Nitrogen depletion on microalgae culture for lipids production – a continuous process facilitated by acoustic settler

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Abstract

Nitrogen-depleted environment during some lipogenic microalgal strains cultivation induces an additional accumulation of lipids, which can improve ecological and economical sustainability of a microalgae lipids production. Although, currently implemented in batch-mode cultures, with inherent limitations, the nitrogen-depletion could be performed under continuous mode with an effective technology: acoustic settler.

Keywords: Nitrogen depletion, Sonoperfusion, Acoustic filter, Microalgae, Lipids accumulation, Continuous culture.

1. Lipids accumulation induced by N-depletion on microalgal strains

Several studies (Breuer et al. 2012; Negi et al. 2016) about microalgae show that under various stress, we can induce some accumulation of interesting biochemical compounds. In the narrow field of microalgal biodiesel, some strains are put in the spotlight for their high content in lipids and/or for their capacity of lipids accumulation under stresses like nitrogen starvation, high salinity, or low illumination.

In this research, we focus on three strains of eukaryotic microalgae known for their lipids content, but also their facility of culture: *Chlorella sorokiniana*, *Scenedesmus dimorphus* and *Scenedesmus obliquus*. These strains were put to nitrogen privation after a growth phase in 3N-BBM, a nitrogen-rich medium. The lipids accumulation under N-starvation is validated for the three strains and some variations in delay and gain were observed.

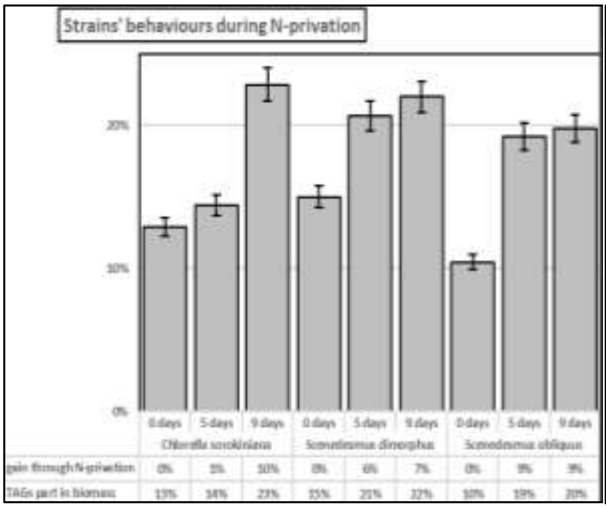

Figure 1 : Lipids content during N-deprivation

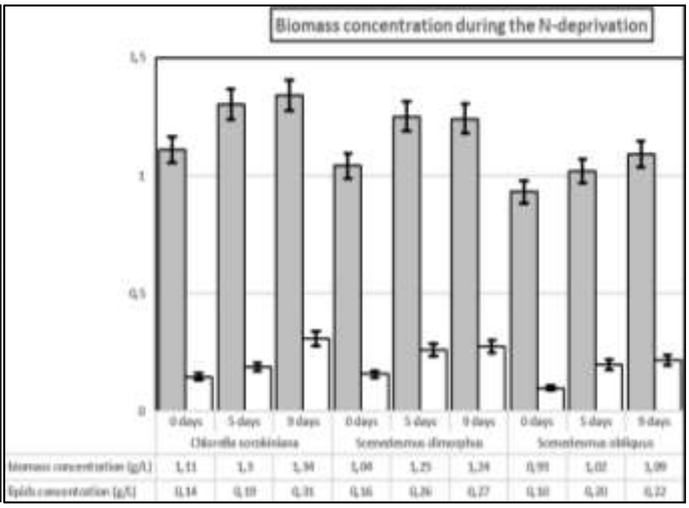

Figure 2 : Biomass and lipids concentration during N-deprivation

For example, *Chlorella sorokiniana* takes few more days than the two others but manages to gain as much as them in lipid content. Since its biomass concentration is slightly higher, its lipids concentration is superior at 9th day.

Even though we observe a weak growth during N-deprivation,the accumulation stage cannot be simultaneous with the growth stage. Thereby, the production must have at least two steps: a "growth" and an "accumulation" step. Existing technologies are quite ineffective in case of continuous culture processes. On one hand, chemical-induced separation like flocculants addition can pollute medium and biomass and can induce permanent aggregation(González-Fernández and Ballesteros 2013). On the other hand, physical filters can be either obstructed by biofouling or need to be implemented in batch conditions. It is also mostly time- and power-consuming, and non-compatible to a continuous culture. With this in mind, we turn to a technology unconventional with microalgae cells: acoustic settler or "sonoperfusion".

