Antioxidant hyper-accumulation and CO_2 optimization in microbial fermentation

Key words: microalgae, yeast, antioxidant, CO_2 toxicity, bioreactor

Background

Functional foods and nutraceuticals have the potential to improve the human health status as well as support economic growth in rural communities. A major focus of the functional foods and nutraceuticals market is antioxidants, as damages caused by reactive oxygen species is considered to be the basis for the progression of aging and several diseases including chronic kidney disease, cardiovascular disease and cancer... A wide variety of food sources including animal, plant and microorganism have been identified to possess antioxidant properties. Among the microorganisms, yeast and green microalgae show great potential for numerous application (energy and environmental applications, source of molecules for food and feed, pharmacology or cosmetics). Their use can be traced back to the first breadmaking approximately 4500 years ago in ancient Egypt. Today, we aim, with this internship, at producing antioxidant compound by employing these two microorganisms: yeasts and microalgae.

Still, before molecules can be produced at an industrial scale, many challenges still need to be met. Indeed, a relatively long development time in the laboratory is necessary before moving to industrial scale. This work covers steps such as strain selection, determination of the conditions for growth, the conditions for production of the molecules of interest and the purification of these molecules. During the bioprocess at an industrial scale, the presence of excessive CO_2 (resulting from cellular growth) plays the role as an inhibitor in the fermentation broth. Hence, to optimize reaction conditions, a better understanding of the CO_2 hazard conditions and mechanism is essential.

Two goals therefore lie behind these objectives. First, we aim to find out a set of operating conditions that would stimulate the microalgae and yeast to increase the production of the antioxidant, without slowing down their growth. The balance is often subtle and difficult to achieve. For this purpose, we developed a specific process (patent pending) which can be used to induce the oxidative stress. It should be adjusted not bring additional harmful components to the culture. This has already been proven successful. Owing respect to its simplicity, it also provides feasibility for further industrial production and purification steps. Then, we will focus on better understanding of yeast and algal culture behavior by exposure to different CO_2 conditions. To do so, a control and monitoring system has been set up according to a thesis in the host team. For this study, specialized mini bioreactors have been developed (8 units, Figure. 1) in the previous project. These bioreactors can be operated in parallel to allow for fast screening.

Figure 1: 500-milliliter bioreactors

The objective of this internship is twofold: 1) to find the conditions for microalgae and yeast to produce antioxidants. For this, it will be necessary to carry out microbial cultures, monitor the growth, and analyze the level of antioxidants under the corresponding conditions. 2) to study the culture behavior under difference CO_2 conditions. Still, to make this purpose, monitoring culture behavior, and determination of the relevant molecules or ions will need to do.

Intern Missions

In order to better understand this internship subject, the student will start with a bibliographical research phase focused mainly on the effect of excessive accumulation of CO_2 on the microbial broth, the assays of antioxidant, and cytometry (principle of operation and viability markers) and their application to microalgae.

At the same time, the trainee will be able to start the microalgae and yeast cultivation. For this, cultures will have to be conducted in sterile mini bioreactors (8 units, Figure 1). For training purposes and depending on the trainee's curiosity and the realistic operability, the trainee may be introduced to the various analyses that can be performed to monitor a microalgal culture, such as following the cell growth, the pH, the consumption of sugar and nitrogen sources, and the cellular ROS.

In a second step, the antioxidant assays will take place. To do so, it will be necessary to be able to find out the potential antioxidant and corresponding detection methods. The methods for detecting antioxidants have been reported for reference (Kesraoui et al. 2011). For this, the host team has already identified a series of promising protocols, however, in the light of its bibliographic study, the trainee may propose another protocol. Several tests have been completed in the preliminary condition exploration. After a few batches of antioxidant assays, the experiments of CO_2 inhibition on microbial culture will be performed. In order to achieve this goal, it will need to better understand the pathways of carbon dioxide inhibition on yeast and algal, and the carbon dioxide behavior in aqueous systems (Chen and Gutmanis 1976, Dixon and Kell 1989). Then therefore to reveal the variation of the potential molecules or ions.

Finally, a time will be devoted to the student so that he can format his results and write his internship report.

Reference

Kesraoui O, Marzouki MN, Maugard T, Limam F. In vitro evaluation of antioxidant activities of free and bound phenolic compounds from Posidonia oceanica (l.) Delile leaves. African J. Biotechnol. 2011;10(16):3176–85.

Chen, S. L., & Gutmanis, F. (1976). Carbon dioxide inhibition of yeast growth in biomass production. Biotechnology and Bioengineering, 18(10), 1455-1462.

Dixon, N. M., & Kell, D. B. (1989). A review–the inhibition by CO2 of the growth and metabolism of microorganisms. Journal of Applied Bacteriology, 67, 109-136

Desired profile

- Training at the level of Bac +4/5
- Mastery of laboratory techniques
- Knowledge in biotechnology is necessary for the successful completion of this internship.
- Practical experience in the cultivation of microorganisms would be appreciated.
- On an individual level, critical thinking, autonomy and rigour are expected qualities.

Host laboratory

The CentraleSupélec BiotechnologyChair, inaugurated in November 2010 and hosted by the European Center for Biotechnology and Bioeconomy (CEBB), is active in three areas of expertise:

- Characterization & conversion of lignocellulosics.
- Biotransformation.
- Separation techniques.
- Transversal base for modeling, simulation & visualization.

The research focuses on the development and multi-scale modeling of innovative processes for biomass valorization. Backed by the Process and Materials Engineering Laboratory, the Chair provides a close link between its parent institution, CentraleSupélec, and the economic and academic players in the region, by putting its R&D expertise at the service of innovative projects. These two laboratories have developed strong expertise in the subject matter. This expertise is available from two angles:

- Knowledge in microalgae growth with equipment ranging from vial growth to pilot reactor,
- A competence in modeling applied to complex systems (hydrodynamics, living, ...) and to the design of experimental devices.

Practical information

The internship/project will take place at the CEBB in Pomacle (Marne, 51). The internship will last between 4 and 6 months and can start between February to March 2021. Remuneration will be awarded.

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