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THE DIGITALGAESATION PROJECT

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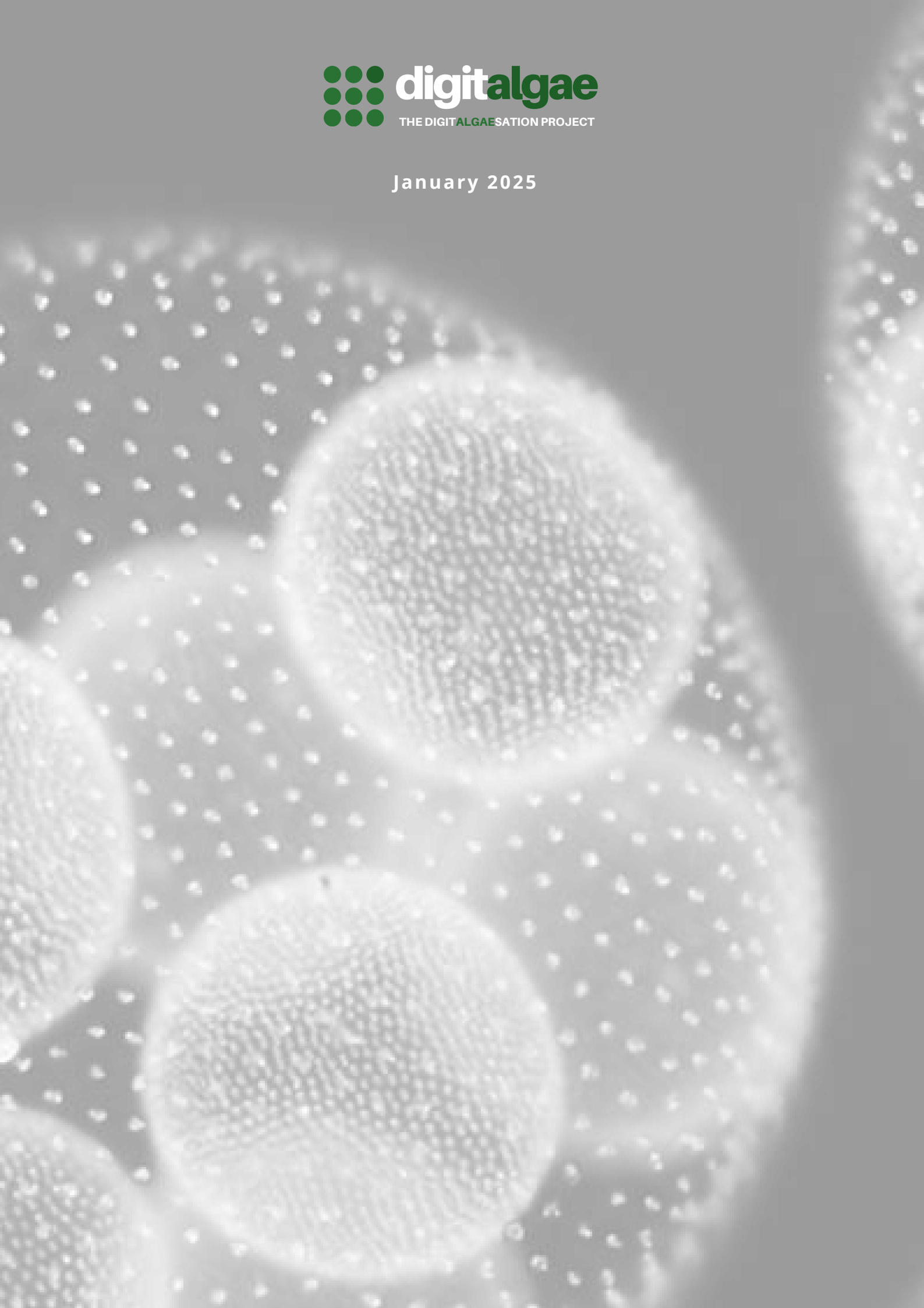
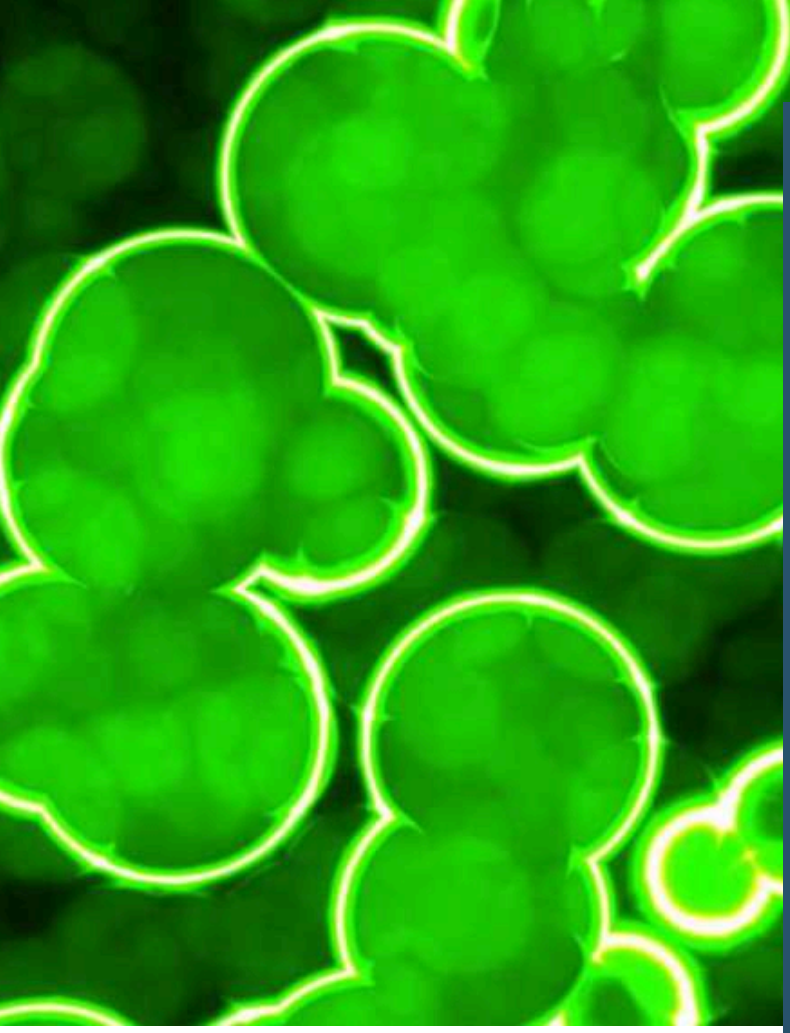
January 2025





The objective of digitalgaesation project is to propose a digitalisation approach to optimising control and operation of microalgae cultivation processes, so as to maximise their light conversion efficiency and to drive the sustainable portfolio of microalgae-based processes towards commodities and energy markets.

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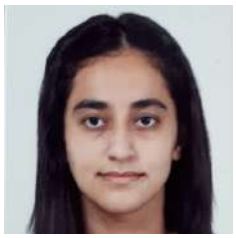
Tehreem Syed

Rosaria Tizzani

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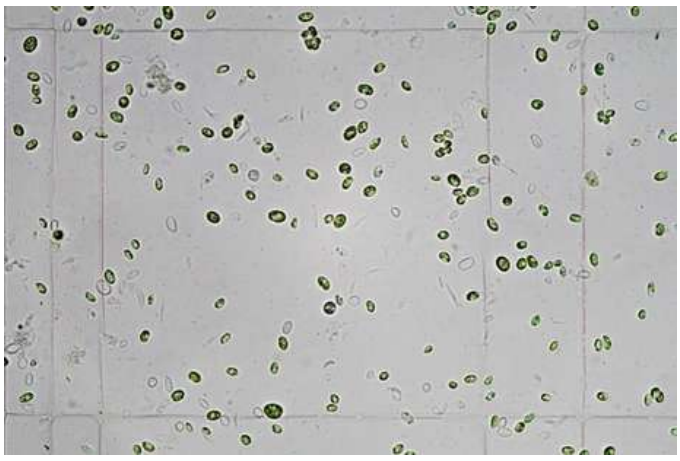
Development of a continuous microphotobioreactor for rapid model identification

Beatriz Felices Rando - January 18, 2022

Microalgae have multiple applications in many diverse sectors that already make their cultivation profitable, such as pharmaceuticals, cosmetics or food supplements. One of the most appealing applications of microalgae lies on the energy sector because of microalgae's high lipid content and renewable nature.
However, the biofuels obtained from microalgae's biomass are still not competitive with classical fuels due to the low productivity and high cost of microalgae cultivation processes. In order to exploit microalgae's potential to the full, it is necessary to optimize process design and operation of microalgae cultivation and biomass productivity. For this purpose, we need models that we can identify and calibrate, which is quite a challenging task, as these models often contain complex equations with a large number of parameters that are frequently correlated, hindering the process of model identification.
Therefore, the goal of this project is the development of a microphotobioreactor that would help us overcome identification problems such as limited system observability, noisy measurements and variable biological responses. Working on a microscale brings the possibility to reduce the time and cost required to evaluate the single effect of multiple variables in microalgae growth, also making control easier. It also helps reducing some side-effects that hinder the comprehension of the process. For example, a self-shading effect between the cells can limit the understanding of how light interacts with the microorganisms, and the bigger the scale, the bigger this effect becomes.
Some of the assets of the reactor under development are: the capability of supporting long-term culture of algae cells, allowing the setting of different temperature levels and complex light dynamics and being compatible with on-line imaging. The evaluation of the reactor's performance is carried out through cellular counting, fluorescence measurements and protein, carbohydrate and pigment content analysis.

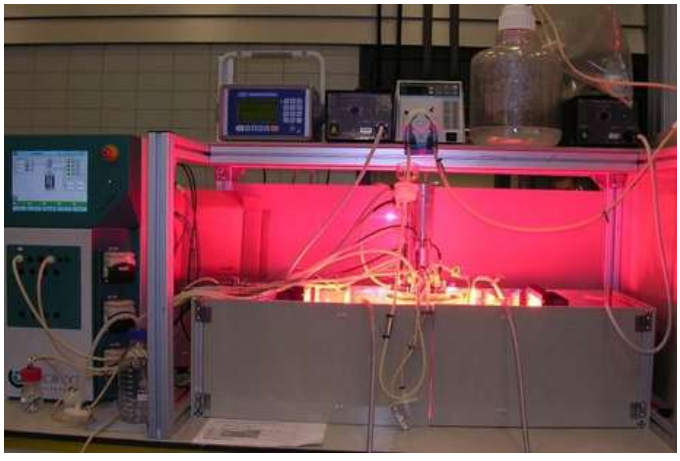


Scenedesmus obliquus

Sensor technology fusion for on-line monitoring and control

Henrik Niklas Geltner - January 18, 2022

The establishment of algae based production processes for application in different goods such as food, feed, nutritional supplements, medicine, fuel or platform chemicals is increasingly gaining more attention. A main reason for this development might be due to the crescent need to replace conventional industrial processes with more sustainable ways of production.
The ongoing increase of computational and sensoric capabilities opens new options to establish monitoring systems that allow to get better insights, statistical certainty and control over various process parameters in order to improve process performances rapidly. Experimental investigation of algae cultivation in laboratories however depicts itself quite far from being automated or digitalized. Typical work tasks during cultivation processes involve a frequent removal of sample volumes out of the reactor vessel to be analyzed with various methods to obtain the process describing data.
In many cases the analysis is done after the experiment has ended. Each of this samples taken poses the risk of a contamination and therefore a disruption of the whole experiment. Additionally it is required to take multiply samples at a time in order to guarantee a satisfactory statistical certainty of the dataset. On the other hand the amount of samples possible is limited by the small working volumes of a few liters in lab-scale reactor vessels. The aim of this project therefore lies on the development of an experimental set up for on-line monitoring. This will consist of suitable sensors, a data acquisition infrastructure and signal processing software for the on-line monitoring of relevant process parameters.
The description of the process state for example can be done by mass-balancing elemental components of the microalgae like carbon, oxygen and nitrogen to determine the time specific production and consumption rates and monitor them in real time.



On-line monitoring system

Modelling and control of the photoacclimation response in an industrial environment

Rosaria Tizzani - January 18, 2022

The optimization of microalgae cultivation has been occurring on two levels, genetically modified strains, and advanced control systems. On one side, the genetic modification of microalgae is aimed to enhance or modify the metabolic activity for biotechnological exploitation.
The selected microalgae species, mutants, or wild type, show higher photosynthetic efficiencies. They are also used as sustainable biofactories for enhanced production of added value components. The design of the production biosystem does not rely only on genetic tools, but a variety of nutritional and environmental causes has been shown to influence the physiological characteristics of microalgae.
On the other side, several aspects of process optimization are under the spotlight to increase productivity at the industrial level. The design at a large scale must take into account several aspects like nutrient feeding, thermal regulation, pH adjustment, and mixing. Ideally, closed photobioreactors (PBRs) should enable optimal control of culture conditions minimizing the practical and economic constraints. Energy requirements and construction costs of closed PBRs still hinder their application for large-scale production. Therefore, the big challenge is to provide maximal performance and user-friendly tools for industrial exploitation. Different approaches to optimize bioprocesses can lead to enhanced productivity. Assessing PBR geometries suitable for the cultivation of specific strains is crucial since microalgae do not have a single standard response. Long-term modeling efforts also provide engineered operation strategies for the predictable massive production. Analytical relations that can easily be applied to predict biological behavior are valuable information in a preliminary engineering phase. The control problem concerning microalgae biomass production in large-scale PBRs is composed of different control levels because, depending on the final use of the biomass, different control objectives (productivity, economic issues, quality aspects) need to be fulfilled during the production process.

Gauditana cultivation

Modelling and control of the photoacclimation response in an industrial environment

Rosaria Tizzani - January 18, 2022

Modeling microalgae growth and photoacclimation under different conditions at the industrial level is the core of the project. The effects of several light regimes (dark-light cycle, light mixing, and light pulsing) and temperature will be studied to evaluate the effects on the photosynthetic activity and other metabolic processes. Advanced remote-control systems will be developed and integrated in PBR with different geometries.
Currently, the cultivation of different species has been tested in cylindrical plastic bags with an integrated remote-control system. It is a cost-effective and flexible method to test different operational conditions to produce raw biomass and valuable components like lipids, polyhydroxybutyrate, accessory pigments, and exopolysaccharides from different strains.
In conclusion, multilevel approach still needs to take priority over the purely biological and engineered solutions. The combination of genetically modified microalgae strain, altered growing conditions, modeling of bioprocesses, and advanced control strategies will provide effective solutions to optimize large-scale production.

Advanced techniques for model-based experiment design under uncertainty

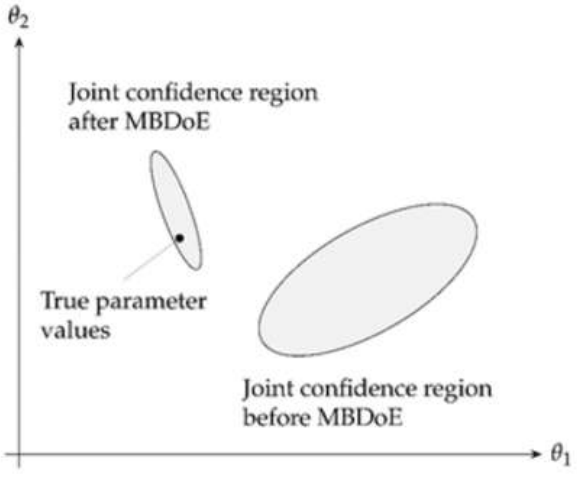
Marco Sandrin - May 7, 2022

The use of first-principles mathematical models to describe bio-chemical phenomena is paramount to the effective development of microalgae processes. Model-based design of experiments (MBDoE) represents a valuable tool providing several methodologies that assist the model-building strategy of such complex mathematical models. The objective is to design experiments (or campaigns of experiments) that will provide the highest information content possible, thus reducing the time and resources needed to develop and validate models.

Model-based design of experiments can be mainly used towards two distinct but highly related goals: model discrimination, which aims to select the most appropriate model whenever multiple competing models have been proposed to describe a certain system, and improving parameter precision, which corresponds to improving the reliability of the estimates of the adjustable parameters once a specific model has been identified.

The initial focus of this research project has been directed to MBDoE for improving parameter precision. Several criteria are available in the scientific literature, but the ones that are most commonly used are not free from drawbacks. Local design criteria involve a linearisation of the model around the currently available estimates of the model parameters to predict the information content of the experiments being designed. This can lead to the design of suboptimal (non-informative) experiments, especially at the early stage of the model development procedure when parameter estimates may be highly uncertain or not available at all. Several criteria have been proposed that are more robust to the initial guesses of the model parameters, but they are often too computationally demanding.

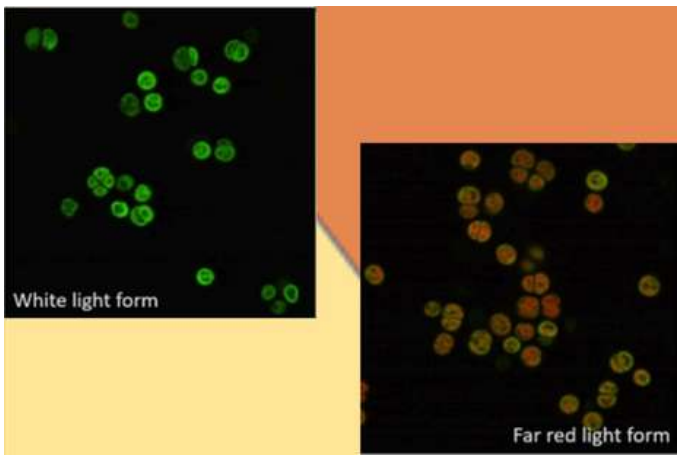
The current research work involves attempting to combine local criteria with other approaches to the experiment design problem, with the aim of providing a methodology that can be used to design the most informative experiments possible. The design of campaigns of parallel experiments, in place of single experimental runs, is also currently under investigation.



Costs and benefits of far red light photoacclimation in cyanobacteria

Giovanni Consoli - May 7, 2022

Photosynthesis is the process that harnesses the energy of photons that hit the Earth's surface to split water and produce molecular oxygen that energizes the atmosphere while forming the biomass that sustains all the food chain in the form of biomass, and our economies and lifestyle in the form of fossil fuels.
Most oxygenic photosynthetic organisms are tailored to use the same portions of the solar spectrum, not far from the range of radiation that we can see with our eyes. Wavelengths below 400 nm are too energetic to be employed safely, while wavelengths above 700 nm simply cannot deliver enough energy to drive the process. The wavelength range used by most organisms is called photosynthetically active radiation (PAR).
This changed forever with the discovery of two types of "low energy" photosynthesis that allow different organisms to perform the same tasks but with less energetic photons. The first, discovered in 1996 was the cyanobacterium Acharyochloris marina, that containing mostly chlorophyll *d*, is able to use radiation up to 740nm. The latter, then named chlorophyl *f*, is now known to be present in at least 50 cyanobacterial species spread around different ecological niches. When these organisms sense a reduction of the amount of white light compared to far red light, chl *f* is synthesized along with subunits of photosystems and antennas, allowing them to transiently remodel their photosynthetic apparatus and to extend their absorption spectra up to 750nm in an acclimation process known as FaRLiP (far-red light photoacclimation).
Changes in the absorbance of these organisms vary from species to species, but this acclimation process enhances light harvesting in wavelengths comprised between 700 and 750nm, accounting for a potential 19% increase in available photons, providing scope for improvement to the theoretical maximum efficiency of photosynthesis.



Confocal microscope fluorescence of white light adapted and far red light adapted C. thermalis, a cyanobacteria containing the FaRLiP cluster. In green: chlorophyll a fluorescence, in red chlorophyll f fluorescence.

Costs and benefits of far red light photoacclimation in cyanobacteria

Giovanni Consoli - May 7, 2022

The possibility of expanding the absorption range of photosystems and antennas to limit the amount of self-shading has created both in academia and industry a renewed interest in trying to couple it with existing photosynthetic bioprocesses in order to achieve to higher productivities

However, to be able to effectively design biotechnological applications of far-red photosynthesis, we first need to better understand what the functional penalties are to this improved harvesting capabilities, since these could range from enhanced sensitivity to photodamage to losses in efficiency of excitation energy trapping. Moreover, a better understanding of the acclimation process itself is needed to be eventually able to tune its triggers, speed and overall effects on the photosynthetic apparatus to be used in agricultural or biotechnological processes.

Under the supervision of Prof. Rutherford and Prof. Chachuat, I am developing a controlled growth environment for the “far red” strain, while trying to develop a model that predicts photosynthetic parameters of far red acclimated cyanobacteria.

Microalgal population dynamics related to changes of environmental

Yob Ihadjadene - May 7, 2022

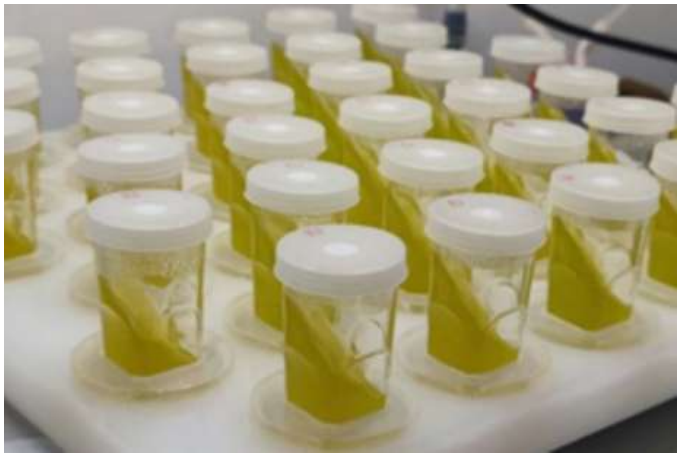
Microalgae constitute the basis of life, in a variety of marine and freshwater ecosystems. They are efficient cell factories, the majority operating photosynthetically to turn light energy and atmospheric carbon dioxide (CO_2) with simple inorganic nutrients into biomass. Moreover, they have great potential for the production of industrially relevant high-value products such as proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins and pigments that have numerous health benefits and trading potentials.

Due to the use of photons as energy source and the gaseous C-source CO_2, phototrophic bioprocesses in particular are characterized by a high degree of heterogeneity directly affecting the physiology of the cell population (e.g. cell metabolomics, cell cycle phase) – a fact that has been neglected in many studies so far.

It has been shown that the amount of synthesized bioactive compounds is directly related to two major aspects, namely the environmental conditions and the cell cycle patterns of the microalgae species. The first aspect has been the subject of many studies for several decades already. Nevertheless and according to our knowledge, the concept of population heterogeneity has not been the subject of any in-depth study to date, due to both its complexity and the absence of already established techniques and protocols.

A special feature of many microalgae is cell division by multiple fission, which differs from classical bacterial cell division by the number of daughter cells. In contrast to binary cell division (2 daughter cells), multiple fission can yield 2n daughter cells depending on the environmental conditions.

This means that there is a considerable influence of the process conditions on the biomass production.

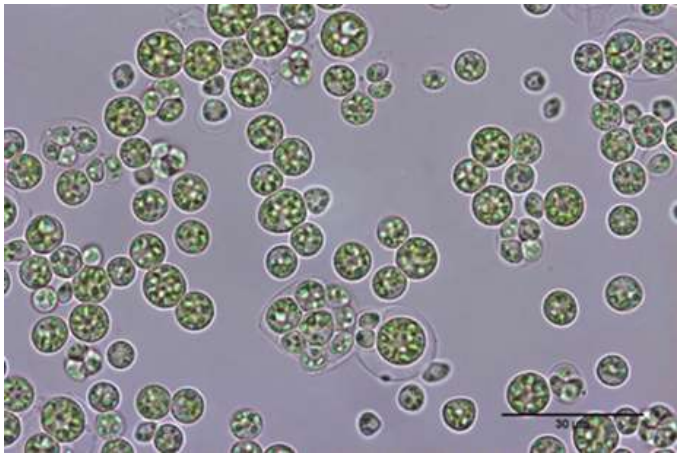


Chromochloris zofingiensis cultivation in the CellDeG screening system.

