

Productivity and lipid accumulation of a novel acidophile microalgae

Mari Carmen Ruiz¹, Isabel Vaquero¹, Virginia Obregón², Mayca Márquez³, Benito Mogedas¹ and Carlos Vílchez¹

INTRODUCTION

Microalgae are definitely the way to a sustainable, easily reproducible natural green source for highly demanded products. These are seen as a renewable source for fatty acids that can be converted into biodiesel. Increasing the intracellular concentration of fatty acids, with emphasis in those saturated ones, should be the first step in the algal biodiesel production process.

In this work, we intend to show that nutrient limitation, namely either N starvation or P starvation, can be used increase the total fatty acid content per microalgal cell and to increase the saturated to unsaturated fatty acids ratio. The study was carried out with a novel acidophile microalga that grows selectively at pH 2.5 and that was isolated by our group (Algal Biotechnology Group) from acidic drainages. This *Chlamydomonas acidophila* microalga is able to accumulate large quantities of lutein, this can be enhanced by the addition of Cu (II). High productivities can be obtained during semi-continuous cultivations in tubular reactors with this microalgae.



Figure 1. Tinto river. An acidic river with extreme conditions for life in the southwest of Spain, where this strain of microalgae was isolated.

MATERIALS AND METHODS

Chlamydomonas acidophila strain was grown on a K9 modified medium. The cultures were grown on lab conditions. The pH of the medium was 2.5. As carbon source was used 5% CO₂ in air (v/v).

A photosynthetic active radiation irradiance of 160 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were provided by fluorescent lamps and the temperature was 27°C.

The growth rates were calculated by measuring the optical density at 750nm by UV-VIS spectrophotometer and the dry weight of biomass using 47 mm GF/F glass microfibre filters.

The pigment content of the microalgae was determined by HPLC after extraction with organic solvent.

The fatty acid content was determined by GC following the Shovon Mandal (2009) method (BIO OILS).



Figures 2 and 3. Tubular bioreactor, air fluidized-bed bioreactors and conical flasks erlenmeyers used in the experiments.

