EPS were observed by fluorescent microscopy (Fig. 1). Thus, *C. calida* EPS reveals to be a promise option in phytoremediation area to remove microplastic from contaminated water.

Keywords: Microplastic, Cyanobacteria, Extracellular polymeric substances, Heteroaggregation.

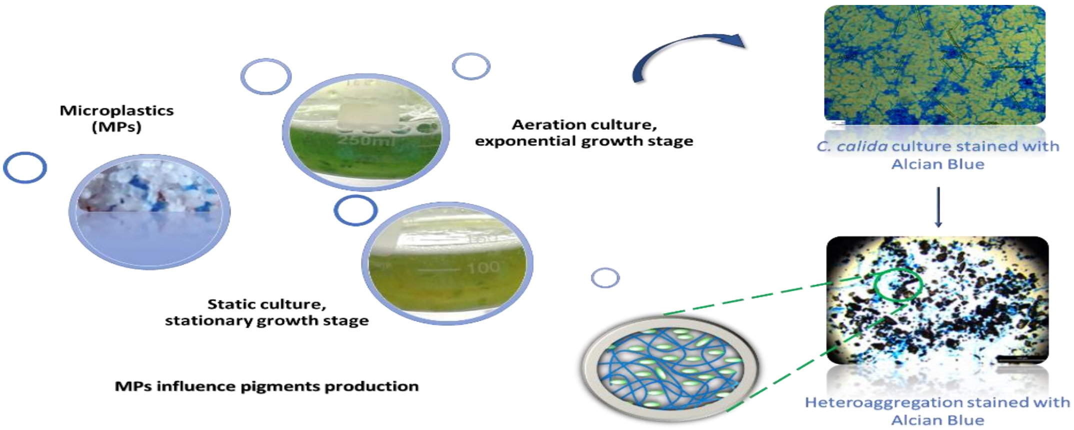

Fig. 1. Effect of microplastics in the different growth phases of *C. calida* under static and aeration conditions.

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ELECTROCOAGULATION-FLOTATION OF MICROALGAE SUSPENSIONS – EFFICIENCY AND PROCESS INTEGRATION

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ABSTRACT

Among applicable technologies for microalgae/bacteria suspension pre-concentration, electrocoagulation-flotation (ECF) has been suggested as an effective alternative to conventional metal-salts addition. In this presentation, a summary of collected experiences - either from a comprehensive analysis of a dataset of 190 records from 29 research papers or from direct lab-scale testing- on using ECF for microalgae pre-concentration will be discussed. Conflicting data are reported about the optimal values of the operational parameters and on how relevant factors affect process performances. By considering only experiments performed under efficient operational conditions (specific electric consumption and metal dosage), a typical range for the main process parameters (applied current density, potential, process time) was retrieved. Ranges for the main KPIs (separation efficiency, specific electric consumption sEEC, specific metal dosage) were computed and distinguished according to anode metal (Al/Fe) and microalgae type (marine/freshwater). Al was found to be more efficient than Fe as for all KPIs. For Al anodes, sEEC was found to be one order of magnitude lower when ECF is applied to marine microalgae. Additional aspects were considered, related to the toxicity of metals and the partitioning of metals between the solid/liquid phase. Photosynthesis inhibition was tested building dose-response curves. The recovery of photosynthesis inhibition was also quantified to evaluate the possibility of reusing/recycling the harvested biomass. Relevant modifications to the medium optical properties suggested a strong effect of iron with implications of dosing metallic flocculants in algae/bacteria processes. Finally, suggestions on improved protocol for ECF testing will be provided for a more effective data interpretation.

Keywords: Electrocoagulation-Flotation, Microalgae/bacteria, process efficiency, inhibition

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EXTRACELLULAR POLYMERIC SUBSTANCES AS POTENTIAL BIOFLOCULLANT FOR MICROPLASTICS WATER REMOVAL

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ABSTRACT

The contamination of aquatic environments by plastic residues caused by disintegration into small fragments of lower molecular weight polymer - microplastics (MPs) has been increasing around the world. The scientific community trends to minimize this impact through the develop of sustainable methodologies for the removal. The flocculation technique comprises a conventional approach to remove the small solids in wastewater treatment with high efficiency. Extracellular polymeric substances (EPS) produced by microbial organisms displayed good flocculants properties due their unique physico-chemical properties [1]. This research work intended to assess the bioflocculant activity of cyanobacteria-based EPS to remove MPs of contaminated water. *Cyanocohniella calida* and *Chroococus submarinus* EPS were extracted and purified from axenic cultures grown under aeration. EPS were characterized in terms of molecular size, morphological and structural properties. Different parameters were evaluated on the bioflocculant activity (Fig. 1): EPS's/MPs ratio (1/2, 1/2.5, 1/5, 1/6.6), 1/10, 1/25, 1/250); pH (3, 3.5, 4, 5, 6, 7); salinity (0, 15, 37 ‰); cation type (Fe^{3+}, Ca^{2+}, Mg^{2+}, K^{+}); MPs type (polystyrene-PS- and polymethylmethacrylate-PMMA) and cation concentration (0, 0.03, 0.05, 0.3, 0.5, 0.72, 0.95%). The EPS bioflocculant activity from cyanobacteria was compared with commercial EPS (alginate and xanthan gum). The highest bioflocculant rate of 79.4% was achieved for the EPS from *C. calida* and of 72.1% for *C. submarinus* to remove the PS-MPs of contaminated water in the presence of the trivalent cation, Fe^{3+} (0.05% (w/w), pH 3.5 at 25±1ºC). Thus, cyanobacteria EPS from *C. calida* and *C. submarinus* reveals to be potential bioflocculant agents suitable to be applied in wastewater treatment.

Keywords: Extracellular polymeric substances; Cyanobacteria; Microplastics; Flocculation; Wastewater treatment.

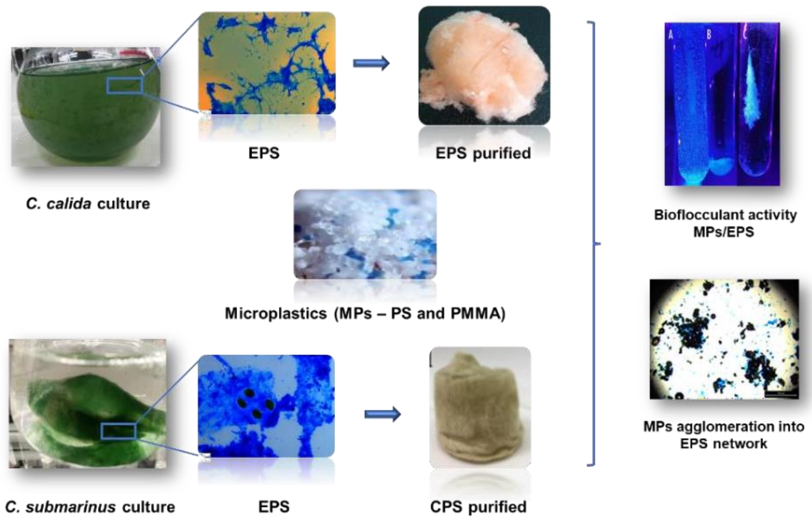

Fig. 1. Extracellular polymeric substances and their potential for aggregation/flocculation of MPs in the network.

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Marisa Faria has a degree in Biochemistry since 2010 and a master’s degree in Applied Biochemistry since 2016 from the University of Madeira. Currently, Marisa Faria has a PhD Scholarship from FCT (BD/6615/2020) is a PhD student in Chemistry at the University of Madeira. Her research focuses on exploring an effective natural source to replace current synthetic materials to minimize the negative impact on the environment, and the development of sustainable, biodegradable, and biocompatible biotechnological methodologies to obtain biomaterials from natural sources with biomedical potential. She is coauthor of twenty-one scientific articles with international recognition and carries supervision functions of curricular internships at the University of Madeira.

The financial support has been provided by the Foundation for Science and Technology with a PhD grant (BD/6615/2020), through CIIMAR (UIDB/04423/2020 + UIDP/04423/2020) and by the European Territorial Cooperation Programme PCT-MAC 2014-2020 through project REBECA-CCT (MAC/1.1.B/269).

NUTRIENT REMOVAL FROM GREENHOUSE DISCHARGE WATER BY *NANNOCHLOROPSIS LIMNETICA*.A WIN-WIN APPROACH

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ABSTRACT

In large parts of Mexico as well as in many other places in the world water is scarce and efficient water use is of utmost importance. This is also very relevant for the Mexican, food producing, greenhouse industry where nutrient rich water often needs to be discharged. In our approach we used the freshwater, n-3 poly unsaturated fatty acids accumulating algae *Nannochloropsis limnetica* to reduce the amounts of nitrogen and phosphorus in the generated discharge water. Simultaneously safe algal biomass, rich in omega-3 fatty acid was produced which was up to 3% (w/w) included in poultry feed in order to generate omega-3 enriched eggs. *N. limnetica* was produced in a tubular vertical photobioreactor which was connected to a greenhouse (Finka location) near Queretaro in central Mexico. In our calculations the production of algae on discharge water can reduce the nitrogen content in the water from 16.6 to 9.5 mM. A further reduction could be achieved when the algal water obtained after centrifugation would be reused for a new batch of algal production. Such an approach, however, should also consider other relevant nutrients such as phosphorus and micronutrients. Furthermore, as algae production strongly varies during seasonal changes, crashes, maintenance, the amount of nutrients that would actually be removed can vary.

Keywords: Nannochloropsis limnetica, n-3 long-chain polyunsaturated fatty acids, nutrient removal, greenhouse discharge water.

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Company info:
Wageningen Research: The Research Institutes of Wageningen University & Research carry out application-oriented and field-based research. They are commissioned by the government, commercial businesses and non-profit organisations. WFBR performs applied research for sustainable innovations in healthy food, fresh food chains and biobased products.
Finka Ahuehuetes S.A. de C.V.Finka is a vegetable production company, producing different species of tomato and cucumber in greenhouses on an area 0f 18,5 Ha. It focusses on high productivities with high sustainability goals.

PIV4ALGAE PROJECT RESULTS: BIOREMEDIATION OF WASTEWATER FROM DIFFERENT SOURCES USING MICROALGAE

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ABSTRACT

The PIV4Algae project aimed at using wastewaters in microalgal cultures, with a drive to reduce biomass production costs while promoting effluents' remediation. Although the use of microalgae for wastewater remediation has been extensively studied, several aspects need improvement. Some characteristics of the effluent (e.g., high colour and turbidity, high heavy metals content, etc.) may limit microalgal growth and, hence, their bioremediation efficiency. Therefore, the PIV4Algae research team has been working in the optimisation of microalgal cultivation using wastewaters from different sources (paper industry effluents, landfill leachates and urban wastewaters) (Porto et al. 2020; Porto et al. 2021). The work carried out demonstrated that it was possible to achieve promising biomass productivities (16-110 mg_{dw} L^{-1} d^{-1}) and remediation efficiencies (up to 90% nitrogen removal and up to 70% phosphorus removal). Moreover, the selection of the adequate microalgal candidate, the modulation of the effluent load and the design of a suitable photobioreactor configuration has significantly improved microalgal growth, offsetting the negative impacts of using real effluents in microalgal cultivation. Additionally, the use of microalgae in the remediation of a primary-treated urban effluent has significantly enhanced the removal of native bacteria, reducing the time required (only six days were needed) to achieve the legal limits for nitrogen and phosphorus discharge in aquatic environments. Besides these promising results, a techno-economic assessment was also carried out to evaluate the viability of a microalgal unit for the treatment of a paper industry effluent and bioenergy production. The study demonstrated that the project is economically viable and contributes to the achievement of seven of the United Nations Sustainable Development Goals (Silva et al. 2021).

Keywords: Biomass production; Effluent treatment; Microalgae; Process optimisation; Sustainability

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Acknowledgements:

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Company profile:

The Faculty of Engineering of the University of Porto (FEUP) is a public institution of higher education, which aims to train world-class engineers driven by an R&D environment of excellence. FEUP has over 8000 students and 442 professors and researchers across 9 departments. The Department of Chemical Engineering houses LEPABE.

PHYTOREMEDIATION CAPABILITY OF THE MARINE MICROALGAENANNOCHLOROPSIS GADITANA TO PHTHALATES

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ABSTRACT

Phthalic acid esters (PAEs) are a main group of plasticizers present in many commercial compounds. Phthalates have been of great concern as they are ubiquitous contaminants in the marine environment and toxic to many marine organisms like phyto or zooplankton, fish and arthropods [1]. To evaluate the physiological response and the phytoremediation potential of *Nannochloropsis gaditan*a to PAEs, this microalga was exposed for 7-days to contaminated water with diethyl phthalate (DEP), di-n-buthyl phthalate (DBP) and di(2-ethylhexyl) phthalate (DEHP). Low (0.1 mg L^{-1}) and high (10 mg L^{-1}) concentrations of each of the pollutant, and of the combination of the three, were used. Physiological parameters and PAEs concentrations were studied after 96 h (acute stress) and 7-days (chronic stress) of exposure. PAEs had impact on *N. gaditan*a, where DBP is the one that most affects the growth and pigment production. In low concentrations PAEs have a growth stimulant effect especially for carotenoids. Thus, for aquaculture purposes the presence of these contaminants should be taken in consideration. To study the applications in phytoremediation, the bioconcentration and biodegradation percentage have been determined [2]. PAEs were extracted from both water and algal phases and analysed by GC-MS. The results show that marine microalgae, as *N. gaditana* can represent a valuable tool for depuration of seawater from PAEs, but can be toxic to species from higher trophic levels, when seen from a trophic web viewpoint

Keywords: Phthalate acid esters; Phytoremediation; Biodegradation, Bioconcentration, GC- MS, *Nannochloropsis gaditana.*

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POSTER RESENTATIONS

Biostimulant, Biocontrol

ALGÆUROPE2021

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ANTIBACTERIAL ACTIVITY IN SEVEN MICROALGAE SPECIES ISOLATED FROM LAGOONS IN WESTERN GREECE

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ABSTRACT

In the present study, the antibacterial activity of seven microalgae species isolated from lagoons in Western Greece was studied: *Amphidiniumcarterae*, *Asteromonas gracilis*, *Tetraselmis* sp. (red var.), *Tetraselmis* sp. (red var., Pappas), *Tetraselmis* sp. (red var., Kotichi), *Tetraselmis* sp. (palmella) and *Tetraselmis marina* (var. Messolonghi). *Chlorella minutissima* was used as a reference species, as it has shown antibacterial properties in earlier studies (Makridis et al., 2006). The fish pathogenic bacteria used were *Vibrio anguillarum*, *Aeromonas hydrophila,* and *Vibrio alginolyticus*.

Microalgae cultures were treated with a mixture of antibiotics (Munro et al., 1995) to kill bacteria present in the cultures. This was verified by plating on tryptic soy agar dishes. The antibiotic was removed after successive dilutions over 3-4 weeks. At the onset of the experiments, small volumes of 5 mL were inoculated with different bacterial pathogens. The growth of the added pathogens was followed 0, 1, 2, 4, and 6 days after inoculations by spreading 10-fold dilutions on TSA agar dishes, and the colonies were counted after incubation for 7 days (Kokou et al., 2011). The experiments were performed both in the presence and absence of light.