Microalgal population dynamics related to changes of environmental

Yob Ihadjadene - May 7, 2022

In this perspective, one of the main objectives of our project is to develop new single-cell analysis techniques and methods to study the population heterogeneity of the non-conventional microalgae Chromochloris zofingiensis, a promising natural producer of high value carotenoids such as astaxanthin.
By analyzing the cell cycle regulation, i.e. the formation of daughter cells as well as the metabolic activity in the individual cell cycle phases, on external environmental conditions, a substantial optimization of biomass and carotenoid production is aimed at this project. The impact of the environmental conditions on the population heterogeneity is integrated into population models, which can extend widely-used growth models.



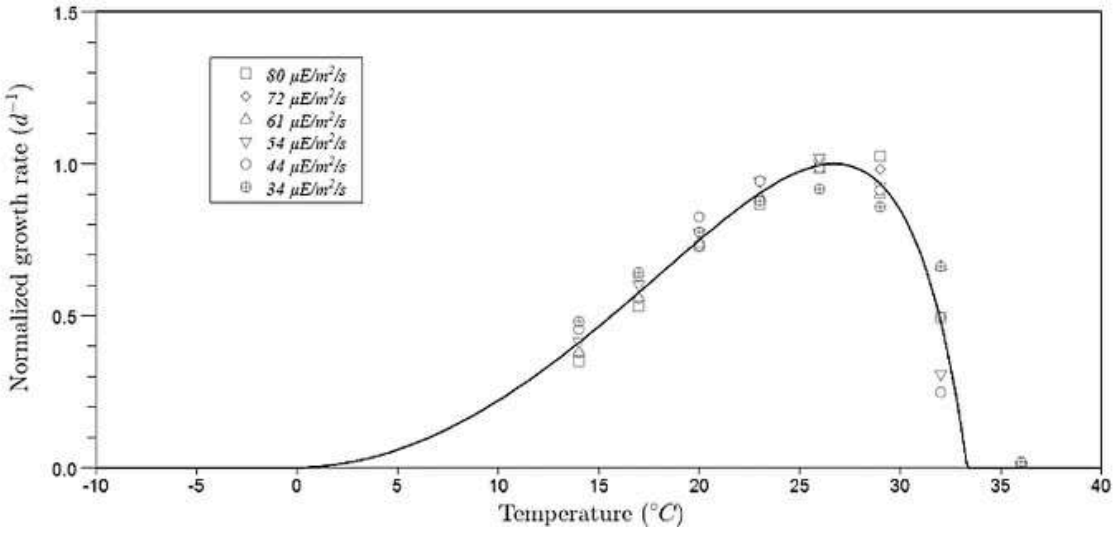
Cell population of Chromochloris zofingiensis showing a high heterogeneity (e.g. cell cycle phases).

Adaptive Model for temperature control

Ali Gharib - July 15, 2022

Due to the complex dynamics of the growth of microalgae a sophisticated control method is needed, to achieve the maximum yield out of a microalgae plant.
Model-predictive control (MPC) is a powerful tool gaining a lot of attention in the field of modern control systems.
With the help of an underlying model predicting the system behavior, an optimal working mode is determined for maximizing a productivty criterion.
Temperature and light are two variables having a significant influence on the growth of microalgae. In various systems sunlight is the only light source. Temperature however, has mostly to be controlled, since microalgae optimally grow in a specific range of temperature and the yield varies along this range. Above a specific temperature, they die.
A temperature model evolution is crucial to predict the temperature in a plant. Data on the air temperature, light inflow from weather forecasts, can be used to predict the temperature of the microalgae medium. In existing literature heat transfer models achieve high accuracy. However, these models are complex, have many parameters and accurate knowledge about the plant. Due to their highly computational effort, they are also not usable for MPC.
The initial step is to create an adaptive temperature model, that has less parameters and has higher computational efficiency than the existing heat transfer models in literature, so it can be used for MPC. Afterwards this model has to be validated with data from different systems, to ensure an accurate temperature (prediction) submodel as part of the microalgae growth model, that will be used to achieve the optimal yield.

Growth rate of Nanochloropsis oceanica with respect to temperature



Adaptive Model for temperature control

Ali Gharib - July 15, 2022

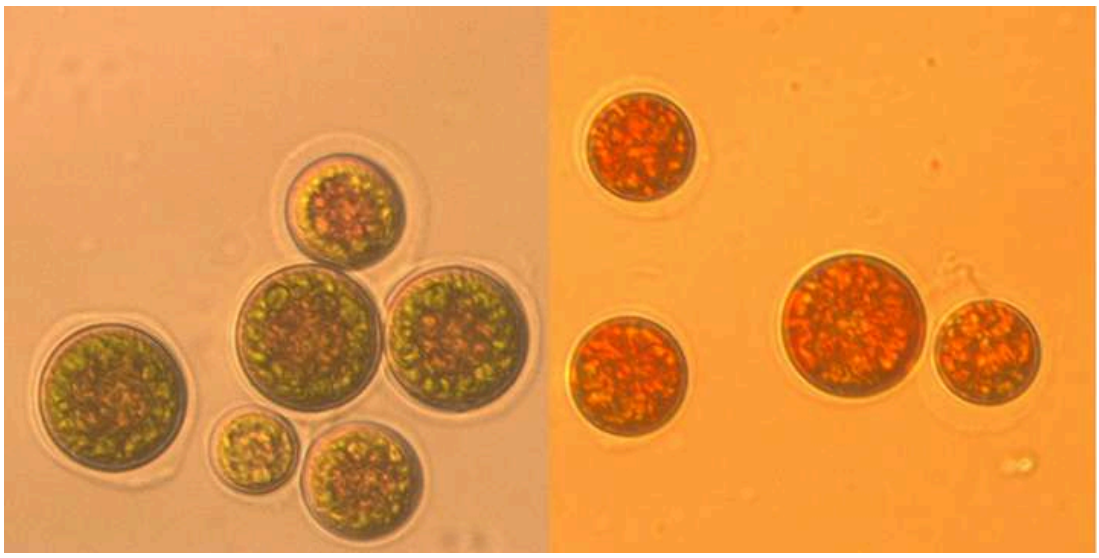
Under the supervision of Prof. Olivier Bernard and Dr. Walid Djema, I am developing a model-predictive control with auto-adaptation, which takes benefit of future meteorology to track both the culture density and the temperature along the day.
Afterwards the work of the project provides the implementation and testing of the control strategy on a pilot plant as well as the benefits assessments in terms of productivity, water use, energy use and manpower requirement.

A modelling approach for high-value products from microalgae

Lars Stegemüller - May 7, 2022

Microalgae have a broad spectrum of potential applications, such as for human consumption, aquaculture feed, nutraceuticals and pharmaceuticals. The utilisation of microalgae offers a sustainable approach due to their ability to fixate CO2 and uptake inorganic nitrogen and phosphorous sources from wastewater.
The freshwater microalgae Haematococcus pluvialis is of explicit industrial interest due to its high content of astaxanthin. Astaxanthin is a secondary carotenoid with a bright red colour and high potential as a food supplement due to strong antioxidant, anti-inflammatory and antitumoral properties.
Astaxanthin is meant to protect the cell from various stress conditions. The most common stress factors H. pluvialis faces are intense illumination, salt stress and nutrient deprivation. Given that the growth under stress conditions is very limited, a two-stage production is often applied where the cells are grown under favourable conditions in their green motile stage and afterwards exposed to one or several stress conditions.
Translating the behaviour of cells with respect to growth and product accumulation into a mathematical model can give valuable insights and help to improve dynamic processes. The most important input parameters for microalgae growth models are light intensity, nutrient concentration, temperature, CO2 availability and, in the case of a mixotrophic system, the concentration of additional carbon (e.g. glucose, acetate).
In general, there are many different ways to approach a microalgae growth model. A model can simulate the behaviour at a molecular level or focus on an entire population. ›

Microscope picture of Haematococcus pluvialis in dark green stage (left) and red, astaxanthin-rich stage (right)



A modelling approach for high-value products from microalgae

Lars Stegemüller - May 7, 2022

The latter requires some simplifying assumptions and makes use of empirical correlations (e.g. Monod kinetics). However, it requires less computational power and can give accurate results if calibrated and validated correctly.
Building a reliable model for optimisation and decision support requires not only appropriate mathematical expressions but also accurate data. This data can often not be obtained by a single experiment, especially when the model should account for several input parameters. A thoughtful selection of experiments and accurate work in data acquisition is therefore key to success.
In conclusion, modelling approaches can result in optimised and automatized processes. This can guide us towards a world where microalgae play an important role in sustainable production processes (e.g. astaxanthin from H. pluvialis). However, a thoughtful plan and accurate collection of data is required in order to succeed.

Hybrid Modeling Platform for Process Monitoring and Optimization

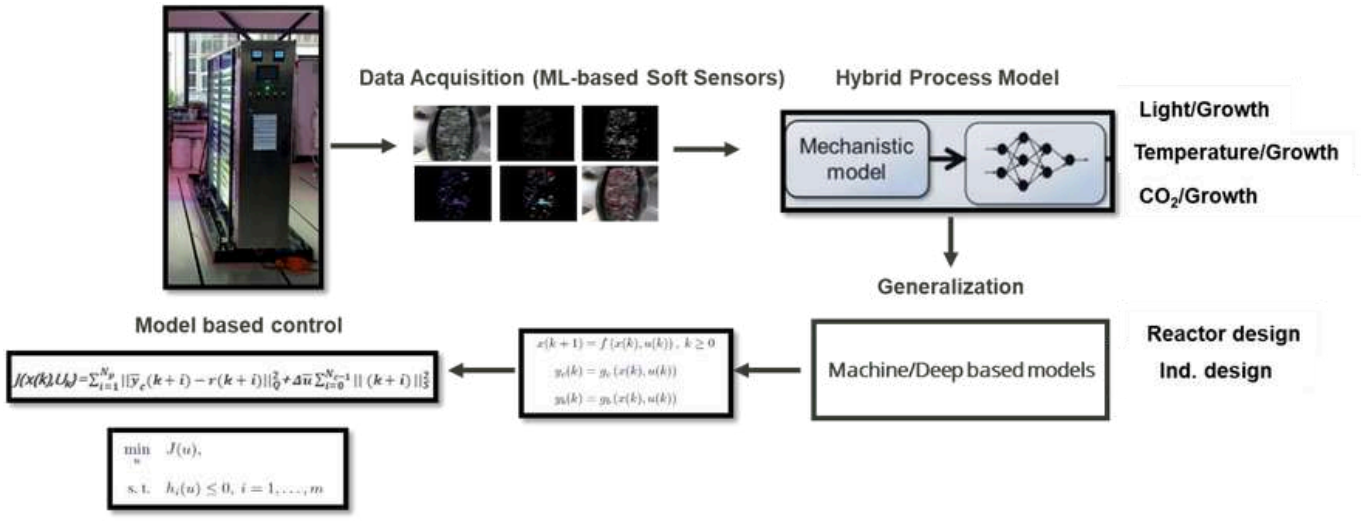
Tehreem Syed - July 15, 2022

Microalgae are the principal producers of biomass in aquatic environments, ensuring the survival of life on Earth. They have gained significant attention worldwide due to their vast biopharmaceutical, nutraceutical, and renewable energy applications. Open and closed photobioreactors (PBRs) are used to cultivate microalgae, which is a complicated method where biological, environmental, and design variables affect the system, and those biological variables have complicated dynamics to be modeled and controlled.

Several predictive models have been published that account for the complexities of bioprocesses. The fermentation processes and algal photo-production systems have been simulated, optimized, and scaled up by employing Monod and the Droop model. However, recognizing an appropriate model structure to evaluate physical knowledge is complex and usually demands massive production time. Therefore, machine learning (ML) and deep learning (DL) have been used for bioprocess dynamic modeling and online optimization. These models can capture complex process behavioral patterns in a particular operational range without prior physical knowledge. However, they have numerous weaknesses, including difficulties in extrapolating an extensive range of metabolically controlled process behavior and the risk of model overfitting.

In recent years, a third modeling approach such as hybrid modeling has been proposed to address these issues. This approach integrates non-parametric (black-box) and parametric (white-box) into a hybrid model system to benefit from mechanistic and data-driven models.

The flow of hybrid modeling for monitoring and optimization



Hybrid Modeling Platform for Process Monitoring and Optimization

Tehreem Syed - July 15, 2022

Therefore, this project aims to implement hybrid modeling to monitor, predict, optimize, and control microalgal processes. The data will be acquired from different photobioreactors and will cover information about the interaction of the algae metabolism and the process operating conditions.
The physics-based mechanistic models on the relations between process parameters such as light, temperature, and dissolved oxygen and the algae's behavior serve as a starting point for designing the platform architecture and as objective functions for the ML and DL methods. The trained, reactor-specific models serve as state observers for monitoring and provide a basis for optimal control and Model Predictive Control schemes (MPC) applied to algae plants.

Assessment of light dynamics on growth performance and definition of model- based protocol for algae selection and genetic manipulation

Tim Michelberger - October 25, 2022

Over the last two decades, microalgae have become an increasingly popular source for the sustainable production of pharmaceuticals, food and feed, and other goods. However, the cultivation of microalgae and the production of valuable products still has limitations that have not yet been overcome. For instance, the cultivation of microalgae in industrial plants compels the algal cells to live in an environment that differs strongly from their natural habitat.

During cultivation in industrial photobioreactors, microalgae reach extremely high cell densities whereas in nature they live in rather dilute communities. In photobioreactors cells can therefore experience strongly variable light conditions due to mutual shading, as well as nutrient limitation.

Moreover, the chemical composition of microalgae is often not completely congruent with our desired pipe dreams, forcing scientists to often choose their microalgae species by trading-off between optimal growth rates, ease of cultivation and preferred cell composition.

To address these challenges, genetic engineering of microalgae strains can be a powerful tool to adapt algal cells to industrial cultivation or to alter their chemical composition towards our desired profiles.

There is a plethora of techniques for genetic engineering; however, most of them rely on deleting or overproducing gene products which can then affect photosynthetic efficiencies or metabolic pathways.

Importantly, the behavior of genetically engineered microalgae strains often strongly depends on their culture conditions including for example light intensity, temperature, nutrient availability, or the geometry of the photobioreactor.

Moreover, the same genetic alterations can lead to highly diverse effects in different species.

Microalgae culture for lab-scale experiments

Assessment of light dynamics on growth performance and definition of model- based protocol for algae selection and genetic manipulation

Tim Michelberger - October 25, 2022

For this reason, we need to investigate differences in the photosynthetic apparatuses of microalgae to identify interesting targets for genetic engineering.
Under the supervision of Prof. Tomas Morosinotto, I am generating mutants of different players involved in photosynthesis in microalgae of the genus Nannochloropsis, to analyze their effect on the photosynthetic efficiency. Furthermore, I aim to alter the chemical composition (e.g., protein composition) of these microalgae to adapt their nutritional value and optimize them for industrial use.
Subsequently, I will characterize the generated mutants in variable cultivation systems to identify the growth conditions under which we can exploit a putative positive effect of our genetic alteration the most.

Modeling microalgae growth: challenges and perspectives

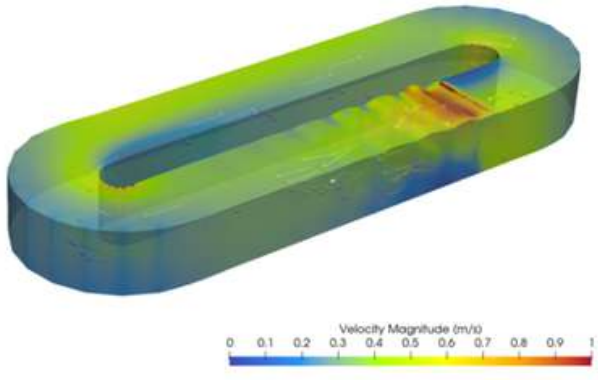
Ignacio Fierro - October 25, 2022

Microalgae growth is highly affected by the light intensity that they receive. Lack of light drives slow growth, whereas over-exposition to strong light can damage certain proteins on the cells and slow down the photon harvesting process. Typically, both conditions are present in photobioreactors: one single cell can perceive low light in the deepest part of the culture and suddenly, due to mixing, go to the most illuminated part driving photoinhibition. We do not fully understand how the exposition to a strong light gradient in the culture affects the growth of each individual cell, yet some mathematical models are able to predict the production of microalgae in some conditions.

In addition to the fact that microalgae face a strong light gradient in bioreactors, they have evolved adaptive mechanisms to overcome photoinhibition, the so-called photoacclimation, in which each cell can modify the amount of pigments in order to avoid damage to the photosynthetic machinery.

Both phenomena have different timescales: while the photodamage and recovery processes have timescales of minutes, photoacclimation has a timescale of days. Both phenomena affect the growth rate of the microalgae. Coupling two different models for different phenomena is challenging, especially if they have different timescales.

Mathematical models are calibrated on the basis of available experiments. The conditions under which these experiments are performed are also important. Not all bioreactors have the same geometry and operating conditions; some of them have more than one species of microalgae, and the resulting interactions cannot be neglected. Moreover, some mathematical models can be very accurate in some conditions and useless in other situations. Modeling the growth rate of microalgae can be particularly complex.



Velocity field simulated in a CFD program. Cell positions are tracked to compute the light that they perceive; the growth rate of each cell can then be estimated

Modeling microalgae growth: challenges and perspectives

Ignacio Fierro - October 25, 2022

A relevant issue is related to the scale-up from laboratory conditions to the industrial scale, where several processes must be optimized. Mathematical modeling plays an important role in this situation, where an accurate model can be used for monitoring, control, and optimization. Combining computational fluid dynamics (CFD) and biological models to estimate the growth rate in a bioreactor is getting more attention. By doing so, we can track the light history that a single algal cell perceives as it moves through the bioreactor (see image). We can then estimate the growth of a single cell and extrapolate to the entire bioreactor productivity. There are some obvious limitations: running a computational fluid dynamics model is expensive in terms of computational resources; moreover, since biological models were developed using experiments that have different configurations, there is no model that can perfectly describe the growth rate of a single cell.
To summarize, modeling the growth of a microalgae culture is a highly complex task, especially at industrial scales where many factors must be considered. These factors can be biological or specific to the bioreactor operation. Our goal is to create a model that can be used for monitoring and optimization of processes to obtain the best performance of the culture taking into account biological and operational factors.

Modeling and control of microalgae-based wastewater treatment

Rebecca Nordio - October 25, 2022

Microalgal biomass is increasingly gaining interest in new emerging sectors such as biogas and biodiesel production, animal and aqua feed, biopesticides, pharmaceuticals and biofertilizers. Apart from biomass production, starting from the 1960s, microalgae were studied as a new water remediation technology, with the advantage of guaranteeing fewer greenhouse gas production, less energy demand and costs, and less production of residues (i.e., sludge). Moreover, the coupling with wastewater treatment can be considered a cost-effective algal production, as the nutrients necessary for the microalgal growth come directly from the wastewater and do not have to be provided by chemicals or fertilizers. In sewage, microalgae live together with bacteria typically classified as nitrifying and heterogenic. Thanks to their synergy, it is possible to guarantee the degradation of organic matter and the recovery of nitrogen and phosphorous. In a few words, microalgae produce the oxygen that is used by bacteria, while bacteria degrade the organic matter and produce the organic carbon necessary for microalgal life; moreover, two different groups of nitrifying bacteria (ammonia-oxidizing bacteria and nitrite-oxidizing bacteria) enhance the nitrogen recovery, using the ammonia (NH_4^+) and transforming it into NO_3^-, used by microalgae.
Although the potentiality of this remediation process is already proven at laboratory scale, the process still needs to be further optimized in terms of productivity and nutrient recovery at pilot and industrial scale dimensions, as it still is facing challenges related to costs and efficiency. The most used design for wastewater treatment is the open pond, which ensures low capital investment and easy maintenance, permitting the treatment of high water volumes thanks to its high volume-to-surface ratio.

Raceway reactors installed at the University of Almeria

Modeling and control of microalgae-based wastewater treatment

Rebecca Nordio - October 25, 2022

However, this technology allows a narrow control of the most impacting parameters, since the system is exposed to culture contamination, and variables like light and temperature continuously change based on the weather variation. Apart from developing successful technology for biomass production, further optimization needs to be performed for the other process parameters that can be regulated, such as pH, levels of oxygen dissolved, culture level, and harvest/dilution strategy. Therefore, it is fundamental to study their impact on the culture performance and the microalgae-bacteria equilibrium to define the optimum conditions for nutrient recovery and the process remediation efficiency. This objective can be carried out by optimization analysis in large-scale systems, permitting the provision of valuable information and further leading future work to enhance process optimization and automation. Moreover, mathematical modeling proves to be a very powerful tool because it provides a process description depending on the process parameters and also allows to perform simulations (to better understand the process) and the implementation of advanced control strategies.
The objective of the research project at the University of Almeria, under the supervision of Prof. Gabriel
Acién and Prof. José Luis Guzmán, is to optimize and model microalgae wastewater treatment processes. The project will be carried out outdoors in pilot-scale raceways reactors available in the pilot plant of the University of Almeria. In these facilities, optimization analysis and development of new control strategies and new virtual sensors for culture monitoring and control will be done. Moreover, mathematical models will be developed allowing to describe the microalgal growth and the remediation efficiency and permitting the implementation of simulation tools.

Spectroscopic on-line biosensors for monitoring and control of microalgal cultivations

Luis Porras-Reyes - January 17, 2023

Microalgae are well known photosynthetic microbes used as cell factories for the production of relevant biotechnological compounds (e.g. pigments, unsaturated fatty acids) and environmental applications (e.g. wastewater treatment, lipids for biofuel). Despite these outstanding characteristics, industrial scale production of microalgae is still at an early stage of development. Moreover, the economically viable support of the sustainable large-scale production of microalgae at the present state of technology is a challenge.

One important bottleneck in this support is the lack of suitable on-line sensors for the reliable monitoring of biological parameters, mostly concentration of intracellular components, in microalgae bioprocesses. The use of spectroscopic sensors and non-optical methods (e.g. NMR, Mass spectrometry) provides interesting alternatives to overcome this situation. Sensors based on optical methods (e.g. spectroscopy of absorption, reflectance and scattering, fluorescence, microscopy, and multispectral imaging) have proven their applicability and reliability to target relevant biological variables in microalgae bioprocess for example biomass, cell concentration, nutrient consumption (e.g. NO3, glucose), intracellular products (pigments and lipids), physiological state (e.g. photosynthetic efficiency), cell morphology, and presence of contaminants.

Together with spectroscopic sensors, the application of software sensors, i.e., estimators based on model and data driven process models, using physical signal inputs of multiple sensors could complement the on-line monitoring as it allows the estimation of the desired biological process variables in real or almost real time which are often hidden in the spectroscopic data and only indirectly accessible.

Raceway reactors installed at the University of Almeria

Spectroscopic on-line biosensors for monitoring and control of microalgal cultivations

Luis Porras-Reyes - January 17, 2023

But which techniques are used in the development of a software sensor? For that purpose, different techniques are employed including artificial neural network (ANN), deep neural network, adaptive interval observer, Luenberger observer, Kalman filter, Principal Component Analysis (PCA), and PLS regression model. Also, these techniques could be employed in combination, in parallel or in sequence. For example, multiple linear regression in combination with mechanistic model or support vector regression together with random forest regression.
Considering the forgoing, the development of a non-invasive optical on-line biosensors represents an interesting opportunity within the context of microalgae bioprocesses. The expected results in this work include the development of a novel on-line sensor system combining hardware sensors and advanced real-time data processing, using software sensors, for a fast automated estimation of different relevant biological process parameters.

Data mining approaches for monitoring and decision support of indoor microalgae production

Shyam Krishnan - January 17, 2023

Microalgae are rich sources of protein, lipids, carbohydrates, and pigments. During the last decades, they emerged as a sustainable source of raw materials in the food, feed (aquaculture), nutraceuticals, medicinal and cosmetic industries. The high production cost associated with industrial microalgae manufacturing is a pressing concern that challenges their adoption as a viable source of green energy fuels and raw materials in other industries. The question of variable algae quality associated with open outdoor production can be addressed using a closed indoor production strategy with better control systems. Yet the burden of production cost is more severe in an indoor production system. The microalgae production cost can be reduced by improving photosynthetic efficiency, process optimization and automation. Real-time multi-parameter optimization for maximal growth and efficient automation with process control techniques for reduced labor cost are interesting solutions to explore from an industrial engineering perspective.
A major focus of this research is to develop data mining approaches for monitoring and decision support of indoor algae production in the proprietary vertical flat panel photobioreactor system (ProviAPT) developed by Proviron, Belgium. At Proviron, fully automated microalgae cultivation is functional across lab scale, pilot scale and production scale units. All three units are equipped with Siemens PLCs, Arduino and Raspberry Pi modules integrated with a supervisory control and data acquisition system ('OpenSCADA'). Despite high productivity and automation levels, there is room for improvement, especially in growth parameter optimization and further improving biomass productivity. The large volume of online data generated by sensors and actuators archived by the SCADA system enables the application of data mining techniques and machine learning models for prediction and process control strategies.

