

Growth and lipid accumulation kinetics of *Coccomyxa melkonianii* SCCA 048: experimental and modeling

Santina Soru¹, Veronica Malavasi¹, Alessandro Concas^{2*}, Giacomo Cao^{1,3*}

1 Interdepartmental Center of Environmental Science and Engineering (CINSA), University of Cagliari, Via San Giorgio 12, 09124 Cagliari, Italy; 2 Center for Advanced Studies, Research and Development in Sardinia (CRS4), Loc. Piscina Manna, Building 1, 09010 Pula (CA), Italy; 3 Department of Mechanical, Chemical and Materials Engineering, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy

*Corresponding authors: <u>aconcas@crs4.it; giacomo.cao@dimcm.unica.it</u>

Highlights

- Preliminary results of the autotrophic growth and lipid production of *C. melkonianii*.
- Experiments were performed in batch lab-scale.
- Quantitative (SPV) and qualitative (GC) evaluation of the lipid content.
- A simple mathematical model is proposed.

1. Introduction

The identification of new microalgal strains capable of accumulating significant amounts of lipids and highvalue bio-products, while growing in low-quality waters today represents a crucial issue for the sustainable exploitation of microalgae technology. A new species, Coccomyxa melkonianii SCCA 048 [1], found in natural waters contaminated by acid mine drainage phenomena, shows high adaptability to extreme environmental conditions such as high heavy metal concentration and various pH values. It has been welldocumented that nitrogen deficiency might represent the most preferable strategy to induce lipid biosynthesis due to its high-efficiency and universal applicability amongst microalgae. However few studies involved deeply the lipid accumulation in Coccomyxa genus (Trebouxiophyceae) under nitrogen starvation conditions [2-4-7]. The assessment of the effect of nitrogen starvation on lipid biosynthesis was carried out on the basis of similar studies [e. g 5]. In this work growth and lipid accumulation kinetics of C. melkonianii in batch reactors is quantitatively investigated for the first time in the literature. Finally, a simple mathematical model has been developed to the aim of interpreting the experimental data and to preliminary assess the possibility of transposing the obtained results on industrial scales [3 - 4 - 7]. The model, which is not shown here for the sake of brevity simulates temporal evolution of microalgae, nutrients and lipid concentration as well as the light intensity distribution within the growth medium. To this aim, comprehensive kinetics have been considered along with the suitable material balances for batch photobioreactors.

2. Methods

An authentic freshwater strain *Coccomyxa melkonianii* SCCA 048, obtained from Sardinian Culture Collection of Algae at the University of Cagliari [6] was investigated in this work. In order to assess the effect of nitrogen starvation on lipid productivity, *C. melkonianii* was cultivated in 2 L batch stirred bottles where the initial concentration of dissolved nitrogen was suitably changed. In the base case experiment the growth medium BBM was used (BBM standard with NO₃ initial concentration 250 gNaNO₃/m³). Subsequently the nitrogen concentration was varied by increasing five times the initial concentration (BBM 5N) and by reducing it by one-fifth (1/5N-BBM) respectively. The growth of microalgae was monitored spectrophotometrically (OD) and the biomass concentration (Cb) was calculated by taking advantage of a suitable Cb vs OD calibration curve. A slightly modified colorimetric method based on the use of Sulpho-Phospho-Vanillin (SPV) was adopted to quantify the lipid content of microalgae and the lipids were extracted from wet biomass according to the procedure described by [5].



3. Results and discussion

The identification of the suitable nitrogen initial concentration is crucial for the optimization of productivity in terms of biomass and lipids. Preliminary results indicated that the cells growth rate (accumulation of biomass) of *C. melkonianii* depends on nitrogen availability in medium, although it can be observed that no significant effect on the biomass production kinetics derives from the increase of nitrogen content (5N BBM) with respect to the one of the standard BBM medium. On the other hand, the experimental results obtained using an initial nitrogen concentration reduced by five times (1/5N-BBM) show that both final biomass concentration and growth rate are decreased (Fig. 1a). It can also be observed that the lipid synthesis kinetics is boosted when the nitrogen content is lowered by five times (1/5 N BBM) (Fig. 1b), thus indicating that nitrogen-limitation concentrations can effectively stimulate lipid synthesis. Further details about the fatty acid methyl esters composition of extracted lipids and the comparison of experimental data with model simulations will be provided in the final version of the paper.

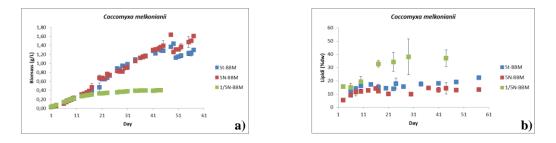


Figure 1. Evolution in time of total biomass concentration (a) and lipid content (b) during batch experiments when varying the initial concentration of nitrogen in solution.

4. Conclusions

Extremophile algae are considered to be highly tolerant and adaptable to severe environmental conditions including low-quality waters or nutrient deficiency. These first results show that lipid accumulation can be triggered in *Coccomyxa melkonianii* by modulating nitrogen concentration in the growth media [4]. Specifically, high lipid productivities are achieved when operating under nitrogen starvation. Therefore, these preliminary results corroborate the assumption that *C. melkonianii* might be exploited for biotechnological applications even when cultivated in low quality waters. Analysis of the fatty acid methyl esters composition of the extracted lipids, including EPA and DHA, is currently under way in order to evaluate the potential exploitation of the strain for the nutraceutical market.

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Keywords

Kinetics; Coccomyxa melkonianii; lipid productivity; FAMEs.