INFLUENCE OF ILLUMINATION ON THE GROWTH AND LIPID PRODUCTION BY

*Chaetoceros calcitrans*

**Natalia T. Ribeiro¹, Daniela A. Nogueira¹, Natalia T. Ribeiro¹, Juliane Machado da Silveira¹, Évelin Vidal¹ e Carlos André V. Burkert¹**

¹Federal University of Rio Grande, School of Chemistry and Food, Rio Grande-RS, Brazil (e-mail: nogueiradaniali@yahoo.com.br)
INFLUENCE OF ILLUMINATION ON THE GROWTH AND LIPID PRODUCTION BY Chaetoceros calcitrans

Natalia T. Ribeiro¹, Daniela A. Nogueira¹, Natalia T. Ribeiro¹, Juliane Machado da Silveira¹, Evelin Vidal¹ e Carlos André V. Burkert¹

¹Federal University of Rio Grande, School of Chemistry and Food, Rio Grande-RS, Brazil (e-mail: nogueiradaniali@yahoo.com.br)

Abstract

Microalgae have been highlighted in biotechnological studies by presenting relevant capacity for the production of lipids that can be used for biodiesel production and can be applied in different ways in the production of proteins, lipids, carotenoids, chlorophyll, enzymes and vitamins. The microalgal growth and lipid production are dependent on the species of microalgae used, favorable growing conditions and the organic carbon source. Chaetoceros calcitrans is a microalga that has been studied for this purpose, but there are few studies using the raw glycerol as a source of organic carbon in its cultivation. The aim of this study was to evaluate different illumination conditions in the cultivation of C. calcitrans, verifying the effects on biomass and lipid content. Cultivations were performed at 30°C, with a raw glycerol concentration of 3.37 g.L⁻¹ and two photoperiod regimes (12: 12 h and 24 h light : dark). Control assays were also performed, without raw glycerol addition. At the end of 10 days of cultivation, lipids were quantified in order to establish the most adequate conditions for lipid production by C. calcitrans. With the use of a cycle 24 h light and a raw glycerol concentration of 3.37 g.L⁻¹ it was obtained the highest lipid content (45.1 ± 1.9%).

Keywords
Raw glycerol; biomass; Chaetoceros calcitrans; lipids.

INTRODUCTION

Microalgal biomass has been gaining prominence in the world market and can be applied in different ways for the production of proteins, lipids, carotenoids, chlorophyll, enzymes, esters, antibiotics, vitamins and hydrocarbons (Richmond et al., 2004). The increasing demand for products produced by microalgae is due mainly to the fact that they have substances with antioxidant activity, polyunsaturated fatty acids (PUFAs), immunologically effective proteins and virostatic compounds (Molina et al., 1999).

The cell growth rate of microalgae are affected by a combination of environmental parameters like light intensity, photoperiod, temperature and nutrient composition of the culture medium (Kitaya et al., 2008). In addition, modifications in the culture conditions, particularly variations in salinity and carbon dioxide concentration, can directly affect the growth rate and production of lipids by these microorganisms (Damiani et al., 2008).

The main biofuels used in Brazil are ethanol obtained from sugarcane and, in an increasing scale, biodiesel, which is a clean and renewable fuel whose properties are similar to diesel and it is defined as a mixture of mono methyl or ethyl esters of fatty acids from animal fats or vegetable oils (Ma et al., 1999). However, during the production of biodiesel is generated glycerol as a byproduct (Mota et al., 2009). This usually has 55-90% purity and a growing surplus is generated by the current market
(Garcia et al., 2000). An alternative to the use of such glycerol is its utilization in biotechnological processes as a carbon source, aiming at the sustainable biodiesel production. Studies show that glycerol may be used as an organic carbon source by microalgae for obtaining lipids and other high value-added compounds (Chen et al., 2011; Chi et al., 2007).

*C. calcitrans* is an unicellular microalgae belonging to the class Bacillariophyceae, widely used as feed for marine crustacean larvae and bivalve molluscs cultivation (Shei et al., 2008). Also, several species of *Chaetoceros* have relevance in obtaining biotechnological products, such as thiamine (Brown et al., 1997), antibiotics, enzyme inhibitors, pharmacological active compounds and toxins (Lincoln et al., 2003) and fatty acids (Shamsudin, 1992).

Under mixotrophic conditions, some microalgae increase their growth rate and their biomass (Garcia et al., 2000). Thus, the use of an alternative carbon source, such as glycerol, can contribute for the reduction of the costs of the culture media regarding the use of traditional carbon sources, and can lead to an increase in biomass production and also add value to the biodiesel production chain. In this context, the aim of this work was to evaluate different illumination conditions in the cultivation of *C. calcitrans*, verifying the effects on biomass and lipid content.