Indoor microalgae production unit at Proviron, Hemiksem, Belgium

Data mining approaches for monitoring and decision support of indoor microalgae production

Shyam Krishnan - January 17, 2023

This demands the design and development of a centralized data warehouse. Based on unit-level data acquisition, the online data available from the SCADA system is currently collected at four distinct levels from the lab and pilot-scale photobioreactors. Reactor unit level data (sensor output and actuators input), industrial log data, feed unit data (nutrient recipes, feed volumes), calculated (real-time and cumulative) growth data, offline measurement data and metadata are cleaned, preprocessed, and stored. A 'R Shiny'-based dashboard is developed on top of the data warehouse to enable statistical analysis, visualization, and monitoring.

The long-term objective of this research consists in developing models that are simple, transferrable, and capable of predicting optimal growth conditions from the real-time monitoring data with high accuracy for an industrial microalgae production environment. The big data volume and disruptive changes in computation power enable the application of statistical, machine learning and deep learning techniques. At any stage, collecting, integrating, and processing data in a uniform way is crucial to developing reliable predictive models. Still, the application of machine learning techniques on bioprocess data is not a simple task. Hybrid deep learning models or a combination of machine learning models with mechanistic models are interesting strategies we plan to explore in this scenario.

In an industrial production environment, the quality of data from the sensors and actuators are important parameters to consider. Data quality validation based on statistical outlier detection on industrial and bioprocess variables in the early stage of the research is a significant task. Unsupervised multivariate outlier detection models can be used to identify bioprocess anomalies and detect and flag abnormal industrial process variable behaviors. The insights from these models can be further used for identifying and predicting sensor and actuator failures and planning maintenance schedules.

Upon optimizing the data integration procedure using data transformation and feature engineering techniques, we can expand the predictive models to include the various growth conditions and discover patterns between growth conditions and outcomes. The generated models should be evaluated for their capability to support an operator in defining improved growth conditions in an industrial production environment. The results should be validated based on two criteria:

1.What growth conditions are expected to achieve the maximal yield?

2.What growth condition combinations should be evaluated next to allow to improve the model most efficiently?

The outcomes can be further used as feedback to improve the model.

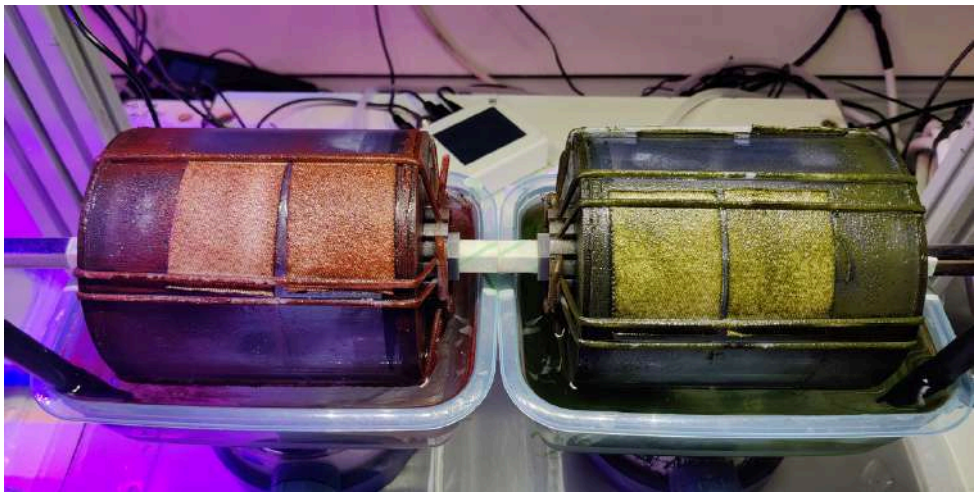
Rotating green to red: a biofilm-based approach to astaxanthin production

David Pereira - January 17, 2023

Microalgae are photosynthetic microorganisms able to convert light energy and inorganic nutrients into high-value compounds such as fatty acids, polysaccharides, or carotenoids. Astaxanthin, a red carotenoid pigment, is a very attractive molecule due to its antioxidant properties, which are significantly stronger than those of other carotenoids and vitamins. It has numerous applications in various industries, including cosmetics, aquaculture, nutraceuticals, food, feed, and pharmaceuticals. While most astaxanthin on the market is produced synthetically, the main natural source of astaxanthin, which still represents less than 1% of the total commercialized amount, is the microalgae Haematococcus pluvialis.

Scaling up the commercial production of astaxanthin from the microalgae H. pluvialis presents several challenges due to its unique metabolism. The biosynthesis of astaxanthin in H. pluvialis is a complex process that is highly up-regulated in conditions of stress and which coincides with the accumulation of triacylglycerols (TAGs). Thus, optimization of the various culture conditions such as nutrient level, light, pH, and temperature is necessary to achieve maximum biomass and maximum astaxanthin production. However, most of these optimal conditions are exclusive to each phase (biomass or astaxanthin). As a result, the current industry standard for cultivating these microalgae for astaxanthin production involves a two-phase strategy. First, in the "green" vegetative growth phase, cells are initially grown under favourable conditions like low light and excess nitrogen. Once optimal biomass concentration is reached, the "red" inductive production phase is triggered by exposing the microalgae to stress conditions such as high light, salinity, or an imbalanced carbon-to-nitrogen ratio, enhancing astaxanthin synthesis. Under one or a combination of these factors, H. pluvialis is capable of accumulating astaxanthin up to 5% of its dry weight.

Rotating biofilm-based system at CentraleSupélec, with Haematococcus pluvialis biofilm in both red and green stages



Rotating green to red: a biofilm-based approach to astaxanthin production

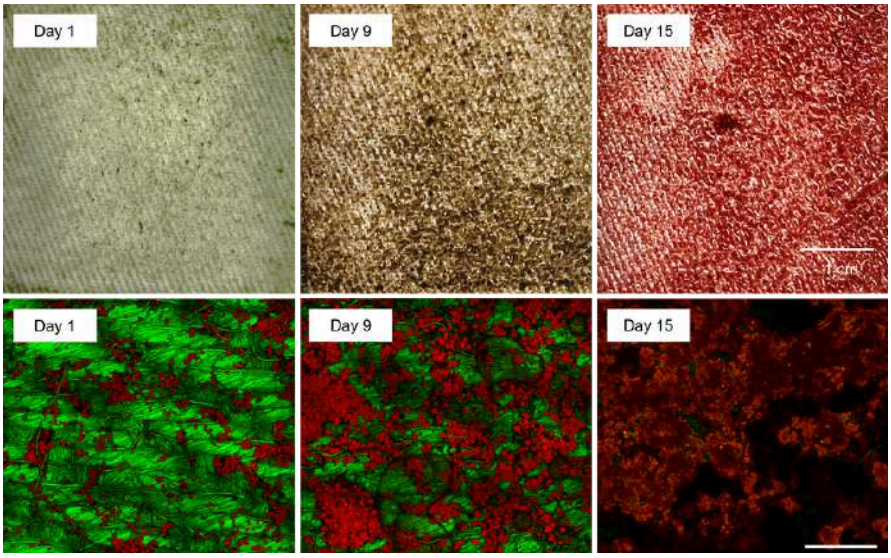
David Pereira - January 17, 2023

While the two-phase process has been shown to be more productive than one-phase strategies in the literature, it also comes with significant drawbacks. Specifically, in the case of producing astaxanthin, half of the cultivation period is unproductive, lowering overall productivity. These challenges are further exacerbated when using traditional suspension cultivation methods, where microalgae cells are grown in liquid nutrient media before being harvested for molecule extraction. One approach to overcome these issues is the use of biofilm-based systems, where microalgal cells are closely associated with a substratum, separating most of the biomass from the bulk culture medium.

The key advantage of using a biofilm-based system in the production of astaxanthin is the significant reduction in the reactor and medium volume required to cultivate an equivalent amount of biomass. The high density of dry biomass eliminates the need for dewatering before downstream processing, making the process much more energy-efficient. In contrast, in planktonic cultures, which typically have low biomass concentrations of less than 10 g/L, the majority of the culture is represented by water rather than algal biomass, resulting in significant energy demand for harvesting. As a result, these systems are characterized by low productivity, high energy demand and high operating costs associated with the production and harvesting steps, becoming a bottleneck for commercialization.

To answer all these questions, our project aims to optimize the production of astaxanthin in H. pluvialis, in a rotating biofilm-based system using a combination of experimental, modelling and control strategies.

Images of the cotton fabrics on days 1, 9, and 15: accumulation of astaxanthin over time (from green to brown to red). Confocal laser scanning microscope images of the same fabric supports: cells (in red) attached to a cotton fabric (in green).



Combined effect of temperature and light intensity on microalgae growth in small scale photobioreactors

Beatriz Felices-Rando - April 19, 2023

Temperature and light intensity are the main factors affecting microalgae growth in unlimited nutrient conditions. The approach of assuming temperature and light intensity as two independent factors in photosynthesis models is generally accepted — however, a weak coupling between both variables is already observed at low light intensities, while limited data about the joint effect of temperature and light intensity are available under photoinhibiting light intensities.

At low light intensities, the photosynthetic rate is limited by the rate of photon supply, which is considered to be independent of temperature. At high light intensities, photosynthesis is no longer limited by photon supply, while temperature, on the other hand, can affect the rate of photosynthesis dark reactions, causing a potential imbalance between energy supply and energy consumption and affecting the threshold for saturation. A stronger coupling between light and temperature could therefore be expected at higher light intensities. Besides the short-term effects of temperature on growth rate, long-term effects may as well occur (acclimation to temperature) as well as changes in cell morphology.

An ongoing project at University of Padova is aiming to study the coupled effect of light and temperature at photosaturating light intensities on the microalga Scenedesmus obliquus. Figure 1 shows two small scale photobioreactors (PBRs) that are being used to test different combinations of light and temperature on acclimated steady state continuous microalgae cultures. Reducing the scale of PBRs can accelerate the process of data acquisition while easing the control of the manipulated variables and reducing the experimental effort.

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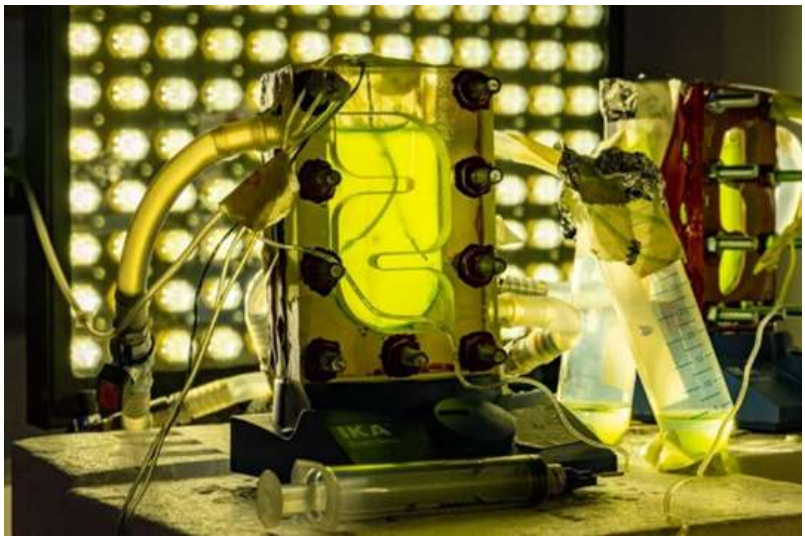
Figure 1. Milli-photobioreactors used for the experiments (volume = 45 mL; thickness = 15 mm).



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Beatriz Felices-Rando - April 19, 2023

Around 48h after inoculating the reactors with the cells (in batch conditions), exponential growth phase is reached, and the reactors are connected in continuous operation. Optical density at 750nm (OD750) is measured every day to track stability. After 3 days with a stable biomass concentration, dry weight, cell count, pigment analysis, and image analysis are performed to characterise every sample.
Provisional results seem to indicate that the coupling between light and temperature is actually not very strong even at photosaturating light intensities; nevertheless, fitting the obtained data with an uncoupled model (i.e. a model that considers temperature and light intensity to be two independent factors) would be the final test to confirm this hypothesis.

Accounting for transient changes in bioprocess conditions for robust process monitoring and improved control of microalgal processes

Henrik Geltner - April 19, 2023

Microalgal biotechnology and the production of microalgae-derived products are promising technologies in the industrial transformation to create a more circular and biobased economy with net zero carbon footprint.
For the production of microalgae, many process conditions have to be controlled according to specific environmental requirements by the microalgal strain of interest. These requirements include for example the control of process acidity and temperature to be maintained in identical range to the biological niche of the microorganism in nature. Aside from these more general process conditions, it is necessary to supply the microalgal cells with enough nutrients and energy to maintain their vitality and growth. Typically, energy supply of algae is achieved by illumination and consequent harvesting of light energy by the photosynthetic apparatus of the algae. The growth processes of microalgae cultures and multiplication of cells over time has the consequence that more nutrients and energy are required to maintain the culture and provide conditions for growth. As it is not possible to infinitely augment the supply of energy and nutrients proportionally to the increasing number of cells, this ultimately leads to process conditions of limited growth, where one or more factors are less available per cell and thereby limit the speed of cell growth. Such shifts from growth assuring into limiting conditions often trigger the cells to not only grow slower but also undergo changes in cell internal processes and in their morphology. Some of these changes are due to evolutional traits of microalgae and can be seen as a microalgal skill set that can actively be exploited. By helping microalgae in adjusting to their constantly changing natural environments which are governed by daily and seasonal fluctuations in light, temperature, and nutrient levels, these response mechanisms give algae their versatility and robustness.

Figure 1. Batch cultivation of Nannochloropsis oceanica.

Accounting for transient changes in bioprocess conditions for robust process monitoring and improved control of microalgal processes

Henrik Geltner - April 19, 2023

In many cases these mechanisms are employed intentionally in industrial productions. This way, by actively changing certain process conditions, one can force the algae cultures into metabolic states where the production of a byproduct of interest is enhanced. However, not all such changes in cell properties seen in microalgae are a consequence of internal reaction mechanisms: transient cell alterations might also be a result of the degradation of vital microalgal components and consequently cell death. For these reasons, the task of process monitoring is of major importance to the design of industrially feasible microalgal productions.

High levels of control which are demanded in industry raise the urge for monitoring techniques that supply operators constantly and reliably with information on all important process parameters at a fast rate and with minimal time delays between measurement and control action. Accuracy and reliability of measurements for general biological quantities must not be susceptive to changes in cell state, as cells might change their properties without changes to their overall quantity. At the same time, the monitoring systems should also detect fluctuations in cell properties in order to identify whether metabolic shifts occur and to distinguish between intentionally exploited mechanisms and unwanted processes happening such as culture deaths.

These ambiguous requirements to monitoring growing cell cultures cannot be satisfied by analyzing and describing the process through one single measurement technique only. For instance, the analysis of dry weight concentrations, which is a commonly employed method to describe developments of bioprocesses, does give a good estimate about the total biomass being produced. However, this measure does not differentiate whether an increase in biomass happened because of cell division and growth or due to the enrichment of cells with byproducts like carbohydrates that expand them in size and mass without further cell division.

The assessment of biological growth through elemental mass balancing and design of biomass and bioproduct predictors by a software sensor-based approach are promising approaches for the design of metabolic state sensitive monitoring of microalgal processes.

While elemental mass balancing, which is based on laws of mass conservation, allows to describe biomass enhancements as a general measure of quantities, it enables to certain degrees the differentiation of biomass into characteristic groups. The increased productivity of such classes of molecules can simultaneously be detected and described through changes in the ratios between different elemental conversion rates. The estimation of built products and byproducts is subsequently done based on stoichiometric model assumptions of growth and respiration.

Accounting for transient changes in bioprocess conditions for robust process monitoring and improved control of microalgal processes

Henrik Geltner - April 19, 2023

Soft sensing approaches have the advantage of using multiple parameters from obtained sensor networks data for estimation of parameters. Thereby they allow not only prediction of otherwise unmeasurable factors to the process but also they can be designed to account for changing process conditions, rendering them robust parameter estimators.

In this project we develop software sensors to monitor elemental conversion rates during microalgae growth. By employing elemental mass balancing approaches to these conversion rates, we aim to simultaneously estimate biomass evolution and biomass differentiation on-line and reduce disrupting effects of changing cell properties and process conditions. The focus of this project is set to oxygen, carbon dioxide and nitrogen as these compounds account for a mass fraction of ~90% of microalgal biomass.

Nitrogen is one of the essential macronutrients for balanced growth of microalgae. Its uptake rates are closely tied to accumulation of other byproducts such as lipids and carbohydrates in microalgae. Optimal microalgal biomass and lipid production therefore will require strategies with precise control of nitrogen levels to meet the optimal trade-off between promoting microalgal growth and enhancing lipid productivity. Current strategies of measuring nitrogen uptake require the collection of samples and time-demanding sample processing and analysis, making them unsuitable for automized process control.

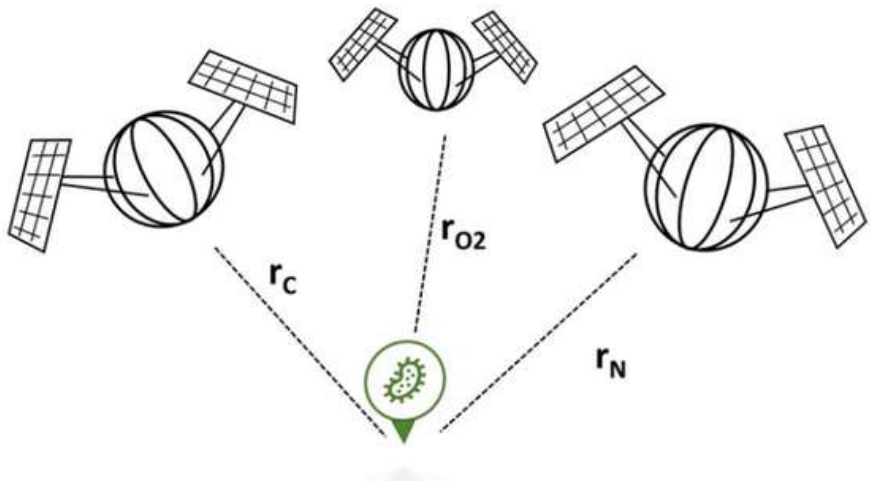


Figure 2. On-line elemental mass balancing employs multiple conversion rates to determine process states and development of microalgal growth processes.

Accounting for transient changes in bioprocess conditions for robust process monitoring and improved control of microalgal processes

Henrik Geltner - April 19, 2023

The investigated approach for soft sensing nitrogen uptake rates in microalgae relies on interaction of charged nitrogen species such as nitrate and ammonium and the overall charge balance of process medium. The uptake of the two molecular nitrogen species changes the equilibrium of charge balance and can thereby cause protonation or deprotonation of culture medium, which can be seen by shifting pH values during growth and nitrogen uptake processes. These shifts might be corrected by a pH dependent strategy of nitrogen supply in form of acids or bases to simultaneously tackle protonation and deprotonation of the process.

Acidophilic microalgae: the new alternative for phycocyanin production at industrial scale

Rosaria Tizzani - April 19, 2023

Polyextremophile acidophilic microalgae are a unique class of microorganisms that thrive in extreme environments with extremely low pH levels and high temperatures. These organisms are capable of surviving in very harsh conditions, making them potentially useful, for instance, in the mining and metal recovery industries. The acidity of the growing media hinders the development of bacterial contamination, an impacting problem in microalgal fermentation processes that involves the use of organic carbon. This unique acidic environment represents a huge benefit for food-grade production on an industrial scale. Ongoing research into their molecular biology and metabolic pathways proves that these microalgae are a promising area of study for both basic science and practical applications.

Galdieria sulphuraria is a unicellular red alga belonging to the class Cyanidiophyceae and is found in acidic hot springs with temperatures up to 56°C and a pH range of 0.5 to 4.5. Galdieria is an extremophilic red alga that can grow in extremely acidic and hot environments, such as geothermal springs and volcanic sites, using a range of metabolic pathways. Its growth and metabolism are greatly influenced by the availability of organic carbon sources and the presence of light. One of their unique metabolic pathways is the use of the C4 pathway for carbon fixation, which helps them to survive in low-CO2 environments. Additionally, Galdieria is known for its ability to perform mixotrophic metabolism, which involves utilizing both photosynthesis and heterotrophy to obtain energy and nutrients. This allows them to grow in a wide range of conditions, including those with limited light and nutrients.

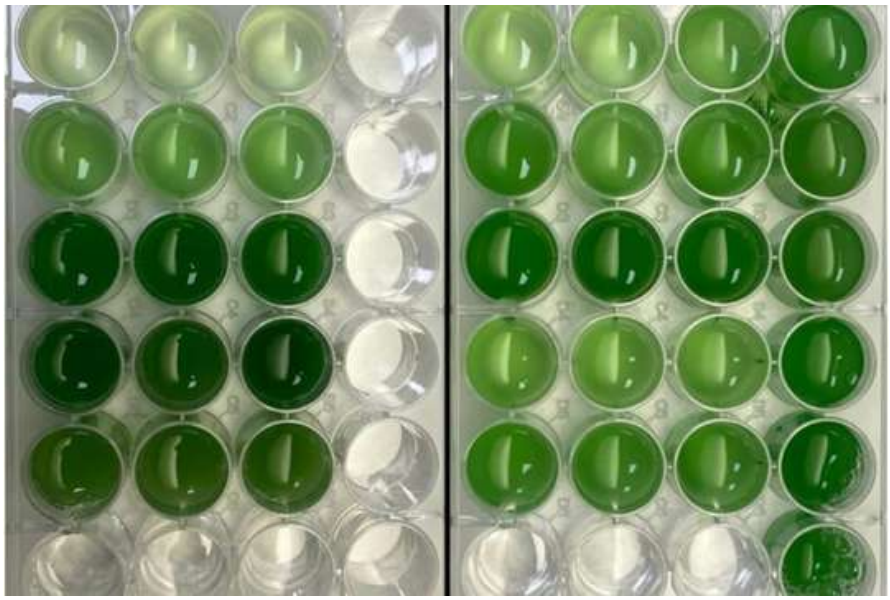


Figure 1. Screening of different concentration of glucose cultivating Galdieria sulphuraria in a 24 microwell plate

Acidophilic microalgae: the new alternative for phycocyanin production at industrial scale

Rosaria Tizzani - April 19, 2023

Organic carbon sources, such as glucose or pyruvate, are used by Galdieria to generate energy through glycolysis and the tricarboxylic acid (TCA) cycle. Galdieria's metabolism is characterized by a high rate of respiration, allowing it to thrive in oxygen-rich environments. Studies have shown that the availability and type of organic carbon source can affect the growth and metabolic activity of Galdieria. For instance, when grown on glucose or pyruvate as the sole carbon source, Galdieria has a higher growth rate and produces more pigments than when grown on inorganic carbon sources like CO2.

Furthermore, the type of organic carbon source affects the composition of Galdieria biomass. For example, when grown on sugars like glucose or sucrose, Galdieria produces more lipids and starch, whereas when grown on organic acids like acetate or lactate, it produces more proteins and amino acids. The availability and type of organic carbon source can have significant effects on the growth and metabolism of Galdieria, with important implications for potential biotechnological applications, such as in the biofuels and bioproducts industries.In this study, we performed a screening of three organic carbon sources to identify the best option and concentration applicable at an industrial scale. Algae growth under photoautotrophic conditions was optimized to boost biomass productivity in the first step. In the second step, different conditions were applied to induce the production of high-value metabolites, like phycocyanin. Glucose at 4.5 g/L was the best option among the three organic carbon sources tested at five different concentrations in presence and absence of light. The increased content of phycocyanin was related to the enhanced growth rate under mixotrophic conditions. Abiotic factors such as the C:N ratio and the light intensity play a role in the enhancement of phycobiliproteins, making Galdieria an exceptional alternative to phycocyanin-producing microalgae like Spirulina. Acidophilic algae are a fascinating subject for scientific investigation and a potential candidate for biotechnological applications.