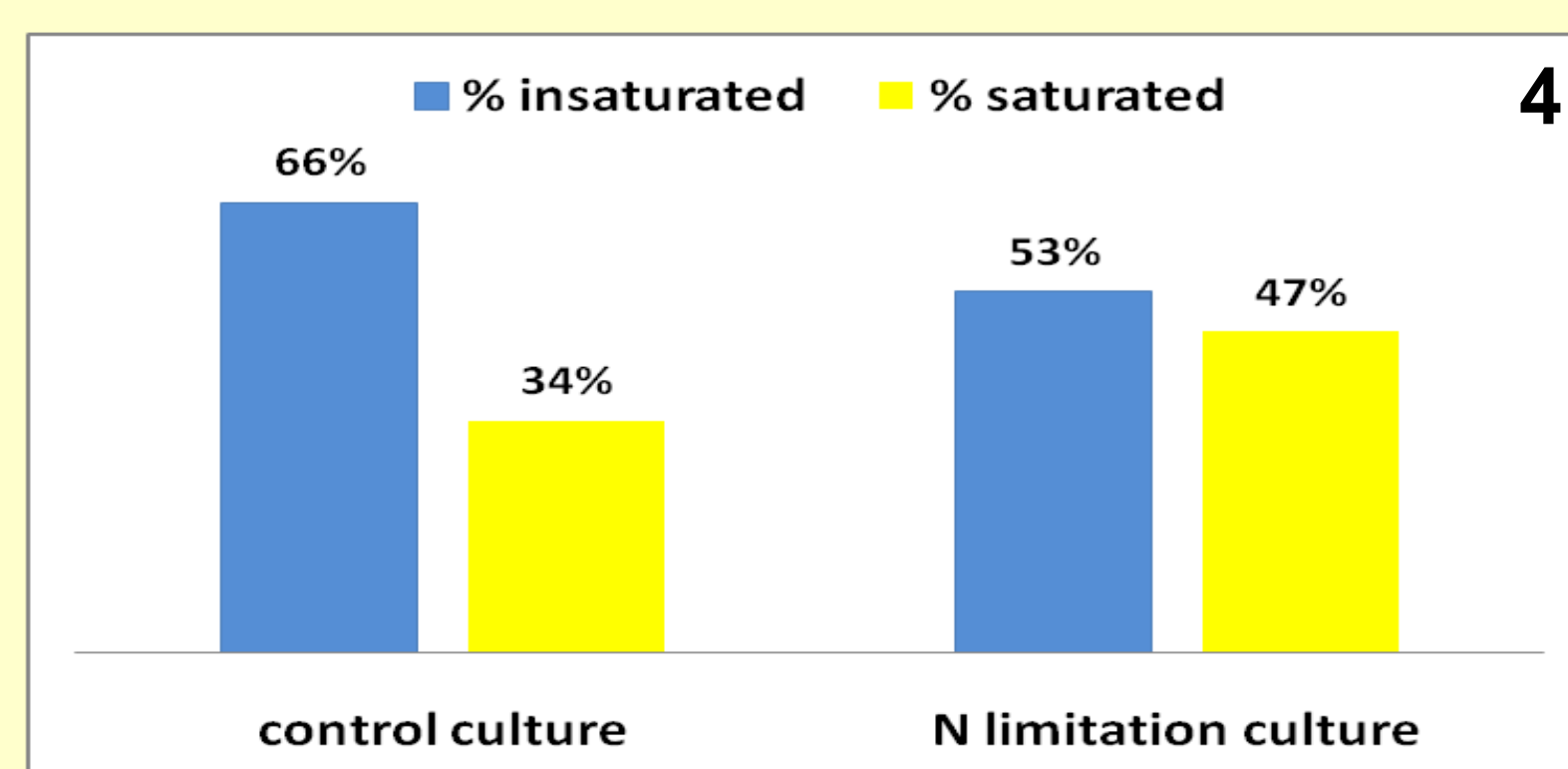


Figure 4. Content of Fatty acids (saturated and insaturated) obtained from the cultures of *Chlamydomonas acidophila* incubated in fresh standard culture medium (control culture) and under nitrogen starvation.