All microalgae inhibited the growth of bacteria compared with the control treatments (sterile seawater with Walne Medium), where the numbers of bacteria increased exponentially. In general, the antimicrobial activity was higher in light, especially in the case of *Asteromonas gracilis*, for all three species of pathogenic bacteria. A weaker antimicrobial activity was observed in cultures of *Asteromonas gracilis*, palmella and *Tetraselmis* sp. (red var., Pappas). In cultures of *Amphidinium carterae* no difference between light and dark was observed, although antimicrobial activity was lower than for the other microalgae species. Among the *Tetraselmis* sp. species, *Tetraselmis* sp. (red var.) exhibited the highest antimicrobial activity in light conditions.

The microalgae *Amphidinium carterae, Tetraselmis* sp. (red var.) and *Asteromonas gracilis* reduced dramatically the numbers of *A. hydrophila* compared with sterile seawater added the pathogen after 6 days as shown in Figure 1, where *A. gracilis* reduced the numbers of pathogen cells by more than 99%.

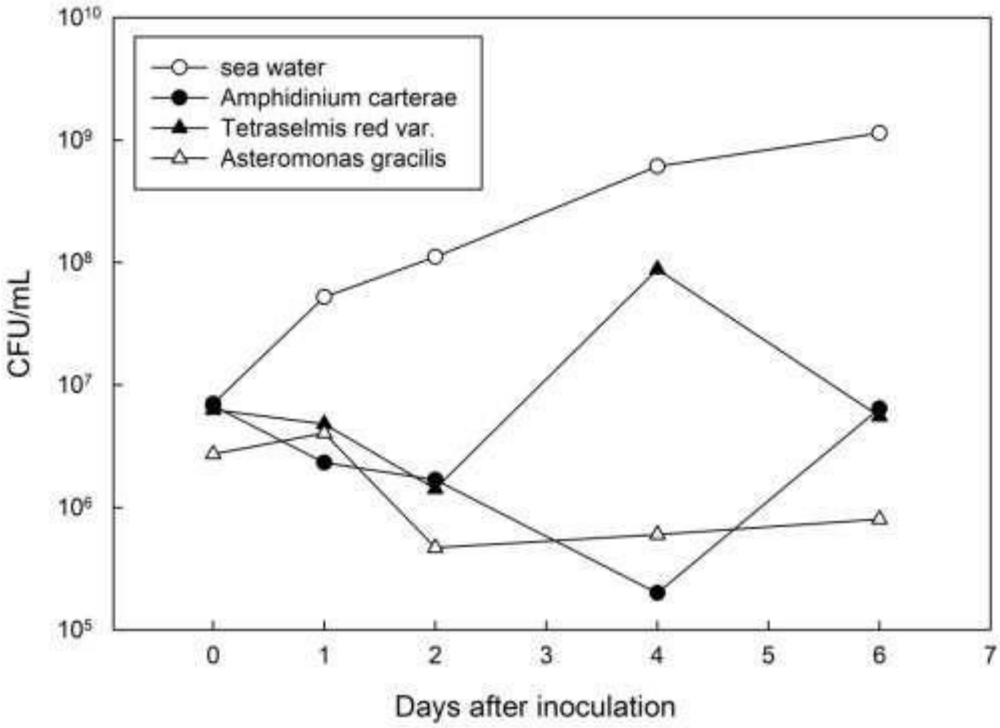

Figure 1. Number of colonies per mL (log scale) of *A. hydrophila* in experiments performed on microalgae *Amphidinium carterae*, *Tetraselmis* sp. (red var.) and *Asteromonas gracilis* compared with sterile seawater, through time in light conditions.

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BIOPESTICIDES BASED ON MICROALGAE VIA CIRCULAR ECONOMY APPROACH – (EMFF- ALGAENAUTS PROJECT)

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ABSTRACT

Guaranteeing a safe and sustainable food production is a major challenge for a growing population worldwide, including Europe.

Microalgae, especially cyanobacteria, can be considered one of the main biological agents for the control of pathogenic fungi and soil-borne diseases in plants because they produce biologically active compounds with antifungal, anti-biotic, toxic activities and against nematodes[1].

During the implementation of the H2020-SABANA Project (Sustainable Algae Biorefinery for Agriculture aNd Aquaculture), microalgae strains (freshwater and seawater) have been evaluated for agricultural purposes. According to the results based on antifungal, antibacterial and biostimulant activity, the most promising strains for developing biopesticides and biofertilisers were chosen. The sustainable production of these strains has been evaluated and proven using seawater and wastewater (urban wastewater and pig manure), and also it has been developed methods to process the biomass into biopesticides extracts at pilot scale.

ALGAENAUTS is an EMMF-BEW-2020 innovation project aims at developing a new line of sustainable and eco-friendly biopesticides line of products for agriculture from microalgae biomass sustainability cultivated. ALGAENAUTS has been designed from a circular economy approach with integral utilization of biomass. Residual biomass from biopesticides production process will be used for biofertilisers formulation.

The ALGAENAUTS innovativeness is a real contribution to a more sustainable and circular economy with almost zero environmental, human and biological impact. Unlike harmful practices, ALGAENAUTS constitutes as an alternative for the new sustainable agricultural sector because it derives from 100% natural environments and products, thus preserving the biological ecosystem.

Keywords:
Circular Economy, Biofertilisers, Biopesticides, Food Production, Biorefinery, Microalgae, Biostimulants, Sustainable Processes, Large Scale, Bioeconomy.

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BIOGRAPHY

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Dr. Joaquín Pozo Dengra studied Chemisrty at the University of Almería where he received his PhD in 2010 in the area of biochemistry and molecular biology. He has been working for 6 years as Head of Biotechnology area and Director of analytical services department at TECNOVA Technology Center. He is expert in project management as principal investigator and project manager. He is partner and R&D Director in Biorizon Biotech. Co-supervisor of 4 Master thesis, co-author in 11 articles published in refereed journals, 1 chapter of book, 5 patents, more than 30 congress presentations and 3 sequences submitted to GenBank.

Company info:
Biorizon Biotech SL is a biotechnological company located in Almería (Spain). Biorizon Biotech is the world's pioneer in developing and producing agricultural products based on microalgae (biostimulants and biofertilisers). Biorizon has successfully marketed microalgae-based products for agriculture in worldwide with subsidiary companies in Ecuador, Chile, Peru, mexico and Morocco.

SEAWEED EXTRACT PRIMING AND INDUCTION OF HOST RESPONSES IN SOLANACEOUS VEGETABLE CROPS

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ABSTRACT

The archaic overuse of chemical pesticides has become a paramount issue of environmental pollution, disproportionately affecting small islands and developing countries. The hazardous use of chemical pesticides pollutes the environment, causes human health problems, puts a strain on the economy and leads to pathogen resistance. Pioneering sustainable agricultural practices through new creative green systems are necessary. Fortuitously, frequent influxes of seaweed biomass and vastly unexplored seaweed species in the Caribbean basin serve as a treasure trove of immense underutilized potential. The current study exemplifies this through the investigation of the effects of seaweed species for their bioefficacy in both greenhouse and field cultivated tomato and sweet pepper crops. A multi-faceted approach revealed the upregulation of key defence and growth signalling pathways and a significant increase in defence enzyme content after treatment with seaweed extracts. Furthermore, microbiome studies revealed the enhancement of certain groups of beneficial microorganisms in crops with the biostimulant treatments. This study clearly highlights the vast potential of seaweed extracts from species as an organic alternative through classical growth assays and in-depth molecular investigation. Overall, the study's findings support the use of seaweeds as viable economic inputs as a means of achieving long-term sustainability and the delicate balance of managing crop production and reducing inorganic chemical dependency, especially in small island countries.

Keywords: seaweed extracts, biostimulant, priming, tropical, tomato, sweet pepper, phytopathogen, sustainable agriculture.

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Biography:

A precocious scientist who aspires to be a "game-changer" in the scientific world with an emphasis on sustainability. "Take something negative and transform it into something positive."

POSTER RESENTATIONS

Cosmetics

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

CHARACTERISATION OF MARINE MICROALGAE STRAINS FROM GREEK TRANSIENT WATERS FOR COSMETIC APPLICATIONS

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ABSTRACT

Microalgae from transient marine ecosystems exhibit high growth potential with promising properties in various sectors of industry (Tzovenis et al., 2009). In this context a sampling in various Greek coastal lagoons is under way and here we report preliminary results for strains characterised and assessed for their potential in the production of extracts for cosmetic use. Several strains were isolated by either plating or multiple-dilutions and single cell isolation methods. The isolates were characterised with morphological, biochemical, biokinetic and molecular methods to identify them and assessed their industrial potential. The strains AthU-Al Dun32 and AthU-Al Mes17 (preserved in the Athens University Algae culture collection of the NKUA) were chosen for more detailed analysis. Several extracts of each strain were prepared with effective cell disruption protocols, using both organic solvents and solvents that can be used for cosmeceutical applications. The antioxidant potential of all extracts was evaluated by Ferric Reducing Antioxidant Power Assay, DPPH radical scavenging method and ABTS-radical scavenging capacity activity. Further characterisation for cosmetic use was carried out with inhibitory assays, against tyrosinase and skin extracellular matrix (ECM) degradation enzymes, to explore for potential specific beneficial activities on skin care. Finally, *in vitro* toxicity assays were employed to assure the safety of these extracts for human cosmetic use. Results highlighted the potential of the microalgal extracts as promising feedstock of value-added extracts for cosmetic applications indicating the need for further investigation on this prospective.

Keywords: microalgae, cosmetics

References: Tzovenis, et al., 2009. Screening for marine nanoplanktic microalgae from Greek coastal lagoons (Ionian Sea) for use in mariculture. Journal of applied phycology, 21(4), pp.457-469.

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SCENEDESMUS RUBESCENS PRODUCTION STRATEGIES FOR ADDED VALUE BIOMASS

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ABSTRACT

Microalgae have attracted interest worldwide due to their potential to be used in several applications as biofuels, pharmaceuticals, feed and cosmetics[1,2]. One of the most high-value products from microalgae are carotenoids[3]. This group of pigments are known for their powerful antioxidant action[4]. Also, these pigments have been claimed to reduce the risk of cardiovascular diseases, macular degeneration and certain cancer types[3]. For these reasons, carotenoids present high commercial value, being of great interest for industrial production. In this way, this work aimed to produce highly concentrated biomass of *Scenedesmus rubescens* (0037SA) in heterotrophic conditions and, in a second stage, to induce carotenoid production.

Media optimization by DoE resulted in a global productivity improvement by 0.92-fold (from 1.94 to 2.79 g L^{-1} day^{-1}) and the specific growth rate by 1.26-fold (from 0.90 day^{-1} to 1.13 day^{-1}). Scale-up was then evaluated in 7L stirred-tank reactor, achieving a maximum biomass concentration of 72 g L^{-1}.

The culture from the 7L fermenter was used to test carotenoid production in heterotrophy also applying a DoE approach. The highest value obtained were 1.05, 0.87 and 0.22 mg g^{-1} of dry weight for lutein, canthaxanthin and astaxanthin, respectively.

Thus, in heterotrophic conditions, a high-density *S. rubescens* culture with a high carotenoid content can be obtained. This conclusion points towards the feasibility of using of this species for industrial high value applications.

Keywords: *Scenedesmus rubescens*, heterotrophic, high-density cultures, DoE, carotenoids,

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Gonçalo Espírito Santo is currently a research fellow at Allmicroalgae since 2021, January. He holds a degree in biology from the Instituto Superior de Agronomia and a master's degree in biotechnology at the Instituto Superior Técnico, where he accomplished his master's thesis at Allmicroalgae. In his thesis work, he acquired experience in auto and heterotrophic microalgae cultivation. Gonçalo believes in microalgae as the key to a more sustainable future, being one of the biggest motivations to work in this area.

POSTER RESENTATIONS

Feed

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

BIOACTIVE MOLECULES FROM MICROALGAE FOR AQUACULTURES

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ABSTRACT

Aquaculture is one of the most important sources of our future food supply and the production of fish in aquaculture systems is increasing rapidly. One of the main challenges aquaculture is facing are diseases caused by pathogenic microorganisms [1]. Microalgae possess the possibility to synthesize different kinds of bioactive molecules that show an antibacterial, antifungal, or biofilm inhibiting effect. Not only the microalgae, but also their surrounding microbiome, composing of a large variety of microorganisms, can influence the synthesis of such biomolecules [2]. The AquaHealth project is investigating microalgal resources and their microbiome for bioactive substances that show an antibacterial, antiviral, or biofilm inhibiting effect with regard to aquaculture systems. The international consortium of the Hamburg University of Technology, Universität Hamburg, Sea & Sun Technology as well as SINTEF AS and Aalborg University will deliver fundamental new insight into microalgae and their associated microbiomes. Integrated 'omics approaches will be applied and further, sequence- and function-based screening in search for new bioactive and prebiotic candidate molecules. The Hamburg University of Technology is involved in the scale up of the cultivation process for selected strains producing promising targets of interests and in the development of appropriate downstream processing strategies. Economic, environmental, and social impacts of novel product candidates will be assessed, as well as routes towards commercialization of identified biomolecules and technologies. Overall, the AquaHealth project will contribute significantly to a positive development of sustainable land-based aquaculture, securing high-quality food supply for the world's growing population while simultaneously reducing environmental impact.

Keywords: microalgae, bioactive molecules, blue biotechnology, aquaculture

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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 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.

EFFECT OF THE LIGHT SPECTRUM AND ITS INCIDENCE TIME ON THE PRODUCTION OF PHYCOCYANIN IN *ARTHROSPIRA PLATENSIS* - PHYCOFARMING

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ABSTRACT

PhycoFarming is a project which aim is to create a new value-added chain for agricultural enterprises, to make the still young industry of algae production in Lower Saxony (Germany) competitive in the long term and to tap the great market potential of algae as a primary producer. The project concentrates on processing the fresh *Arthrospira*- (Spirulina-) biomass produced directly at the production site into a phycocyanin extract. The farmer, who has previously only been a producer of biomass, will be able, at the end of the project, to extract from microalgae biomass optimized for the target substance directly at the production site in a field-tested pilot plant. The residue biomass will be analyzed and tested for its feasibility as a feed supplement for aquaculture and in the pharmaceutical industry.

A vital part of the production of phycocyanin is the quality of light used by *Arthrospira*. Therefore four different strains of *Arthrospira platensis* were cultivated under different light spectra: LED blue (465 nm), LED red (625 nm), white LED 5500K, alone and in different combination. The use of different incidence times (photoperiods) – light phase/blue or red LED phase/dark phase – was evaluated as well.

The results for the production of phycocyanin show that these different light conditions strongly influence the final product/extract.

These results and the knowledge that the value of the extracted phycocyanin is many times higher than the raw material price for dried biomass, indicate that the cultivation of *Arthrospira* offers an innovative, competitive alternative for agricultural enterprises. In this project, research, agriculture and downstream companies work together in an economically sensible way for the first time to produce quality microalgal products in Germany.