Photochemistry on a budget: solving the Cryo-EM structure of a long-wavelength photosystem I

Giovanni Consoli - July 17, 2023

In the last decades, different photosynthetic organisms were identified which can exploit longer wavelength photons to drive photosynthesis; both academia and industry showed a renewed interest in trying to couple it with existing photosynthetic bioprocesses, with the objective of achieving higher productivities. These organisms benefit from an extended absorption range (up to 750 nm) that would account for a 19% nominal increase in available photons, and allow improving the theoretical maximum efficiency of photosynthesis. However, photosystems (PS) conduct extremely energy-insensitive redox chemistry, in which most of the energy absorbed from photons is consumed to handle the dangerous chlorophyll a photochemistry, to limit the damage incurring from side- and back-reactions, and at the same time maintain the yield of forward electron transfer as high as possible. Using lower-energy photons to perform the same reactions, as in the case of chlorophyll f containing photosystems, puts even more stringent bioenergetic requirements.
To characterize these huge protein complexes, research in the fields of bioenergetics and photosynthesis has always relied on spectroscopic and modelling techniques, and since the advent of structural biology, on high-resolution protein structures which represent a key element in elucidating the chemistry, mechanisms and energy fluxes of the molecular machineries of interest. With the recent advancement in the field of cryogenic electron microscopy single particle analysis (Cryo-EM SPA)—an ensemble of data processing techniques that enable the determination of protein electrostatic potential maps from transmission electron microscopy images of a monodispersed solution of the protein of interest—it is now possible to obtain high-resolution structures of very large complexes using considerably fewer samples, lower time and optimization than those required by protein crystallography. What a decade ago was thought to be impossible is now becoming a predominant approach in many fields. Cryo-EM Single Particle Analysis (SPA) is collected at liquid nitrogen temperature (77 K).
Consequently, the structure of multiple PSI and PSII supercomplexes from cyanobacteria, algae, and plants were determined within the last five years, showing the extreme diversity of evolution in the organization of the antenna around the highly conserved cores. The importance of these contributions not only lies in the identified structures; the possibility to understand the in vivo organization of complexes in the thylakoid membranes also represents a paramount aspect. A new era for structural biology has started, in which Cryo-EM will likely play a dominant role in determining the structures of large multi-subunit membrane complexes. In this project, we focused our effort in solving the structure of a far-red light adapted photosystem I, the second light driven protein complex in the photosynthetic electron transport chain.

Photochemistry on a budget: solving the Cryo-EM structure of a long-wavelength photosystem I

Giovanni Consoli - July 17, 2023

Harnessing the energy deriving from photons, photosystem I has the role of oxidizing plastocyanin, a soluble copper electron carrier on the luminal donor side reducing ferredoxin on the stromal acceptor side, generating one of the most negative redox potentials in nature.
The structural information obtained will help us assign the 8 sites containing the long-wavelength pigments, allowing us to mathematically model excitation energy transfer and charge separation in the complex, bringing us one step closer to understanding the functional penalties of long-wavelength photosystems, and recognizing their possible biotechnological applications.

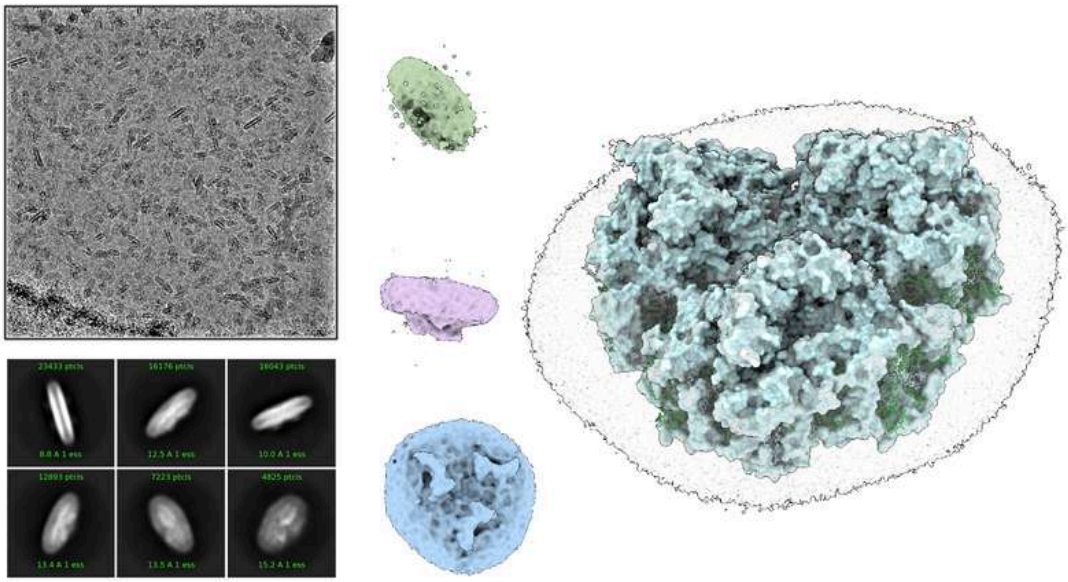


Figure 1. Main processing stages of the structure of far-red PSI obtained through Cryo-EM SPA. Left: the original images, the 2D classification of the particles selected, and the 3D volumes obtained. Right: the molecular model fitted to the experimental data.

Exact designs of optimal experiment campaigns

Marco Sandrin - July 17, 2023

Model-based design of experiments (MBDoE) for parameter precision can greatly accelerate the development of predictive mechanistic models. A particular focus has been on sequential MBDoE, where (possibly dynamic) experiments are designed one-at-a-time using gradient-based techniques. For nonlinear models, the resulting optimisation problems are typically nonconvex, and thus prone to converging to local optima. Numerical failure caused by singular information matrices is also commonplace. Such computational challenges hinder the design of experimental campaigns comprising multiple parallel runs.

Effort-based methods overcome these challenges by discretising the experimental space into a finite set of candidate experiments. They determine the fraction of the total number of experiments (the “effort”) associated with each candidate, with the objective of selecting the combination of experiments that will generate the highest information content. Treating the efforts as continuous variables leads to a convex formulation that can be solved efficiently using convex optimisation techniques. However, the optimised efforts typically assume fractional values, which need to be rounded to integer values in a subsequent step. This a posteriori effort rounding can result in large suboptimality, especially for campaigns with a small number of experiments.

As part of the research project, a discrete-effort methodology has been developed for the exact design of experiment campaigns comprising a finite number of experiments. Integrality constraints on the number of experiment replicates are enforced directly through pure integer programming, with a convex objective function and linear constraints. The experimental design space is discretised using low-discrepancy sampling, and the Fisher information matrix is computed for each candidate experiment. The resulting optimisation problem considers one of the standard experiment design criteria (e.g., D-optimality, A-optimality) applied to the overall information matrix of the campaign.

Two prototype implementations of the proposed approach are under development: the first one as a new experiment design solver withing the gPROMS modelling framework, the second in Python by leveraging a pre-existing package developed at Imperial College London. Several optimisation techniques were evaluated for the solution of the pure integer program; these include the classical outer-approximation algorithm used in mixed-integer nonlinear optimisation, as well as more advanced algorithms that attempt to capture the curvature of the objective function. Outer-approximation proved to be the most reliable option due to significant number of optimisation variables involved, and several modifications were implemented to tailor the solution algorithm to the considered optimisation problem and effectively speed up the computations.

Exact designs of optimal experiment campaigns

Marco Sandrin - July 17, 2023

The application of the proposed methodology on selected case studies has shown that experiment campaigns obtained by solving the exact design case lead to better objective function values than those obtained by the combination of continuous-effort formulations and apportionment, and the benefit is particularly relevant when considering low number of experiments. The method prioritises experiments with exceptional information content, whereas continuous-effort designs attempt to include as many candidates with nonzero effort as possible. In addition, the increase in computational effort with respect to continuous-effort methods is often negligible.

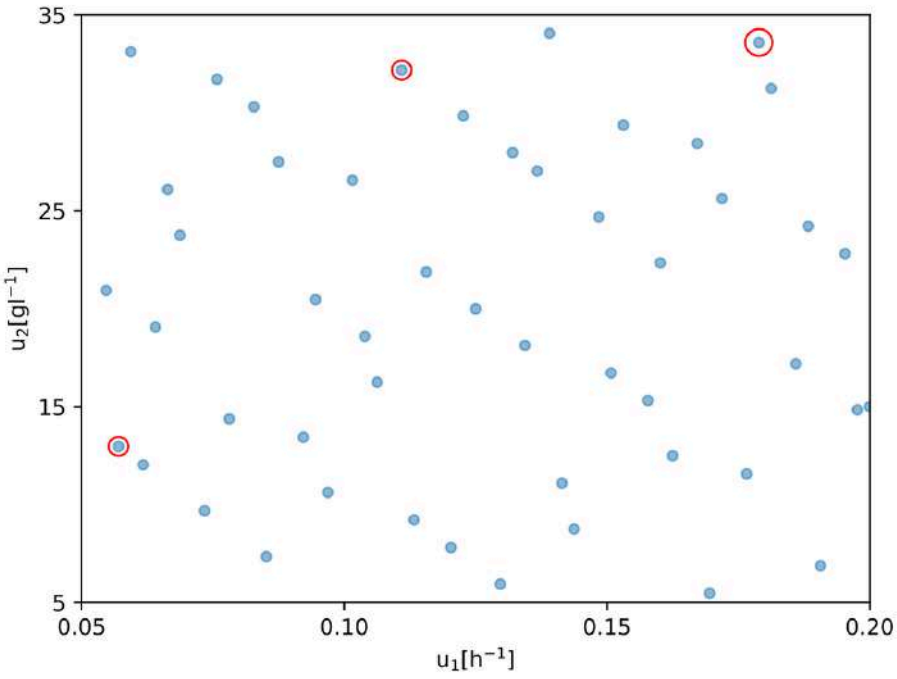


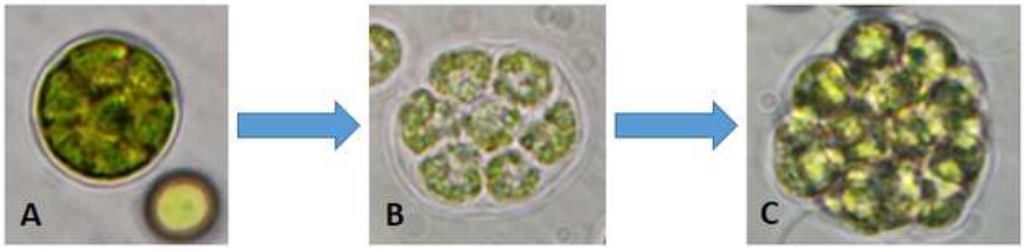
Figure 1. Results from the application of the methodology to a case study related to a bio-chemical process, showing the discretisation of the experimental design space and the selected experiments that maximise the information content of the campaign.

Study and monitoring of the multiple fission cell cycle of Chromochloris zofingiensis: a high-throughput approach

Yob Ihadjadene - July 17, 2023

Microalgae are photosynthetic microorganisms that typically live in freshwater, soil or marine habitats, but also more extreme habitats, such as salt, sulfur-rich lakes or on snow surfaces. They are becoming increasingly important in the bioeconomy and biotechnology sectors as an attractive, sustainable source of value-added products, owing to their enormous potential for the production of industrially relevant, high-value products, e.g., pigments with antioxidant and antibacterial activity such as carotenoids (astaxanthin, canthaxanthin, β-carotene and lutein), polysaccharides, polyunsaturated and omega-3 fatty acids. Some microalgae species grow and divide through binary fission, in which one cell divides into two cells, while others divide through multiple fissions, in which a cell can produce up to several daughter cells; in some cases, both binary and multiple fission can be executed in response to changes in ambient conditions or life cycle phase.

Chromochloris zofingiensis is a unicellular freshwater microalga, considered as a promising natural producer of high-value carotenoids such as astaxanthin. It can reproduce itself via asexual multiple fission cell cycle, under which DNA replication and nuclear division are executed multiple times prior to cell division. Thus, allowing the observation of polynuclear cells (PNC) (Figure 1), in which the number of daughter cells within a cell is determined by the number of DNA replications and nuclear division events before cell division. Though, as C. zofingiensis cells progress through their cell cycle, they grow, commit to divide, execute DNA replications and nuclear divisions, therefore creating the PNC sub-populations in which 2n daughter cells are produced, with the exponent “n” indicating the number of DNA replications that occurred and representing a degree of heterogeneity within the same population (Figure 2). ›



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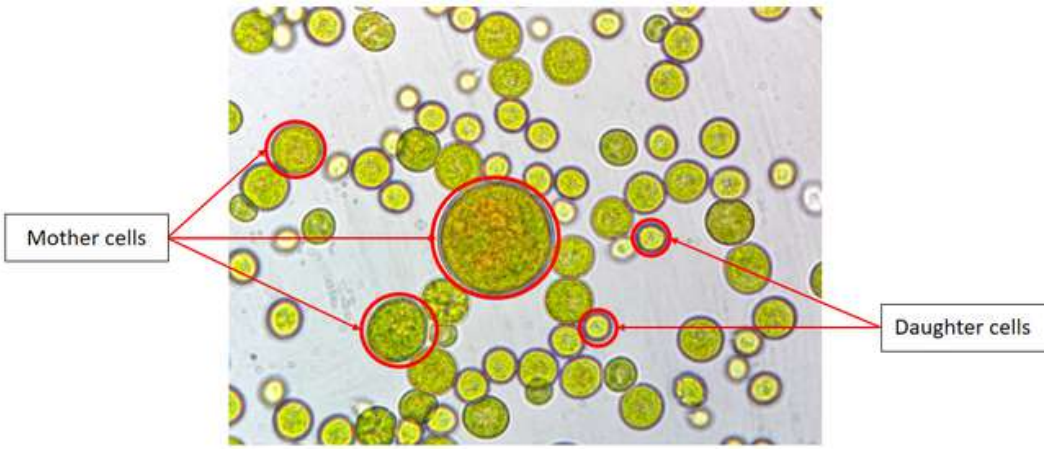

Figure 2. Microscopic observation showing high cell population heterogeneity of C. zofingiensis cultivated in non-limited photoautotrophic conditions.

Precise and quick monitoring of key microalgae population features such as cell count, cell size, cell morphology, and DNA content is crucial for applications in cell culture research to develop feasible algae-based bioprocesses.

Thus, analysis and examination of these different physiological state parameters must be precise, accurate, and characterized by quickly measurements. In this perspective, one of the main objectives of our project is to develop new single-cell analysis techniques and methods to study the population heterogeneity of the non-conventional microalgae C. zofingiensis.

Based on Flow Cytometry (FCM), a method that allows high-throughput analysis and acquisition of key data on cell populations at a single-cell level.

Study and monitoring of the multiple fission cell cycle of Chromochloris zofingiensis: a high-throughput approach

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Therefore, a sensitive routine is currently being developed to allow us to detect and recognize microalgal population heterogeneity as well as different phases of the cell cycle, by combining DNA staining dyes with flow cytometry and image cytometry analysis.
The latter offers visual inspection of the cell population and exact quantification of sub-populations proportions since it provides different images for each analyzed cell (Figure 3).

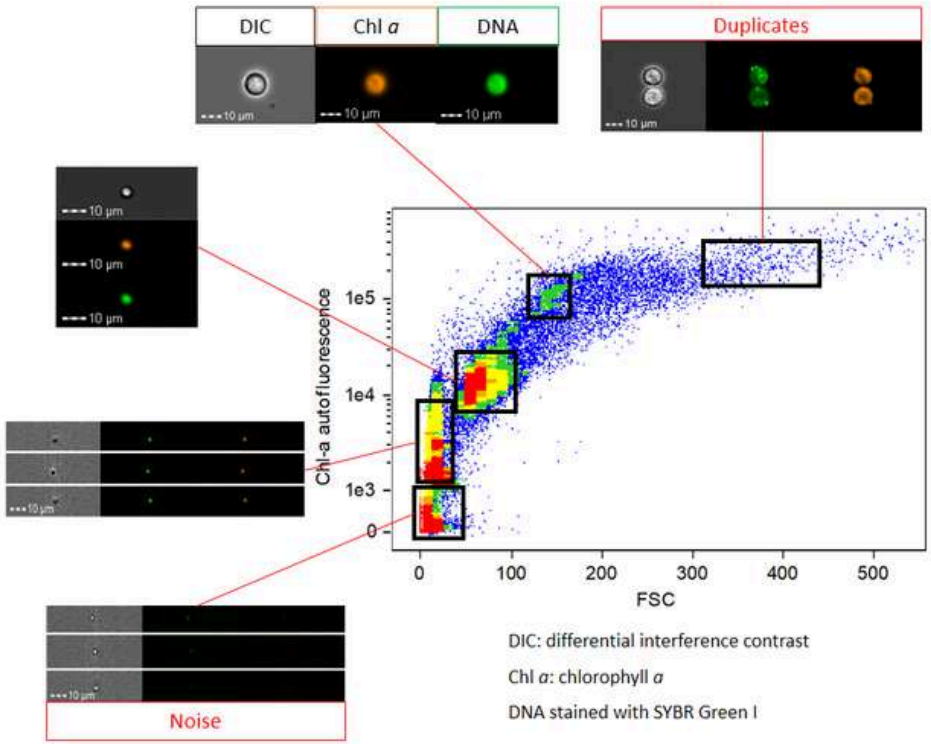

Figure 3. High throughput Image Cytometry analysis of C. zofingiensis population heterogeneity at the single-cell level.

Advancing microalgae cultivation through precision temperature modeling: Achieving optimal growth with adaptive models and model predictive control

Ali Gharib - October 17, 2023

In the realm of sustainable solutions, microalgae stand out as versatile organisms with the potential to revolutionize multiple industries. From biofuels to pharmaceuticals, these microscopic powerhouses hold the promise of eco-friendly innovation. However, unlocking their full potential depends on understanding their environment, particularly the influence of temperature.

Temperature modeling, a powerful tool in this endeavor, offers precise control over microalgae growth. At its core, this technique is a mathematical representation of how temperature variations impact microalgae behavior. It provides the means to predict and control their growth with precision.

In controlled environments like raceway ponds, temperature plays a critical role. These large, shallow pools of water serve as the platform for microalgae to harness sunlight and nutrients for their growth. Maintaining the optimal temperature is crucial for achieving this balance.

Temperature modeling involves the processing of data and mathematical equations. It incorporates inputs such as weather data, such as sunlight intensity and air temperature, as well as pond-specific details like dimensions and depth. The model then determines the ideal temperature for microalgae growth.

Why is this endeavor important? Microalgae, though tiny, hold immense potential as biofactories. They can produce various valuable substances, from biofuels to pharmaceutical ingredients. However, they require a tailored environment, starting with precise temperature control, to operate efficiently. The power of this model lies in its adaptability. It can transition seamlessly between different ponds, accommodating various sizes and environmental conditions.

To achieve this precision, the adaptive model undergoes an identification process. This involves calibrating the model using a small dataset spanning just four days. The model's adaptability extends beyond raceway ponds; it can seamlessly predict temperature in different cultivation systems, such as tubular reactors, and even within a greenhouse.

This adaptability comes from the model's underlying principles. It focuses on the most critical aspects of temperature dynamics, emphasizing factors such as radiation, evaporation, and convection. By targeting on these fundamental components, the model streamlines the prediction process, making it more agile and responsive.

Moreover, this temperature model is not an isolated achievement. It will serve as a cornerstone in the implementation of Model Predictive Control (MPC). MPC optimizes processes by predicting future behavior and adjusting control inputs accordingly. By incorporating the temperature model, MPC will fine-tune microalgae cultivation systems to maximize productivity and sustainability.

Advancing microalgae cultivation through precision temperature modeling: Achieving optimal growth with adaptive models and model predictive control

Ali Gharib - October 17, 2023

MPC's benefits are far-reaching. It enables real-time adjustments based on changing conditions, enhancing efficiency and reducing resource consumption.
In conclusion, the integration of precision temperature modeling and Model Predictive Control represents a significant step toward sustainable microalgae cultivation.

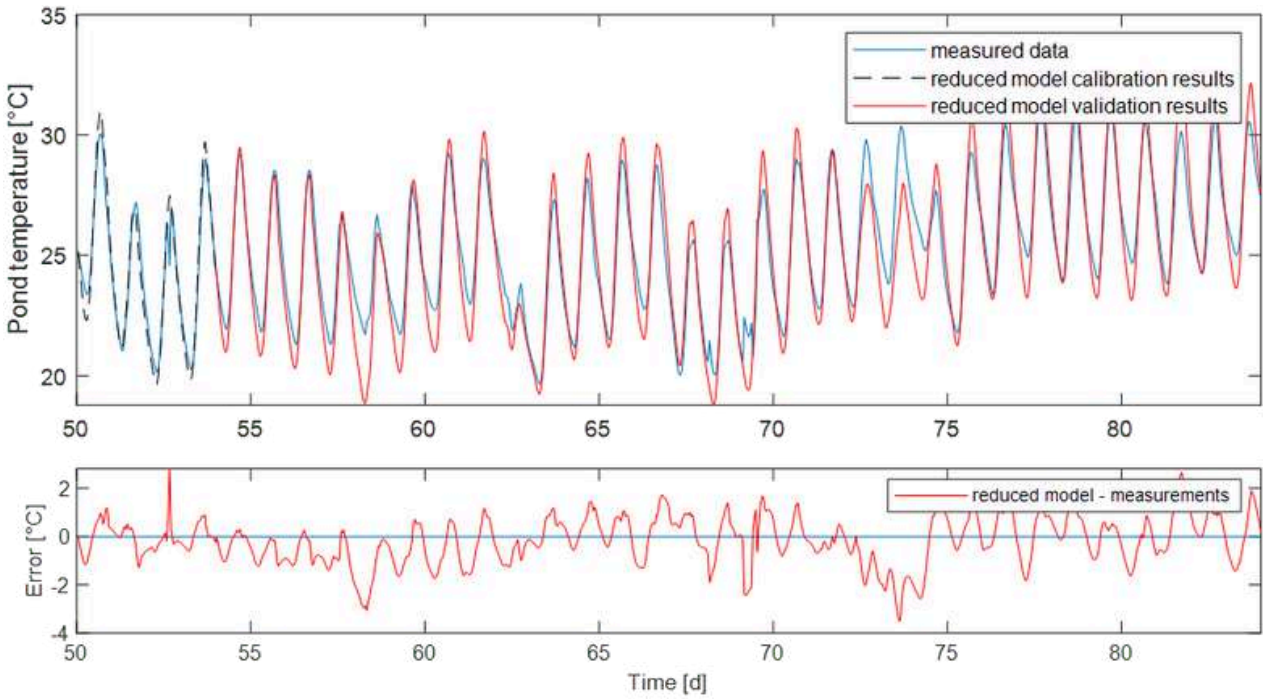

Figure 1. Temperature prediction of the adaptive heat transfer model using a dataset of 4 days.

The impact of nutrients on microalgae growth and what this means for biological models

Lars Stegemüller - October 17, 2023

Depending on the strain, microalgae are rich in protein, carbohydrates, lipids and offer highly valuable components such as astaxanthin or β-carotene. Moreover, microalgae take up CO2 and can grow solely on sunlight with high areal productivities. Currently, microalgae biomass is mainly produced for high-value applications related to human consumption, including nutraceuticals and pharmaceuticals. However, microalgae also showed potential for other applications e.g. for bulk chemical production, wastewater treatment and aqua feed. High production costs of 5 to 50€ per kg make a wider application spectrum unfeasible, which is partly due to the significantly lower production rates on industrial scale compared to lab-scale. To solve this problem a better understanding of interactions between the different growth input parameters is required. Specifically, the carbon, nitrogen and phosphorous metabolisms play a key role in the growth of microalgae. Nitrogen is required to synthesize protein, RNA and DNA, while phosphorous is essential to RNA, DNA, ATP and phospholipid synthesis. Since protein structures are omnipresent in every aspect of the cell such as in transporters, enzymes or cell organelles, nitrogen is often considered the most important nutrient for cellular growth. In the following, we will explore the effect of nitrogen and phosphorous on the microalgae Haematococcus pluvialis. An interesting candidate for biotechnological production of astaxanthin, since it can accumulate up to 4% astaxanthin inside the cell. Astaxanthin is a high value carotenoid with beneficial properties for human health. Due to strong antioxidant and anti-inflammatory effects it appears to be a suitable supplement to improve overall health and has even shown positive effects for cancer prevention and treatment.
However, the cell cycle and therefore the growth of H. pluvialis is complex and the differences in morphology during different stages are exceptional (Figure 1). These changes are directly related to the availability of nutrients and can be observed by microscopy, flow cytometry or other visual analysis of the cells.

