High added value metabolites production in photobioreactors (PBR) using microalgae encapsulated in hybrid material (VALOALGUE* project)

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CONTEXT

Currently, several researches threat about culture of microalgae due to their ability to produce valuable metabolites such as lipids or saccharides. This kind of mass culture should allow the production of bio-sourced molecules technologically interesting (bioethanol, biofuel, biopolymers…)

On the other hand, the market for bioactive molecules such as recombinant proteins is growing worldwide. As others organisms, some microalgae can produce, in small quantity, high added values metabolites, but with the benefit that microalgae are autotrophic. These metabolites can be produced and excreted in the culture media naturally or resulting from a genetic transformation. The only problem is to recovery metabolites diluted in a free cell culture. Filtration or purification seem to be undesirable because of clogging risks. So, the immobilization of microorganisms inside a porous material should be a solution to obtain a good metabolites recovery.

PURPOSE

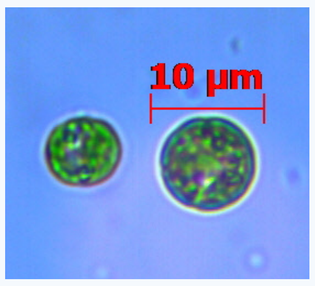
The two main objectives in this project are the selection of one or several microalgae producing interesting bioactive molecules, and the improvement of the porous material for microalgae encapsulation. The material has to be biocompatible, to hold in microalgae, allow the diffusion of nutrients and excreted metabolites, and finally to be able to withstand mechanically for a sufficient time production.

Then, the optimization of the process (design and influence of various parameters) will be a great part of the study. About the design, there are many kinds of photobioreactors (PBR): flat, in column, tubular,… The agitation can be realized by fluidized bed, bubble column, airlift or mechanical stirring. Several parameters such as culture medium, beads diameter, beads/culture medium ratio, illumination,… have to be tested.

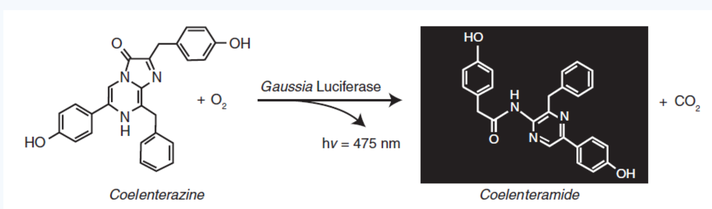
STRAINS AND METABOLITES

The main strain currently studied is a wall-less mutant of *Chlamydomonas reinhardtii* producing and excreting *Gaussia luciferase*, a recombinant protein. This microalgae is a freshwater strain (TAP medium) able to growth under autotrophic or mixotrophic conditions:

Chlamydomonas reinhardtii mutant



Gaussia luciferase bioluminescence reaction



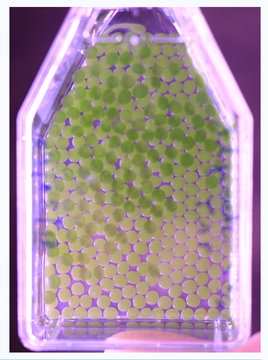
Haddock, S.H.D., McDougall, C.M. and Case, J.F., The Bioluminescence Web Page, http://lifesci.ucsb.edu/~biolum/ (created 1997; updated 2005).

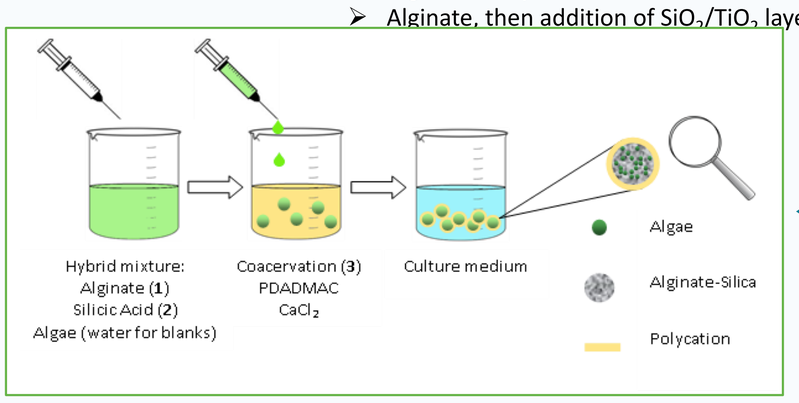
A second strain, *Haslea ostrearia* is a marine diatom microalgae producing naturally a blue pigment called marennine. This polyphenolic pigment presents diverse characteristics: allelophatic, antioxidant, antibacterial, antiviral,…

BEADS PRODUCTION*

Beads are mostly composed of alginate. But, in order to consolidate its structure and its lifetime, there is different ways to improve the recipe:

- Composition of the hybrid mixture:
  - Only alginate
  - Addition of SiO_2
  - Addition of TiO_2
- Composition of the coacervation solution:
  - Polydiallyldimethylammonium chloride (PDADMAC)
  - $CaCl_2$
- Recipe:
  - Alginate or hybrid mixture
  - Alginate, then addition of SiO_2/TiO_2 layer by layer





Keep in mind:

- Bio compatibility
- Diffusion of metabolites and nutrients

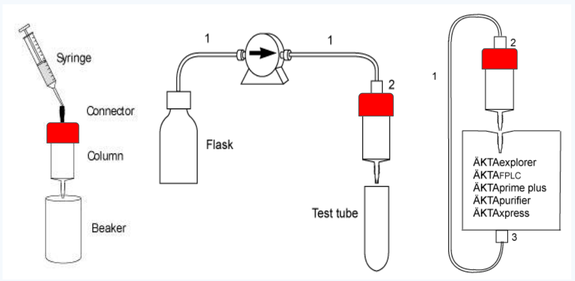
* Only for freshwater strain

ANALYTICAL METHODS

For the recovery of *Gaussia luciferase*, two analytical methods are being developed:

- PURIFICATION step:

→ The *Gaussia luciferase* produced by *Chlamydomonas reinhardtii* has the particularity to be tagged by a StrepTag II (a sequence with 6 specific amino acids). So, the purification step become convenient with specific columns purification for this tag:

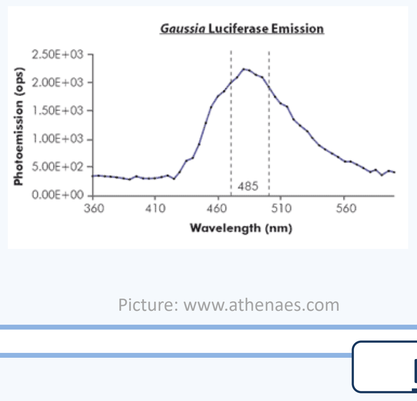


Pictures: www.gelifesciences.com

- QUANTIFICATION step:

→ By total proteins quantification after purification (Kit BCA from G-biosciences) or by bioluminescence quantification (Synergy H1 from Biotek)

Picture: www.gbiosciences.com



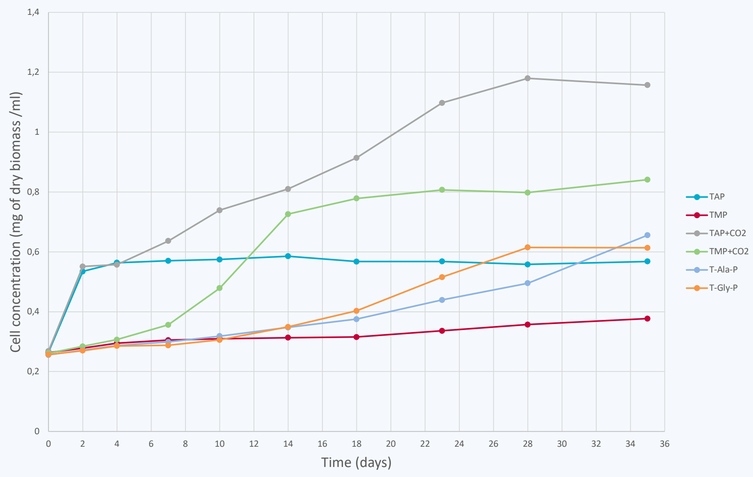
Picture: www.athenaes.com

PROCESS OPTIMIZATION

Several parameters have to be tested. A first study is about the culture medium of *Chlamydomonas reinhardtii*. In Fact, this microalgae is usually grown under mixotrophic conditions. Acetate is the organic carbon source of the medium, but represents a contamination risk by others microorganisms (bacteria, fungi). So, some culture medium were tested in order to substitute the acetate:

- TAP = with acetate
- TMP = without acetate
- TAP+CO_2 = with acetate and a pH control by bubbling CO_2
- TMP+CO_2 = without acetate and with a pH control by bubbling CO_2
- T-Ala-P = alanine substituting acetate
- T-Gly-P = glycine substituting acetate

Evolution of cell density as a function of time in the various medium tested



Best compromise:

Use acetate in the beginning of the culture and control the pH control by bubbling of CO_2

→ Allows to limit the interventions number (pH controlled)

→ CO_2 constitutes a carbon source after the acetate consumption

DISCUSSION

The first results are hopeful: two strains producing interesting metabolites have been selected by ULiège. Some recipes of encapsulation are studying in collaboration with UNamur. Design of photobioreactors are studying in collaboration with ULiège and analytical methods are being developed by UMons. These results are a good starting point in order to continue the research. The diffusion and the study of bio compatibility of porous material represent an important part of tests to be conducted. Then, a lot of parameters influencing the process have to be tested firstly at laboratory scale and secondly after scale-up.

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