RESULTS AND DISCUSSION

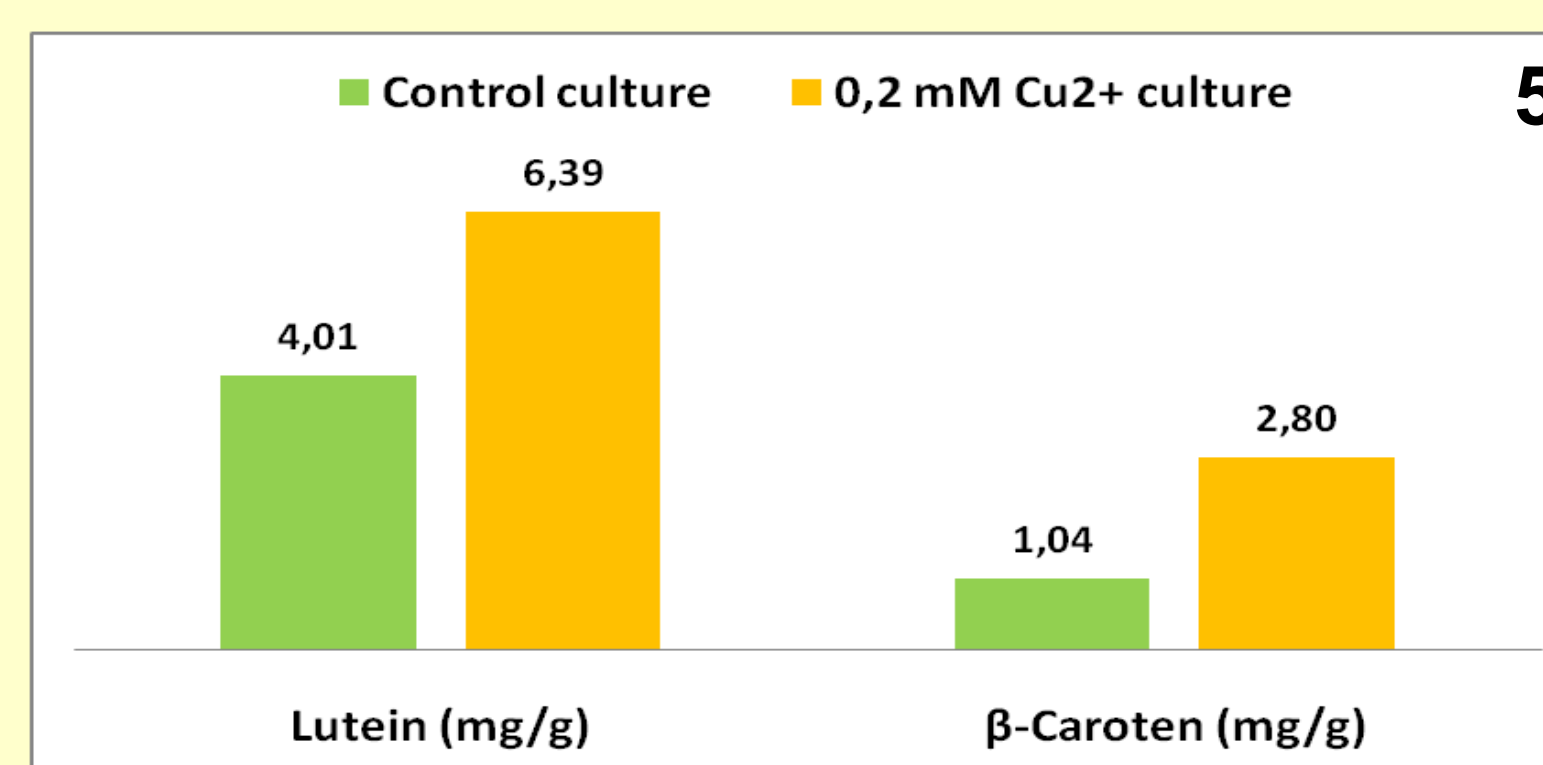


Figure 5. Lutein and β -carotene concentration (mg/g) obtained from the cultures of *Chlamydomonas acidophila* grown in semicontinuous regime and incubated in fresh standard culture medium (control) and culture medium containing the best Cu²⁺ concentration previously determined (0,2mM).

CULTURE	GROWTH RATE (d ⁻¹)	VOLUMETRIC PRODUCTIVITY (g·l ⁻¹ ·d ⁻¹)
1	0.301	0.097
2	0.481	0.155
3	0.622	0.201

Table 1. Growth rate and volumetric productivity of biomass calculated from the cultures of *Chlamydomonas acidophila*. Culture 1 carried out in fluidized-bed bioreactor, Culture 2 in flat-panel bioreactor and Culture 3 in tubular bioreactor.