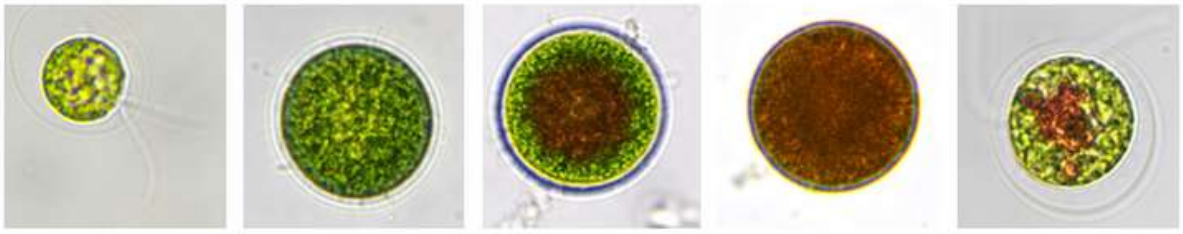

Figure 1. Life cycle of Haematococcus pluvialis.

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Lars Stegemüller - October 17, 2023

The different cell stages vary from ellipsoidal vegetative cells with two flagella, called zoospores to red spherical cysts, called aplanospores. In addition, there are several transition stages with unique morphological characteristics. In the case of H. pluvialis, the cell cycles are of great importance, because astaxanthin is only formed in red cysts. Despite changes in color and shape, the cells vary considerably in size. While the vegetative zoospores are usually smaller, the red cysts can easily be three times bigger. One reason the cell changes from zoospore to an aplanospore is a lack of available nutrients such as nitrogen or phosphorous. Therefore, a zoospores to aplanospores transition is often observed in batch cultures once nutrients become scarce. Considering a batch cultivation limited only by nutrients we can observe an exponential increase in biomass until a plateau is reached, very similar to bacterial growth. However, unlike bacterial growth, this plateau is not reached once nutrients are fully consumed but only at a later stage. This can be easily explained by looking at the cell cycle. As the cells turn into aplanospores, the internal ratio of nutrients to carbon decreases as the cells continue with the uptake of carbon. The carbon is usually stored in the form of carbohydrates such as starch or in the form of lipids.
Most biological models for bacterial growth use the Monod equation to describe the effect of nutrients or substrate on growth. Hence, one might think that this would also be a viable solution to describe microalgae growth. However, a limitation of Monod based models is that once the dissolved nutrients are depleted, given that the Monod term goes to zero once the nutrients are depleted, it results in zero growth at this point. Although microalgae usually also stop dividing at this point, the biomass concentration still increases due to the increasing size of the individual cells. Thus, a different mathematical description is required. A popular solution is the Droop equation, which considers internal nutrients instead of the dissolved nutrient concentration (Figure 2).
The simulation above clearly shows that the Droop model is the better choice when calculating microalgal growth in respect to nutrient availability. However, the implementation of this model is more challenging. Since the model considers not only dissolved nutrients but also an internal nutrient quota, additional measurements for model calibration are required. Performing measurements of internal nutrients is significantly more difficult since the nutrients are bound in many different ways (see above) and therefore can´t be measured as easily as dissolved, inorganic nutrient sources such as ammonium or phosphate. Instead, methods are required in which the cells are either completely digested or burned at very high temperatures while supplying enough oxygen.

The impact of nutrients on microalgae growth and what this means for biological models

Lars Stegemüller - October 17, 2023

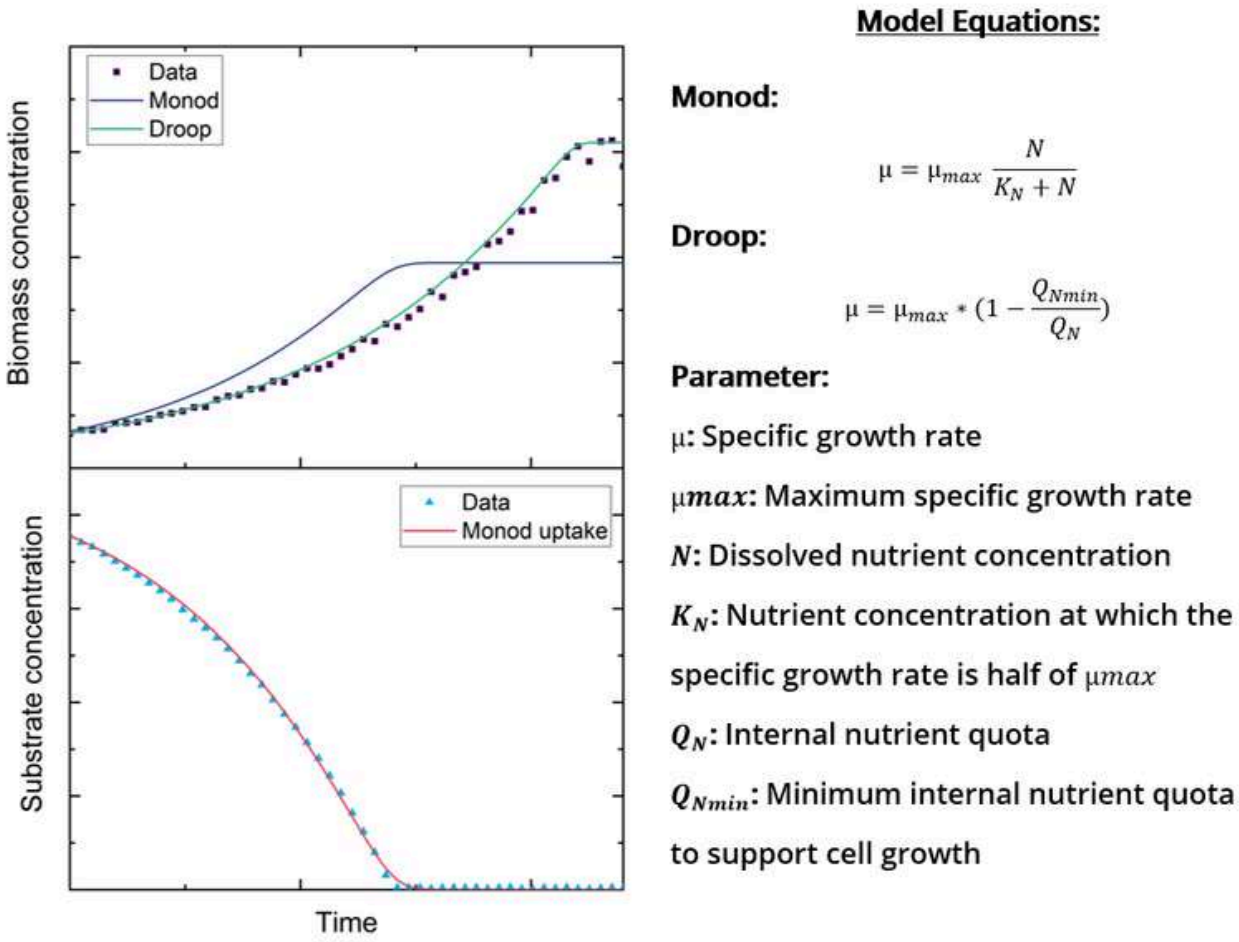


Figure 2 Comparison of the Monod model and the Droop model for biological modelling of microalgae growth.

Prediction of microalgae growth using Machine Learning models

Tehreem Syed - October 17, 2023

Recently, unicellular microalgae have attracted considerable interest due to their ability to utilize CO2 in the photosynthetic process and produce various valuable compounds such as biofuels, bioplastics, pigments, and vitamins. Microalgae are a promising source for a sustainable replacement for fossil fuels in transportation and power generation. Their efficient use solar energy utilization and capacity to generate energy-rich biofuels through advanced thermochemical techniques contribute to this. An overview of the microalgae cultivation process for the production of different products is shown in Figure 1.

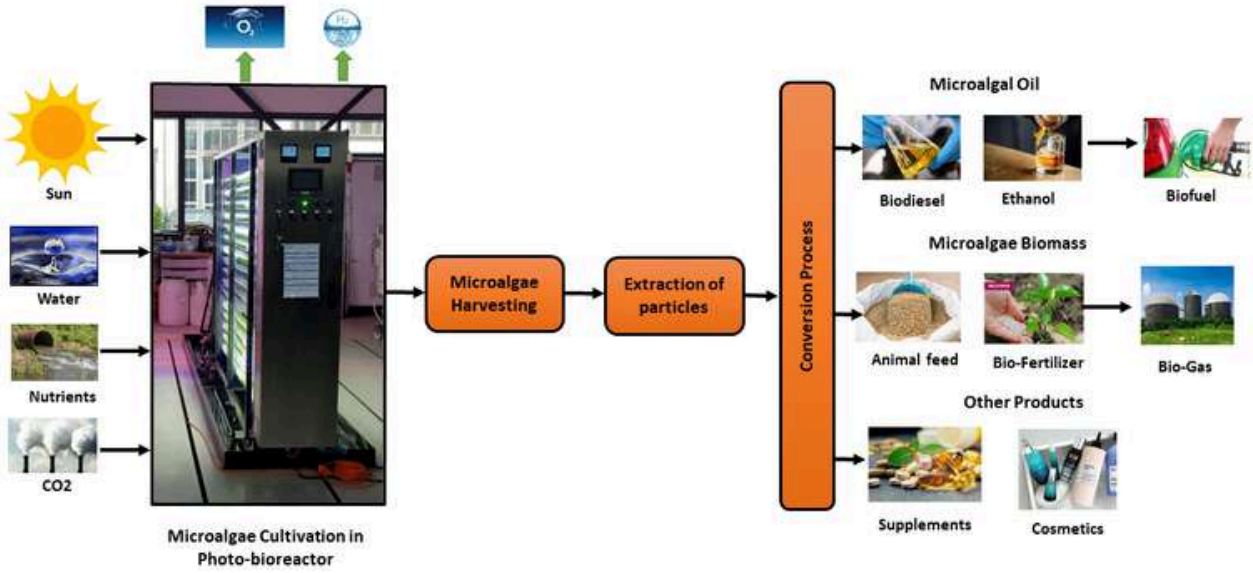


Figure 1. Microalgae cultivation principle, downstreaming with different bioproducts obtained

Microalgae can be cultivated under phototrophic conditions, utilizing either artificial lighting indoors or natural sunlight outdoors. Closed photobioreactors (PBRs) for outdoor cultivation have been regarded as a feasible choice for large-scale microalgae cultivation. This method utilizes natural sunlight within a controlled, enclosed system, reducing the possibility of contamination. Nevertheless, the biological processes involved in outdoor cultivation become complex due to variations in light and temperature, which are fundamental factors for microalgae cultivation.

Prediction of microalgae growth using Machine Learning models

Tehreem Syed - October 17, 2023

In this work, we investigate the influence of sunlight dynamics on the growth of Phaeodactylum tricornutum. Growth models play a crucial role in understanding the mechanisms of microalgal growth and improving production efficiency. Based on first principles, several conventional models have been proposed to describe the growth dynamics of microalgae concerning light intensity. However, most of these models need further validation in the context of outdoor cultivation. In addition, the existing models omit the light acclimation phenomenon, which describes how microalgae cells adjust their biochemical structures in response to varying light intensities to improve their light utilization.

The light acclimation effect is important for the growth of microalgae, affecting factors such as changes in the chlorophyll a to carbon ratio, the composition of accessory pigments, the abundance of photosynthetic proteins, the parameters of the photosynthesis-irradiance curve, and the coupling between light absorption and electron transfer. In higher light conditions, microalgae decrease their pigment content to maintain a balance between light absorption for energy and the requirements for growth and carbon fixation. Consequently, the incorporation of the light acclimation effect into growth models is crucial for accurate predictions of microalgal growth dynamics. Numerous researchers have attempted to incorporate the light acclimation effect into growth models, aiming to improve the accuracy and applicability of these models. Nevertheless, the majority of these models have been developed using controlled indoor laboratory experiments and have not been verified in outdoor cultivation settings utilizing natural sunlight. Therefore, validated growth models that consider the light acclimation effect in outdoor cultivation of microalgae are required.

Machine learning models have attracted considerable attention from various fields in recent decades. In contrast to traditional models that rely on explicit mathematical equations, machine learning algorithms are designed to obtain knowledge from data, thus classifying them as data-centric models. Although machine learning has shown success in areas related to microalgae, such as genetic engineering, species identification, and medium screening, its use for growth modeling in microalgae cultivation remains limited. In addition, existing studies have not adequately considered the effect of light acclimation.

In order to consider the influence of light acclimation, we integrated a time sequence of light-related information as an additional input into the machine learning models. This allowed the prediction of microalgae growth to account for the history of light exposure. We employed support vector regression (SVR) and long short-term memory (LSTM) networks for addressing the regression task, given their widespread use in time series analysis in machine learning.

Prediction of microalgae growth using Machine Learning models

Tehreem Syed - October 17, 2023

In the SVR and LSTM models, we used a light history for growth prediction within the machine learning frameworks. The LSTM and SVR models were trained using the current biomass (X) and the light history time series data (I) as inputs. The results demonstrate that the LSTM model performs exceptionally well in predicting microalgae growth within the test dataset compared to SVR. However, the SVR model shows signs of overfitting as its performance on the training dataset significantly outperforms its performance on the testing dataset.

Optimal controls related to microalgae cultivation

Ignacio Fierro - January 18, 2024

In recent years, there has been a notable increase in the industrial cultivation of microalgae, accompanied by the development of new technologies such as photobioreactors, sensors, and controllers. These new technologies are capable of estimating biomass inside the photobioreactor. When the data is recorded, mathematical models can be fitted in order to forecast the biomass taking into account different factors, as light intensity, dilution rate, pH, nutrient concentrations, etc. In industrial production levels, several questions are of interest in order to make the most of our resources and constraints. For example: What is the optimal performance of the system involved? How should the photobioreactor be operated to maximize the production of biomass or any compound of interest produced by the microalgae? Which operational parameters should be constant? Do some biological phenomena specific to microalgae influence production?

Accurate mathematical models describing biomass evolution in a photobioreactor play a crucial role in addressing these questions. However, mathematics cannot answer all the questions, especially when a factor is not considered, or its influence is not well stipulated, or worse, when two factors together act differently than when they act separately. Modeling the behavior of biological organisms present an additional challenge: acclimatization and adaptation. Microorganisms, in particular microalgae, are able to acclimate to certain external factors, e.g., they can adjust the amount and composition of pigments depending on the amount of light they perceive. This acclimatization can impact growth in the photobioreactor and subsequently affect the model's prediction.

But let's forget, for the moment, the limitations of the models and let's assume that we have one that describes very well the biomass evolution in our photobioreactor which can be operated in continuous mode, considering the influence of light and dilution rate. While other factors are kept under control, such as pH and temperature, other factors are not assumed to be limiting, as nutrients (they have enough nutrients for growth). We now are interested to study the best performance of our system using some mathematics.

Before getting into this topic, a definition is needed. Control theory is a branch of engineering and mathematics that deals with adjusting a system's behavior to achieve desired outcomes. This manipulation is called control. In the context of a photobioreactor, controls may include light intensity, nutrient inlet concentration, temperature, pH, medium volume, CO2, or dilution rate. The questions posed above can be formulated in the optimal control language.

First, define the objectives. These may include maximizing biomass production, optimizing nutrient utilization, or enhancing the production of specific compounds.

Optimal controls related to microalgae cultivation

Ignacio Fierro - January 18, 2024

Second, the key controls or operational parameters should be determined, for instance, pH is maintained at constant levels injecting CO2 to ensure the survival of the species concerned, but light intensity can be controlled depending on the concentration of the biomass to avoid photoinhibition and trigger the photosynthetic machinery of the culture. These factors vary depending on how the microalgae are being cultivated. In addition to this, to study an optimal control problem, we must also consider the limitations of the model. For example, if our model does not consider the influence of nutrients, we will not be able to consider this factor as a control, it just makes no sense.

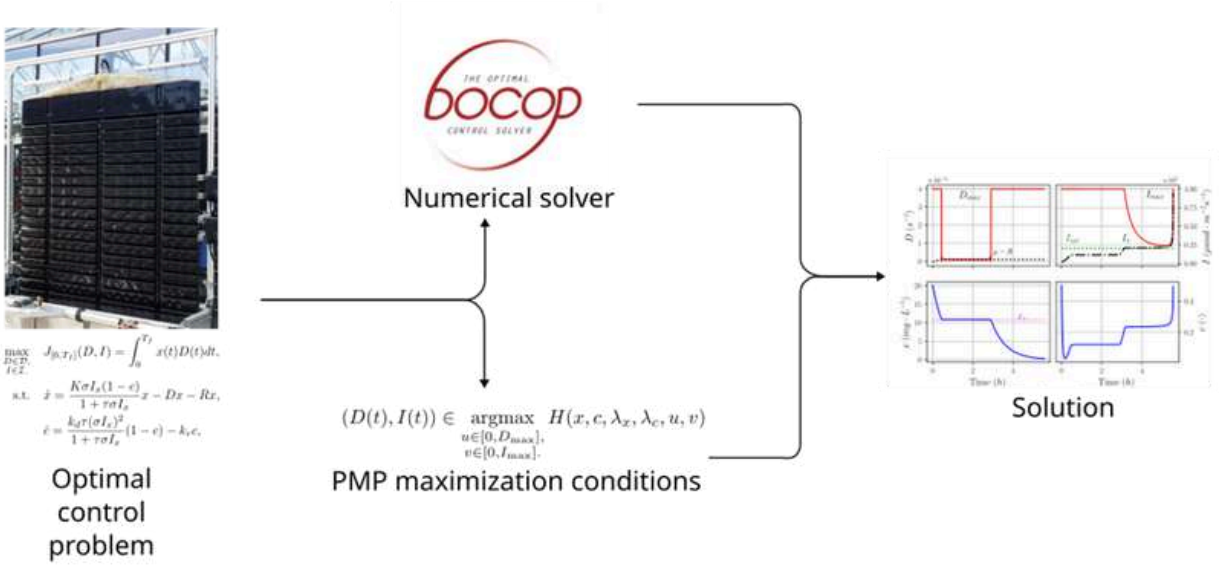
Then, given a certain objective, for example, to maximize biomass production, the question is: how do we set our controls? The Pontryagin's maximum principle (PMP) is a mathematical tool that can be used to answer these new questions. When a mathematical model of our system, written as a set of ordinary differential equations, has well-defined the influence of these controls, PMP can be applied to find the optimal control. The principle provides the necessary conditions for a control to be optimal in the presence of constraints. However, these necessary conditions are not easy to decipher and most of the time one must resort to a software that is able to solve an approximation of the problem. By combining the theoretical results delivered by PMP and the numerical simulations of some software, we can gradually discover the structure of our optimal control. Why gradually? Discovering the secrets of our unknown optimal control is like piecing together a puzzle. At the beginning, a few conditions can be derived from the PMP, for example, the optimal operation of the dilution rate can take three values, zero, at its maximum value and a third value that's unknown. Numerical simulations can help us determine this unknown value.

The engineering challenge does not end after finding the solution to our control problem. This solution has to be evaluated and interpreted. Perhaps the solution is outside the margins in which our model was tested and we are not sure if the predictions will be as expected, since the photobioreactor has never worked in those conditions, or even worse, it is not possible for the photobioreactor to work in these conditions.

In conclusion, control theory emerges as a valuable framework to address questions related to optimizing system performance in industrial-scale microalgae cultivation. By formulating optimal control problems and leveraging mathematical tools like the Pontryagin's maximum principle (PMP), researchers can navigate the intricate relationships between operational parameters and desired outcomes. However, the applying PMP requires careful interpretation. This often involves using numerical simulations to uncover optimal control strategies.

Optimal controls related to microalgae cultivation

Ignacio Fierro - January 18, 2024



To understand an optimal control problem in a microalgae-based bioprocess it is crucial to formulate the system on differential equations, pinpointing the controls in order to apply the Pontryagin maximum principle optimality conditions and utilize a numerical solver.

Optimizing large-scale microalgae-based wastewater treatment: a comprehensive modelling approach

Rebecca Nordio - January 18, 2024

Microalgae-based wastewater systems are highly complex from a biological point of view. In wastewater, microalgae coexist with thousands of different microorganisms in a unique ecosystem that allows the efficient recovery of nutrients (such as nitrogen and phosphorus) naturally present in this type of medium. In these systems, microalgae interact not only with bacteria, but also with fungi, potential pathogens, and viruses. Consequently, one of the key challenges related to the correct management and operation of these systems is handling co-existing populations, as their interactions significantly impact effectiveness and stability.

In this context, mathematical modelling emerges as an invaluable tool for guiding decision-making within the framework of optimal operation. An accurate mathematical model enables the understanding of microbial population interactions, the evaluation of process variables, optimization of the process through the implementation of control strategies, and improvement of the design of production systems, especially for large-scale production.

In the framework of modelling microalgae growth, the literature provides numerous examples where the growth rate is correlated with light, temperature, pH, and nutrient availability. However, to establish validity in a large-scale system, it is necessary to consider the totality of these factors, in the so-called "comprehensive models," defined as models that consider the effect of multiple process parameters and biological mechanisms. Additionally, in the case of wastewater systems, it is necessary to include the interaction with other microorganisms present in the medium and their competition for nutrient consumption. However, to develop a model applicable to large-scale production, it must strike a balance between accuracy and complexity, focusing only on relevant mechanisms to avoid exceeding computational demands. Therefore, selecting the right microorganisms to model is one of the fundamental steps.

A study carried out in Denmark that uses next-generation sequencing technologies revealed that in a wastewater treatment plant, up to 3000 different microbial species can be present. However, most of them are in very low abundance and, presumably, are not relevant to the treatment processes. This fact resulted in focusing only on a few bacterial groups that are in high abundance and influence nutrient removal, typically nitrifying (AOB, NOB), and heterotrophic bacteria. In fact, the first bacterial group contributes to the consumption of ammonium, in competition with microalgae, and the production of nitrate, while the second contributes to the degradation of the chemical oxidation demand (COD) present in wastewater.

After defining the system to be modelled, a new comprehensive model for microalgae-bacteria systems, named the ABACO-2 model, has recently been developed and validated (Figure 1).

Optimizing large-scale microalgae-based wastewater treatment: a comprehensive modelling approach

Rebecca Nordio - January 18, 2024

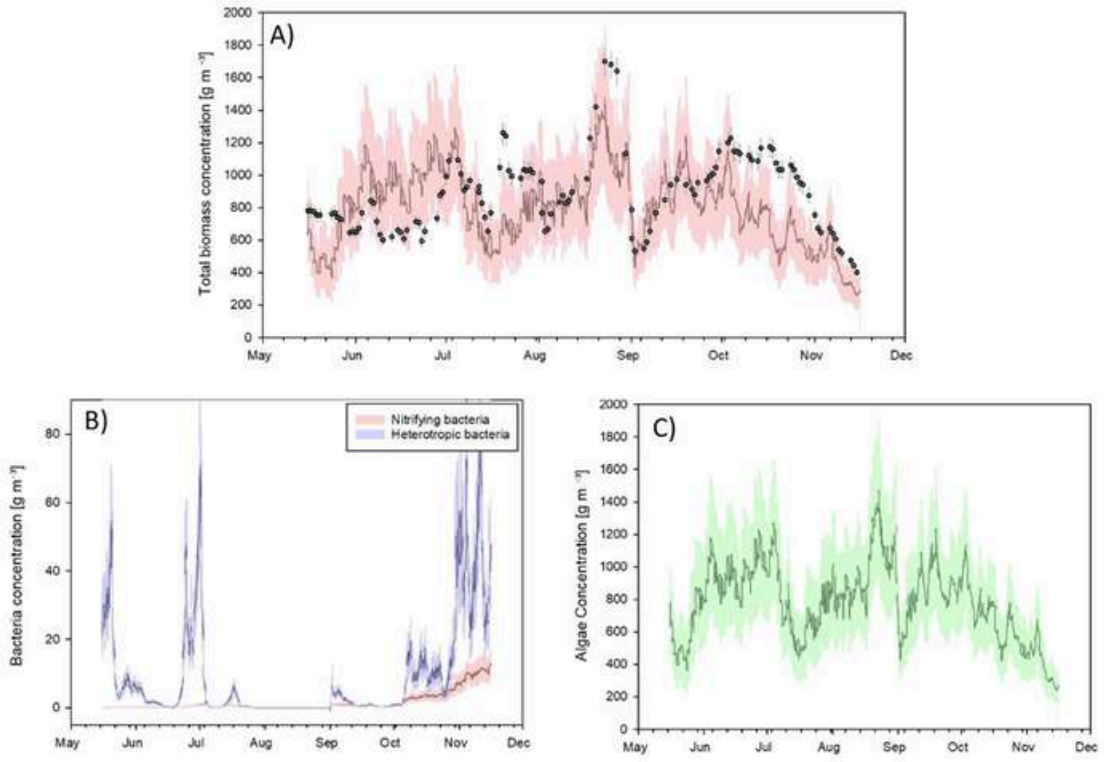


Figure 1: Validation of ABACO-2 model. A) total biomass concentration; B) Bacteria concentration; C) Algae concentration

This model effectively represents the interaction between bacteria and microalgae in wastewater treatment, including biomass concentration and nutrient evolution in the medium. Moreover, it has successfully been validated over an extended period of 8 months for a pilot-scale raceway reactor of 80 m2.