**MATERIAL AND METHODS**

In the experiments, we used the microalgae *C. calcitrans*, supplied by Marine Biology and Environmental Biomonitoring Laboratory (LABIOMAR) of the Federal University of Bahia. The volume of inoculum added corresponded to 10% of the volume of sterile medium. The microorganism was kept in 1 L Erlenmeyer photobioreactors containing 900 mL of Conway medium (Walne, 1966), using seawater with salinity of 28‰ and the addition of raw glycerol, supplied by BS Bios (Passo Fundo, Brazil). The photobioreactors were disposed in an incubator with controlled illumination (Eletrolab EL-202, Brazil), provided by fluorescent lamps type daylight, with irradiance of 3000 Lux (2,22×10⁵ μmol.m⁻².s⁻¹), with direct and constant injection of sterile atmospheric air through a pump system. The initial biomass concentration was 0.55 g.L⁻¹ and the raw glycerol concentration was 3.37 g.L⁻¹, with purity of 82.09%.

The cultivation temperature was 30°C. For luminosity two photoperiod regimes (12: 12 h light : dark) were tested. Control experiments were performed without addition of raw glycerol.

The experiments were performed in triplicate and aliquots were taken each 24 h for determination of biomass, by measuring the absorbance at 680 nm and subsequent conversion to the dry weight concentration (g.L⁻¹) by a previously constructed calibration curve. At the end of the 10 days of cultivation, lipid content was determined using the method of Bligh and Dyer (1959). The differences between the illumination types and between the presence and absence of raw glycerol were evaluated by t-Test with 95% confidence level, using the Statistica 5.0 software (StatSoft Inc., USA)

**RESULTS AND DISCUSSION**

Figure 1 (a) shows the biomass growth of the microalga *C. calcitrans* without the addition of raw glycerol (control assays) and it can be observed a significant increase in the maximum biomass from 0.22 ± 0.01 g.L⁻¹ to 0.54 ± 0.14 g.L⁻¹ when using a cycle 24 h light instead of a 12 h light/12 h dark.
When raw glycerol was added (3.37 g.L⁻¹) as a carbon source for \textit{C. calcitrans} (Figure 2b), there was a significant increase in the maximum biomass concentration from 0.63 ± 0.06 g.L⁻¹ (12 h light/12 h dark) to 1.59 ± 0.25 g.L⁻¹ (24 h light).

![Figure 1: Biomass of \textit{C. calcitrans} in the control condition (a) and with the addition of 3.37 g.L⁻¹ of raw glycerol (b).](image)

In the control assays, with the change of photoperiod regime from 12 h light/12 h dark to photoperiod regime 24 h light, a significant increase in lipid content was observed, from 6.0 ± 1.2% to 13.5 ± 2.4%. With the addition of raw glycerol (3.37 g.L⁻¹), the same behavior was observed, with the lipid content increasing from 36.6 ± 2.6% to 45.1 ± 1.9%.

It was also observed that the addition of raw glycerol had a positive impact on biomass concentration and lipid content for both illumination conditions used. It was observed a significant increase in maximum biomass concentration from 0.22 ± 0.01 g.L⁻¹ to 0.63 ± 0.06 g.L⁻¹ (12 h light/12 h dark ) and from 0.54 ± 0.14 g.L⁻¹ to 1.59 ± 0.25 g.L⁻¹ (24 h light); and a significant increase in lipid content from 6.0 ± 1.2% to 36.6 ± 2.6% (12 h light/12 h dark ) and from 13.5 ± 2.4% to 45.1 ± 1.9% (24 h light).

**CONCLUSIONS**

From the results achieved in this work, it was observed that the production of lipids by \textit{C. calcitrans} was affected by cultivation variables such as glycerol concentration and illumination. The addition of raw glycerol resulted in higher lipid accumulation and biomass production, when compared with the control assays. The cultivation condition established for illumination was a cicle 24 h light and the addition of raw glycerol in a concentration of 3.37 g.L⁻¹, obtaining a lipid content of 45.1 ± 1.9% and a maximum biomass concentration of 1.59 ± 0.25 g.L⁻¹. \textit{C. calcitrans} was capable of assimilating glycerol, demonstrating its potential utilization in biotechnological processes as a carbon source for the production of products with higher added-value.

**ACKNOWLEDGEMENTS**
The authors thank the financial support from CAPES and CNPq.

**REFERENCES**