Keywords: microalgae, *Arthrospira*, phycocyanin, light spectrum

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Ricardo is since 2018 a Research Associate at the Aquaculture Research Group of the Alfred-Wegner Institute in Bremerhaven, Germany. Currently he is working within the PhycoFarming project where he specializes in the cultivation of *Arthrospira* and the extraction of phycocyanin as a business perspective for agricultural enterprises.

Ricardo has a MSc. in Microbiology from the Portuguese Catholic University (Porto - Portugal). He has experience with open and close cultivation systems, production of valuable compounds by microalgae (i.e. antioxidants, polyunsaturated fatty acids, pigments and antimicrobial molecules), harvesting of biomass and treatment of wastewater using algae-bacteria symbiotic biomass.

Company profile:

The Alfred-Wegener Institute (AWI) is one of the largest Marine and Polar institute in Germany. The Aquaculture Research Group (AQF) has extraordinary technical expertise, along with extremely valuable infrastructures. The subgroup microalgae biotechnology of AQF works on development, improvement and evaluation of microalgae products, participating in national and international projects.

MICROALGAE GROWTH IN WINE AND TOMATO AGRO-INDUSTRIAL WASTE UNDER DARK CONDITIONS

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ABSTRACT

In a world where climate changes are getting progressively worst, there is a need to evolve towards a circular economy more environmentally sustainable, therefor, green alternatives and valorisation of by products is now more important than ever. Annually the agro-food industry produces a large quantity of waste after processing its raw materials, forwarding these residues can be expensive, and even selling to other industries is not profitable [1]. The ProteAlgaFeed project is the use of Portuguese agro-industrial waste resulting from the wine and tomato pulp industries, to produce nutritious substrates for the cultivation of microalgae under heterotrophic conditions, more specifically *Chlorella protothecoides*, to get a quality protein for animal feed. To evaluate the composition in glucose and nitrogen of the syrups, and their consumption during the assays, the two following analyses were performed: Reducing Sugars Measurement with DNS method, and nitrogen measurement. Also, to assess the development of the microalgae two samples were taken daily to test cell growth (through wet and dry weight mass from a sample medium and optical density) and contaminations (through microscopical observation). The optimal culture medium obtained through a Response Surface Methodology (RSM) using a Central Composite Design was the combination of the following substrates: Muscatel wine fermentation lees and tomato sludge from reverse osmosis. This optimum, is the ideal to produce a protein rich of dry biomass up to 33%. The production of microalgae combined with these alternative techniques allows for a significantly reduction in the costs of preparing the culture medium, electricity, and other factors, thus having a great potential to obtain a high protein content product in an ecological and sustainable way.

Keywords: Microalgae, agro-food waste, heterotrophic, feed.

References:

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About the author:

Ana Rita Gonçalves Martins, born on December 1, 1995. Since I was young, I have an interest in topics related to science and biology, and this was reflected in the studies I choose since high school to high education, where I graduated in Biotechnology and currently attending a Master in Biological and Chemical Engineering.

My ideals and expectations of being able to contribute, however small this contribution may be, to make the planet more ecological and sustainable, led me to discover themes for which I am now passionate about such as alternative foods and lifestyles, biofuels among others, hence the enthusiasm I share about microalgae as it covers all these areas.

NUTRITIONAL EFFECTS OF ROTIFERS FED WITH MICROALGAE BLEND ON THE GROWTH AND DEVELOPMENT OF ZEBRAFISH

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ABSTRACT

Zebrafish is a highly used model species in research. However, the wide biological variability in feeding protocols often leads to not reproducible results among different facilities. Generally, zebrafish are fed with a mixture of inert and live feed (artemia or rotifers). Rotifers have a low nutritional profile, and it is often necessary to enrich them to meet the nutritional requirement of zebrafish. Microalgae can be used as a viable source of metabolites mainly when cultivated under stress conditions, namely nutrient depletion, increasing proteins, minerals, and omega-3 fatty acids (EPA and DHA). These metabolites can be essential for the proper development of zebrafish. In this study, four microalgae species have been tested (*Tetraselmis verrucosa f. rubens, Nanofrustulum shiloi, Nannochloropsis oceanica Phaeodactylum tricornutum*) and grown under two concentrations of nitrates (2 and 10 mM) with the aim to enrich the rotifers. Biochemical analyses were carried out to characterize the nutritional value (total lipids, proteins, minerals, and carotenoids) of both microalgae and enriched rotifers. The microalgae enriched rotifers will later be used to feed zebrafish larvae and compared with a standard commercial feed product (without life feed). Results will evaluate larval survival, reproductive performance of adults (hatching rate) and growth (length and weight). The results of this work will provide guidelines for novel zebrafish dietary protocols and standardization of feeds.

Keywords: Microalgae, *Tetraselmis, Nanofrustulum, Nannochloropsis, Phaeodactylum*, Rotifers, Fatty Acids, Zebrafish

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About the author:

Filipa Pinheiro is currently working as a fellow in the scope of the Zebrabloom project (commercial product development formulated with microalgae to enrich live zebrafish prey), whose function is to create microalgae with distinct biochemical profiles that meet the nutritional needs of zebrafish, will also characterize the biochemical profile of microalgae and rotifers. 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:

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.

PHAEODACTYLUM TRICORNUTUM – A POTENTIAL FEED INGREDIENT FOR ATLANTIC SALMON

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ABSTRACT

Microalgae are considered as sustainable alternatives to marine and terrestrial ingredients in feeds of Atlantic salmon, the farming of which is rapidly growing globally with the adoption of land-based farming systems. We have demonstrated the suitability of multiple microalgae in salmon feeds. Here we promote the diatom *Phaeodactylum tricornutum,* a good source of omega-3 fatty acids, especially eicosapentaenoic acid (EPA), as a feed component for salmon. We also examined if fish and feed performance was affected when the cell-wall of the diatom was broken. Two studies were conducted, a 9-week feeding trial under controlled conditions and a 7-month trial in a commercial-like setting in sea cages. In both studies, the control group of fish received low-fishmeal alga-devoid feeds while the test groups received broken or whole alga-incorporated (at 7.5% inclusion) feeds. Growth and feed performance of the different study groups in the short-term controlled study did not vary significantly. However, the specific growth rate, and protein efficiency ratio of the 7.5% whole alga fed group had an edge over the other treatments. As for the commercial-scale trial, the feed conversion ratio of alga-fed fish groups was not on par with that of control fish. Although we did not find any differences in any of the proximate composition values of the whole body, there were differences in the fatty acid profiles of the study groups. EPA content was significantly higher while docosahexaenoic acid was lower in the fillet of fish fed on the alga. Disruption of the cell wall of the diatom did not enhance its nutritive value for Atlantic salmon. *Phaeodactylum tricornutum* thus holds promise as a feed ingredient for the growing salmon farming industry.

Keywords: *Phaeodactylum tricornutum*, Atlantic salmon, growth, fatty acids

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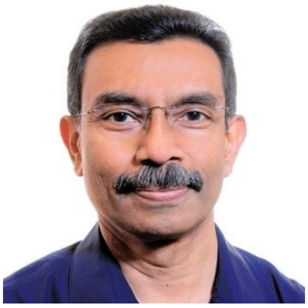
BIOGRAPHY



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POSTER RESENTATIONS

Food

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

ADAPTATION TO SEAWATER OF *ARTHROSPHIRA PLATENSIS* AND *CHLORELLA VULGARIS* NATIVE STRAINS AND OPTIMIZATION OF CULTIVATION AND DOWNSTREAM PROCESS: A CASE STUDY IN GRAN CANARIA

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ABSTRACT

The microalgae *Arthrospira platensis* and *Chlorella vulgaris* are the only two species allowed for human consumption in Europe in unprocessed form. Their biochemical composition can largely improve the nutritional characteristics of conventional food and feed. Gran Canaria (Spain) is a strategic site for microalgae production due to ideal climatological conditions and large seawater availability. The main bottlenecks for the cultivation of freshwater strains are the environmental and economic costs associated with the use of freshwater, fertilizers supplying and management of effluents. The objective of this study was to optimize the cultivation of the native Canarian strains *A. platensis* BEA 1257B and *C. vulgaris* BEA 0441B by adaption to seawater, reduction in nitrogen addition and more efficient CO_2 supply. Cultures productivity and biochemical composition of the biomass cultivated with 10% seawater were similar to those obtained in the freshwater medium for both strains. Nitrogen reduction under a quarter of the concentration in the standard culture mediums did not affect culture productivities and only partially affected biomass composition. The use of a plexiglass hood located at the CO_2 supply site in open raceway culture of *C. vulgaris* increased productivity of 17% at low CO_2 supply flow rates. Harvesting efficiency for *A. platensis* was considerably higher on 15 µm size nylon net with respect to 25 µm stainless-steel net (89.1±5.0% vs 56.3±2.4%, respectively). Rinsing of fresh biomass with freshwater (5:1 water:biomass ratio) and pressing had a relevant effect in ash content reduction (18.7±3.6%, 25.2±3.2% and 32.2±2.6% for rinsing, pressing and both processes together, respectively). Preliminary results suggest that seawater amount in *A. platensis* medium can be further increased (at least up to 50%) while maintaining similar productivities.

Keywords: *Arthrospira* (Spirulina), *Chlorella*, human consumption, cultivation process optimization, seawater, nitrogen reduction

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BIOGRAPHY

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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, Junior Research Scientist at the Biotechnology Department of Instituto Tecnológico de Canarias (https://www.itccanarias.org/web/es/areas/biotecnologia). 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.

ALGAE IN NEW FOOD PRODUCT DEVELOPMENT: MARKET, PRODUCTS AND CLAIMS

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ABSTRACT

The economic value of global algae products market is expected to continue growing at a CAGR of more than 5.1% between 2019-2024 (Mordor Intelligence, 2021). This biobased economy has been supported by US and EU government initiatives to boost algae products production, and by growing consumer awareness to increase consumption of nutritious and protein-rich products with low environmental impact. Algae market can be segmented in food (consumer goods and ingredients), animal and fish feed, fertilizer, industrial application (e.g., pigments, packaging, environmental remediation), biofuel, pharmaceutical, nutraceuticals and cosmetics, and other applications (adapted from Cabane, 2020). Algae products innovation include the use of microalgae (e.g., Chlorella and Spirulina), and macroalgae (e.g., Irish moss, kelp, sea lettuce) as food ingredients, not subject to the Novel Food Reg. (EU) 2015/2283, while several algae strains and products are approved as Novel Food (Reg (EU) 2017/2470). Most popular products include allergen-free products, gluten-free baked goods, egg replacer, vegetarian butter, snacks, burgers, seafood alternatives, nutrient-dense beverages, low saturated fat ice-cream, infant formula and supplements (Siegner, 2017). Algae-based ingredients have been used as food source of protein, polyunsaturated fatty acids (DHA; ALA), macro and micronutrients (Ca, Mg, I, B), vitamins, carotenes, and salt. In the food industry, algae can also be used as stabilizer, thickener and/or gelling agent, food colouring and additive, foaming, and emulsifying agent. Neutral taste and color attributes are obstacles that are being overcome (though deodorizing procedures, new algae strains, and operating systems). Colab4Food will present the market dynamic in Food & Drink Category, in the last three years, as well as the main claims of the new product launches, showing some examples (Mintel GNPD Database, 2021).

Keywords: Allergen free, gluten free, nutrient dense, protein, omega-3 fatty-acids, food & drink categories

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BIOGRAPHY

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About the author:
Denise has an academic background in Food Science and Technology with a BSc from Purdue University (USA) and a MSc from KU Leuven (BE). She has worked in quality assurance and R&D in relation to dairy and soy products, and gum and candy. She also has experience in evaluating bioactivities of plant extracts and *in vitro* testing. She is now a researcher at Colab4Food focusing on research strategy and procuring funding from national (Portuguese) and international sources. She is passionate about the food sector, especially in new product development aimed at promoting health, sustainability, and bringing about an enjoyable experience.

Company profile:
Colab4Food provides consultancy and services in Innovation, Research and Development for agri-food sector, aiming at increasing its competitiveness and resilience through a collaborative strategy between the academic and business entities. It contributes to the development of sustainable processes, innovative solutions and circular economy concepts, supported by a highly qualified team.

BIOCHEMICAL COMPOSITION OF ENIGMATIC GREEN MACROALGAE, *CAULERPA MACRODISCA* DECAISNE (BRYOPSIDALES, CHLOROPHYTA)

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ABSTRACT

Another type of green macroalgae identified as *Caulerpa macrodisca* was newly reported its occurence in Malaysia. Limited information was recorded on the nutritional properties of this particular species. Hence, the present study was conducted to determine the biochemical composition of wild *C. macrodisca* collected from Sabah waters, Malaysia. The biochemical composition analysis involved the determination of proximate composition, fatty acid, amino acid and mineral content, general toxicity level, total phenolic content, antioxidant activity level and caulerpin content. Based on the results, the study reported the nutritional content of *C. macrodisca* with predominantly high amount of protein (20.54%DW) and fibre (21.98%DW). Other than that, high amount of ω6 PUFAs (13.16% of total fatty acids) was detected in this species. Besides, high amount of macrominerals, sodium (6.18 g/100g) and potassium (2.15 g/100g) was also recorded in the species with low Na/K ratio. This species also performed good antioxidant activity level with moderate IC_{50} value of 0.73±0.55 mg/mL. An important bis-indolic alkaloid named caulerpin was also detected in the species with substantial concentration of 5.8±0.12 g/mL that is known to be an antinociceptive and anti-inflammatory agent. Thus, findings from the present study provided baseline nutritional information of another promising *Caulerpa* species, *C. macrodisca* that might be beneficial for the global seaweed industry.

Keywords: Biochemical composition; Nutritional properties; Enigmatic; Green macroalgae; *Caulerpa macrodisca*.

BIOGRAPHY

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About the author:

A person with high curiosity level and keen to explore new things. Completed my BSc. in Molecular Bioscience and Biotechnology in 2012 with a Minor in Business Administration. Completed another degree of MSc. in Aquaculture and currently pursuing another degree for PhD in the same field, soon to finish end of 2021. Experienced with molecular biology, cell and tissue culture previously. Currently, in the making of being an expert for seaweed physical culture. Instead of academic achievement, actively involved in official and freelance emceeing for various events other than volunteering for several community service and youth development.

Company profile:

After recruiting the critical mass of experts, developing infrastructure and conducting a performance audit, Universiti Malaysia Sabah decided to upgrade this research unit in the year 2000 to the status of a fully-fledged Centre of Excellence and named it 'Borneo Marine Research Institute' (BMRI). Since then the Institute has made progressive strides in research and development in various areas within the scope of its theme which is 'Conservation and Sustainable Development of Marine Resources'.

BIOCONVERSION OF SEAWEED BIOMASS INTO PROTEIN AND PREBIOTIC FOOD INGREDIENT TROUGH OF A MICROBIAL ARTIFICIAL CONSORTIUM

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ABSTRACT

The stages of production of proteins of animal origin to satisfy human needs require abundant natural resources and their intake is even associated with health problems. It is necessary to search for appropriate substitutes for animal proteins, proteins healthier, with a lower total cost, and lower resource consumption will guarantee the future of food security.