ABACO-2 is a robust biological model that can be used to assess populations living in wastewater systems. Differentiating experimentally between communities in such systems poses a significant challenge. In fact, the biomass productivity of raceways is evaluated with the dry weight methodology, which includes the contribution of both bacterial and microalgae populations. Separating them remains challenging but significant, as their ratios impact various process outcomes, such as biomass quality and water remediation efficiency. Successive filtrations based on cell size differences have been explored, but they often result in a notable presence of bacteria that attach to microalgae due to cell aggregation. Alternative methods, including flow cytometry techniques (FCM), prove valuable in assessing the relative composition of mixed microorganism populations, which encompass both prokaryotes and eukaryotes.

Optimizing large-scale microalgae-based wastewater treatment: a comprehensive modelling approach

Rebecca Nordio - January 18, 2024

This approach discriminates between groups by analysing the intrinsic characteristics of individual cells, such as size, complexity, and autofluorescence. Additionally, molecular identification techniques, such as the amplification of 16S and 18S rDNA sequences, serve to evaluate the structure of the microbial community. Alongside these methods, photo-respirometry, based on traditional respirometry, has been employed to discern population differences. However, these methods lack a direct correlation in biomass concentration (g·L-1), which is more straightforward to interpret.

In this context, mathematical models offer a useful tool for indirectly studying how the balance between populations evolves. Operational conditions, notably cultivation height, dilution/harvesting strategy, and oxygen removal capacity, have a substantial influence.

Integrating additional aspects into the models, particularly those relevant to the stability of microalgae culture and the disinfection capacity of the system, presents a future challenge. This involves considering factors such as the impact of algae predators (e.g., fungi, viruses) or pathogenic bacteria (e.g., E. coli, Clostridium). In particular, with respect to the last aspect, their reduction is a crucial requirement set by the European Union in the context of water reuse, especially in agriculture. This expansion of the models will contribute to a more comprehensive understanding and effective management of microalgae-based wastewater systems, aligned with regulatory standards and ensuring sustainable practices.

Genetic engineering is a powerful tool – how reduced shading of microalgae strains can improve algal growth in photobioreactors

Tim Michelberger - January 18, 2024

Microalgae are highly interesting organisms for photosynthetic research and industrial applications. These unicellular eukaryotic organisms exhibit the highest photosynthesis and growth rates amongst photosynthetic organisms and produce many interesting compounds such as lipids, pigments, or proteins that find broad application, for example, in the food and feed industries or pharmaceutical products. In addition, they often grow in salt water and can be cultivated in photobioreactors (PBRs) on non-arable land using the sun as sole light source, making them an interesting sustainable crop. However, microalgal strains have not yet been fully domesticated and their cultivation still faces major challenges. Algal cells normally aim to maximize light absorption by increasing their chlorophyll content in the chloroplast. In dense algal cultures, this causes mutual shading of the cells, resulting in light only reaching a small layer in the culture that is close to the light source. In mixed cultures, this makes the algal cells experiencing light fluctuations as they are constantly transported from the illuminated layers into darker areas and vice versa. Under fluctuating light, microalgae cells constantly need to balance photosynthesis and photoprotective mechanisms. This constant switch requires a lot of energy and can still allow photodamage, often resulting in reduced growth.

However, these challenges have also triggered intensive research and the development of technologies for genetic engineering of microalgae to adapt them to the experienced light conditions. Over the last two decades, scientists have created several tools for randomized or targeted genetic deletion, insertion, or mutation. Furthermore, the scientific community is slowly agreeing on roughly 10-20 algal species from different phyla that are commonly used as model organisms and for which most technologies are being developed. Since the discovery of the CRISPR/Cas9 toolkit for genetic engineering in the 2010s years, the development of such technologies has massively accelerated, and we are now equipped with many powerful tools to modify the genomes of several microalgae models fast and simple.

In the laboratory of Prof. Tomas Morosinotto at the University of Padova (Italy), which is part of the Digitalgaesation network, we are working on the optimization of industrially relevant microalgae strains for cultivation in photobioreactors. One strategy to reduce the experienced light fluctuations in PBRs is to reduce the chlorophyll content in the algal cells, allowing light to penetrate deeper into the culture and illuminating more cells at once. Using random mutagenesis, our lab could successfully isolate strains of the microalga Nannochloropsis gaditana that contain less chlorophyll and showed improved growth in small lab-scale systems.

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However, microalgae are highly adaptive organisms that balance their pigment composition according to the ambient light conditions and other factors such as nutrient availability. Thus, it is important to assess the growth of the newly generated strains in other environments. Testing growth in large industry-scale PBRs requires extensive work and is relatively costly. As part of my PhD project, I therefore aimed to characterize Nannochloropsis gaditana with reduced chlorophyll content in a more sophisticated lab-scale environment.

For this, a selected promising strain was cultivated in a fully automated lab-scale photobioreactor system (Figure 1). The PBR featured automated temperature and light controls. Moreover, dilution with fresh medium was automatically regulated as soon as the culture reached a certain optical density to grow the cells in a constant turbidostatic regime. Optimal pH was guaranteed by on-demand CO2 injection.

Figure 1. Microalgae cultivated in an automated PBR system. The Nannochloropsis strain with reduced chlorophyll content was grown in bottle 2 and 4 and allows more penetration of the light. For the image, all bottles were diluted to the same cell number.

We assessed the chlorophyll composition of the strains grown in the PBR and saw that the chlorophyll content was reduced by ~40% (Figure 2, left panel), which is similar to what was observed in lab-scale systems.

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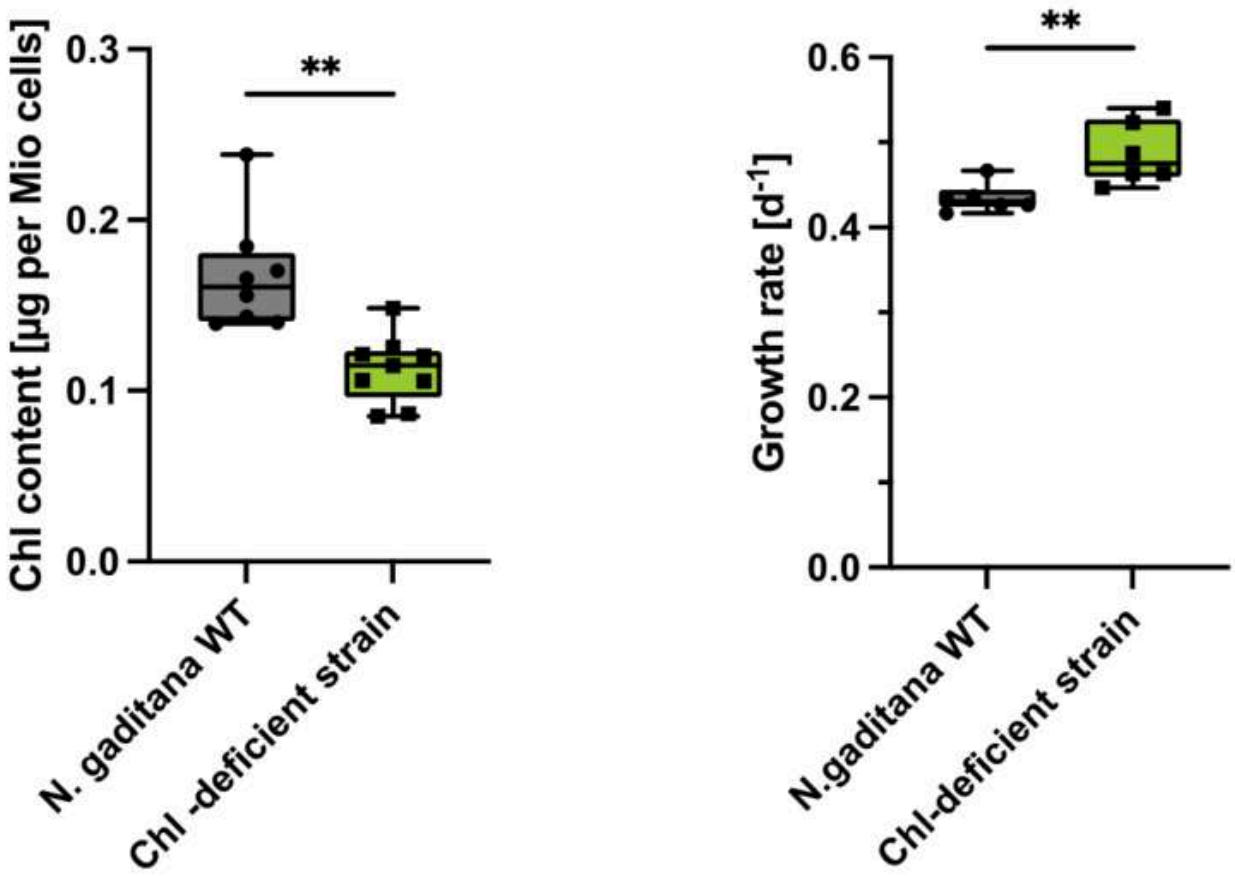

*Figure 2. Reduced chlorophyll content can improve growth of N. gaditana in photobioreactors. Left panel: chlorophyll reduction compared to the WT strain. Right panel: Growth rate of N. gaditana with reduced chlorophyll content is increased in photobioreactors. Significance was assessed using a one-tailed Student's t-test, **p<0.01.*

This suggests that the reduction of the chlorophyll content is stable across different cultivation systems. Investigating the growth performance of the strain, we discovered that the strain with less chlorophyll showed a growth rate that was increased by about 12% compared to the original wild-type (WT) strain (Figure 2, right panel).
These results could validate that the reduction of chlorophyll represents a valid strategy for improving microalgal growth across diverse cultivation systems. In the next steps, beneficial strains need to be tested in big industry-scale PBRs that in general have more depth, possibly potentiating the advantage of the chlorophyll reduction.

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Taken together, we could exemplarily show that genetic engineering is a powerful technology for improving microalgal growth. As we are constantly increasing our knowledge about microalgal cells, the science community is also slowly shifting its focus from enhancing not only the growth but also to exploiting algal cells as biofactories for the production of interesting products. With the development of new technologies in the future, we can then accompany the process of microalgal domestication and usage.

Why and How to Monitor Microalgal Biofilms?

David Pereira - April 22, 2024

Microalgae are an excellent source of valuable compounds for diverse industries, including food, aquaculture, and nutraceuticals. Traditionally, microalgae are cultivated in a planktonic state within photobioreactors (PBRs) and raceways. Recent advancements have led to the development of biofilm-based technologies, which utilize microalgae's ability to form biofilms and grow on substrates. This technique reduces the required water volume and simplifies the harvesting process, resulting in higher biomass yields and enhanced energy and cost efficiency.

However, the industrial production of these valuable molecules can only be optimized if we carefully monitor biological features like biomass and cell traits. In both traditional and biofilm-based cultivation methods, there are currently no standard, easy-to-use systems for online or inline analysis. Monitoring is predominantly done destructively by taking samples for offline analysis, which is both time-consuming and poses a contamination risk. Specifically, in biofilm systems, this method removes a significant amount of the final product as biomass is highly concentrated. Thus, the development of non-destructive techniques to improve productivity and preserve the integrity of the biofilms is highly desirable.

Innovations in the monitoring field have introduced optical methods for in situ monitoring, using techniques like UV-Vis, fluorescence, and vibrational spectroscopy (FTIR and Raman). Yet, for microalgal biofilms, which often develop on opaque carriers like textile fabrics, a new non-invasive monitoring tool is necessary. The opacity of these carriers makes reflectance-based methods more suitable and effective than transmission-based ones.

To address this gap, techniques already in use in fields such as precision agriculture and environmental science can be adapted for microalgal biofilms. Reflectance technology, for instance, has been successfully used for characterizing natural biofilm communities and for measuring primary production in oceans. From a commercial perspective, this sensing technique has proven effective in agriculture for applications such as plant phenotyping and monitoring plant growth and nutritional status. This is accomplished by computing reflectance indices (RIs) based on measurements in the visible (400-700 nm) and near-infrared (700-1000 nm) spectral regions, which are then utilized to develop correlations with the targeted features.

At CentraleSupélec in Paris, our research is dedicated to addressing these monitoring challenges. Haematococcus pluvialis was chosen as the primary experimental microalgae not only because it is the leading producer of astaxanthin – a carotenoid known for its potent antioxidant properties and numerous health benefits – but also because it undergoes significant changes in cell pigments, structure, and composition during its life cycle. These changes make it an ideal candidate for evaluating the robustness and adaptability of a monitoring sensor under varying conditions.

Why and How to Monitor Microalgal Biofilms?

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Therefore, we explored the dynamics of biomass and astaxanthin production in H. pluvialis within a rotating biofilm system. This study involved characterizing key biofilm traits for process optimization, including biomass accumulation, astaxanthin content, and chlorophyll levels under various light and nutrient conditions (Morgado et al., 2023). Subsequently, in the monitoring domain, we developed a protocol using reflectance spectroscopy and specifically tailored reflectance indices that correlated strongly with those key biofilm traits in a non-destructive manner (Morgado et al., 2024).

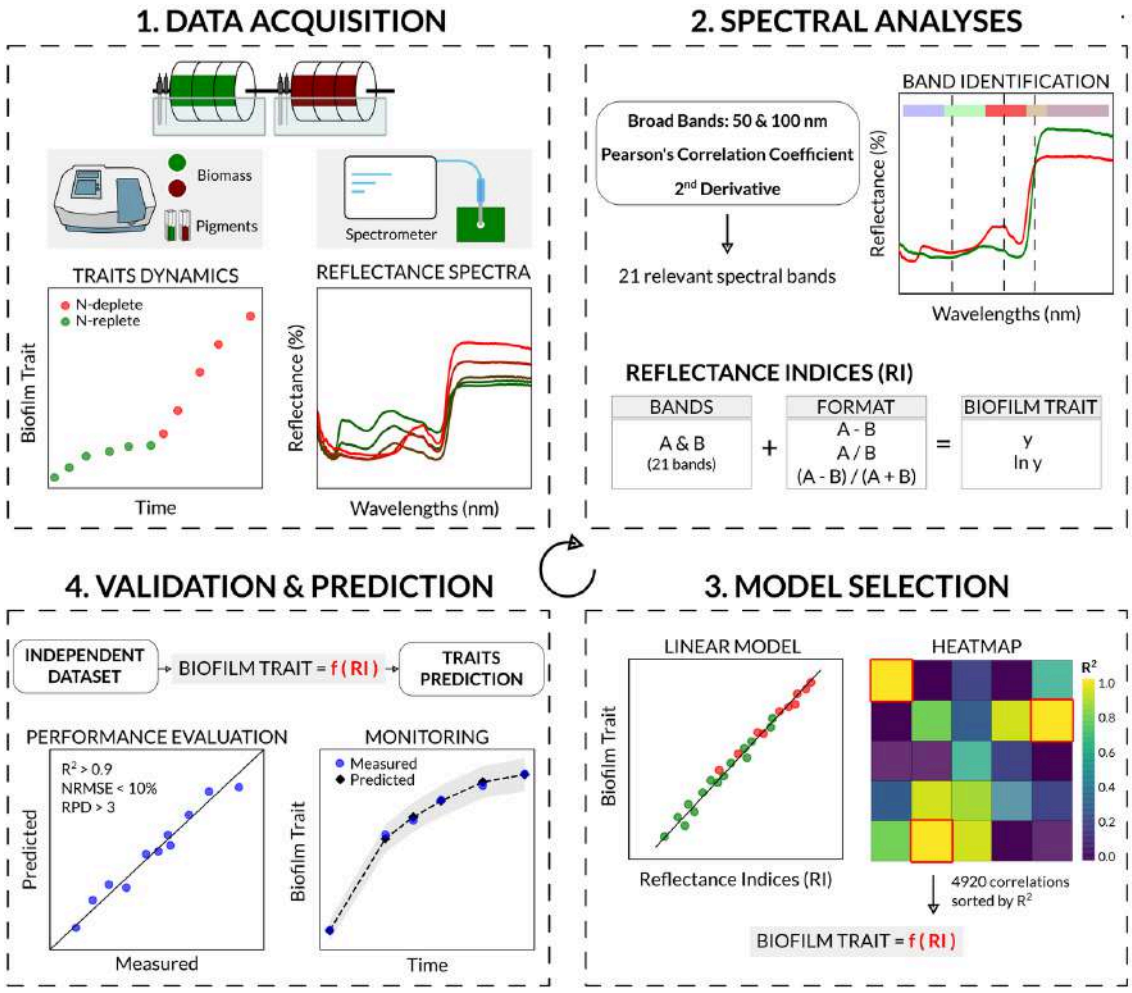

Figure 1. Workflow depicting the protocol utilized to develop and evaluate the regression models based on reflectance spectra.

David Pereira - April 22, 2024

The workflow of that protocol, summarized in Figure 1, involved:

1. Acquiring reflectance spectra simultaneously with each biofilm trait measurement.
2. Identifying relevant bands by applying pre-processing techniques such as the Savitzky–Golay filter and Pearson's correlation, and constructing reflectance indices in three different formats: A-B, A/B, and (A-B)/(A+B).
3. Computing all possible combinations, and selecting the best indices for each biofilm trait. These indices were then used to develop and calibrate linear models.
4. Validating the models on an independent dataset to evaluate their predictive performance and reliability.

Our findings confirmed that the relationships between biofilm traits and reflectance indices (RIs) can be accurately described using linear regression models, demonstrating their strong predictive performance and adaptability under various operational conditions. The implementation of this monitoring technique could lead to an efficient and smarter way of microalgae biofilm farming making it an excellent candidate for scaling up to industrial applications. Looking forward, the focus will shift towards integrating this framework into an online monitoring system, combined with mathematical modeling and control strategies, to further boost the productivity and efficiency of microalgal biofilm-based technologies.

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Fluorescence sensor for the monitoring and control of microalgal cultivations

Luis Porras Reyes - April 22, 2024

Microalgae are well known photosynthetic microbes used as cell factories for the production of relevant biotechnological compounds (e.g., pigments, unsaturated fatty acids) and environmental applications (e.g., wastewater treatment, lipids for biofuels). Despite these outstanding properties, the industrial scale production of microalgae is still at an early stage of development compared to other cell-based bioprocesses. An important bottleneck in this support is the lack of suitable online sensors for reliable monitoring of biological parameters in microalgae bioprocesses.

Fluorescence spectroscopy offers several advantages as a powerful analytical technique in the monitoring of different processes. Its high sensitivity, selectivity, fast measurements, versatility, and non-invasiveness make it a widely used tool for research, quality control, process monitoring, and diagnostic applications in fields such as biochemistry, bioprocess engineering, pharmacology, environmental science, and materials sciences. But first, what do we call fluorescence phenomena? When referring to this concept we have to consider that a molecule, called fluorophore, absorbs light energy which promotes electrons into a temporary excited state (fluorescence lifetime), followed by a return to a basal state in which the excess energy is dissipated through the emission of photons (emitted fluorescence light), i.e., energy whose wavelength is larger than that which generated the excited state, due to energy loss by, e.g., vibration. In order to show fluorescence, fluorophores must contain an aromatic system in their chemical structure.

In biotechnology, fluorescence measurements are commonly used for the detection of biological molecules such as proteins fractions (e.g., tryptophan, tyrosine, and phenylalanine), vitamins, and cofactors (e.g., FAD and NADH) which have been used in the estimation of critical process parameters (CPP) (e.g. biomass, metabolites, substrate consumption, product formation). In microalgae bioprocesses, pigments like chlorophyll and phycobiliproteins should also be considered as candidate fluorophores for the monitoring and control of these microorganisms.

Researchers have reported using sensor software and fluorescence spectroscopy to assess CPPs in microalgae cultures (e.g., biomass, pigment, and lipid content). However, the fluorescence data is still collected through offline measurements. This presents an opportunity for the development of online fluorescence sensors that can provide real-time, non-invasive, and reliable information on the status of microalgae cultures.

Our recent studies focused on developing an online prototype fluorescence sensor to monitor microalgae cultures. We began by screening Chlorella vulgaris cultures to identify the most important intracellular fluorophores (refer to Figure 1). With this basic information, it was possible to design a simple and low-cost sensor prototype that has demonstrated promising results.

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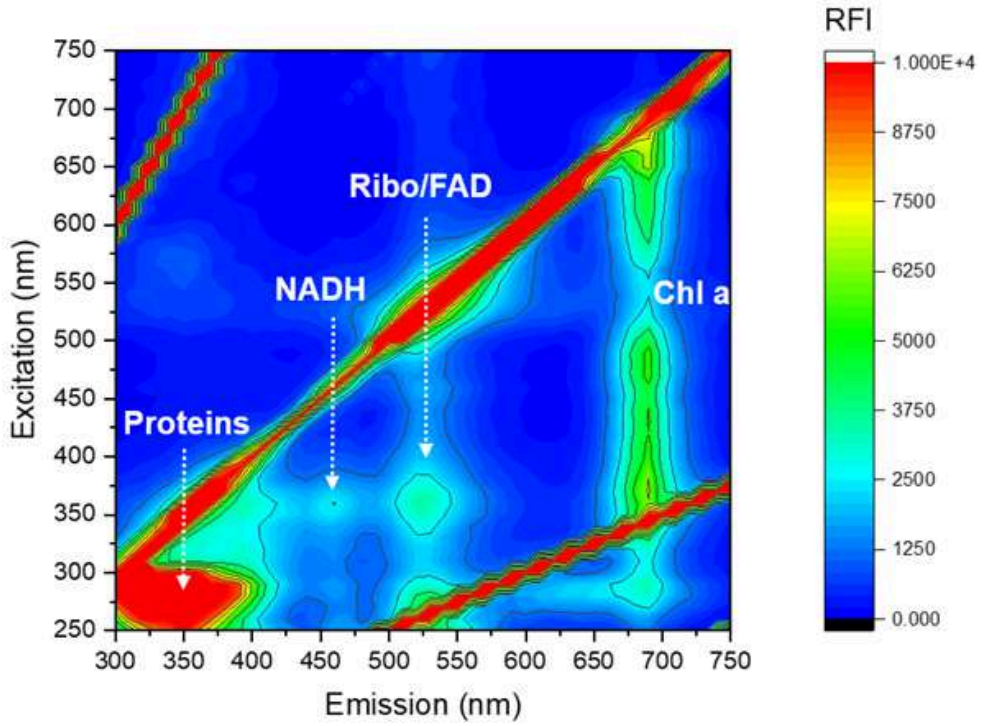

Figure 1. Offline 2D fluorescence spectra of a 13 days C.vulgaris shaking flask cultivation (24°C, 100rpm, BBM medium (pH 6,5), continuous light (55 µmol photons m−2 s−1).

The fluorescence signals obtained with riboflavin (chemical reagent) at different concentrations showed a linear trend with both the on-line prototype and the reference method: fluorescence spectrophotometer (refer to Figure 2). This trend was also observed in the online and offline measurements with NADH (results not shown). Finally, by using dilutions of C. vulgaris cultures, it was able to obtain online fluorescence signals for both riboflavin and chlorophyll. The fluorescence signal intensity decreases significantly when measuring riboflavin, while the measurements associated with chlorophyll are found throughout the sensor intensity detection range (refer to Figure 3). The behaviour of the evaluated dilutions shows a parabolic trend from the most diluted samples to the undiluted samples. These results are consistent in both fluorophores and could be possibly explained due to a signal saturation caused by the accumulation of the sidescatter light.

