Prodigio

Newsletter 2023/24

Developing early-warning systems for improved microalgae PROduction and anaerobic DIGestIOn

EDITORIAL

The production of microalgae as a source of renewable fuel lived its golden years during the 2000s. The high photosynthetic efficiency of microalgae as well as their potential to grow on non-arable land and without the need for potable water attracted enormous interest on the part of the scientific community. Such interest ended up permeating the business world that saw microalgae production as a promising industry: green gold. But all expectations placed on microalgae as a renewable fuel source collapsed by the end of the decade. What seemed like a panacea turned out to be the downfall of many companies that lost their interest in the long-awaited industry of microalgae biofuels. Companies require profit in the short term but, like any other technology that starts from a lab-flask, its large-scale implementation implies overcoming a series of drawbacks, which sometimes means a long way to go.

One of the main drawbacks in this case lies in the fact that tamed microalgae grow in isolation, but in large-scale systems they need to deal with a multitude of pathogens and diseases that reduce their productivity.

PRODIGIO project aims to address this issue by developing early warning systems. These systems, which are common and very successful in many other industries, will allow us to predict when the system is going to fail, giving us time to apply countermeasures. The ultimate goal is to increase the stability of the biological communities involved in the production of microalgae biomass and its subsequent conversion into biogas. The PRODIGIO consortium, made up of world experts in the fields of process engineering, ecogenomics of microalgae and anaerobic digestion systems, and mathematical modelling, aims to renew commercial interest in microalgae as a source of biofuel and thus contribute to building a healthier planet.

Below we present the progress of the activities planned in the PRODIGIO project as well as some intermediate results.

prodigio-project.eu

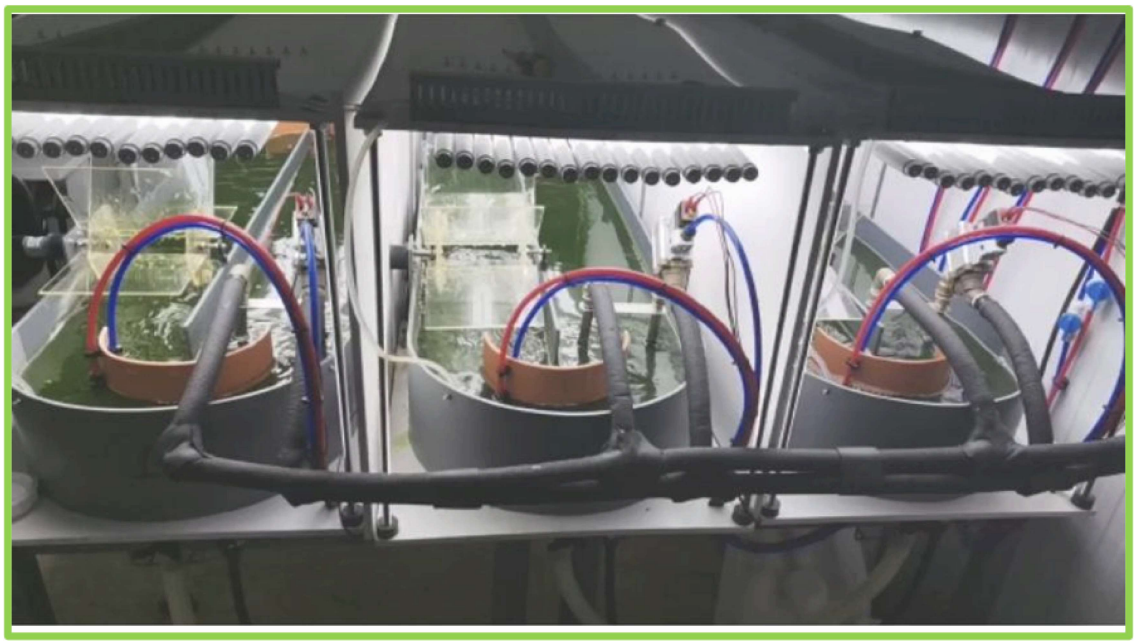
MICROALGAE PRODUCTION SYSTEMS

Failure tests in photobioreactors

The University of Almeria completed the set of experiments planned to evaluate the stability of large-scale microalgae production systems and to

identify the major factors provoking the failure of these systems. It was concluded that more than operational or environmental factors, the main factors behind the failure of these systems are biological. Thus, microbiological analysis performed allows the detection of complex microbiota, especially when coupling wastewater treatment and microalgae production. Experiments were performed both using clean water and fertilizers, but no large differences in terms of stability of the cultures were observed despite this relevant difference. Results are being processed and analysed to find early signals that allow the prevention of these failures and to develop measures to minimize their impact on the performance of the overall production system. Moreover, a new experimental setup has been developed to perform specific trials in this respect, introducing selected contaminants to study the evolution of the cultures under these conditions.