In this research, we obtained an ingredient rich in proteins and ß-D-glucans that can substitute partially or entirely the protein of animal origin. This product is the result of increasing the nutritional value of brown seaweed *Durvillaea antarctica* through a submerged fermentation process with an artificial consortium of marine fungus and terrestrial fungi. We analyze the nutritional composition (carbohydrates, protein, amino acids, total fibre, caloric energy, fat), heavy metals, toxins and pesticides. Additionally, we analyze the phenolic components, beta-glucans and antioxidant activity. The content of total protein increased ~442% (total protein 52 g per 100 g DM) the total amino acids increased ~245,3% (total amino acids 39 g per 100 g protein DM). This product has all essential amino acids, low content of fatty acids (8,7%) and available carbohydrates (2,9%), good content of total dietary fiber (26.8%), no toxic metabolites or mycotoxin, no heavy metals and pesticides. ß-D-glucans was double compared with *D. antarctica* (21%), and higher antioxidant activity (TEAC of 34 µM/g DM). In conclusion, the bioconversion of *D. antarctica* through a microbial artificial consortium results in a product that has high-quality protein and great nutritional value with potential prebiotic due to the presence of ß-D-glucans. Therefore, this product could be used as a promising strategy to replace animal protein.

Keywords: protein, ß-D-glucans, *Durvillaea antarctica*, microbial consortium, bioconversion

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Ph.D. in Chemical Engineering and Biotechnology, with postgraduate training in Renewable Energies, Environmental Management, and Audits, bachelor in Biology. With over 10 years of professional experience in Ecuador and Chile, working in projects research, in the study of bioproducts (food, energy) from biomass and obtaining new value-added products from new microorganisms. Experience in analytical laboratory techniques such as microbiology, molecular biology, biochemistry, chemistry, biotechnological and separation processes, thoroughness and autonomous work, research capacity, proactivity, experience in student training, and preparation to work in a multidisciplinary team. Committed to the creation, dissemination, and transfer of scientific and technological knowledge.

COMPARISON OF *PARACHLORELLA KESSLERI* CULTIVATION IN DEFINED MEDIUM AND MEDIUM BASED ON SALINE WASTEWATER

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ABSTRACT

Desalination of cheese whey by electrodialysis yields saline wastewater (SWW), which has limited use and represents an environmental and financial burden on the dairy industry. The goal was to test pre-treated SWW, supplemented with source of carbon and nitrogen, as the basis of a culture medium and to prove experimentally the concept that it was a suitable resource for heterotrophic cultivation of the microalgae *Parachlorella kessleri* (formerly known as *Chlorella vulgaris* G11). Optimization of nitrogen source was carried out first followed by experiments with simulated salinity inducing conditions of SWW in defined medium (DM) and shake flasks. These results were adopted in shake flask and bioreactor cultivation using pre-treated SWW and diluted pre-treated SWW. Highest volumetric biomass productivities were obtained in defined medium with using urea and yeast extract (0,075 $g.L^{-1}.h^{-1}$ for urea; 0,083 $g.L^{-1}.h^{-1}$ for yeast extract). Tests with salinity showed better growth parameters for lower concentration of salts, which was confirmed by experiment with diluted SWW. Volumetric biomass productivity decreased by 10 % in diluted SWW medium with urea and by 17 % for YE. In bioreactors, the same trends with productivity decreasing were observed. Although the optimized cultivation of microalgae on alternative medium based on SWW resulted in biomass productivities somewhat lower than those on DM, potentially lower production costs of *Parachlorella* biomass and the meaningful use of SWW are the main outcomes of this work.

Keywords: *Parachlorella kessleri*, saline wastewater, cultivation, bioreactor, biomass productivity

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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.

FRESHWATER CRYPTOPHYTES; A SOURCE OF PHYCOERYTHRIN PIGMENT

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ABSTRACT

Phycobiliproteins are pigments with uses in pharmacology, cosmetics, foods, and as fluorescent probes in biochemistry. Cryptophyte microalgae are one possible source of phycobiliproteins as well as other molecules such as omega-3 fatty acids. The use of cryptophytes in biotechnology is currently very limited and especially the potential of freshwater species is poorly documented. For commercial microalgae production it is important to find the best performing strains in terms of growth and yields of the products of interest. Phycoerythrin is a phycobiliprotein with red colour and strong yellow fluorescence. In this study, we evaluate the growth and phycoerythrin production of eight strains of freshwater cryptophytes belonging to the genus Cryptomonas, comparing them to two marine strains. The strains are grown in batch cultures under standardised conditions. Most of the studied freshwater strains have lower growth rates and all of them have lower biomass yields than the marine strains. However, most of them have much higher cellular phycoerythrin concentrations, which in the case of two strains leads them to a significantly higher overall phycoerythrin yield. There is large variation among cryptophytes in growth rates and phycoerythrin content. Our results suggest that freshwater cryptophytes of the genus Cryptomonas may be better sources of phycoerythrin than the more extensively studied marine strains.

Keywords: microalgae, cryptophyte, phycobiliprotein, phycoerythrin, freshwater

BIOGRAPHY

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Léon Mercier is a doctoral student in the Doctoral Programme in Sustainable Use of Renewable Natural Resources at the University of Helsinki. He is also a student member in the Helsinki Institute of Sustainability Science (HELSUS). In his master's thesis, he investigated how the wastewater from baker's yeast production could be used for the culture of *Euglena*. Currently, his main research aim is to understand what kind of role cryptophyte microalgae could play in the bio- and circular economy. More specifically he is focused on the production of pigments and fatty acids, and the use of side-stream waters by cryptophytes.

IMPROVEMENT OF MICROALGAL BIOMASS USING RANDOM MUTAGENESIS

Lisa M. Schüler

Bombo, G., Duarte, P., Santos, T.F., Maia, I.B., Jacinto, R., Schulze, P.S.C, Pereira, H., Greque de Morais, E., Trovão, M., Machado, A., Carneiro, M., Soares, M., Barros, A., Silva, J., Barreira, L., Varela, J.C.S.

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Allmicroalgae Natural Products S.A., Portugal

ABSTRACT

Microalgae are sustainable feedstocks of proteins, carbohydrates, and lipids rich in high-valuable carotenoids and *n*-3 polyunsaturated fatty acids. To facilitate the application of microalgal biomass to food, feed and pharmaceutical markets, microalgal strains with higher biomass / target compound productivities, improved physiological and/or organoleptic properties are needed. In this study, random mutagenesis was used to improve the biomass of two robust, industrially relevant strains, namely the euryhaline microalga *Tetraselmis striata* CTP4 and the freshwater species *Chlorella vulgaris*. Upon random mutagenesis, combined with selection via fluorescence activated cell sorting, two norflurazon-resistant *T. striata* strains were isolated, displaying carotenoid contents increased by up to 1.5-fold as compared to the wildtype (WT). Comparative gene expression analysis revealed the upregulation of several carotenogenic genes, namely those encoding phytoene synthase, phytoene desaturase, lycopene-*β*-cyclase and *ε*-ring hydroxylase in the novel strains compared to the WT. Interestingly, these mutants also displayed higher contents of EPA (4.4 mg g^{-1} DW) as compared to the WT. A similar approach applied to *C. vulgaris* under heterotrophic growth resulted in chlorophyll-deficient mutants with enhanced protein contents by up to 48.7% of DW. Additionally, the norflurazon-resistant *C. vulgaris* strain showed a deficiency in carotenoid accumulation, producing only the colorless carotenoid phytoene. This study revealed that highthroughput selection procedures combined with random mutagenesis can lead to higher contents for high-value compounds in some microalgae, while improving performance and organoleptic nutritional characteristics in others. Furthermore, the study of randomly mutagenized strains provided further insights into the regulation of metabolic pathways. Thus, this strategy seems to be a powerful tool for microalgal strain improvement, in particular when the required changes in the metabolism are complex and pleiotropic.

Keywords: Microalgae, strain improvement, pigments, polyunsaturated fatty acids, FACS, nutritional applications

Funding: Research contract ALGACO$_2$ and FCT through project UIDB/04326/2020.

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Lisa Schüler holds a BSc and MSc in Life Science, University of Hannover, Germany and a PhD in Biotechnology, University of Algarve, Portugal. She is currently part of the GreenCoLab team as a biotechnologist specialized in algal contaminants and strain improvement. In her previous work in the MarBiotech group (Center of Marine Sciences, Portugal), Lisa studied the improvement of microalgae with the focus on high-value compounds using physiological and genetic approaches. Lisa has published 14 articles and one book chapter in international peer-reviewed journals and participated in several international conferences.

Company profile:

GreenCoLab is a collaborative platform between research institutions (CCMAR, LNEG) and industry (ALLMICROALGAE, NECTON, ALGAPLUS and SPAROS) with the common goal of advancing R&D on the cultivation of algae and algal biomass downstream processing to generate a portfolio of new commercial products and services for the industrial partners.

STARCH RICH CHLORELLA VULGARIS: HIGH−THROUGHPUT SCREENING AND UP−SCALE FOR TAILORED BIOMASS PRODUCTION.

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3 Wageningen University and Research Centre, Netherlands

ABSTRACT

The use of microalgal starch has been studied in biorefinery frameworks to produce bioethanol or bioplastics, however, these products are currently not economically viable. Using starch−rich biomass as an ingredient in food applications is a novel way to create more value while expanding the product portfolio of the microalgal industry. Optimization of starch production in the food−approved species Chlorella vulgaris was the main objective of this study. High−throughput screening of biomass composition in response to multiple stressors was performed with FTIR spectroscopy and nitrogen starvation was identified as an important factor for starch accumulation. Further studies were subsequently performed to assess the role of light distribution, investigating photon supply rates in flat panel photobioreactors. Biomass specific photon supply rate proved to have a strong effect on the accumulation of storage compounds and starch−rich biomass with up to 30% starch was achieved in cultures with low inoculation density (0.1 g L^{-1}) and high irradiation (1800 µmol m^{-2} s^{-1}). A final large scale experiment was performed in 25 L tubular reactors, achieving a maximum of 44% starch in the biomass after 12 hours in nitrogen starved conditions.

Keywords: Chlorella vulgaris, starch, FTIR, photon supply rate, microalgae

BIOGRAPHY

About the author:

With a background in Biological sciences (BSc) and Marine Biology (MSc), Giorgia Carnovale started studying microalgal biotechnology through an Erasmus+ internship at the Plymouth Marine Laboratory, working on photobioreactor design and harvesting techniques. From 2018 she is a PhD student at the Norwegian Institute for Biotechnology and Norwegian University of Life Sciences, investigating the response of microalgae to stress with particular focus on species approved for human consumption and the possible industrial applications of the storage compounds they accumulate.

SYNECHOCYSTIS SALINA: FACTORIAL OPTIMIZATION FOR PIGMENTS (CAROTENOIDS AND PHYCOBILIPROTEIN) PRODUCTION

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ABSTRACT

Cyanobacteria, also known as blue-green microalgae, comprise a large group of ancient photosynthetic microorganism. Recognized by their striking aptitude to produce a wide range of biological compounds (including pigments), cyanobacteria have gained importance within the biotechnological sector. Most cyanobacterial pigments (viz. carotenoids and phycobiliproteins) are indeed attractive colorants for the food, feed and cosmetic industries, since they are non-toxic and non-carcinogenic. In addition, they possess pharmacological bioactivities, so extracts thereof are of interest as functional ingredients to nutraceuticals, food supplements, cosmeceuticals, or for biomedical research 1. The market of natural and functional products is increasing worldwide, so there is a window of opportunity to explore cyanobacterial biomass – and accordingly search for optimal conditions in biosynthesis of such pigments.

The cyanobacterial plasticity is influenced by culture conditions, including light, culture media, temperature, salinity and pH. Scarce studies have to date focused on pigment production of cyanobacterium Synechocistis salina – a species that has proven to possess significant biotechnological application, namely antioxidant capacity, and ability to produce phenolic compounds and pigments 2. Hence, this study aimed to improve pigment production of Synechocystis salina LEGE 06155, following a factorial design based on Box-Behnken approach. Parameters preselected for optimization were temperature (15-25 ºC), pH (6.5-9.5) and NaCl concentration (10-40 g.L-1). The resulting pigment content was analyzed spectrophotometrically, with sampling every two days along 22 days of grow for each experiment set of conditions. Carotenoids and phycobiliproteins contents were set as objective functions for the aforementioned optima.

Keywords: Cyanobacteria, pigments, Box-Behnken model, optimization

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BIOGRAPHY

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About the author:

Joana Assunção is currently developing her PhD program in Chemical and Biological Engineering from University of Porto – more specifically at CIIMAR and LEPABE. Her mainfocus is the optimization process of biomass production and downstream processing (metabolite extraction and recovery) of marine cyanobacteria – particularly Synechocystis salina. She holds a particular interest in pigment extraction methodologies based on green principles (e.g. successive extraction, ultrasound, microwave, etc) applied to the concept of Biorefinery.

POSTER RESENTATIONS

Genetics-Synthetic Biology

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IDENTIFICATION AND CHARACTERIZATION OF THE FCP-FUCOXANTHIN CHLOROPHYLL A/C-BINDING PROTEIN GENES IN THE HAPTOPHYTE *TISOCHRYSIS LUTEA*: INFLUENCE OF NITROGEN AVAILABILITY AND LIGHT INTENSITY ON FCP GENES EXPRESSION AND FUCOXANTHIN SYNTHESIS

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ABSTRACT

Tisochrysis lutea is a well-known haptophyte used in aquaculture (Bendif *et al.*, 2013). The quantity of fucoxanthin present in this alga is of interest to the cosmetic, pharmaceutical and health industry. Fucoxanthin is an orange pigment and one of the major carotenoids contributing approximately 10% assessed total production of carotenoids in nature (Matsuno, 2001; Peng *et al.*, 2011; Kim *et al.*, 2012). Here we present a first description of the fucoxanthin (Fx) and chlorophyll (Chl) a/c-binding protein (FCP) of *Tisochrysis lutea*, performed through blast analysis. The FCP family contains 52 *lhc* genes in T. lutea. We observed differences in *lhc* classification among Chromista. We proposed a classification of the *lhc* genes, composed of *lhfr*, *lhcr* and *lhcx*, with one *lhcf* group specific to diatoms and seaweeds and two *lhcf* groups specific to haptophytes. The FCP pigments binding sites were annotated in *T. lutea* by comparison with the model diatom *Phaeodactylum tricornutum*. The expression of *T. lutea lhc* genes was assessed during turbidostat and chemostat experiments, one with constant light and changing nitrogen states, the second with a 12 h: 12 h sinusoidal photoperiod and changing nitrogen states. RNAseq analysis of the 52 *lhc* sequences revealed a dynamic decrease in the expression of genes with nitrogen depletion. The 12 h: 12 h cycle expression revealed *T. lutea* lhcx2 is only expressed at night.