The results are promising for developing an online fluorescence sensor for microalgae cultures. However, future adjustments to the electrical current that feeds the light source for each fluorophore's excitation should be considered. Online fluorescence sensors, when coupled with software sensors, can be used to estimate CPPs relevant in the monitoring and control of microalgae cultures.

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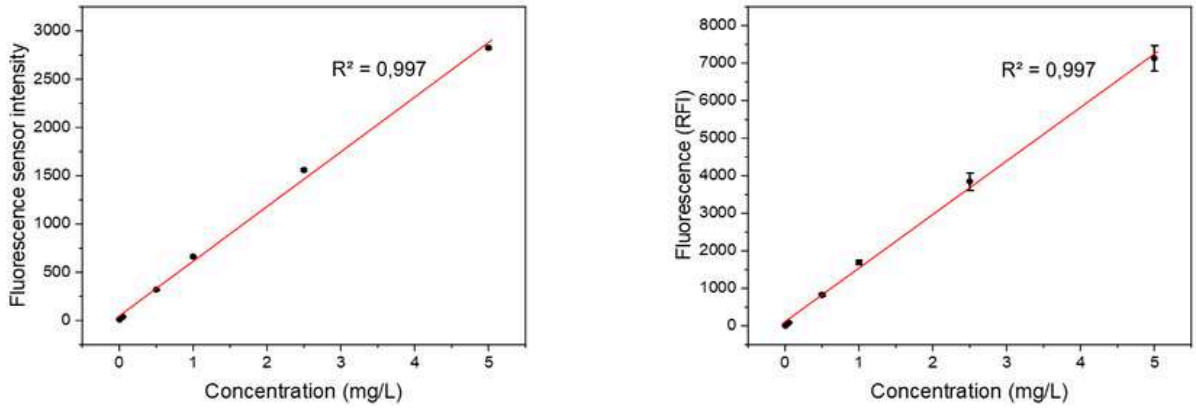

Figure 2. Online (left) and offline (right) measurements of different concentrations of riboflavin (0,005, 0,05, 0,5, 1, 2,5 and 5 mg/L in PBS pH 7,5).

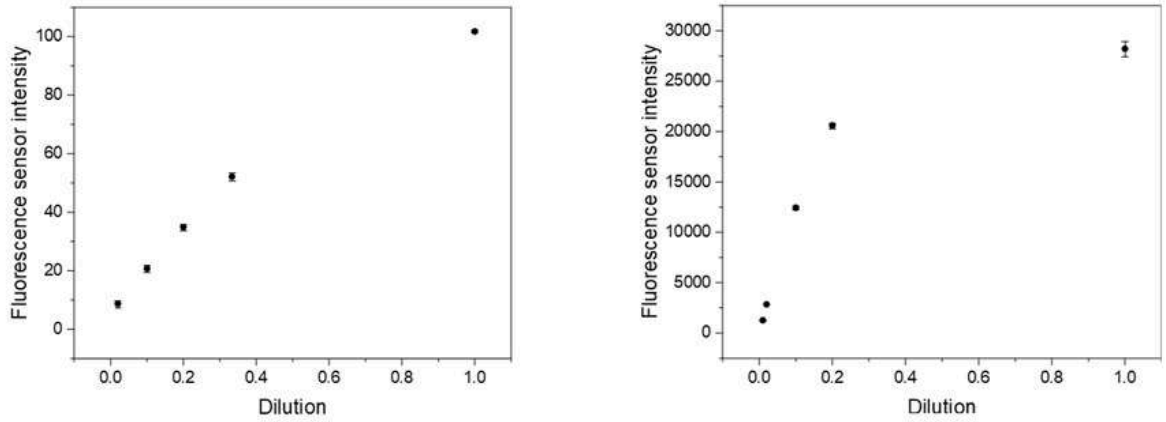

Figure 3. Online riboflavin measurements (left) and online chlorophyll measurements (right) using C.vulgaris cultivated cells at different dilutions (1:50, 1:10, 1:5, 1:3, 1 using NaCl 0,9%).

Enhancing microalgae production through data-driven technologies

Shyam Krishnan Kalliadan - April 22, 2024

In the rapidly evolving landscape of biotechnology, the industrial production of microalgae stands out as a field ripe for innovation, particularly through the application of data-driven technologies. These advancements encompass data management, sophisticated analytical dashboards, predictive modeling, and the integration of machine learning and deep learning techniques. Each of these components plays a crucial role in optimizing the cultivation, monitoring, and control of microalgae production, promising not only to increase efficiency but also to enhance sustainability.

Effective data management is the cornerstone of modern industrial microalgae production. A centralized data warehouse that aggregates both online and offline data offers a comprehensive overview of the production metrics. This integrated approach ensures that data from various stages of microalgae cultivation is readily accessible, thereby enabling timely decision-making and process optimization. With real-time data monitoring facilitated by sophisticated data pipelines, producers can fastly identify and respond to any anomalies or opportunities for improvement in the cultivation process. Dashboards play a pivotal role in the industrial environment by providing a real-time visualization of key production metrics. These tools are not just passive displays but active components of production management, enabling the seamless monitoring of growth rates, nutrient uptake, and environmental conditions.

Predictive modeling stands at the forefront of technological integration in microalgae production. Traditional methods for assessing vital process parameters, like biomass and nutrient concentrations, predominantly rely on offline lab analysis. Although precise, these methods suffer from delays that render them impractical for real-time control and optimization of bioprocesses. To bridge this gap, the development and implementation of advanced machine learning techniques, such as Long Short-Term Memory (LSTM) and LSTM-Transformers, have become increasingly prevalent.

A significant advancement in this area is showcased through the development of LSTM-based soft sensors. This innovative approach leverages the predictive power of deep learning to offer real-time insights into microalgae growth, directly impacting production strategies and operational efficiencies. The LSTM model, a type of recurrent neural network optimized for sequence prediction, is ideal for the time-series data typical in microalgae cultivation. It excels in handling data where the timing of input information is crucial, as it maintains internal states to remember past data, thus providing a more accurate prediction of future events.

Further enhancing the capabilities of traditional LSTMs, the integration of Transformer models in LSTM architectures (LSTM-Transformers) represents an evolution in handling sequence data. Transformers provide an attention mechanism that weighs the importance of different words in a sequence, offering significant improvements in modeling long-range dependencies.

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When combined with LSTMs, this model can effectively capture both the fine-grained details and broader context of time series data, which is essential for accurately predicting the complex biological and environmental interactions in microalgae growth.

In this research, the LSTM was meticulously trained using data from a controlled environment featuring a vertical flat panel photobioreactor. The comprehensive data collection covered various process parameters, including light absorption rates, CO2 concentrations, and other environmental conditions affecting microalgae growth. The ability of LSTM-based models, particularly when augmented with Transformer capabilities, to predict microalgae biomass concentration is highly reliable. These models significantly surpass the performance of earlier benchmark methods, highlighting their superior capability to handle the multifaceted nature of biological systems. By accurately predicting growth conditions, producers can optimize harvesting cycles, adjust feeding rates, and better control light and CO2 exposure—all in real time. This not only maximizes yield but also ensures the highest quality of biomass production by maintaining optimal growth conditions throughout the cycle.

However, the study also acknowledges challenges in applying these advanced models, such as the need for extensive datasets for training and the potential for overfitting. Future research will likely focus on refining these models to handle a broader range of conditions and to further enhance their accuracy and reliability.

In summary, the advancement of LSTM and LSTM-Transformer-based soft sensors represents a paradigm shift in how data is utilized for the cultivation and monitoring of microalgae. This technology not only streamlines production but also enhances the scalability and sustainability of microalgae as a bioresource. As industries continue to seek more sustainable and efficient production methods, the role of advanced data analytics and machine learning models will undoubtedly expand, marking a new era in biotechnological innovation.

The integration of data-driven technologies in microalgae production marks a significant advancement in the field of biotechnology. Through sophisticated data management systems, real-time dashboards, predictive modeling, and the strategic application of machine learning and deep learning, producers can achieve unprecedented levels of efficiency and productivity. These technologies not only streamline production but also open new avenues for innovation in sustainable biotechnology, ensuring that the cultivation of microalgae remains both economically viable and environmentally responsible. As the industry continues to grow, the adoption and refinement of these technologies will be key to its future success, offering promising prospects for both industrial players and the environment.

Enhancing microalgae production through data-driven technologies

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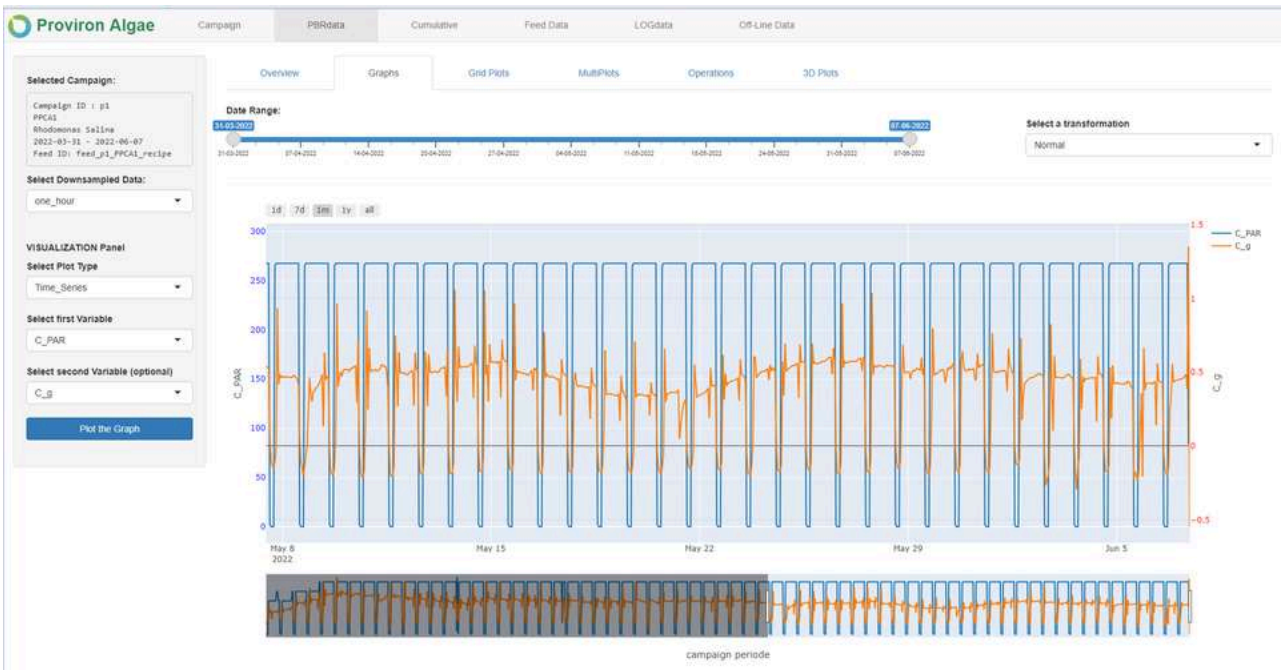

Figure 1. Dashboard interface for algae production monitoring with integrated pipeline and predictive modeling functionality.

Dynamic design of experiments for high-value compound production from acidophilic Coccomyxa onubensis in a high cell density cultivation system

Rosaria Tizzani - July 17, 2024

Microalgae offer a sustainable alternative for producing high-value bioactive compounds, specifically antioxidant pigments. Lutein, a key carotenoid vital for diverse physiological functions, including light-mediated reactions, antioxidation, and ocular protection, provides significant clinical advantages. However, conventional extraction of lutein from marigold flowers poses challenges associated with resource-intensive cultivation, substantial land use, and complex processes. In contrast, microalgal lutein production demonstrates up to sixfold higher extraction rates and reduced resource requirements. The projected growth in the global lutein market underscores a shift towards sustainable production, emphasizing the diverse benefits of lutein in pharmaceuticals, nutraceuticals, and the food sector. Nevertheless, stringent European regulations present challenges for employing microalgae in human applications, limiting production to closed photobioreactors characterized by high automation and significant sterilization costs.

A future challenge involves enhancing microalgae growth for increased productivity while minimizing contamination risks from other microorganisms, notably bacteria. This study focuses on acidophilic microalgae, specifically Coccomyxa onubensis, leveraging its proficiency in acidic conditions to yield high amounts of carotenoids, especially lutein, and prevent bacterial contamination. The implementation of an innovative cultivation system, featuring a hydrophobic membrane, strategically enhances photoautotrophic growth by facilitating CO_2 provision through diffusion. This design not only ensures a stable microenvironment but also significantly improves CO_2 mass transfer, thereby boosting biomass productivity. Employing a dynamic design of experiments (DoDE), the study meticulously controls key variables, including dynamic light intensity profiles, nitrogen feeding strategies, and constant partial pressure of CO_2.

Exploring three-tiered CO_2 partial pressure, with the highest concentration reaching 10%, establishes a correlation with the mitigation of CO_2 emissions from significant industrial sectors. This comprehensive approach aims to uncover the commercial viability of C. onubensis, utilizing a dynamic design to refine microalgal cultivation strategies and provide valuable insights for sustainable bioprocessing, particularly in high-value bioactive compound production such as lutein. From the optimization, different cultivation strategies were developed to optimize the dynamic profile of light intensity and fed-batch feeding for nitrogen to maximize the productivity of biomass, carotenoid, and lutein. For instance, for biomass productivity, the light intensity started from 100 and reached a peak value of 800 $\mu mol/(m^2 \cdot s)$, the nitrogen feeding was 49.99 mg supplied in 5 days, and the CO_2 partial pressure was equal to 6.54%. The biomass productivity with this configuration was 1.42 g/L/d. Regarding lutein, the highest lutein productivity was 2.15 mg/L/d when the microalga was cultivated under high light intensity, from 100 to 800 $\mu mol/(m^2 \cdot s)$, and a CO_2 partial pressure equal to 1%.

Dynamic design of experiments for high-value compound production from acidophilic Coccomyxa onubensis in a high cell density cultivation system

Rosaria Tizzani - July 17, 2024

In conclusion, the application of a dynamic design of experiments coupled with a high-density cultivation system enabled us to find the optimal trends of dynamic and static variables. In light of these results and the microalgae's ability to grow in environments that reduce the risk of contamination, Coccomyxa onubensis is a promising candidate for producing high-value-added compounds in an industrial context. This study demonstrates the potential of using a dynamic experimental design to optimize cultivation conditions, thereby enhancing the commercial feasibility of microalgal-based bioprocessing.

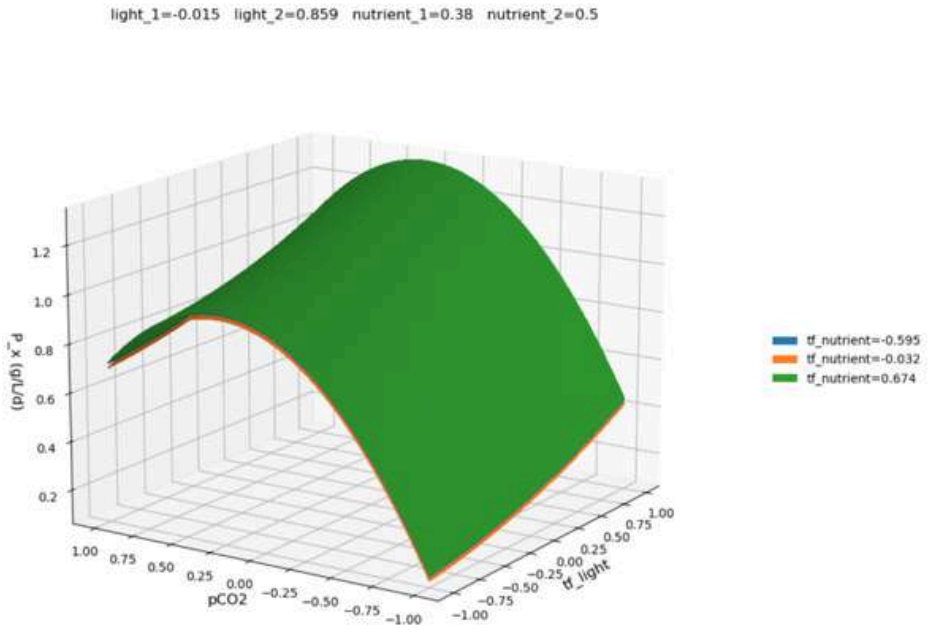

Figure 1. Response surface model for biomass productivity relating the factors of time to reach light peak (t_f light) and duration of nitrogen feeding (t_f nutrient).

Strategies for real-time monitoring of nitrate levels in open-pond cultivation systems

Henrik Geltner - July 17, 2024

As one of the essential building blocks of life, nitrogen is often used to boost crop growth in agriculture. Common fertilizers therefore contain high nitrogen concentrations in the form of nitrate salts. The remaining drain waters from horticulture thereby still contain high levels of nitrate. Discarding such nitrate-rich drain waters into the environment can severely damage the natural balance of rivers by increasing toxicity for fish and invertebrates and causing algal blooms. Therefore further removal of nitrate from the remaining waters is needed before it can be discarded into the environment safely. To achieve this reduction of nitrate different technologies can be employed. One promising approach is cultivating microalgae in the drain water and extracting them before discharging the nitrate-diminished water. Microalgae like higher plants need nitrogen as a key element to build up biomass and grow. Moreover, they can be grown photosynthetically using sunlight as their energy source while consuming carbon dioxide as a carbon source in the process. Utilizing wastewater in this process is especially promising because it combines the upcycling of waste streams to valuable biomass and the reduction of nitrate and carbon dioxide pollution in a natural and environmentally sustainable way.

One of the challenges in optimizing this process is designing optimal process strategies and process controls to sustain efficient nitrate removal rates and high microalgae productivity while keeping process costs low. To do so, a continuous, precise, and fast monitoring of nitrate and biomass concentrations is required to gain a deeper understanding of process characteristics and enable permanent process control under optimal conditions. Unfortunately, current options to continuously monitor nitrate concentrations are scarce and complicated to implement. Therefore new methods have to be developed that allow a reliable and automatic detection of nitrate concentrations in the nutrient solution over time. These monitoring methods should be implementable in open-pond systems which are the most common reactor type for processing high quantities of drain or wastewater due to their low operation costs. By this constraint, the list of potential measurements is limited to in-line sensors directly installed into the culture water or analytic methods performed in a bypass flow from the reactor.

One strategy to estimate nitrate levels can be via measurements of the electrical conductivity in the cultivation broth. Electrical conductivity (EC) is the ability of a material to conduct electric current e.g. transfer electrons. Sensors for electric conductivity are generally robust and span a wide signal range making them very suitable for use in open-pond systems. In aqueous solutions, nutrient salts dissociate into forming ions of opposed charges. These charged molecules in the medium facilitate the transfer of negatively charged electrons through the electric field. In this context, the nutrient solution is also called electrolyte. Nitrate is the negatively charged ion formed in solutions of different salts such as sodium nitrate or potassium nitrate.

Strategies for real-time monitoring of nitrate levels in open-pond cultivation systems

Henrik Geltner - July 17, 2024

At lower salt concentrations the electrical conductivity of the solution increases nearly proportional with increasing ion concentrations. This allows for easy calibration of EC signals to Ion concentrations in low-salinity solutions. The extent to which the electric conductivity and the concentration of a specific ion type relate differs by the type of ion species depending on its overall charge, size, and the total amount of ions already dissolved. At higher salinities, ions interact with each other, for example, by shielding each other's charges and decreasing their free mobility in the medium. By that, electron transport can be dampened. Thus, electric conductivity and Ion concentrations are not correlated linearly for elevated salinities. Various mathematical models have been developed that intend to describe these effects. However, the specificity of many models towards only certain ions and process conditions makes a proper model selection for the broad use case of outdoor algae cultivation difficult. Another challenge of estimating specific ion concentrations in mixed solution by EC measurements is predicting the extent to which further ion species affect the conductivity signals. A proper approximation of nitrate levels by EC can only be achieved if sufficient knowledge of the most contributing chemical processes and additional measurements can be combined. Such a combination of multiple sensing principles and a detailed model of the process and measurements is called sensor fusion.

In microalgae cultivation nitrate is one of the main contributors to algal biomass formation. Thereby, it can be estimated that the consumption of nitrates has a stronger impact on the dynamics of electric conductivity signals and the contribution of many other ions is less important. Still, some other molecules may also interfere and must be measured or approximated. Further species of ion that greatly influence electrical conductivity signals in microalgal processes are protons, hydroxide anions, as well as bicarbonate and carbonate. While the levels of protons and hydroxides can be determined from pH measurements there is no current option to directly measure the carbon dioxide-dependent molecules bicarbonate or carbonate in real-time. These could, however, be approximated knowing either the levels of total inorganic dissolved carbon or dissolved CO_2 and the pH of the medium. As described, many steps in such sensor fusion approaches comprise only estimates of various parameters based on first principle assumptions incorporated in the process model. Here, different algorithms can be applied, for example, Kalman filters or Luenberger-observers to improve the accuracy of the final parameter estimation. These algorithms adjust initial estimates of variables based on the difference between a prediction of measurement signals and real measurements obtained. The sensor fusion principle and software sensor design are powerful tools to enable close predictions of unmeasurable parameters such as nitrate concentrations in real-time applications which are increasingly utilized to advance automation in bioprocess technologies.

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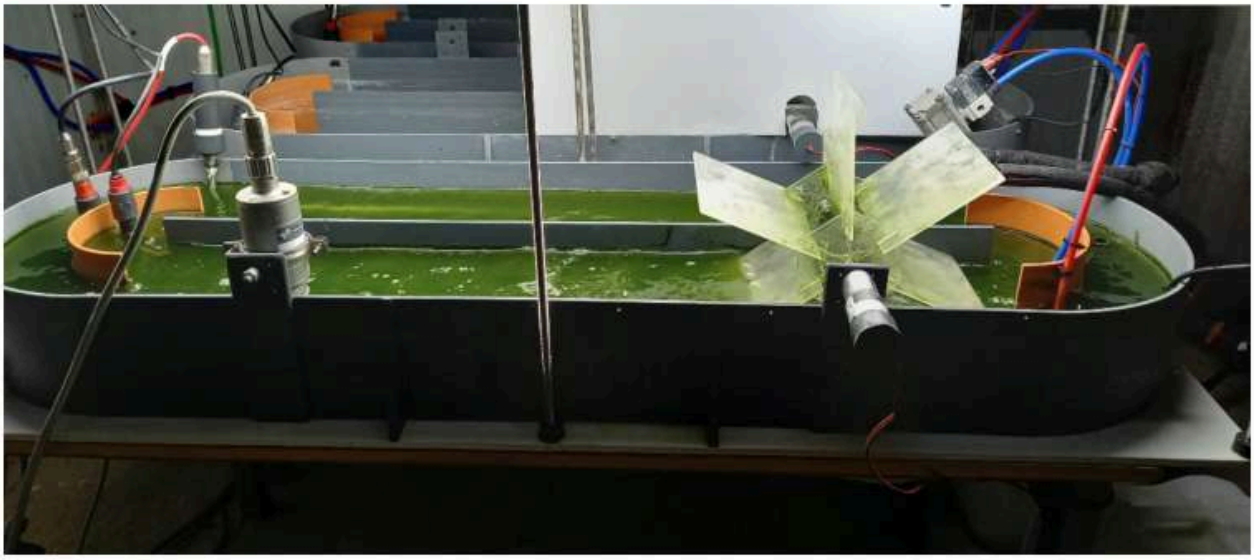

Figure 1. Raceway open-pond reactors equipped with paddle wheel at the pilot plant facilities of IFAPA Institute (Almeria, Spain).

Effect of light on biofilm structure of a green alga and a diatom

Beatriz Felices Rando - July 17, 2024

Microalgal biofilm systems show multiple advantages over classical photobioreactors for biotechnological applications, such as lower water requirements and energy consumption. However, the high biomass densities reached at the most mature stages of the biofilm may limit light and nutrient availability. A better understanding of how the metabolic activity of the cells changes in such a different environment is needed to optimise biofilm-based systems for industrial applications.