Learn more



An experimental setup installed to perform tailored experiments about the stability of microalgae cultures

Modelling the failure of microalgae production

Two 7-month time series have been produced from late May to early December of the microbiomes in open microalgal production ponds fed with wastewater and with clean water plus fertilizers for two consecutive years, 2021 and 2022. The average sampling frequency was 3 times per week (Monday-Wednesday-Friday) and the duration of the time series was 70 to 80 time points. The metabarcoding data and metagenomic data for the selected dates were stored on a NAS station and will be available upon publication in a public repository. The microbiomes were highly diverse and

dynamic, with complex interactions taking place in the ponds. About a thousand different eukaryotic species were found in each of the experiments. In the case of prokaryotes, about 5000 different species were identified in the wastewater treatment and 2500 in the fertilizer treatment, reflecting the high input of diverse microbiota from the wastewater system.

CSIC is currently preparing a manuscript describing the community structure and possible linear interactions derived from network analyses on amplicon sequence variant (ASV) metabarcoding. Interesting results reveal which pathogenic bacteria, parasitic fungi, and other microbiome organisms can affect microalgae cultivation. In addition, CSIC have installed the empirical dynamic modeling (EDM) tools in the high-performance computing cluster located at the ICM and are now dealing with adjustments such as time gaps (on weekends and holidays you cannot enter the sampling facilities) and seasonality in cross convergence mapping (CCM); that is, it is necessary to produce seasonal surrogates of the ASV time series to rule out false positive causal interactions driven solely by the common seasonality of ASV pairs. The EDM analyses will allow us to infer causal relationships between the most recurrent ASVs, the environment, and the biomass produced in the tanks. Specifically, with CCM, the partners will identify the strongest causal relationships, while the multi-view distance (MDR) regularized S-map will allow them to quantify the temporal strength and signs of nonlinear microbial-environment interactions.

Final results from these analyses will arrive no later than the end of January and it is expected of them to significantly advance the ecological understanding of microalgal biomass production systems. Ultimately, these new insights will be used in the development of early warning signals that help anticipate system failures and improve the performance of the microalgae biomass production process. This is essential to accelerate the commercialization of microalgae biomass.

Learn more

Outdoor reactors utilized for the evaluation of failures in large-scale systems

ANAEROBIC REACTOR SYSTEMS

Failure tests in anaerobic reactors

In the last 12 months of PRODIGIO project, IMDEA Energy evaluated microalgae anaerobic digestion (AD) failure against salinity intrusion and the presence of pesticides/antibiotics to identify early warning signals of biogas decline. Moreover, different recovery strategies were studied to amend both perturbances. The results evidenced that antibiotics damaged the AD process to a larger extent than salinity, whereas pesticides did not exhibit a negative effect on AD performance. Antibiotics caused a total halt in biogas production after 5 days of shock, requiring immediate countermeasures to recover the system. Besides, not only methane but also intermediate metabolite production ceased, indicating the disruption of the whole process (hydrolysis, acidogenesis, acetogenesis and methanogenesis). The study of the recovery strategies revealed that AD subjected to antibiotics requires a large volume of reactor re-inoculation with a diversity-rich microbiome to recover the metabolic networks. By contrast, AD was faster restored after salinity intrusion. In this case, re-inoculation of a small volume of an adapted microbiome (lower biodiversity) resumed the biogas production. This was indicative that only the most sensitive microorganisms were affected.

Learn more

Bench-scale anaerobic digesters located at the IMDEA-energy facilities.