Keywords: Carotenoids; Fucoxanthin and chlorophyll a/c-binding proteins; Lhcx, Lhcr, Lhcf, RNAseq analysis, *Tisochrysis lutea*, Turbidostat and chemostat continuous culturing.

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Company profile:

Ifremer is the French Institute of Research and Exploitation of the Sea. Recognized around the world as one of the first institutes in marine science and technology, Ifremer conducts research, produces expertise and innovations to protect the ocean, use its resources responsibly, share marine data and offer new services to all stakeholders.

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ALTERNATIVE NITROGEN SOURCES TO CONTROL CONTAMINATION IN MICROALGAE CULTURES

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ABSTRACT

An important constrain in microalgae cultivation is the appearance of biological contaminations that can lead to total culture loss and thus represent a critical obstacle to increasing the process's productivity and sustainability. In this sense, cultures of *Nannochloropsis* sp. were inoculated with a mixed population of common contaminants composed of other algae (e.g., Chrysophyceae), ciliates (e.g., *Paramecium* spp.), amoeboid organisms, bacteria, and fungi. Control cultures were grown in standard Nutribloom® Plus (NB+; Phytobloom by Necton) medium with nitrate as the primary nitrogen source, and the treatments in NB+ where nitrate was replaced by ammonia chloride. ALGEM systems accurately controled and monitored pH, light, and temperature conditions mimicking outdoor Summer conditions in Olhão, Portugal. Culture growth and fast fluorescence transients (OJIP) were monitored, while contaminant detection was improved using a Flowcam (Benchtop B3 series). On the 5th day of cultivation, total culture loss occurred under the control conditions where the contaminant population proliferated. The population was mainly composed of *Spumella*-like nannoflagelates, *Amoeba radiosa*, bacteria, and fungi. In contrast, the cultures grown in ammonia chloride as the sole nitrogen source remained healthy (F_v/F_m = 0.67 ± 0.01) and reached the stationary phase with a 3-fold increase in cell concentration ($2.5\times10^8 \pm 2.1\times10^7$ cell mL^{-1}). Although several contaminants were still present in the treatment condition, these represented an average of 0.7% of total *Nannochloropsis* sp. cells, mainly composed of *Paramecium putrinum* and Chrysophyceae. These results demonstrate that ammonia chloride may be used as a treatment to hinder the detrimental effect of biological predation of *Nannochloropsis* sp. cultures and avoid total culture crash, thus reducing economic loss by increasing the life expectancy of cultures.

Keywords: *Nannochloropsis* sp.; contaminants; ammonia chloride; nitrate; industrial production.

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Carolina Vela Bastos is a recent graduate of the Master of Marine Biology from the University of Algarve. Her thesis focused on optimizing the cultivation protocols of two high-value diatoms, *Skeletonema costatum* and *Chaetoceros calcitrans.* She is currently working as a Research and Development Technician at the company Necton and the University of Algarve. The most recent work is associated with the PROFUTURE project (Boosting the production and use of microalgae protein-rich ingredients in food and feed), where different strategies to prevent, control, and eliminate contaminants in microalgae cultivations is in development.

Company profile:

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 m3 photobioreactors and a raceway of 200 m3, where it cultivates species including Nannochloropsis, Tisochrysis, Phaeodactylum, Tetraselmis and Skeletonema.

MAJOR IMPROVEMENTS IN THE DESIGN OF RACEWAY PHOTOBIOREACTORS USING COMPUTATIONAL FLUID DYNAMICS (CFD)

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ABSTRACT

The Raceway Photobioreactors have presented in recent years a low level of innovation, these production platforms have a great importance implemented worldwide and a high potential for improvement, some improvements will be presented in this work. Its geometry has been standardized to achieve a design guide for its rapid implementation focusing on its length and width to maintain effective fluid dynamic conditions for a correct transit of the cell path, the air bubble pit has been redesigned to work in conjunction with the paddlewheel. The design of the baffles has been standardized in its curves as the first vertical mixing structure by increasing the tangential acceleration to lift the cells that travel through the deepest zone of the channel. As a second vertical mixing structure, a series of blades installed in the deepest area that generate large diameter vortices has been studied in order to mix cells that have had little light capture and a series of redesigned blades installed in their most superficial area to multiply the number of small diameter vortices in order to increase the speed of rotation of cells to achieve a frequency of integration of light. The Rubio Camacho et al., 2003 model has been used to calculate the effectiveness of the new improvements.

Keywords: microalgae, photobioreactor, raceway, baffles, blades.

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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 10 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 in the research and development industry.

OPTIMIZATION OF CULTIVATION TO INCREASE PRODUCTIVITY OF A *GAMBIERDISCUS EXCENTRICUS* STRAIN ISOLATED IN THE CANARY ISLANDS

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ABSTRACT

Toxic marine dinoflagellates of the genus *Gambierdiscus* are the causative agents of Ciguatera Fish Poisoning (CFP), the most prevalent algal-borne illness worldwide. Ciguatera is caused by consumption of fish contaminated by ciguatoxins (CTXs), which are biotoxins mainly produced by many *Gambierdiscus* species. Cultivation of *Gambierdiscus* spp. is challenging due to its benthic behaviour, slow cell growth rate, and releasing of toxins in the medium. So far, cultivation is carried out at laboratory scale under strictly controlled conditions, while large-scale traditional systems for microalgae cultivation result to be unsuccessful, which limit to achieve even biomass quantities necessary to characterize the microalgal toxic profile. This study aimed at optimizing the cultivation of a native Canarian strain of *Gambierdiscus excentricus* (OCH45) at laboratory to pilot-scale. The microalga was cultured in a Meprolim® polypropylene tray of 2062 cm^2 surface with final volume of 3 L using a modified f/2 medium and the resulting productivities were compared with those obtained in cell flasks. Cells were incubated at 25 °C under fluorescent cool-white lights with a photon flux density of 80 µmol photons m^{-2} s^{-1} with light-dark cycle of 12:12 h. Stable productivities of 60 ± 8.3 cell ml^{-1} day $^{-1}$ where achieved in tray. These results point out that this cultivation system allows to reproducibly reach quantities of *G. excentricus* biomass required for toxic profile analysis (i.e., 50-100 ×10^6 cells) in limited amount of time (approximately 3 months). A stackable and readily scalable polymethyl methacrylate (PMMA) prototype with surface of 2233 cm^2 was also developed, which according to preliminary studies, allows to further enhance *G. excentricus* areal productivity.

Keywords: *Gambierdiscus*, benthic, toxic, cultivation, tray, productivity

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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 areas of Renewable Energies, Desalination and Water Treatment, Environmental Sciences, Medical Engineering and Biotechnology.

POSTER RESENTATIONS

Physiology

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ASSESSMENT OF EFFECTS OF CERIUM OXIDE NANOPARTICLES ON CHLORELLA WITH REFERENCE TO HIGH LIGHT STRESS

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ABSTRACT

Widespread use of cerium oxide nanoparticles (CeO_2-NPs) is likely to result in their escape to aquatic environment. The present study focuses on the effects of CeO_2-NPs (size ~25 nm, triangle and square shaped) on Chlorella under high light stress. While high light reduced the cellular viability of Chlorella, CeO_2-NPs did not have any effect on cellular viability under either light condition, suggesting their non-toxicity. An enhancement in SPY-LHPox fluorescence in high light exposed cells revealed that high light promoted formation of organic hydroperoxides (ROOH). However, in the presence of CeO_2-NPs, ROOH levels in cells remained unaltered under either light condition, suggesting that CeO_2-NPs do not affect the formation of ROOH. High light decreased the total chlorophyll and carotenoid content in the cells, which was further enhanced by CeO_2-NPs in a concentration-dependent manner. Contrary to high light, CeO_2-NPs did not affect total chlorophyll and carotenoid content in cells under low light. In particular, CeO_2-NPs induced a decline in carotenoid pigments neoxanthin, antheraxanthin, zeaxanthin, lutein, and β-carotene in cells. These results clearly suggest the role of CeO_2-NPs in causing damage to the photosynthetic pigments under high light. We propose that ROOH produced in cells under high light are oxidised/reduced by CeO_2-NPs to peroxyl/alkoxyl radicals, which oxidize the photosynthetic pigments.

Keywords: Cerium, Chlorella, Nanoparticles

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I did my Ph.D. in the field of environmental nanobiotechnology from University of Delhi wherein I worked on the mechanisms of generation of nanoparticles by plants. Later, during my post doc at University of Seoul, Korea, I worked on the interaction between nanoparticles and plants. I'm a recipient of Marie Curie individual fellowship at Palacký University and I'm trying to understand the effects of different speciation states of cerium oxide nanoparticles on microalgae under high light stress.

CHALLENGES AND OPPORTUNITIES OF CO_2 REMEDIATION THROUGH ARTHROSPIRA PLATENSIS CULTIVATION

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ABSTRACT

The cyanobacteria *Arthrospira platensis* has proven to be a rich human nutrient source for centuries in Africa and South America. Nowadays, its original nutritional properties and phycobiliproteins profile make it the first photoautotrophic unicellular organism cultivated worldwide, mainly as bio-based blue pigment and food supplement for agro, cosmetic [1] and nutraceutical industries [2] [3]. The Spirulina production is generally estimated around 10,000 tons per year worldwide, mainly from open raceways. Salts of carbonate or bicarbonate are used as mineral carbon sources, as they provide a buffer ideal for Spirulina growth. In our work, we compared different carbon supply strategies. The ultimate goal being to supply carbon as gaseous CO_2, in order to improve the financial equilibrium of Spirulina cultivation and decrease its carbon emission [4]. After a screening of different strains, the SAG-21 was selected as it reached the best growth rate (0.66 d^{-1}) and phycobiliproteines content (183 mg/g) in the reference conditions used (35°C, Zarrouk's medium [5], 200 µE/m²/s 20/24h in 200 ml flasks). Further experiments were performed with an initial medium without carbonate salts but with semi-continuous injections of an enriched gas phase with 4% CO_2 v/v. These experiments demonstrated the possibility to substitute the traditional carbon source with CO_2, by adapting the initial pH. Thus, an initial pH of 13 enabled to absorb about 1 g of carbon/L and to reach a final pH of about 8 suitable for *A. platensis*. These first results are used to manage an open raceway equipped with an airlift vacuum column able to dissolve the CO_2 required by the biomass growth.

Keywords: *Arthrospira platensis* ; *Spirulina* ; Carbon dioxide; CO_2 capture and utilization; mass balance

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Company profile:

CEA is a leading Research and Technology Organization in the field of energy. MicroAlgae Processes (MAP) Platform can produce tailored algal biomass using different cultivation/harvesting technologies, from lab to semi-industrial scale. The Mechanics, modeling and clean process design laboratory (M2P2) is an academic laboratory of Aix-Marseille University. During the last decade, it has developed an expertise on supercritical fluids applied for algae biorefinery.

CONTINUOUS EXTRACTION OF DITERPENOIDS FROM GM MICROALGAE UNDER INDUSTRIALLY RELEVANT CONDITIONS

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ABSTRACT

The MERIT project[1] is supported by the ERA-NET-Biotechnology Program, were Wageningen University/AlgaePARC tested the feasibility and scalability of extracting diterpenoids (DTs: sclareol (Sc) and manoyl oxide (MO)) from the GM microalgal species *Chlamydomonas reinharditii* and *Phaeodactylum tricornutum*. Here, we present our results on this topic. Continuous cultivations were done in 0.2 to 4L production vessels (> 8 different experiments), showing that product formation (DTs) is coupled to growth, i.e. when biomass production is at its highest. Next, we gathered evidence to elucidate the extraction mechanism: the product is transported into the culture broth and sequentially transferred into a dodecane overlay (in-situ extraction); also, dodecane enters the cells, increasing the extraction yields (milking). However, the constant presence of dodecane causes cultures to collapse after 7-10 days. Nevertheless, the in-situ extraction was optimized yielding 18.6 and 5.9 mg/L/d, Sc and MO, respectively; conditions: photo-chemostat at $0.25d^{-1}$, 25°C, pH 7.5 (addition of HCL), irradiance 1835µE, 12:12 D:N, 0.05 L/min CO_2; no aeration to avoid emulsions, 5% dodecane overlay. Alternatively, we tested extraction with hollow fibers (pertraction) of two types: hydrophilic and -phobic membranes. Both showed positive results, with the hydrophilic system showing higher yields for both DTs: 41.2 and 16.3 mg/L/d (Sc) versus 17.7 and 13.7 mg/L/d (MO); these systems were robust and have the potential to be scalable. All in all, we optimized both in-situ and in-stream (pertraction) simultaneous extraction of 2 DTs under industrially relevant (simulated outdoors conditions) set-ups. The conclusions show the potential of these systems for heterologous production and expand the possibilities to scale a difficult process.

Keywords: GM microalgae, diterpenoid production, in-situ extraction, continuous cultivation, simulated outdoor conditions

References:[1]http://merit-project.net/home.php

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Rafael García Cubero was born in Seville (Spain), in 1980. He obtained his BSc in Biology, Master in Molecular Genetic and Biotechnology and PhD in Biology in the University of Seville. After, he has been working in different enterprises developing products from microalgae. His career also comprises a post-doctoral research positions in Wageningen University and University of Almeria. Currently, he holds a position as a research assistant in AlgaePARC (Bioprocess Engineering in Wageningen University) where he helps in different research projects and supervision of students. These steps have allowed him to publish his results in different peer-reviewed journals.

DNA BARCODING OF ULVOPHYCEAE FROM THE CENTRAL MEDITERRANEAN

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ABSTRACT

Recent genetic studies have shown that many algal species hitherto described morphologically possess cryptic diversity. In fact, these have resulted in the discovery of new species, nomenclatorial changes and country or region records of previously overlooked species. Malta's macroalgal species checklist, based on morphological data spanning the last 23 years, contains 339 species, of which 63 belong to the Chlorophyta: 62 Ulvophyceae and 1 Palmophyllophyceae. This study aims to contribute to genetic data from the central Mediterranean while enhancing knowledge of the region's macroalgal biodiversity. The first genetic studies on red and brown algae from the Maltese islands have resulted in five new records (3 Phaeophyceae, 1 Schizocladiophyceae and 1 Florideophyceae). Our present study aims to barcode the green algae from 6 sites around Malta, Gozo and Comino. Samples of the substratum, including dead shell fragments and stones, were processed via the germling emergence method, with subsequent culturing *in vitro*. The algal germlings were studied via a Nikon Eclipse Ti-S inverted microscope connected to a Nikon Digital DS-Fi 1 camera. Sections were cut from the apical, middle and basal regions of the thallus. The present work provides new records of Ulvales (Ulvophyceae) for this region. DNA extraction and barcoding of the *tuf*A, *rbc*L-3P and ITS1+ 5.8S +ITS2 biomarkers confirmed the presence of *Ulva, Ulvella* and *Cladophora* spp., which included *Ulvella leptochaete* and *Cladophora albida*. The heterokont benthic multicellular algae *Ulva torta*, *Ulva californica*, *Ulvella endostraca* and *Blastophysa rhizopus* were recorded from the Maltese islands for the first time. *Ulva californica,* often misidentified as *Ulva lactuca*, is an alien species in the Mediterranean Sea that had only been reported previously from the North Adriatic Sea.

