**Study of starch accumulation dynamic in nitrogen starved *Chlamydomonas reinhardtii* using controlled torus photobioreactor**

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**Highlights**

* High starch accumulation by microalgae
* Controlled torus PBR with dissolved oxygen and pH regulation
* Physiological adaptation to nitrogen deficiency

**1. Introduction**

*Chlamydomonas reinhardtii* is a green microalgae known to accumulate large amounts of starch in nitrogen-limited conditions, up to 50% of its mass content expressed in dry weight of cells (%DW) that could be used as a feedstock for next generation of biofuels production [1]. In autotrophic nitrogen-starved cultures of *C. reinhardtii*, several physiological changes take place in order to adapt their mass content in pigment content and accumulate starch [2]. Starch accumulation in nitrogen starved cells can be affected by other factors such as anoxic conditions [3], light energy availability [1] and inoculum physiological state. The objective of this work is study of starch accumulation dynamic in nitrogen-starved *C. reinhardtii* in controlled O2 dissolved (OD), pH and light availability conditions, prior to a modelling study with a view to develop an optimized starch production protocol in nitrogen-limiting conditions.

**2. Methods**

A wild type strain of *C. reinhardtii* (137H) has been cultivated in autotrophic nitrogen-limited conditions, using Suoeka medium with NH4Cl concentration of 1,87mM, in a torus-shaped PBR, equipped with a pH-temperature and DO sensor, as well as mass spectrometer for gas analyses. The pH of the microalgae culture was regulated at 7.5 with gaseous CO2 injection. DO has been regulated with 4.5% O2 - 95.5% N2 gas mixture injection flowrate. Limited-growth cultures were performed in batch and at different incident light intensities. Inoculum has been cultivated in 1L air-lift PBR in non-limiting autotropic conditions and pH was regulated by CO2 injection. The local fluence rate was calculated along batch cultures using the 2-flux model and the radiative properties of culture were determined experimentally using the methodology developed by Pilon *et al*. [4]. Biotic and abiotic phases have been analyzed measuring the dynamic evolution of key nutriments and intermediate metabolites, as well as the analysis of the produced gas .

**3. Results and discussion**

For nitrogen-limited growth cultures of *C.reinhardtii*, an NH4Cl initial load of 1.87mM was used to sustain biosynthesis up to 0.25g/L of biomass under standard growth conditions, according to the known stoichiometry. This amount is close to the value obtained during the first 20 hours of culture in concordance with the depletion of NH4+ measured (Fig 1A). After that point, an increase in the starch content was observed and a maximum accumulation of 53% was finally obtained after 70 hours of cultivation (Fig. 1A). A stop of cell growth seems to occur at 32 h of culture, once the number of cells remained relatively constant. In contrast, the nitrogen depletion in the medium lead to a remobilization of the total proteins, while reducing their content to 20% (Fig 1A). In the other hand, the pigments content decreased once the NH4+ vanished. The total pigments content was stabilized around 1%. This results in a change within the pigment distribution compared to a standard growth culture without any mineral deprivation.The radiative properties were estimated leading to the calculation the energy light profile within the cultivation system during the starch accumulation phase (70h). This assessment showed that light was not a limiting factor since a fluence value was estimated at 21umol/m2s at the bottom of the PBR (Fig 1B). Throughout the experiment, the dissolved O2 was regulated to 130% in oxygen saturation in the air, in order to avoid anoxic conditions. Nonetheless, after 63 hours the dissolved oxygen falls as a consequence of low photosynthetic activity (Fig 1C).



**Figure 1.** A. physiology response to nitrogen starvation, B. Fluence rate in batch culture q0= 200umol/m2s, C. Dissolved oxygen and rO2 evolution.

**4. Conclusions**

The use of a controlled torus PBR allowed the study of starch accumulation dynamic after a nitrogen deficiency, where neither light transfer nor dissolved gases (CO2, DO) influenced the storage of starch. A maximum starch content of 53% was obtained after 70 hours of cultivation. The physiological impact of nitrogen deficiency also led to the remobilization of 30% of the total protein content and to the adaptation in the content and distribution of pigments. This work will lead to the development of a biochemically structured model for starch storage in *C. reinhardtii*.

**References [Calibri 10]**

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