Modelling the failure of anaerobic digestion

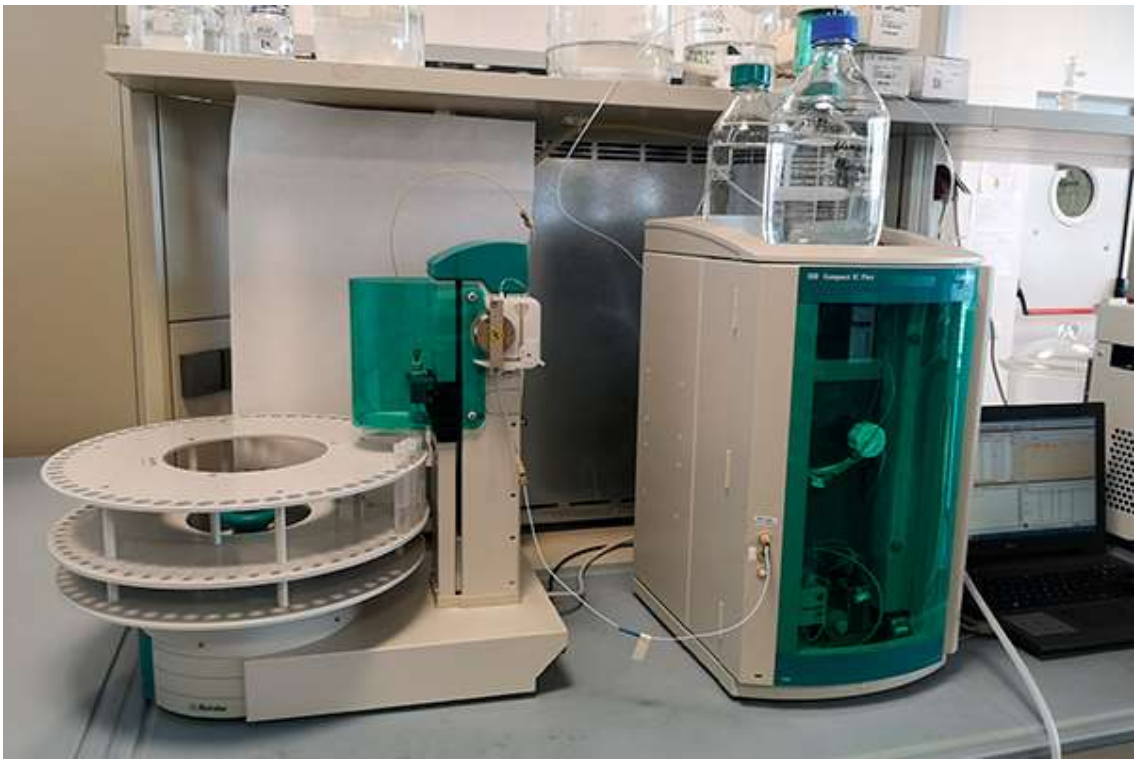
Modelling the failure of anaerobic digestion (AD) has the goal of detecting indicators of process failure. These involve chemical parameters of reactor performance (methane yield and metabolites) but also metabolic shifts within the microbial community thriving inside the reactor. Three experiments were designed to investigate the AD failure including 1) high organic overload, 2) high-protein biomass, and 3) the presence of inhibitors (chemical pesticides, antibiotics, salinity).

Regarding the chemical behavior in AD against shocks, IMDEA Energy has collaborated with LBE-INRAE (Narbonne, France) to model the process response against long-lasting AD overloading. To this end, Silvia Greses enjoyed a 3-months research stay under the supervision of Dr. Jean Philippe Steyer. The results using anaerobic digestion model No1 revealed that the high accumulation of intermediate metabolite caused an inhibition by product in the initial steps of AD, indicating not only the inhibition of methanogenesis but also a limitation in hydrolysis, acidogenesis and acetogenesis.

In relation to the microbial dynamics, NMBU analyzed the 16S microbiome structure as well as perform in-depth analysis of selected timepoints during the reactor perturbations using state-of-the-art metagenomics and metaproteomics techniques, to pinpoint active microorganisms and the enzymes and pathways they utilize. Results so far have shown that

methane production significantly decreases as a response of the perturbances, as desired, and this corresponds not only to a shift in the methane-producing archaea, but also in microbes that are active prior to the methanogenesis (i.e., in hydrolytic and in acetogenic bacteria). Preliminary results using empiric dynamic modelling shows that signatures indicative of AD failure can be observed, and we are currently in the process of elucidating the microorganisms responsible, their functional role and enzymes at play. To this end, NMBU is employing Oxford Nanopore Sequencing of select samples in order to produce high-quality metagenome-assembled genomes and metabolic reconstructions of all microbial players, and link this to the already acquired 16S microbiome structure data.