Keywords: Chlorophyta, Ulvophyceae, Mediterranean Sea, *Ulva, Ulvella, Cladophora*

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Angela G. Bartolo is a PhD student at the University of Aberdeen in the division of Marine Biology. Her research addresses DNA barcoding in Malta. She holds a dual Master's degree MSc/MS from the University of Malta and James Madison University (Virginia) where she focused her research on sea water quality through the use of ecological indicators. Angela's work experience at the Environment and Resources Authority for the past seven years involves working on EU Directives, including the Marine Strategy Framework Directive, Water Framework Directive and Habitats Directive.

EFFECT OF A STRONG HETEROLOGOUS ELECTRON SINK ON THE PHOTOSYNTHETIC APPARATUS IN SYNECHOCYSTIS SP. PCC 6803

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ABSTRACT

Flavodiiron proteins, terminal oxidases, and the cyclic electron transfer (CET) around photosystem I (PSI) mediated by, e.g. NADPH-dehydrogenase-like complex (NDH-1), protect the photosystems from damage caused by an excess of electrons. These alternative electron pathways are in a tightly regulated interplay. Recently, proof-of-concept studies have demonstrated the biotechnological potential of using photosynthetic microbes in whole-cell biotransformation platforms where living cells convert exogenous substrates into desired products using heterologous enzymes and light-driven reductants. Although the effect of heterologous oxidoreductases on photosynthetic yield has been recently reported (Santos-Merino et al. 2021; Assil-Companioni et al. 2020), the detailed feedback effect of the heterologous electron sinks on photosynthetic apparatus is poorly understood. In our study, we used the NADPH dependant ene-reductase YqjM, expressed in *Synechocystis* sp. PCC 6803. We studied the functional status of different photosynthetic apparatus components under photoautotrophic conditions with the focus on the redox state of P700, Fd, PC, as well as the chlorophyll *a* fluorescence and gas exchange. Our results show that YqjM is highly efficient and out-competes CET and the flavodiiron proteins for electrons under the studied conditions by continuously utilising the photosynthetically produced reducing elements needed for its function. Additionally, in the presence of YqjM, far-red oxidation of PSI is enhanced, suggesting an increased electron usage downstream of PSI. These results support the notion that YqjM is a strong electron sink operating downstream of PSI and suggest new strategies for biotechnological applications using biotransformation under photoautotrophic conditions.

Keywords: photosynthesis, cyanobacteria, biocatalysis, flavodiiron proteins, regulation

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Michal Hubacek is a first-year PhD student at the University of Turku, Finland, in the group of prof. Yagut Allahverdiyeva-Rinne. In his research, he focuses on understanding the regulatory mechanisms of photosynthesis in cyanobacteria. His primary interest is modulating photosynthetic reactions to increase electron flow to a targeted pathway or heterologous enzyme.

EFFECT OF BACTERIA-MICROALGA SYMBIOSIS ON *CHLORELLA VULGARIS* BIOMASS

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ABSTRACT

Major biotechnological applications of *Chlorella vulgaris* produced at industrial scale include human nutrition or supplementation to support the increasing demand for non-animal biomass. Microalgae grown in industrial autotrophic production systems has an associated microbiome, mainly composed of bacteria. The microalga-bacteria symbiosis can provide growth- and health-promoting molecules, adding value to the produced biomass and being essential for the maintenance of the industrial-scale production. The main goals of the current project were to monitor, isolate and identify the bacterial communities able to promote growth and enhance *Chlorella vulgaris* biomass cultivated industrially. The microalga production of *C. vulgaris* was conducted in 100 m^3-tubular photobioreactors at Allmicroalgae facilities in Pataias, Portugal, during the autumn/winter seasons and under semi-continuous growth mode. Cultures were followed throughout twenty days from inoculation until harvesting, with sampling every second day. Biomass productivity and biochemical profile (i.e., ash, proteins, minerals, and lipids contents, including the fatty acids profile) were evaluated. From the collected samples, 120 isolates were identified by genetic tools using universal 16S rDNA primers. Bacterial growth was measured during the sampling period by colony-forming units counts and further isolated in plate count agar (PCA). Moreover, the effects of bacterial communities for the enhancement of biomass quality and industrial production are being evaluated.

Keywords: Microalgae; Holobiont; Microbiome; Bacteria; Symbiosis; Biotechnology.

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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 strains of microalgae as a researcher at CCMAR/University of Algarve - MarBiotech Research group. She was awarded a PhD fellowship — Foundation for Science & Technology (FCT). In her PhD project Tamára is researching innovative ways to enhance industrial microalgal biomass production by using metagenomics. Currently, she has co-authored 23 papers published in international peer-reviewed journals and presented 7 poster presentations in international meetings.

Company profile:

The Centre for Marine Sciences (CCMAR) is a multidisciplinary, non-profit research organization for 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 5 years had 15 patents approved and established 2 spin-off companies.

ENERGETIC POTENTIAL OF LIGNOCELLULOSIC MATERIALS: WASTE OR TREASURE? TOWARD THE PROMOTION OF GREEN ALTERNATIVES TO FOSSIL FUEL

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ABSTRACT

Depletion of petroleum reserves, geopolitical tensions and the global climate change have recently accelerated the search for sustainable alternatives to traditional fuels. Lignocellulosic biomasses (either agro-forest and food processing wastes or dedicated energy crops) represent a promising and cheap renewable source of energy, though their full exploitation is naturally limited by plant cell wall recalcitrance to conversion into sugar monomers. We propose the use of sugars derived from lignocellulose to feed microalgae mixotrophic growth to obtain oil to be converted into biofuel. We have evaluated the use of commercial enzymes and of white rot fungi, well known to efficiently degrade lignocellulose, to obtain monosaccharides that have been subsequently employed to feed *Chlorella vulgaris*. Significant algal biomass production was obtained with digestates from barley straw and citrus processing wastes. The ability of *C. vulgaris* to use the most abundant monosaccharides released from these biomasses, and their impact on photosynthesis was evaluated. Moreover, we have characterized the secretome of white rot fungi grown in the presence of different biomasses to identify enzymes useful for improving hydrolysis efficiency of the overall process without release any toxic compounds. Our results suggest that different biomasses can be used to support algal growth, providing a sustainable way to valorize agro-food wastes.

Keywords: Biofuel, microalgae, alternative energies, lignocellulosic biomass

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Francesca Angelini is a Ph. student, attending last year of Ph.D. in Cell and Developmental Biology at La Sapienza University. Her doctoral project relies on the evaluation of lignocellulosic waste biomass potential in feeding microalgae to produce biofuel. Her gained experience in lab is mostly related to chromatographic technics and algae biometrics analysis. In 2017, she graduated with a dissertation on "the role and homeostasis of cell wall damage-associated molecular patterns in *Arabidopsis thaliana*" with final mark 110/110 *cum laude*.

Company profile:

Laboratory of Physiology and Plant Biochemistry, Dept. of Biology and Biotechnology "Charles Darwin"- "LA Sapienza" University; Piazzale Aldo Moro, 5, 00185 Roma RM. #171 in QS Global World Rankings 2022.

IMPORTANCE OF PHENOTYPIC CHARACTERIZATION TO IMPROVE BIOPRODUCT PRODUCTION IN MICROALGAE

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ABSTRACT

There has been a growing interest in microalgae for sustainable production of bioproducts, such as lipids. The phenotypic characterization of mutant cells lines as well as of their corresponding parental cells is determinant to evaluate their ability to produce bioproducts of interest and to enhance yields. Mutations in RuBisCO, the most abundant plant enzyme and responsible for CO2 fixation in photosynthesis, profoundly affects cell metabolism. In the present study, we perform a comparative analysis using two lines of *Chlamydomonas reinhardtii*, one mutated in RuBisCO and a non-mutated control. In autotrophy the mutants display great difficulty to grow, overcome, although slowly, by exogenous carbon source (acetate), resulting in less dry biomass than the control strain. In mixotrophic conditions the mutant showed lower chlorophyl content, lower O_2 evolution rates and deficiency in photosystem II operation, compared to the control line. Phenomenological energy fluxes analyses of the fluorometric parameters, revealed that mutated cells exhibited a higher energy dissipation (DIo/CS) and lower photochemical efficiency detected by low number of reaction centers (RC/CS), significant decreases in the amount of energy absorbed (ABS/CS), entrapped energy (TRo/CS) and electron transport flux per cross section (ETo/CS). Fatty acid composition and contents analysis, also varied between the two lines. The high ratio between the total fluorescence with Nile Red and the chlorophyll *a* fluorescence indicates that the mutant cells accumulate more neutral lipids than the control. This enhanced storage lipid production is corroborated by the up-regulation of key lipid metabolism genes. Our results suggest that RuBisCO mutants, under mixotrophic growth conditions, have a decreased investment in the photosynthetic machinery and their metabolism is redirected towards the production of storage lipids with potential biotechnological applications.

Keywords: Phenotypic characterization; microalgae; photosynthesis; lipids production.

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Biography: Maria da Glória Esquível Finished the PhD 1995. She is Assistent Professor in Instituto Superior de Agronomia, University of Lisbon. Her scientific career has always been combined with training. She is a member of the Centre Linking Landscape, Environment, Agriculture and Food (LEAF); Develops her research in the areas of Biological Sciences with an emphasis on metabolic engineering with microalgae, abiotic stress, RuBisCO CO2 fixation. She has 28 papers in international scientific periodicals with referees. She has more than 65 co-authors in her international publications and book chapters.

Company profile:

LINKING LANDSCAPE, ENVIRONMENT, AGRICULTURE AND FOOD – is a research centre funded by national funds through FCT – Fundação para a Ciência e Tecnologia. LEAF is positioned to conduct studies on the whole agro-food chain, dealing with main issues on a variety of scales, from cells and microorganisms to landscape design.

IMPROVED METHODS FOR PIGMENTS EXTRACTION FROM *ARTHROSPIRA PLATENSIS*

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ABSTRACT

The clinical studies show that pigments work as antioxidants and has anti-inflammatory, cholesterol reducing and other health-improving properties, therefore pigments have high commercial value. A.platensis ir well known food and feed supplement. Strain, growth parameters and drying regime can affect pigment content in algae. So it is important to develop methods for producing spirulina with high pigment content. However to purify pigments for accurate qualitative and quantitative analysis there is no universal method for all algae and literature data are contradictory. Therefore the aim of this study was to compare several methods to find most appropriate for extracting pigments from *A.platensis*. Freezing and Ultrasonication pre-treatment was used. The combination of both was more effective. Ultrasonic probe with 26 Hz was distupting cells more effective than 40 Hz ultrasonic bath. For the extraction of chlorophyll *a* and carotenoids (fat-soluble compounds) the most appropriate solvent was 100% acetone, comparing to 80% acetone in water solution. For the extraction of phycocyanin (water soluble compound) the most appropriate solvent is a 1,5% solution of CaCl2 in water, which is 5 and 57 times more effective than phosphate buffered saline or distilled water, respectively.

Keywords: Extraction, pigment, Spirulina, Arthrospira

Acknowledgement:

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I am chemical engineer. My PhD was on developing new implant material for bone replacement. For more than 10 years I was running practical works for students in course “Unit operations in chemical engineering”, later also in “Mass transfer”. For last 5 years I work on biotechnology, on spirulina indoors cultivation technology development.

IMPROVED PULSE PROTOCOL FOR THE SUSTAINABLE H_2 PHOTOPRODUCTION IN GREEN ALGAE

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ABSTRACT

Hydrogen, if produced from renewable sources, is considered the cleanest energy carrier for the future with significant demands from the global fuel market (1). The photobiological water splitting process, which is inherent to many species of cyanobacteria and green algae, is the most promising and environmentally friendly approach for industrial H_2 gas generation (2). Our previous studies revealed that H_2 production in green algae could be sustained by a train of strong white light pulses interrupted by longer dark phases (pulse protocol), in which each light pulse was short enough to minimize the flow of photosynthetic reductants to CO_2 fixation and to limit O_2 accumulation produced by photosystem II (3). Although the process has a theoretical potential to displace fossil fuels, photosynthetic H_2 production in its current state is not yet efficient enough for industrial applications. Thus, further research on increasing the algal capacity to produce hydrogen is indispensable. In the present study, we further optimize the pulse protocol for the sustainable H_2 photoproduction in photomixotrophic algal cultures. We show that a combination of strong light pulses with the continuous low background illumination gives almost 20-times improvement in the H_2 photoproduction yields upon decreasing the H_2 partial pressure and preventing the excess of intracellular O_2 accumulation.

The work was supported by the Kone Foundation, EU FET Open project FuturoLEAF and NordForsk Nordic Center of Excellence “NordAqua”.

Keywords: Green algae, H_2 production, pulse protocol, low background illumination

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Project title: “Regulation of photosynthesis under different environmental conditions and the biodiversity of Nordic microalgae for the production of biofuels and high-value products”
Project leaders: Prof. Yagut Allahverdiyeva-Rinne and Dr. Sergey Kosourov
Degree title: Doctor of Philosophy (2014-18)
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Thesis title: “Carotenoids affect the structure and functions of the cyanobacterial photosynthetic complexes”
Supervisors: Dr. Zoltan Gombos and Dr. Tunde Toth

INFLUENCE OF LIGHT QUALITY ON THE PRODUCTION OF PHYCOERYTHRIN IN CRYPTOPHYTE ALGAE

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ABSTRACT

In recent years, commercial and scientific attention has boosted the interest in natural products from microalgae. Cryptophyte algae are one of the major groups of microlage and important primary producers in both freshwater and marine habitats. However, their potential contribution in biotechnology has remained untapped. The aim of this study was to optimize the biomass yield and production of phycoerythrin (PE) pigment of cryptophytes under different light conditions, aimed at introducing cryptophytes as a promising alga for industries. PE is present in red algae and cryptophytes as an accessory to the main chlorophyll pigments responsible for photosynthesis. Algal phycoerythrin are applied as food colorant and additives in the food industry, natural dye in cosmeceuticals, and fluorescent probes in biomedical science. For this study, four freshwater cryptophyte Cryptomonas strains (*C. ozolinii*, *C. ovata*, *C. curvata*, and *C*. sp. (CPCC 336)) and one marine strain (*Rhodomonas salina*) were cultivated under three LEDs with different intensities and wavelengths including white, blue and green lights. Species-dependent responses to changes in light conditions were observed such that light changes significantly affected the growth rate, biomass productivity, and the production of PE. Furthermore, the highest growth rate and biomass productivity were under white LED. Nevertheless, optimum light condition to extract the maximum phycoerythrin content was different for each strain.

