Doctoral fellowship: Modelling astaxanthin production in Biofilm-based systems

<u>Keywords</u>: microalgae, high-valuable compounds, biofilm, biofilm-based technologies, bioproduction, multi-scale modelling.

Microalgae production, recognized for its applications within the blue economy, currently occupies a small niche in the global market. One of the primary constraint in expanding microalgae production lies in the complexity of scaling up cultivation facilities to meet industrial demands. This includes challenges from the inoculation of the cultivation system to the maintenance of a stable culture until the extraction and purification of bioproducts. There are various cultivation systems available for microalgae production, from open ponds to closed photobioreactors, both characterized by low productivity and high water and energy demand.

Biofilm-based cultivation systems have emerged lately, in response to those drawbacks, presenting a more efficient solution with higher productivity, simplified harvesting processes, and improved light availability. Various algal biofilm cultivation strategies are in place, including submerged, intermittently submerged, and perfused systems. Among them, one can highlight the rotating systems which are easily up-scaled and are now mainly used for wastewater treatment (Figure 1).

Recently, astaxanthin production using this kind of technology has been demonstrated in our laboratory (Morgado et al., 2023), with productivities comparable to those of other biofilmbased systems, even without targeted system optimisation (Shah et al. 2016; Patel et al., 2022; Kiperstok et al. 2017). In this context, modeling approaches are powerful tools to predict and optimize productivities in microalgae production systems (Polizzi et al., 2022; Gao et al., 2023).

The objective of this thesis is to develop a spatial-temporal model for the production of a high-value compound (astaxanthin) using *H. pluvialis* biofilms. The model is intended to simulate and optimize the production of astaxanthin in a rotating biofilm-based system. Experiments will be also performed to determine the model parameters and to validate the predictions.

The hired PhD student will work in the context of a national project (ANR PEPR Galaxy).

A biological model describing microalgae behaviour, including growth and response to stress, will be combined to a biofilm spatial model. The main metabolic pathways will be formulated and solved at the cell level (La et al. 2020, González-Hernández et al. 2022). Physical mechanisms at biofilm scale will be considered to obtain the fields of growth conditions (light attenuation, diffusion-reaction of substrates, CO₂ and O₂, spatial constraints). The biological model (micro-scale) will be implemented as a discrete model (cellular automata or multiagent model) while the physical aspects will be treated by a continuous formulation. These two models will interact in a two-way coupling. A 2D approach is proposed as starting point (one dimension along the film thickness and one dimension to deal with the film roughness and detachment). It might be extended to 3D in the course of doctoral work.

Experiments will be also carried out to characterize biofilms dynamics (structure and activity through photosynthesis and respiration) in order to better understand biofilm formation mechanisms (from adhesion to detachment), to obtain input data for the multi-scale model

and global indicators to validate the model predictions (Fanesi et al., 2022; Fanesi et al., 2021; Li et al., 2023).

At the end, the coupled model will be used to simulate the process to predict biomass and astaxanthin productivity as a function of operating parameters (including light intensity, ...) (Angeles-Martinez & Hatzimanikatis, 2021; Cole et al., 2015; Polizzi et al., 2022; Warren et al., 2019).

This project will be carried out at CentraleSupélec/University Paris-Saclay in the Laboratory of Chemical Engineering and Materials (*LGPM; Bioprocess team* Gif-sur-Yvette and *Chaire de Biotechnologie* Pomacle).

Required skills: physical/biological formulation, computer programming with experience or motivation for microbial culture)

PhD: starting in the beginning of 2024.

PhD supervision: Prof. Filipa Lopes; Co-supervision: Prof. Patrick Perré

Contact: Filipa Lopes (<u>filipa.lopes@centralesupelec.fr</u>) for further information.

How to apply? Please send a CV and a cover letter to Filipa Lopes using the above mentioned contact information.