Learn more



SUSTAINABILITY ASSESSMENT

Environmental Life Cycle Assessment

To evaluate environmental aspects using Life Cycle Assessment (LCA), ARMINES (OIE) has relied on parameterized models built in Python programming language using LCA-specific libraries **Brightway2** and **lca_algebraic**. The parameterized models require several variable input parameters that serve to estimate, through mass and energy balances, the life cycle inventory on consumed elementary substances and emitted

pollutants, as well as the resulting environmental impacts.

In the last 12 months, a protocol has been developed to convert a static LCA model of the microalgae culture and biogas production, built as a baseline model, into a dynamic model. Firstly, the static model was established by implementing mathematical relationships between the variable input parameters based on discussions with PRODIGIO partners and their previous experience that were complemented by the existing literature. In the second step of the protocol, a global sensitivity analysis method was used to identify the parameters exposed to significant temporal variations that had a remarkable influence on the LCA results. Then, the initial static model was adapted to convert it into a dynamic model that uses time-series as input data for the influencing time dependent parameters.

To apply the models, eight scenarios were defined: two baseline scenarios not exposed to any perturbation and six scenarios defined considering the most common perturbations the cultivation and the anaerobic digestion can have. Among these scenarios, three scenarios consider the perturbation in the absence of the Early Warning System developed in PRODIGIO, whereas the other three scenarios consider the perturbations when these EWS are in place. The perturbations consist in contaminations of the cultivation by pests and the decrease of the organic loading rate during anaerobic digestion due to the decrease of microalgae production or broken pumps.

Social Life Cycle Assessment

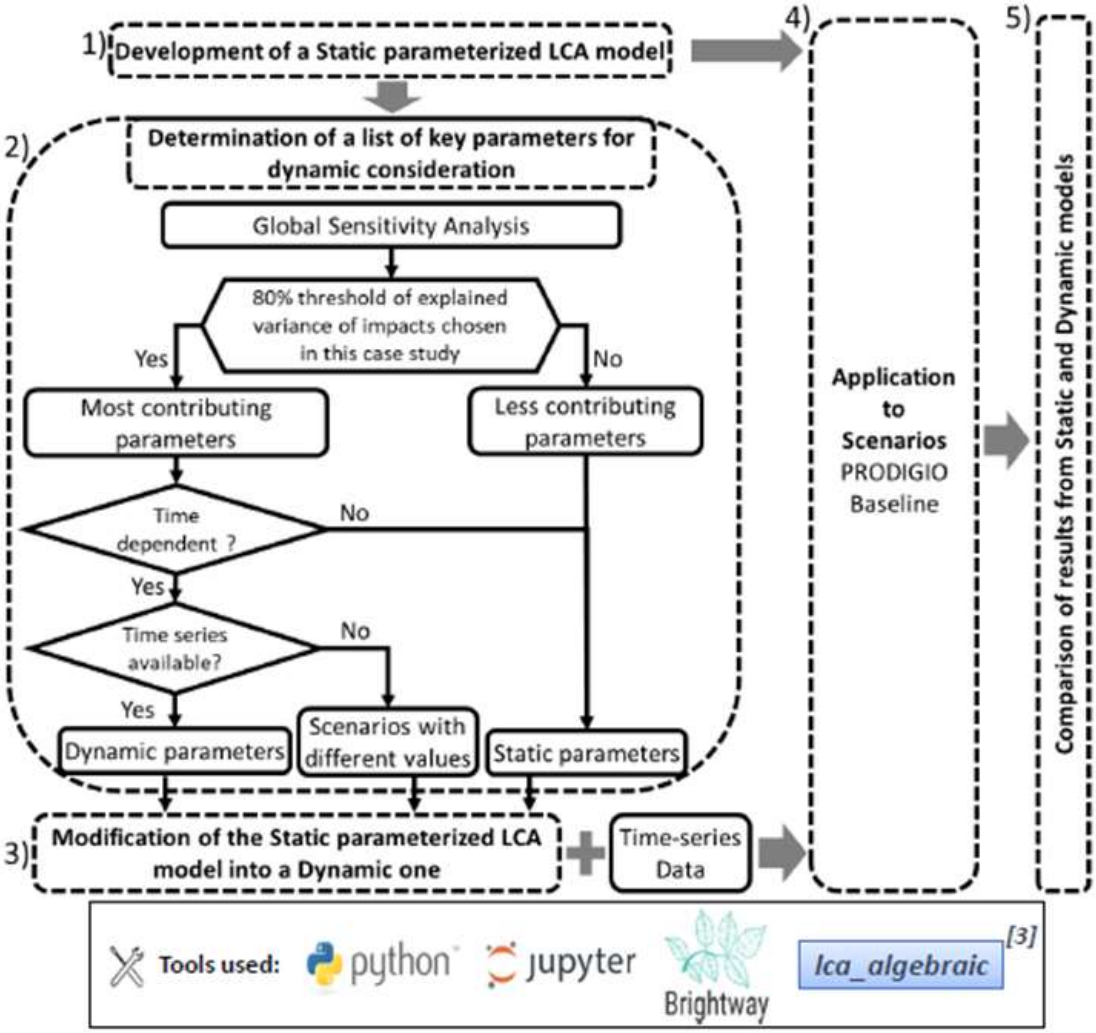
Social Life Cycle Assessment (S-LCA) is a complementary methodological framework built upon the principles of the previously developed environmental LCA to include social and socio-economic aspects to the assessment of sustainability. In this period, a literature review has been conducted to identify relevant stakeholders and impact subcategories related to bio-based processes. Workers and social community where the two stakeholder groups found to be linked to about 80% of all the impact categories considered in available studies.