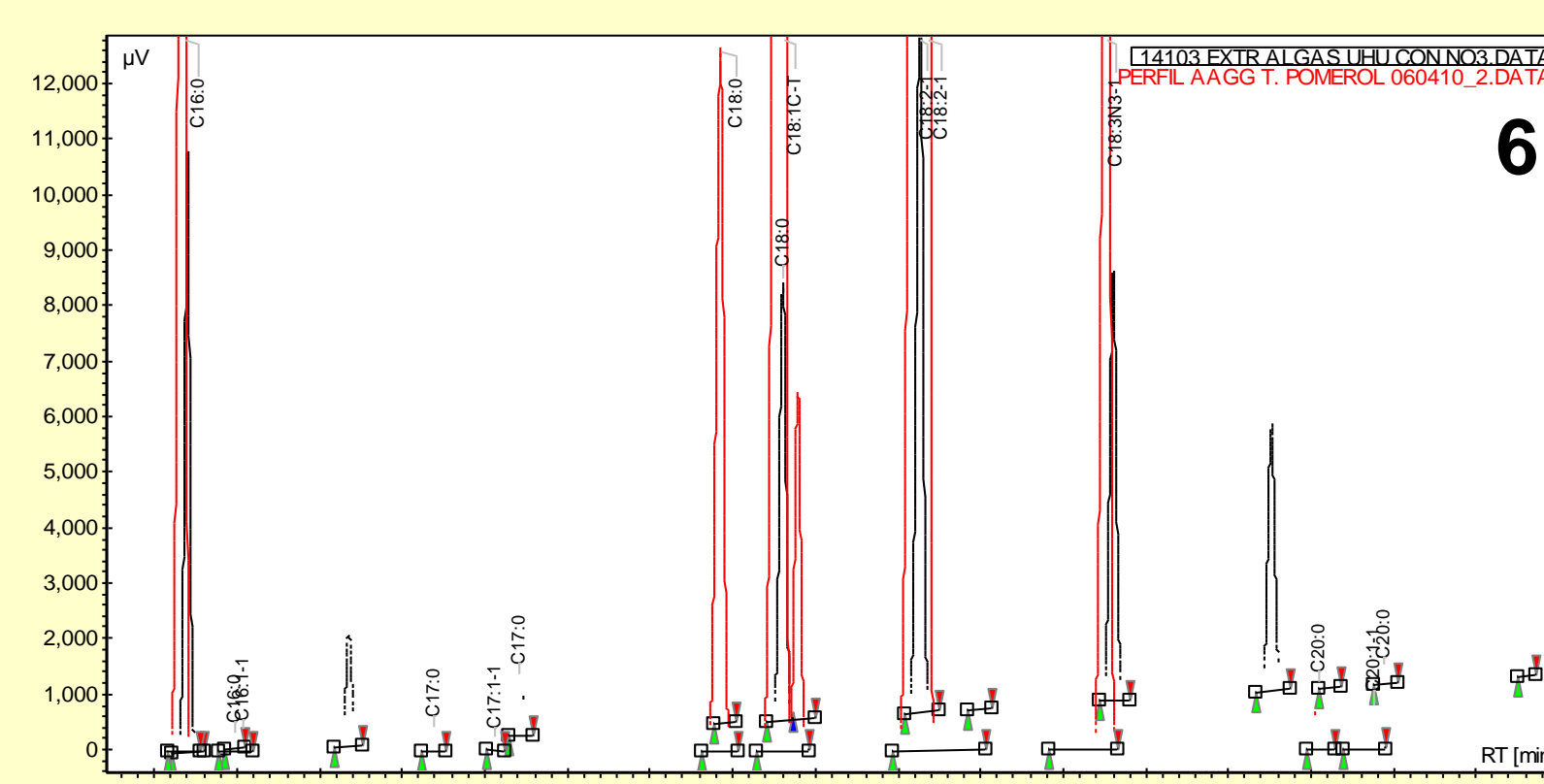


Figure 6. Fatty acid profile obtained from a culture of *Chlamydomonas acidophila* (black) and soya (red).

It is possible to increase the saturated to unsaturated fatty acids ratio using nitrogen starvation. These conditions led to 13 % more saturated fatty acids than control cultures of *C. acidophila*. This fact might be used for the algal biodiesel production process.

- The fatty acid profile in *C. acidophila* is very similar to the soya one, with some differences. This leads to *C. acidophila* would be a possible producer of useful oil for biodiesel.

- In addition of Cu (II) cultures, *C. acidophila* is able to accumulate higher quantities of carotenoids (more than 50% in lutein, and double β -carotene).

The photobioreactor system used greatly influences the growth rate and therefore the productivity (about 50%) of lutein-enriched biomass.

The tubular bioreactor with small light path has been found to be the best culture system to obtain high productivities, compared to the fluidized-bed bioreactor.

¹ Algal Biotechnology Group, Faculty of Sciences, University of Huelva, 21007 Huelva, Spain.

² Bio-Oils Huelva, S.L., Polígono Industrial Nuevo Puerto, 21810 Palos de la Frontera, Huelva, Spain

³ International Center for Environmental Research (CIECEM - UHU), Matalascañas - Huelva, Spain.