2. Acoustic settler or "sonoperfusion"

An acoustic settler allows to concentrate cells of a culture by settling it thanks to acoustic waves. It is a soft, temporary and stressless method to aggregate cells and it induces no biofouling at all. Usually applied in animal cells production to renew the culture medium without inducing stress on cells or biofouling of a filter (Chotteau 2015), we want to implement this technology to concentrate microalgae cells in a very small volume before reintroducing them in a N-deprived medium. The supernatant pulled apart can be reinjected to the initial culture or discarded. Although, this technology could have various uses like a preliminary step of the harvesting process (Bosma et al. 2003; Hincapié Gómez and Marchese 2015) or the extraction of an extracellular compound release in the medium.

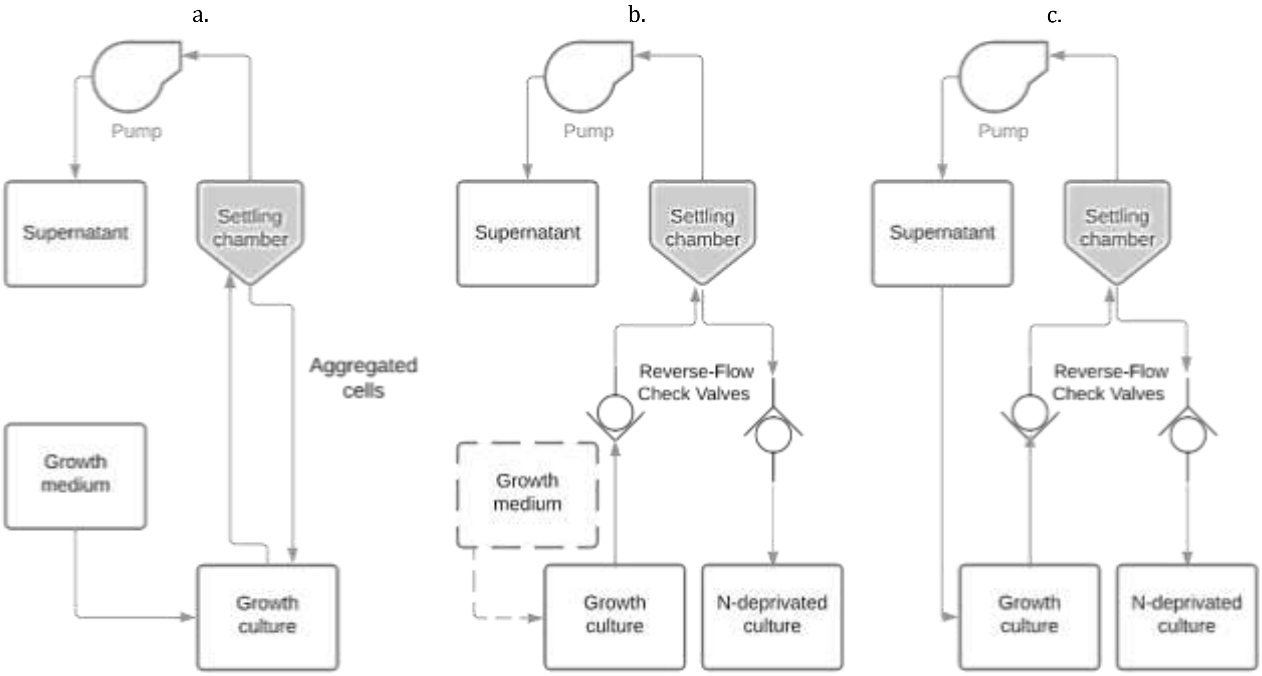

Figure 3 : Several experimental setup (a. use in animal cell cultivation, b. proposition 1 for N-depletion : optimising growth culture, c. proposition 2 for N-depletion : reusing of culture medium)

In spite of the difference of size between animal cells and eukaryotic and prokaryotic microalgae cells, the retention yield is more than 90% of eukaryotic and prokaryotic microalgal cells depending on the strain and the cells concentration.

3. Implementation of the acoustic settler on microalgal culture process

To transfer the cells from a nitrogen-rich medium to a nitrogen-deprived medium, the goal is to transfer the most cells with the least nitrogen-rich medium used for cells growth. Some preliminary assays with a microalgae culture at 3g/L biomass have been done and after only 2 hours, the biomass concentration in the settling chamber reached almost 14g/L and preliminary estimations allow us to hope for a concentration of 40g/L after between 10 and 15 hours of process. Considering the growth rate of microalgae in continuous mode and high productivity photobioreactor, it appears to be a promising solution.

4. Conclusions and perspectives

Acoustic settler can be used with microalgae and allows very effective concentration. We are testing currently the implementation of the acoustic settler on high-productivity flat-panel twin photobioreactors for a two-steps cascade continuous culture. We also consider completing the tests on prokaryotic and eukaryotic microalgae with tests on yeast and mixed culture of microalgae and yeast.

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