Life Cycle Costing

To complete the environmental and social analyses with an economic assessment, a model was developed, integrating the culture section and the anaerobic digestion section. Data for the culture section were based on experimental measurements from the University of Almeria and completed

with information from the literature, whereas data for the anaerobic digestion considered the information provided by IMDEA Energy from lab-scale experiments and complemented with an Aspen model to estimate the scale-up effects. The analysis included the net present value and the payback period. The results showed the strong dependence of the feasibility of the process with respect to both the costs of the equipment and the operation and the prices of the product (i.e. biogas) and co-products.

Learn more



Graphical representation of the protocol to build and compare the results of static and dynamic LCA models

NEWS

PRODIGIO Activities

[The PRODIGIO Consortium convenes in Barcelona for concluding meetings]

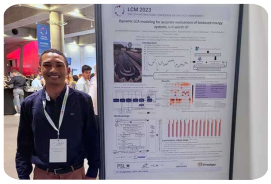
[Growing sustainable biogas in Europe with microalgae]

[Green Gas Mobility Summit abre las puertas de La Nave a proyectos de innovación]

Partners' Activities

[The PRODIGIO project was presented at the meeting of the PARAQUA COST Action]

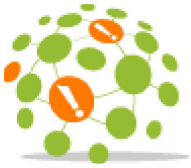
[IMDEA Energy is participating in the 15th Mediterranean Congress of Chemical Engineering (XV-MECCE) in Barcelona, Spain]



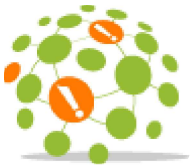
[ARMINES (OIE) engages in Life Cycle Management discussions at LCM 2023 Conference in Lille, France]

[NMBU Presents the PRODIGIO Project at the Nordic Proteomics Society]

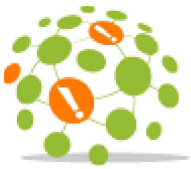
Scientific Publications



Use of airfoils for enhancement of photosynthesis rate of microalgae in raceways



Influence of pH and dissolved oxygen control strategies on the performance of pilot-scale microalgae raceways using fertilizer or wastewater as the nutrient source



Long-term assessment of the nutrient recovery capacity and biomass productivity of Scenedesmus almeriensis in raceway reactors using unprocessed urban wastewater

Don't miss our "**Stakeholder Platform**" section,
featuring news and updates on projects and initiatives where PRODIGIO aims to provide solutions for challenges in the microalgae production industry and anaerobic digestion.

If you are interested in collaborating with PRODIGIO,
please register through our "**Get Involved**" form.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N. 101007006