Keywords: Cryptophytes, LED lights, Phycoerythrin,

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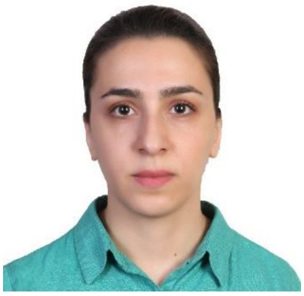

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OPTIMIZING HIGH VALUE LIPID PRODUCTION OF MARINE BENTHIC DIATOMS SPECIES FROM NORTH-EAST ATLANTIC MUDFLATS USING POROUS SUBSTRATE PHOTOBIOREACTOR

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ABSTRACT

Marine benthic diatoms isolated from the mudflats French Atlantic coast have promising lipid value for biotechnology. Screening and mass production trials reveal the preference of some species for minimally disturbed culture conditions over traditional microalgal suspension systems. This project aims to optimize the growth and lipid production of six species of marine benthic diatoms (*Nitzschia laevis*, *Haslea ostrearia*, *Amphora* sp., *Nitzschia alexandrina*, *Opephora* sp., and *Staurosira* sp.) from the Loire estuary and surrounding mudflats using a culture system that conforms to their relatively sedentary living conditions. A low-maintenance laboratory-scale culture system for benthic diatom cultivation was developed, adapted from previously described vertically oriented porous substrate bioreactor (PSBR) designs. Preliminary results for *Nitzschia laevis* show a very rapid growth in biomass from 1.17±0.12 mg (day 0) to 4.90 ± 0.96 mg (day 5) using the PSBR. By the end of the culture period, the photosynthetic efficiency (F_v/F_m) remained to be > 0.5, indicating favorable culture conditions for the biofilm cells. Total lipid made up 25% of the total dry biomass, which is higher than values previously measured in benthic diatoms.

Keywords:

marine benthic diatoms, lipid, photobioreactor, attached,cultivation,microalgalbiotechnology

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About the author:

Mary is currently a full time PhD student at University of Nantes doing a project on the ecophysiology of benthic diatoms for valorization in health, cosmetics, and food.

PRODUCING LIPIDS AND DHA FROM THE HETEROTROPHIC MICROALGA *CRYPTHECODINIUM COHNII*, USING GLYCEROL AS CARBON SOURCE

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ABSTRACT

New alternative energy sources have been searched, to reduce the carbon dioxide emissions. Biofuels are renewable fuels made from oil crops, waste cooking oil and animal fat. However, biodiesel derived from such sources cannot satisfy the existing demand for transport fuels. Oleaginous microalgae are considered a promising feedstock for biodiesel production and, some of them, also synthesize omega-3 (ω-3) compounds. The marine oleaginous heterotrophic microalga *Chrypthecodinium cohnii* produces significant amounts of lipids (up to 50% w/w) which have been totally directed for docosahexaenoic acid (DHA) production, a high value ω-3 compound used in pharmaceutical formulations. A way to reduce the process costs consists of using low-cost substrates in the medium formulation, such as industrial byproducts. Glycerol is a biodiesel industry byproduct that can be used as carbon source in media formulations. However, this substrate may contain inhibitory compounds which may negatively affect the microalgal cells, thus reducing the process yield. Therefore, it is crucial to evaluate the microalgae cell stress response when growing on these substrates. The present work studied *C. cohnii* growth and lipid production (DHA and biodiesel) using biodiesel industry derived glycerol, previously distilled. The microalgae cell response was evaluated by flow cytometry, monitoring the cell membrane integrity and enzymatic activity.

Keywords: *Crypthecodinium cohnii, lipids, DHA, glycerol, Flow cytometry*

References:

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Acknowledgements:

This work has been supported by the project PTDC/EAM-AMB/30169/2017, entitled “OMEGAFUEL – New platform for biofuels and omega-3 compounds production, from the marine microalga *Crypthecodinium cohnii* sustainable biorefinery” supported by FCT (Fundação para a Ciência e a Tecnologia)

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About the author:
Researcher at LNEG Bioenergy Unit. She holds a degree in Chemical Engineering, a master's degree in Biochemical Engineering / Biotechnology and a PhD degree in Chemistry. She has been working in the field of biotechnology / bioenergy, microbial production of biofuels and high value added lipid products, using flow cytometry to evaluate the physiological response of microorganisms to stress environments. She is currently coordinating the national OMEGAFUEL project, being involved in several national and European projects, namely CONVERTE, BRISK2, AMBITION and BET (H2020).

Company profile:
The National Laboratory of Energy and Geology (LNEG) is a State Laboratory that makes RD&D oriented to the needs of society and enterprises, investing in a sustainable research, along with the international best practices. LNEG's mission consists of promoting technological innovation science and technology oriented for economic development.

THE GREATER AVAILABILITY OF NITROGEN INCREASES THE AMOUNT OF EPA-RICH POLAR LIPIDS INCREASING THE ADDED VALUE OF *PHAEODACTYLUM TRICORNUTUM*

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ABSTRACT

Polar lipids rich in omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) are a focus of interest in the production of microalgae because they are a source of bioactive compounds with several applications in human and animal nutrition, cosmetics, and pharmaceuticals. *Phaeodactylum tricornutum,* a unicellular marine diatom, is considered a potential source of EPA (eicosapentaenoic acid), which reinforces its importance for biotechnological purposes. This work aims to evaluate the fatty acid and polar lipid profiles in *Phaeodactylum tricornutum* cultivated under different nitrogen concentrations (12mM, 8 mM, 5mM and 2.5 mM) in the culture medium. To achieve this objective, it was determined the variation of the total content of lipids, phospholipids and glycolipids. The polar lipid profile of *P. tricornutum* was characterized using Liquid Chromatography coupled with high-resolution mass Spectrometry (LC–MS) and for fatty acid (FA) analysis Gas Chromatography-Mass Spectrometry (GC–MS) was used. The *P. tricornutum* biomass obtained with growth media enriched with 12mM nitrogen was characterized with the highest abundance of EPA. In addition, for these growth conditions, the greatest amount of phospholipids and glycolipids was obtained. Recent research suggests that marine polar lipids, such as phospholipids or glycolipids, may be more effective in delivering ω-3 PUFAs to target tissues than, for example, triglycerides. This fact highlights the importance of cultivating *P. tricornutum* with a high nitrogen concentration as this will lead to obtaining biomass that can provide more health benefits and have a higher added value for biotechnological applications.

Keywords:
Phaeodactylum tricornutum, Mass spectrometry, Lipidomics, Fatty acid Profile, Microalgae

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BIOGRAPHY

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Company profile:

GreenCoLab, Portugal, is a non-profit private organization, 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.

THE IMPACT OF LIGHT REGIMES ON *CHLORELLA VULGARIS* BIOFILM STRUCTURAL DYNAMICS AND PHYSIOLOGY

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ABSTRACT

Biofilm-based microalgae technology are attracting more and more attention for its potential to improve sustainability and cost-effectiveness compared with suspension-based systems. Mixing is then replaced by a mechanism to rotate the biofilm so that cells have periodically access to light, and high light intensisities are diluted in time. It becomes then key, for optimizing the process, to understand the impact of the light/dark regimes on the growth and structure of microalgal biofilm. Here, we study the dynamics and physiology of *Chlorella vulgaris* biofilms in a flow-cell subject to various lighting conditions. Light cycle (T) lasting from 15 *s* to 3 *min* at constant average light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$) were compared with the equivalent continuous light. The highest specific growth rate (0.31 d^{-1}) was obtained with the shortest light period (5 *s*). By contrast, growth penalties were observed with extended light exposure times of 30 *s* and 1 *min*. The F_v/F_m data was consistent with the growth performances. Biofilms present thicker and smoother 3D structures over days in general. Cell size responded impressively to the peak light intensity, while cell volume-based chlorophyll-*a* content reacted to the average light dose within the examined light regimes. These data were used to calibrate the Han model and propose an optimal design together with operating conditions. Photoinhibition can be mitigated within the light regimes investigated by applying time cycle lower than 15 *s* under intense light. Predictions indicate that biofilm growth and productivity can then be 7-fold enhanced by high light dilution rate under intense light, but respiration in the dark for a high biomass load can severely reduce the expected gains.

Keywords: Microalgae, biofilm, light regime, Han model, growth, productivity.

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POSTER RESENTATIONS

Process-Automation

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NEW METHODOLOGY FOR MEASURING BIOMASS CONCENTRATION

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ABSTRACT

The growth control of microalgae culture is a key factor in several microalgal biotechnological applications. Regardless of the specific purpose, optical density (OD) is the best-suited method for a fast, cheap and non-destructive estimation of microalgal biomass (Nielsen and Hansen, 2019). Several wavelengths inside the photosynthetically active radiation (PAR) and the infra-red (IR) have been used for the measurement of OD of microalgal cultures. However, there is little consensus in the literature about which wavelength to use for microalgal cultures (Nielsen and Hansen, 2019). It has been demonstrated that errors are greater using a wavelength within the range of maximal absorbance by chlorophyll (Griffiths et al., 2011; Pahija and Hui, 2021). With the aim to develop a more accurate technique and to minimize errors we designed a lab system that uses not only one wavelength but a wider spectrum. In particular, we used a sensor sensible at wavelengths between 500 and 610 nm with a sensibility pick at 555 nm as the receiver. In this way the sensor measures the light that is not absorbed by chlorophyll when a white light passes through a microalgal sample. As a result, the light measured by the sensor is only affected by the absorption related to the concentration of algal biomass. In the tested system we used as light source a submergible white led lamp (4000 K). This lamp was used both for biomass growth and for biomass measurement of different samples of S*pirulina platensis* with concentration from 0.3 to 3 g_{dry_weigh}/L. The obtained coefficient of determination (R^2) of the linear regression (0,998) shows the excellent response of this technology.

Keywords: Microalgae; optical density, biomass, dry weight

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Idea bioprocess technology (idea-biotech) produce innovative, reliable and high-quality Modular Lab Equipment for lab tests and experiments in biotechnology related applications and processes. Regarding microalgal processes idea-biotech has its own lab-scale photo-technologies such as the microalgal respirometer, the submergible led lamp and the customizable photobioreactors.

RECENT ADVANCEMENT IN MICROALGAE CULTURE WITH DAIRY WASTE REMEDIATION USING VISION IMAGING FOR BIOMASS GROWTH MONITORING

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ABSTRACT

The Fourth Industrial Revolution refers to the continuous automation of conventional manufacturing and industrial operations via the use of new smart technologies. Apart from automation, industrial progress also evolves in optimizing the re-use of waste material into the production process, and both the concerns will be covered in this study. Firstly, the study investigates on optimizing the proportion of industrial dairy food processing waste powder substituted into BG-11 as a source of cultivation medium for the growth of Chlorella vulgaris. Various percentages of food processing waste medium, B were substituted in the inorganic medium to analyse the algal growth and biochemical composition. The use of 40B combination was found to yield highest biomass concentration (4.11 g/L), lipid (260.44 mg/g), protein (263.93 mg/g), and carbohydrate (418.99 mg/g) content compared with all the other culture ratio combination. Secondly, the exploitation of colour acquisition was performed onto Chlorella vulgaris growth phases, and a novel photo-to-biomass concentration estimation was conducted via image processing for three different colour model pixels. RGB (Red, Green, Blue), CMYK (Cyan, Magenta, Yellow, Black), and HSL (hue, saturation and lightness) colour model was evaluated for its colour correlation alongside with the growth of microalgae. Based on linear regression analysis, it was discovered that the RGB colour model can read and interpret its colour variance more precisely than CMYK and HSL. Hence, digital image processing using RGB colour model can be explored for future automated microalgae growth monitoring development.

Keywords: Colour acquisition; Colour model; Biomass growth; Dairy food processing waste; Culture medium.

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Angela Paul Peter research interests covers from upstream bioprocessing, microalgae technology. She has industrial research working experience in glove industry. She is also a registered “graduate engineer” from the Board of Engineering Malaysia, 2018. She has published 5 papers (59 citations), and written a book chapter from “The Prospect of Industry 5.0 in Biomanufacturing” (2021). She was also selected as the top 20 researcher participant for IPID-Asia Summer School, 2020 across Asia. She has also participated in the MOSTA - Oil and Fats International Conference, 2021) and an international webinar on Industrial Innovations and Entrepreneurship Opportunity in Algal Biotechnology, IIEA-2021).

Company profile:

The University of Nottingham Malaysia was established 21 years ago and is ranked 103 in the world and (18 in the UK) out of more than 1,000 universities in the QS World University Rankings 2022. With campuses in Malaysia, China and the UK, UNM is truly a global instituti

POSTER RESENTATIONS

Process-Bioactivity measure

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DYNAMIC FOLLOW-UP OF LIPID ACCUMULATION BY MICROALGA AT PILOT SCALE: A CASE STUDY FOR FUTURE PROCESS OPTIMIZATION

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ABSTRACT

Microalgae constitutes a promising feedstock for third generation biofuel due to the high productivity and high lipid content repeatedly demonstrated at laboratory scale. Common strategies to induce lipid accumulation in microalgae consist in putting the microalgae culture under stress such as a nutrient stress (e.g. nitrogen starvation) or high light exposition. However, such stresses result in a loss of biomass productivity and consequently of overall lipid productivity, thereby explaining why micro-algae based biofuels are, to date, not available on the market. The dynamics of lipid accumulation was studied in parallel to the photosynthetic carbon-fixation capacity for the microalgae *Chlorella vulgaris* NIES 227 during batch-fed cultivation over 4 months in a 300L pilot scale photobioreactor (Jumbo XL Synoxis™, France). As biomass concentration reached up to 6.8 $g \cdot L^{-1}$ with a content of fatty acid methyl esther of 56 %, the photosynthetic activity rate of the micro-algae gradually decreased about 20 folds compared with exponential growth phase. The photosynthetic activity rate mapped for the varying microalgae physiological state monitored throughout this study enabled the development of a predictive tool of microalgal biomass productivity during nutrient starvation. Such prediction of biomass productivity loss during nutrient starvation could critically enhance our understanding of the dynamics of lipid production in photo-bioreactor and enable much needed process optimization.

Keywords: Microalgae; Fatty acids; Mathematical modelling; *Chlorella vulgaris*; Photosynthesis; bioprocess engineering

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RECOVERY OF EXCRETED TERPENOIDS FROM GENETICALLY ENGINEERED GREEN ALGAE: INTEGRATING LAB-BASED DATA AND PROCESS MODELLING

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ABSTRACT

Different types of microalgae produce diverse biomass profiles and by extension, their lipid (hydrocarbon and complex lipids) profiles and potential for bioproducts are also varied. But with the use of genetic manipulation or process engineering, a particular product could be targeted and produced by the microalgae of choice. A photosynthetic system capable of generating secreted fuel and other high valued products is highly attractive as it is possible to eliminate costly harvesting processes while allowing carbon fixation. This has a direct implication to our climate and the sustainability of the production platform in the long term. In this study, the application of biocompatible solvents to extract the targeted products from the algal culture allows for continuous expression of said products by the algae, eventually imitating 'single cell biofactories' in which minimal resources are provided to maintain the viability of the algae. The integrated cultivation and separation system was developed and tested to measure the effectiveness of product recovery. These experimental data were then used to design an ASPEN Plus simulation of the entire production process, in which the energy requirements at a pilot plant capacity were determined. In conclusion, this study presents practical techniques and methodologies that may be used in the future to investigate the growth of different microalgal species, the production of various terpenoid products and other configurations of the separation technique in different environmental settings. This allows for quick evaluation of different algae-based production platforms with the eventual aim of scaling-up and commercialization.