The effect of light is of particular importance, as for many industrial cultivation systems, light is supplied artificially to avoid the inherent fluctuations of natural light, which may hinder biomass production and compromise the quality of downstream products. Choosing the right light intensity for cultivation is thus not a triviality in terms of energy management: biofilms tend to be light limited as they grow thicker, since the cells at the higher layers will be attenuating light for the cells at the lower layers, which will eventually stop being photoproductive. Higher light intensities, on the other hand, can be useful to prevent this issue but may as well be causing photodamage to the cells. Unlike suspended cultures, cells growing in biofilm systems are not subject to the mixing-induced light-dark cycles that characterise suspended cultures, which make the latter more tolerant to high light intensities than biofilm systems.

A recent study performed at CentraleSupelec in collaboration with the University of Padova addressed the investigation of the effect of light on two different microalgal species along two weeks of biofilm development. The two strains under study were the green alga Tetradesmus obliquus and the diatom Phaeodactylum tricornutum. Online dissolved oxygen measurements, reflectance spectra, confocal laser scanning microscopy, optical coherence tomography and image analysis were applied to analyse the characteristics of the different biofilms at each light condition for both strains. The main variables analysed were biofilm biovolume, average thickness, porosity and pigment content.

Besides proving that biofilm growth is highly species-specific, results also showed that the times for reaching a plateau by the biofilms in terms of biovolume and biofilm thickness vary strongly depending on light intensity. Pigment content was also strongly affected by light intensity and maturity of the biofilm.

The results obtained from this study provide valuable insights into process design and optimisation of biofilm-based technologies, particularly for choosing the optimal light intensity conditions according to the desired biomass composition and for optimising biomass harvesting times according to growth conditions.

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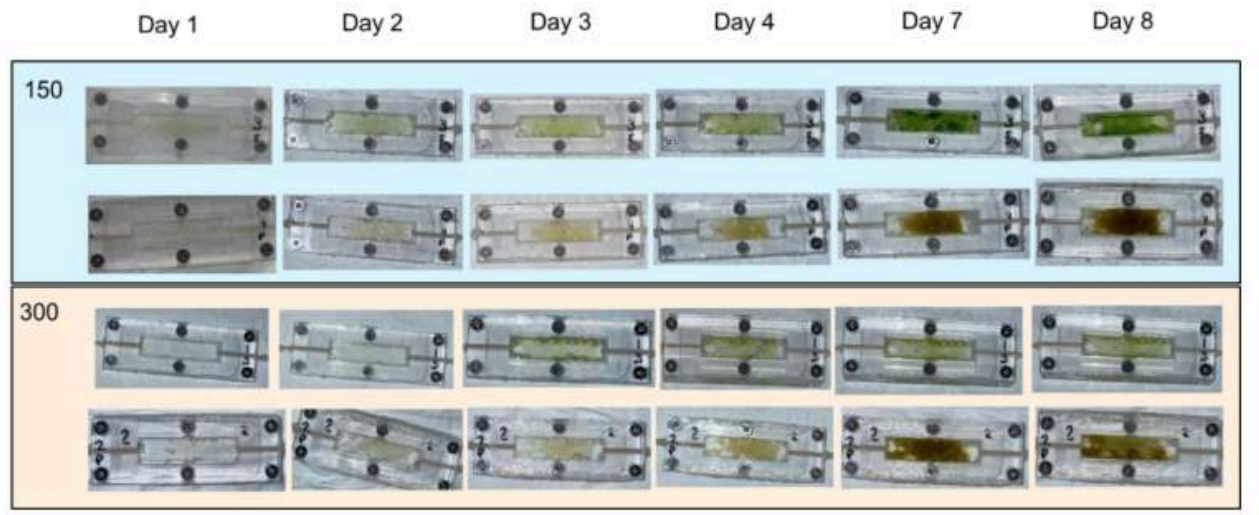

Figure 1. Flow-cells containing T. obliquus (in green) and P. tricornutum (in brown).
Top section: incident light intensity of 150 µmol m-2 s-1.
Bottom section: incident light intensity of 300 µmol m-2 s-1.

CryoEM structure of a red shifted antenna complex

Giovanni Consoli - July 17, 2024

Phycobilisomes are a structurally and functionally diverse family of light harvesting complexes, that binds open-chain tetrapyrroles known as bilins with the aim of absorbing photons and funneling excitation energy to photosystems, mainly photosystem II in cyanobacteria. The extreme variability in size, arrangement and absorbance profile deeply reflects the evolutionary adaptation of this family of antenna complexes to a number of ecological niches.
Given the small number of long wavelength pigments present in Chl f containing photosystem, a red-shifted antenna capable of efficiently funneling far-red photons into far-red Photosystems is crucial to increase the antenna size for wavelengths above 700nm. The FaRLiP gene cluster contains five phycobiliprotein paralogues, that have been confirmed to assemble, together with other subunits in common with white light, into a bicylindrical complex, but in literature no intact structure of this complex is present.
For this reason, we focused on purifying this antenna while attached to photosystem II. We then collected a Cryo electron microscopy dataset of this protein complex to try and solve its structure through single particle analysis. Unfortunately, the flexible nature of the connection between this antenna and the photosystem has prevented us from obtaining a fully intact structure, but we still managed to obtain a map at a resolution of 2.60 Å of the cylindrical complex alone.
This structure is going to greatly impact our understanding of excitation energy transfer from the antenna to the reaction centers in far red light adapted organisms.

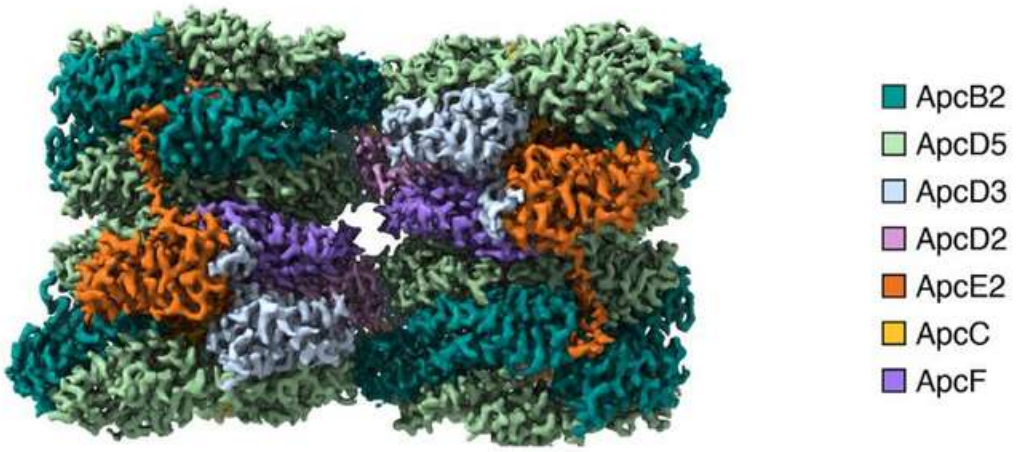

Figure 1. CryoEM single particle analysis reconstruction of the structure of a bicylindrical antenna complex from the cyanobacterial C. thermalis PCC 7203.
Every subunit type has been coloured differently.

Robust experiment design for model discrimination

Marco Sandrin - October 17, 2024

Model-based design of experiments for model discrimination (MBDoE-MD) is a powerful tool that can greatly accelerate the development of first-principles mathematical models. After a preliminary collection of experimental data, multiple model structures are typically proposed to describe the system under investigation and its underlying phenomena. By leveraging the model equations and values of the parameters, MBDoE-MD aims to design experiments that maximise information, facilitating the comparison between rival models and allowing the most appropriate model structure to be selected.

Similarly to model-based design of experiments for parameter precision (MBDoE-PP), MBDoE-MD uses an optimisation framework to maximise a certain information metric. In standard MBDoE-PP approaches, the formulation revolves around the concept of Fisher information matrix, computed from the sensitivities of the measured variables with respect to the model parameters, in an attempt to maximise these sensitivities and reduce the size of the inference regions of the parameters. In MBDoE-MD, instead, the goal of the optimisation problem is to maximise the divergence between the predictions of different models. Since MBDoE-MD is meant to be used as the beginning of the model development process when only little information (if any) is available on the values of the model parameters, it is critical to take parametric uncertainty into account and make the experiment design problem formulation as robust as possible.

A study on the topic was recently conducted as part of a collaboration between Imperial College London and the University of Padova, to develop a framework enabling rapid model selection with a particular focus on microalgae growth processes. A prototype tool has been implemented in Python to tackle the design of dynamic experiments. It involves a standard gradient-based search over the space of the experiment decision variables. The user can specify two or more models exploiting the Pyomo.DAE library, which provides an easy-to-use interface to the integrators in the SUNDIALS suite. The nonlinear programming optimisation problem is solved via SciPy using a trust region algorithm. The experiment design problem can be formulated by specifying the relevant design variables, including the duration of the experiment, initial conditions, constant or time-varying inputs, and sampling times for the measurements.

Parametric uncertainty is included in the formulation of the optimisation problem by considering a set of uncertainty scenarios, sampled from the prior distribution of the parameters using effective space-filling techniques. The model discrimination tool implements three distinct approaches, which differ in the way they account for the different uncertainty scenarios:

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- worst-case approach: aims at designing the most informative experiments for the least informative values of the model parameters;
- expected value approach: calculates the average value of the information criterion over the entire set of uncertainty scenarios;
- conditional-value-at-risk (CVaR) approach: attempts to design the most informative experiments for a (user-specified) percentage of scenarios corresponding to the tails of the parameter distribution. This concept is borrowed from portfolio optimisation.

All methodologies have been thoroughly tested with promising results. Compared to the worst-case design, the expected value and CVaR methods are less sensitive to "bad" parameter values as they account for multiple uncertainty scenarios, and the CVaR approach offers a more averse attitude towards risk. Future work will entail integrating the formulations into a framework for designing campaigns comprising multiple parallel experiments.

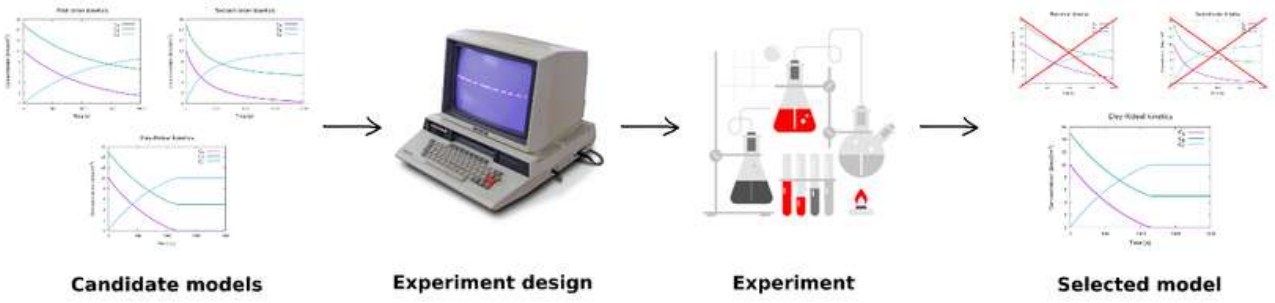

Figure 1. Model discrimination workflow: candidate models are passed to the experiment design tool; the experiment is then performed, and the most appropriate model structure is selected based on the experimental data collected.

Sensor based on flow cytometry for population monitoring of Chlorella zofingiensis at different process conditions

Yob Ihadjadene - October 17, 2024

Microalgae are becoming increasingly important in the bioeconomy and biotechnology sector as an attractive, sustainable source of value-added products. Thus, to develop feasible algae-based bioprocesses, it is crucial to study their intrinsic characteristics, such as their physiology, metabolism and their response to factors influencing their growth. However, the design and optimization of these processes are usually focused on optimizing biomass productivity, while neglecting the impact of cell-to-cell heterogeneity. In many bioprocesses, cell population heterogeneity is accepted and overlooked, nevertheless, in research, consideration of different cell stages is essential, especially for quantitative omics studies, since it has been demonstrated that cell-cycle events directly affect major culture parameters, including cell size, cell density and amount of storage compounds, such as starch, lipids, and polyphosphates.

Therefore, characterizing microalgae sub-populations is of central importance for advancing microalgae-based biotechnology, but traditional methods are often time-consuming and inefficient. To address this, the development of precise and rapid analysis methods is necessary. Thus, flow cytometry (FCM) offers a powerful, high-throughput solution for single-cell analysis, allowing researchers to efficiently study key characteristics like physiology, metabolism, and responses to growth conditions, significantly improving the speed and accuracy of microalgae research.

This work focuses on developing various FCM methods to study and monitor how different environmental conditions, mainly variations of light spectra (blue, red and green), and induced stress conditions (nitrogen depletion and osmotic stress), affect population dynamics and subpopulation structure of the unicellular green microalgae Chlorella zofingiensis, a natural source for lipids, proteins and carotenoids such as astaxanthin, an important pigment for biotechnological applications. It was found that cell division was negatively affected by osmotic stress, regardless of the light spectrum. Similar pattern was observed under nitrogen depletion and in combination of both conditions. On the other hand, cell division was positively affected under optimal nitrogen conditions, regardless of the light spectrum, and reached the highest division rate under red light spectra.

In terms of population heterogeneity, we observed high cell-to-cell heterogeneity in optimal nitrogen condition for red light and blue light illumination, however, this was not the case at green light, where a strong cell-to-cell heterogeneity occurred in combination of osmotic stress with optimal nitrogen concentration. Furthermore, the established FCM approaches enabled for the first time to closely monitor the temporal evolution of the metabolic characteristics in terms of pigment synthesis at the single-cell level, thus providing information on the relative contribution of different subpopulations to the overall bioprocess under the studied conditions.

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These results demonstrate the robustness of flow cytometry technology as a tool for microalgal populations' analysis, and highlight the importance of considering microalgae cell-to-cell heterogeneity and their contribution to the bioprocesses in future studies.

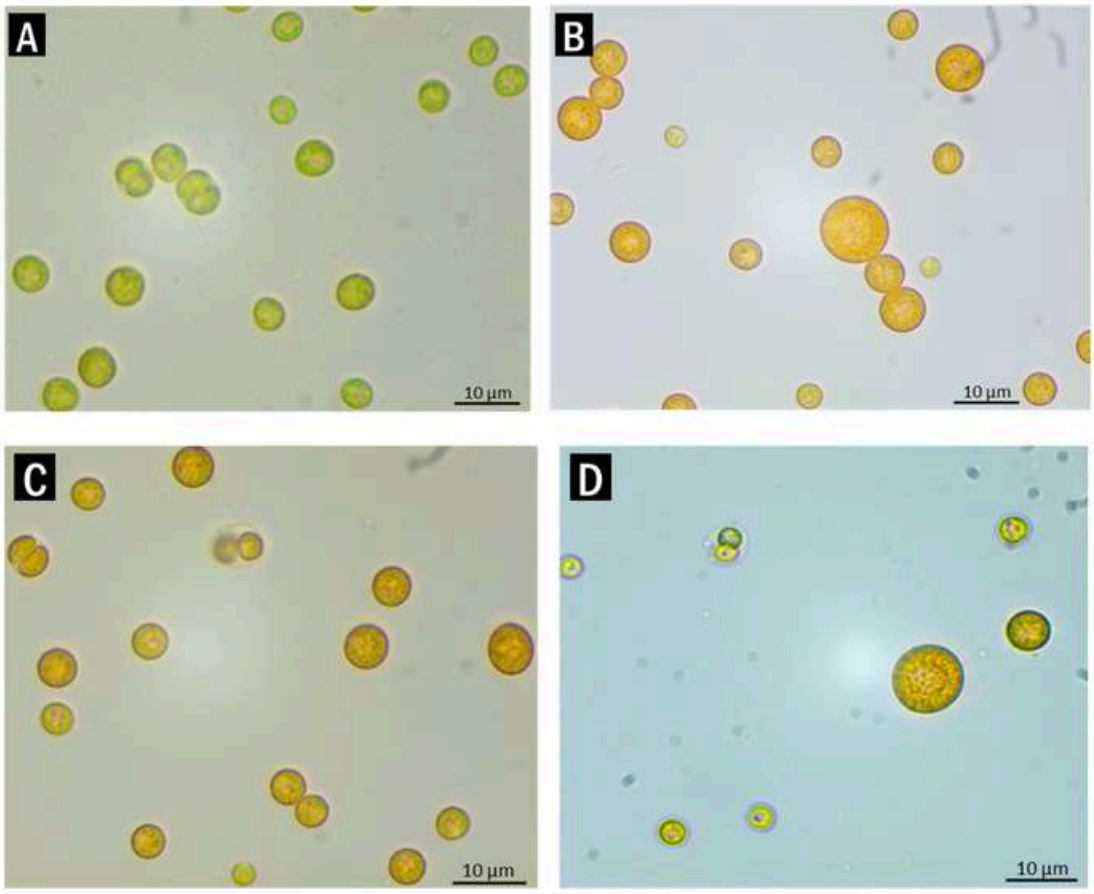

Figure 1: Figure 6. Light microscopy images of cell size distribution at (A) day 0, then day 5 for N-S+ condition under: (B) red light, (C) blue light, and (D) green light spectra.

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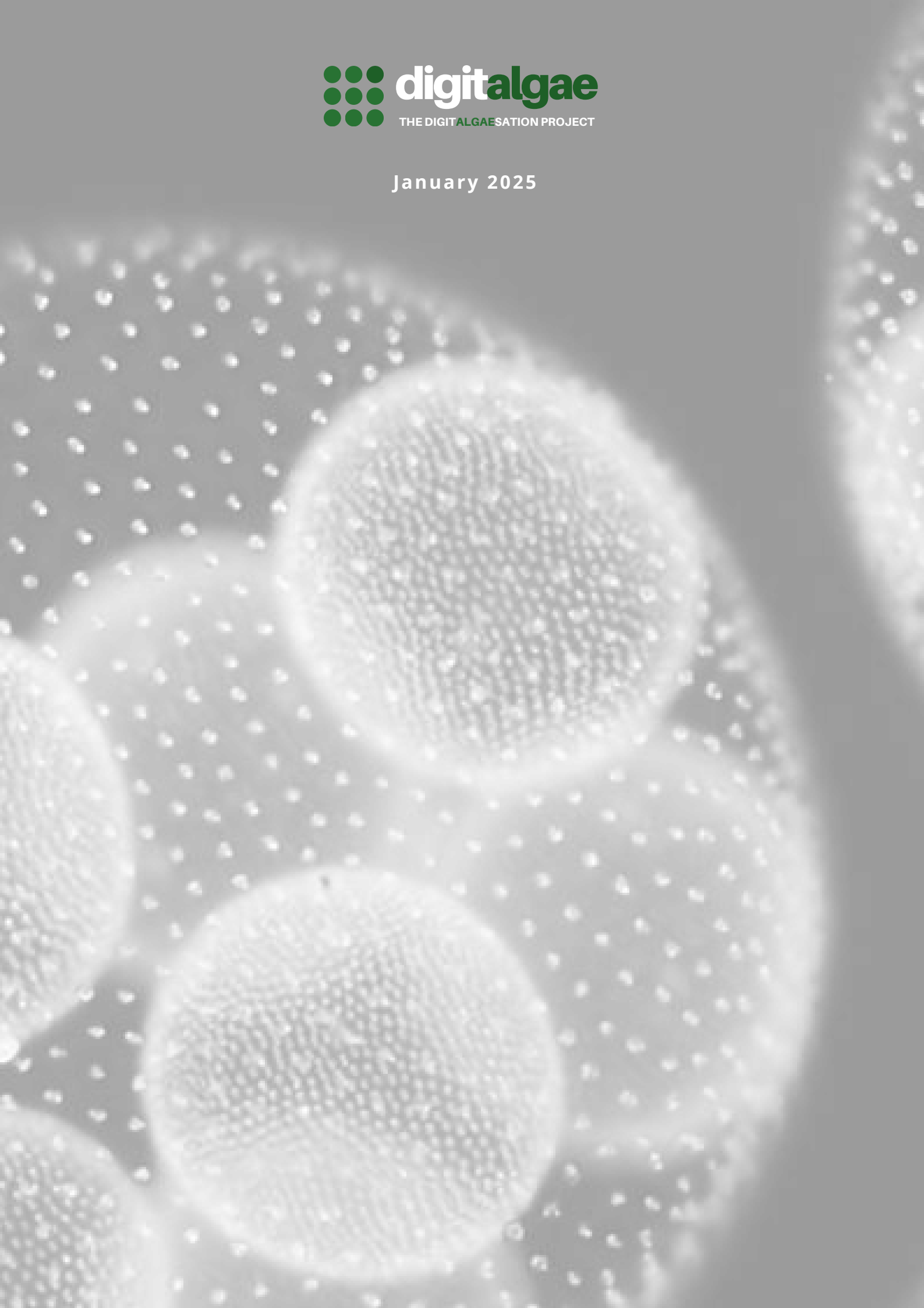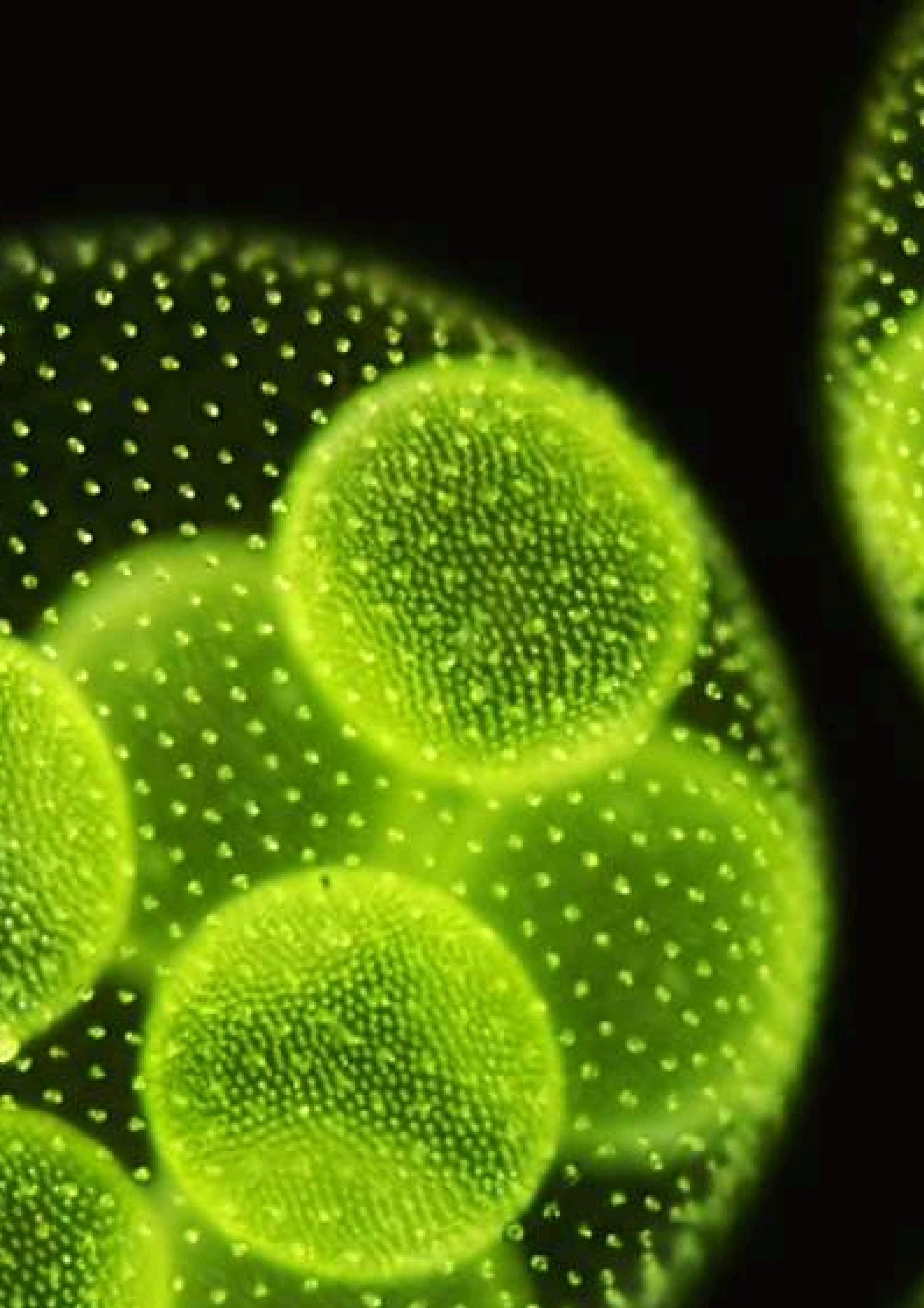
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