Figure 1 – Astaxanthin production (in red) in open-ponds (Figure on the left). Rotating biofilmbased systems (Figure on the right), (Gross et al., 2015; Bernstein et al., 2014)

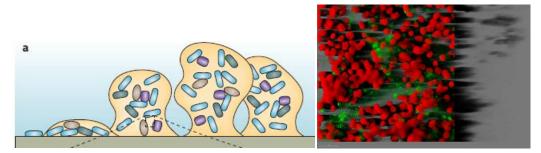


Figure 2 – a) Biofilm development on a solid support (Flemming and Wingender, 2010) – General processes governing biofilm development including cell adhesion, cell growth and polymers excretion on the surface; b) structure of biofilm given by confocal microscopy (microalgae in red, bacteria/polymers in green)

References:

Angeles-Martinez, L., Hatzimanikatis, V. (2021). Spatio-temporal modeling of the crowding conditions and metabolic variability in microbial communities. *PLoS Comput Biol* 17(7), e1009140.

Cole, J.A., Kohler, L., Hedhli, J. (2015). Spatially-resolved metabolic cooperativity within dense bacterial colonies. *BMC Systems Biology* 9, 15.

Fanesi, A., Lavayssière, M., Breton, C., Bernard, O., Briandet, R., & Lopes, F. (2021). Shear stress affects the architecture and cohesion of *Chlorella vulgaris* biofilms. *Scientific reports*, *11*(1), 4002.

Fanesi, A., Martin, T., Breton, C., Bernard, O., Briandet, R., & Lopes, F. (2022). The architecture and metabolic traits of monospecific photosynthetic biofilms studied in a custom flow-through system. *Biotechnology and Bioengineering*, 119(9), 2459-2470.

Gao, Y., Bernard, O., Fanesi, A., Perré, P., & Lopes, F. (2023). The effect of light intensity on microalgae biofilm structures and physiology under continuous illumination.

González-Hernández Y., Michiels E., Perré P. (2022). A Comprehensive Mechanistic Yeast Model Able to Switch Metabolism According to Growth Conditions. Fermentation, 8(12), 710.

Gross, M., Mascarenhas, V., & Wen, Z. (2015). Evaluating algal growth performance and water use efficiency of pilot-scale revolving algal biofilm (RAB) culture systems. *Biotechnology and bioengineering*, *112*(10), 2040-2050.

Kiperstok, A. C., Sebestyén, P., Podola, B., & Melkonian, M. (2017). Biofilm cultivation of Haematococcus pluvialis enables a highly productive one-phase process for astaxanthin production using high light intensities. *Algal Research*, *21*, 213-222.

La A., Du H., Taïdi B., Perré P., 2020 - A predictive dynamic yeast model based on component, energy, and electron carrier balance, Biotechnology and Bioengineering, 117: 2728-2740.

Li, S. F., Fanesi, A., Martin, T., & Lopes, F. (2021). Biomass production and physiology of *Chlorella vulgaris* during the early stages of immobilized state are affected by light intensity and inoculum cell density. *Algal Research*, *59*, 102453.

Li, S., Fanesi, A., Martin, T., & Lopes, F. (2023). Physiological transition of Chlorella vulgaris from planktonic to immobilized conditions. *Algal Research*, 103354.

Morgado, D., Fanesi, A., Martin, T., Tebbani, S., Bernard, O., & Lopes, F. Exploring the dynamics of astaxanthin production in Haematococcus pluvialis biofilms using a rotating biofilm-based system. *Biotechnology and Bioengineering*.

Patel, A. K., Tambat, V. S., Chen, C. W., Chauhan, A. S., Kumar, P., Vadrale, A. P., ... & Singhania, R. R. (2022). Recent advancements in astaxanthin production from microalgae: A review. *Bioresource Technology*, 128030.

Polizzi, B., Fanesi, A., Lopes, F., Ribot, M., Bernard, O. (2022). Understanding photosynthetic biofilm productivity and structure through 2D simulation. *PLoS Comput Biol* 18(4), e1009904.

Shah, M. M. R., Liang, Y., Cheng, J. J., & Daroch, M. (2016). Astaxanthin-producing green microalga Haematococcus pluvialis: from single cell to high value commercial products. *Frontiers in plant science*, *7*, 531.

Warren, M.R., Sun, H., Yan, Y., Cremer, J., Li, B., Hwa T. (2019). Spatiotemporal establishment of dense bacterial colonies growing on hard agar. *Elife* 8, e41093.