Keywords: algae; terpenoids; simulation; biocompatible extraction; solvent extraction

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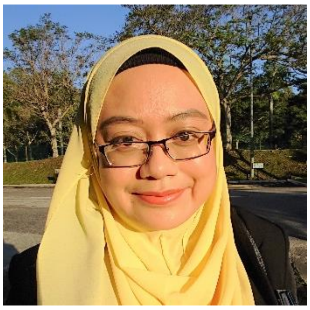

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Dr Irina Harun is currently a Senior Lecturer at Department of Environment, Universiti Putra Malaysia. She graduated with a PhD from Imperial College London (ICL) under the supervision of Prof Klaus Hellgardt, affiliated with the Energy Futures Lab and the Chemical Engineering Department, and was involved with an EU Horizons 2020 project PHOTOFUEL, a €6 million project which focuses on biocatalytic production of solar fuels involving Volkswagen, Neste, Bielefeld University, Uppsala University, Universita Firenze, A4F, Karlsruhe Institute of Technology and Volvo. She is an expert of algae bioprocess, biomass valorization technologies, bioseparation processes and green energy.

Company profile:
Universiti Putra Malaysia (UPM) is one of Malaysia's leading research Universities. Founded in 1931 as the School of Agriculture, the University today combines impressive modern facilities and a dynamic approach to teaching and research with its proud heritage of quality services and achievements.

TUNING THE SOLID RETENTION TIME TO BOOST THE PRODUCTIVITY OF MICROALGAL BIOMASS IN AN INDUSTRIAL PILOT SCALE LED PHOTOBIOREACTOR

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ABSTRACT

Microalgae industrial exploitation has been a trending topic in the last few years. However, low productivity and high production costs seem to be major causes that prevent companies to invest in this market. Biomass productivity (Px) can be enhanced by applying a chemical engineering approach, aimed at working in continuous systems, under properly controlled operating conditions. Up to now, the use of a semi-continuous system is more widespread even if less performing, mainly due to the current state of downstream technologies. To overcome this problem and bring semi-continuous system performance closer to a continuous one, the main operating variable to be adjusted is the solid retention time (SRT) which should be set as closer as possible to the residence time where the maximum of the biomass productivity obtainable. Conversely, the hydraulic residence time (HRT) should be set so to minimize the nutrient loss. Modeling and simulation can help to define the best operative conditions to be set in terms of residence time. In this work, a previously implemented model was validated in a 3.4 m^3 pilot plant and the best operative conditions were defined in terms of SRT. As a result, a deeper control of the cultivation variables was then applied to the pilot-scale plant, aiming at enhancing biomass productivity. This new approach allowed obtaining a biomass productivity of 0.59 g L^{-1} d^{-1}, 2.5 times higher than that obtained in a system operated with standard procedures (0.24 g L^{-1} d^{-1}). The quality of the microalgae strain used in this study, *A. platensis*, was checked by measuring the protein content, confirming that the biomass composition can be also managed by adjusting the SRT.

Keywords: *Arthrospira platensis*, LED light, SRT, protein content

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Borella L, Sforza E, Bertucco A. Effect of residence time in continuous photobioreactor on mass and energy balance of microalgal protein production. N Biotechnol 2021;64:46–53. https://doi.org/10.1016/j.nbt.2021.05.006.

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Master degree in Industrial biotechnology at the university of Padova in 2016, since than I worked at the Interdepartmental Centre Giorgio Levi Cases as a research fellow/scholarships on industrial applications of microalgae. Some of this scholarships were in collaboration with private companies interested in optimizing microalgae growth in their plants. My main research fields are: wastewater treatment with microalgae or microalgae-bacteria consortia; study of growth kinetics parameter by traditional or innovative techniques and process optimization.

TWO STEP GAZING CONCEPTS FOR PHOTOBIOREACTORS

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ABSTRACT

During photosynthesis, dissolved Oxygen is being accumulated in the microalgae cultivation fluid while CO2 is being removed. Hence, after a certain period of cultivation, the concentration of oxygen must be reduced while the CO2 concentration must be increased in order to maintain the optimal cultivation mixture. In some cases where the availability of CO2 is not an issue, it is fair to accept a certain loss of excess CO2. However, it is clearly beneficial to have a precise overview of the CO2 content in the reactor to ensure that the CO2 introduced into the cultivation will be transferred into biomass.

In case of limited access to CO2, controlling the influx of CO2 becomes ever more important. By this measure, the amount of CO2 lost –unused for the cultivation– may be drastically decreased. The combination between Oxygen degassing and CO2 introduction is challenging. First and foremost, since the saturation of CO2 is 180 times higher than that of Oxygen.

Those effects led us to separate the process of oxygen degassing from the process of CO2 gassing by usage of a two-stage gassing unit. The strict separation of those two processes allows us to implement individual process steps in order to control parameters such as pH value, the remaining oxygen level and others. In sum, this practice for a more precise control of those elementary parameters for the increase efficiency and reduce the CO2 loss.

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

References:

Our own tests with reactors that have been equipped with the described technology.

BIOGRAPHY

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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.

WHO MOVED MY CHEESE ALGAE?

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ABSTRACT

Commercial scale microalgae cultivation platforms are increasingly integrating various non-invasive and online measurements to monitor microalgal processes such as growth, biomass and lipid accumulation. In recent years, numerous online sensors including optical and infrared probes have been deployed for real-time monitoring of algal growth. Fourier Transform Infrared (FTIR) technology is one such non-invasive technique that, in combination with multivariate approaches, has proved its potential for in situ species discrimination and relative quantitation of bio-molecules such as lipids, proteins and carbohydrates (Girard et al., 2013; Sigee et al., 2002). However, the discrimination ability of FTIR has not been leveraged to screen for potential invaders, microalgal predators, in algal cultures (Deore et al., 2020). The present study implements a FTIR-based approach to identify signature infrared wavenumbers in microalgal, *Dunaliella tertiolecta*, cultures infested with a zooplankton predator, *Oxyrrhis marina*. *D. tertiolecta* cultures with incremental loads of the predator were screened using a portable bench-top FTIR instrument. We report changes in the absorbance of FTIR-based signature wavenumbers, at 1346, 1363, and 1382 cm^{-1}, as function of increased predator load. Based on a partial least square regression (PLSR) model ($R^2 = 0.894$), the signature spectra indicate the presence of *O. marina* at a concentration of 5×10^2 cells mL^{-1} and at least 72 h prior to the culture crash (Deore et al., 2021). As opposed to offline grazer monitoring tools, the potential for FTIR-based flow-through design in combination with multivariate methods will enable real-time and non-invasive means of early detection of algal grazers.

Keywords:contamination detection; culture crash; Fourier Transform Infrared (FTIR) technology; non-invasive screening

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BIOGRAPHY

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About the author:

Pranali was awarded a joint-PhD degree in Microalgal Biotechnology in the year 2020. Her PhD research focused on developing a range of tools for non-invasive monitoring of microalgal predator as an early warning signs of the microalgal culture crash. Her PhD research was jointly supervised by experts in Reliance Industries Limited; Indian Institute of Technology Bombay, India and Monash University, Australia. Pranali has 7+ years of experience in microalgal transgenics, cultivation, photosynthesis, predator control and detection. Currently, she is a post-doctoral research fellow working on algae-bacteria symbiosis at the University of Melbourne, Australia.

Company profile:

The IITB-Monash Research academy is a joint-research collaboration between the Indian Institute of Technology Bombay, India and Monash University, Australia which incubates cross-disciplinary research and offers a dual badged PhD degree focused to answer various global problems.

POSTER RESENTATIONS

Transversal-Business

ALGÆUROPE2021
07-10·DECEMBER·ONLINE

A COMPARATIVE ECONOMIC EVALUATION OF SCENARIOS FOR THE EXTRACTION OF AN OIL CONTAINING FUCOXANTHIN AND EPA FROM *P. TRICORNUTUM* USING FPA-PBR WITH ARTIFICIAL LIGHT OR NATURAL SUNLIGHT IN GERMANY.

Sebastian Weickert

University of Hohenheim

ABSTRACT

This study determined and analyzed the costs of a process for the cultivation of *P. tricornutum* using artificial lighting or natural sunlight and the subsequent extraction of an oil rich in eicosapentaenoic acid (EPA) and fucoxanthin in four scenarios. The calculations were based on experimental data obtained at laboratory (downstream process) and pilot scale (upstream process) in Germany. In the calculated scenarios, the use of artificial illumination was found to be of economic advantage. The decisive factors for the production costs of the process as a whole were found to be the electricity and associated energy requirements for either artificial lighting (32-35%) or temperature control for cultivation in sunlight (19-23%). Without a permanent artificial light supply, the costs of providing the cultures with CO2 also shift into focus (approx. 17%). The results are however highly dependent on location (electricity prices, wage costs, emission factors).

Keywords: *TEA, artificial illumination, fucoxanthin, EPA, P.tricornutum.*

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About the author:

I studied biotechnology in Saxony and Berlin and moved to Stuttgart in 2012 to work as a scientist and project manager for Subitec GmbH. In 2016, as a pre-doctoral fellow at the University of Hohenheim in the Department of Agricultural and Food Policy, I changed my focus from process development to ecological and economic issues. In 2019, I joined the department of Biobased Resources in the Bioeconomy to work on the project “Economic and ecological evaluation of a biorefinery concept to produce fucoxanthin and EPA on a pilot scale and transdisciplinary developed scenarios on an industrial scale in Germany.”

THE CULTURE COLLECTION OF ALGAE AND PROTOZOA (CCAP); WHO WE ARE, WHAT WE DO, AND OUR EXCITING NEW UPDATES!

Ross M.E.

Allen, R., De Boever, F., Davey, M., Field, J., Green, D., MacKechnie, K., Saxon, R., Stanley, M., Thomas, N., Rad-Menéndez, C.

Culture Collection of Algae and Protozoa (CCAP), Scottish Association for Marine Science, Oban, Argyll, UK, PA37 1QA

ABSTRACT

The Culture Collection of Algae and Protozoa (CCAP) is the world's most diverse service collection for living strains of micro- and macro-algae, cyanobacteria, protozoa, and algal pathogens. CCAP maintains more than 2,500 strains and has a global customer base in both the academic and industrial sectors. CCAP provides a range of services to the community including strain and media provision, cryopreservation, patent deposition, taxonomic and genomic studies, consultancy, and teaching/training workshops. In 2021, CCAP has gone big! We have expanded the services that we offer, by launching two new services to our repertoire. Firstly, to address the growing interest in state-of-the-art algal biotechnology, CCAP is opening the Algae Research, Innovation and Environmental Science centre (ARIES). This facility will allow us to cultivate and harvest larger volumes of algae (>200 L) using photobioreactors and membrane harvesting systems. CCAP-ARIES also provides cutting-edge analytical capabilities, including genomics, metabolomics, and biochemical analyses. Furthemore, to facilitate protistan research and continue the taxonomic curation of the collection, CCAP has collated all nucleotide sequence data for CCAP strains generated by CCAP, collaborators and other researchers. These data are accessible from the CCAP Bioinformatics Gateway and the main CCAP webpage, where users can browse and access sequence data associated with the CCAP strains. A key future development aims to provide a comparative functional analysis of protistan genomes. With these research and developments, as well as ongoing activities (e.g. Darwin Tree of Life project), CCAP will continue to build upon its world-class research and service provision, with which to investigate the unique and underexplored diversity of protistan organisms for the understanding of the natural world and the development of new biotechnological applications.

Keywords: Algal Culture Collection, Large-scale cultivation, Bioinformatics, Metabolomics, Genome Sequencing

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I am the Manager of the Culture Collection of Algae and Protozoa and a Senior Researcher in Algal Biotechnology. I have a broad range of interests relating to biotech and the circular economy. These interests span applied algal research relating to food and feed, bioenergy, bioremediation of nutrients toxic elements, and emerging microcontaminants, CO_2 sequestration, and to produce high-value compounds such as carotenoids. In addition to a growing interest in microbial ecology, taxonomy, genomics and metabolomics.

Company profile:
The Culture Collection of Algae and Protozoa (CCAP) is a Biological Resource Centre located within the Scottish Association for Marine Science (SAMS) on the scenic west coast of Scotland. CCAP is supported by the Natural Environment Research Council (NERC), part of UK Research and Innovation, as a National Capability service.

POSTER RESENTATIONS

Transversal-LCA

ALGÆUROPE2021

07-10·DECEMBER·ONLINE

CLIMATE CLIMATE EFFECTS OF SEAWEED ACROSS THE VALUE CHAIN

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ABSTRACT

Seaweed cultivation and processing industries could contribute to blue growth and the European bioeconomy. Seaweed is often touted as an example of a resource with climate benefits, but to what extent are climate benefits generated, really? We investigate a Swedish production system and present a cradle-to-gate environmental LCA of a kelp supply chain that accounts for carbon capture at sea and includes the hatchery, a 2-ha longline cultivation, and four alternative preservation methods (hang-drying outdoors, heated air-cabinet drying, ensiling, and freezing). The study found that as a result of carbon capture (bioremediation) by seaweed, more CO2 equivalents are (temporarily) absorbed than emitted by the supply chain. The extent of emissions is most affected by preservation methods with greatest impact shares result from freezing and air-cabinet drying, both the two most energy-intensive processes, followed by the cultivation infrastructure, highlighting strategic optimization opportunities. Hatchery processes, harvesting, and the low-energy ensilage and hang-drying outdoors were found to have relatively small impact shares. However, beyond carbon capture and supply chain emissions, there is a bigger system. Following the carbon from cradle to grave, climate effects can be divided into 1) at sea processes, 2) production supply chain emissions, 3) consumption phase with temporary carbon storage and substitution of alternative products; and 4) end of life with emissions or long-term storage. Each of these phases are subject to complexity, uncertainty and context-specificity. We give an overview of existing knowledge. Putting the results of the LCA in this broader perspective, we conclude there is still little evidence available to suggest that these value chains perform much better (or worse) than those of other biomass options when considering the whole product system.

Keywords: Blue carbon, LCA, carbon footprinting, climate, seaweed

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About the author:

Jean-Baptiste's research involves assessing the sustainability of the seaweed industry. His doctoral thesis included a wide range of analytical methods including an Energy and Greenhouse Gas Systems Analysis, Life Cycle Analyses, a Public Perception Survey, a Geographic Information Systems based Multi-Criteria Analysis, and an economic Cost-Benefit Analysis, which together form a collection of insights on the sustainabiltiy of a burgeoning Swedish seaweed industry. He is now on a Post-Doc with a focus on economic and environmental supply chain optimisation. His academic background is in Geography (Ba) from King's College London and in Sustainable Technology (MSc) from KTH, Stockholm.

Company profile:

Founded in 1827, KTH Royal Institute of Technology is one of Europe's leading technical and engineering universities, and a key centre of intellectual talent and innovation. We are Sweden's largest technical research and learning institution and home to students, researchers and faculty from around the world dedicated to advancing knowledge.