Toulouse Biotechnology Institute
Bio & Chemical Engineering

PhD Offer (3 years)

Developing a selective cell sorting process based on flotation in a microalgae/yeast consortium used for biofuels production

I. Description of the subject

Microalgae have been identified in recent years as a promising biomass source for biofuel production, due to their high lipid production capacity and rapid growth rate compared to terrestrial plants. However, at present, large-scale production of biofuels from microalgae faces a number of challenges that have made its development economically unviable. These challenges are mainly associated with large-scale issues, where monocultures are difficult to maintain, particularly in the case of open production systems such as raceways. Indeed, such open systems often result in low biomass production due to the difficulty of CO_2 supply at these scales, combined with a large gas-liquid interface with the atmosphere that results in significant CO_2 outgassing.

A promising strategy to solve these problems is to grow microalgae in mixed cultures with yeast cells. In this type of microbial consortium, microalgae and yeast establish a mutualistic relationship based on *in situ* gas exchange. The high levels of CO_2 produced by yeast directly in the culture medium enables faster autotrophic growth of microalgae, which in turn produce high levels of O_2 for yeast growth. This virtuous circle eliminates the need for an external CO_2 supply to avoid CO_2-induced growth limitations, resulting in a simpler but more productive system capable of achieving higher biomass and lipid production on a large scale.

However, an important limitation in this type of consortia is that yeast grow faster than microalgae, and can thus take over the culture. The idea developed in this project is thus to develop a cell-sorting process, selective and implementable at large scale, to remove the yeasts continuously throughout the culture and maintain the stability of the consortium. For this, the technique used will be flotation separation, and the strategy explored will be to functionalize the surface of bubbles with a molecule that will allow a specific and strong attachment to yeasts. The hypothesis on which this project is based is that a polysaccharide can promote such an interaction.

II. Proposed work

The work will first consist in setting-up a mixed microalgae/yeast culture. To this end, different species of microalgae and yeast will be tested, in different media and under different culture conditions, in order to find optimal conditions. Then, the mixed cultures will be characterized by measuring cell growth as a function of culture conditions, and characterize the interactions between the different cell types and between the cells and the surface of bubbles using atomic force microscopy (AFM) and Fluidic force microscopy (FluidFM). Then, the polysaccharidic composition of the cell walls of cells will be determined by HPLC and solid-state NMR. With this knowledge, a yeast-specific polysaccharide will be selected and

chemically modified to act as a surfactant and modify the bubble surface. Functionalized bubbles will be tested for their selective attachment to yeasts using FluidFM experiments. Finally, the flotation separation process will be used to perform cell sorting in mixed cultures at laboratory scale; a demonstration of the possible implementation of this process will be performed at a pre-industrial scale.

Thus the following techniques will be used during the three years of the contract:

- Microbiology techniques
- Atomic force microscopy (AFM)
- Fluidic force microscopy (FluidFM)
- HPLC analysis
- Chemistry reactions and analysis (NMR)
- Flotation separation

This work is expected to have an important impact by providing a method that can be used on a large scale to maintain the stability of yeast/microalgae consortia, which will allow significant progress towards the production of microalgae-based biofuels.

III. Bibliographic references related to the project:

- Demir-Yilmaz I, Ftouhi MS, Balayssac S, Guiraud P, Coudret C, Formosa-Dague C (2023) Bubble functionalization in flotation process improve microalgae harvesting, *Chem Eng J*, 452, 139349
- Demir I, Lüchtefeld I, Lemen C, Dague E, Guiraud P, Zambelli T, Formosa-Dague C (2021) Probing the interactions between air bubbles and (bio)-interfaces at the molecular scale using FluidFM technology, *J Colloid Interface Sci*, 604:785-797
- Demir-Yilmaz I, Schiavone M, Esvan J, Guiraud P, Formosa-Dague C (2023) Combining AFM, XPS and chemical hydrolysis to understand the complexity and dynamics of C. vulgaris cell wall composition and architecture, *Algal Res*, 72, 103102.
- Demir-Yilmaz I, Guiraud P, Formosa-Dague C (2021) The contribution of Atomic Force Microscopy (AFM) in microalgae studies: a review, *Algal Res*, 60:102506

IV. Candidate profile

We seek for high level candidates with a Master degree in biophysics, chemical engineering, or microbiology, and a first experience in research (in an academic laboratory or in a research & development department of an industrial company), as requested by the Doctoral School (Cf. http://www.ed-megep.fr/). The candidate must be highly motivated and wanting to do multidisciplinary works, experimental sciences and biophysics. The interest for conducting experiments and working with AFM techniques must be important (a previous experience with AFM is not necessary). English speaking and writing is compulsory.

V. Host laboratory

The trainee will work at TBI within the EAD7 Transferts, Interfaces, Mixting team (https://www.toulouse-biotechnology-institute.fr/poles/equipe-tim/), part of the Sustainable Chemical Engineering department. The PhD will be carried out as part of a funded project by the Agence Nationale de la Recherche (project SORCELBUB), and will be conducted in collaboration with the CBMN laboratory in Bordeaux, the LAAS-CNRS and Softmat labs in Toulouse, and the GEPEA lab in Nantes/St Nazaire.

VI. Contract terms

Thesis duration: 36 months, starting date from September 1st to December 1st, 2024.
Employer: SAIC INSA de Toulouse
Doctoral School: Mechanics, Energetics, Civil and Process Engineering (MEGEP, ED 468)
Salary: Application Engineer level corresponding to a gross salary of ~2000€/month according to experience
Funding: secured, grant from the Agence Nationale pour la Recherche (ANR)

VII. Application steps

Candidates must send a single PDF file containing a Curriculum Vitae, Master 1 scores, Master 2 scores if available, a motivation letter in English, and a reference letter from the previous supervisor by e-mail to Dr. Cécile Formosa-Dague and Prof. Pascal Guiraud. Depending on the matching of the candidate to the profile, an interview can possibly be organized and supplementary information can be requested. Applications will continuously be examined until a candidate is chosen.

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