



Universidade do Minho

Escola de Engenharia

## Semana da Escola de Engenharia October 24 - 27, 2011

### UTILIZATION OF DAIRY INDUSTRY BY-PRODUCTS AS SUBSTRATES TO ENHANCE THE PRODUCTIVITY OF MICROALGAE PRODUCTION SYSTEMS

Fernandes, B., Dragone, G., Teixeira, A., Vicente, A.  
Department of Biological Engineering  
E-mail: brunofernandes@deb.uminho.pt

#### KEYWORDS

*Chlorella vulgaris*, mixotrophic cultivation, source, cheese whey, biochemical composition.

#### ABSTRACT

The mixotrophic growth of the microalgae *Chlorella vulgaris* was studied using diverse substrates, namely cheese whey (CW) which is a by-product from dairy industry, cheese whey hydrolysate (CWH), lactose and a glucose/galactose mixture. Growth parameters and biochemical profile of cells were monitored. Our results demonstrate that the use of CW and CWH as substrate for mixotrophic growth, stimulates the growth of *Chlorella vulgaris*, leading to the achievement of high biomass productivities. In terms of biochemical composition, the results indicate that mixotrophic growth influence the pigment content, but does not have a significant influence on lipid, starch and protein content, thus mixotrophy it is only responsible for a considerable increase in the productivity in terms of these constituents, but not for an increase of its content.

#### INTRODUCTION

Traditionally, microalgae have been cultivated with carbon dioxide and light as carbon and energetic sources, respectively, in open outdoor ponds or in closed photo-bioreactors (PBR) (Carvalho et al., 2006). This mode of cultivation, known as photoautotrophic, presents several drawbacks, with consequent limitation on algal productivity: (1) light penetration

limitation, aggravated when the cultivation is intensive and causes self-shading; (2) growth parameters are dependent on local weather conditions; (3) harvesting is laborious, costly, and sometimes limited by low cell densities; (4) in PBR, development of algal biofilm on surfaces limits light penetration into the culture; (5) the high concentration of oxygen accumulation is a problem; and (6) continuous maintenance is required (Perez-Garcia et al., 2005). Strategies to improve the efficient use of light or eliminate its requirement by cells and so reduce the cost of microalgal biomass production, involve heterotrophic, photoheterotrophic or mixotrophic growth of algae (Perez-Garcia et al., 2005). The ability of mixotrophs to process organic substrates means that cell growth is not strictly dependent on photosynthesis, therefore light energy is not an absolutely limiting factor for growth (Andrade and Costa 2007), as either light or organic carbon substrates can support the growth. Cheese whey (CW) is a by-product derived from cheese-making and casein manufacture in the dairy industry and it has been traditionally regarded as a polluting waste stream. Its major components are lactose (45 - 50 kg m<sup>-3</sup>), proteins (6 - 8 kg m<sup>-3</sup>), lipids (4 - 5 kg m<sup>-3</sup>), and mineral salts (8 - 10% of dried extract), constitutes an inexpensive and nutritionally rich raw material. Thus, the present study was conducted to determine if our strain of *Chlorella vulgaris* can growth mixotrophically in a medium supplemented with cheese whey (CW) or cheese whey hydrolysate (CWH) and how growing conditions affect its kinetic growth and its composition in terms of major constituents (lipids, proteins and starch).

#### METHODS



Universidade do Minho

Escola de Engenharia

## Semana da Escola de Engenharia October 24 - 27, 2011

**Microorganism:** A thermophilic *Chlorella vulgaris* (strain P12) was used for cultivation.

**Culturing conditions:** The control growth medium used during autotrophic growth was based on the elementary composition of algal biomass. Conditions of mixotrophy was induced by adding cheese whey powder or cheese whey hydrolysate solution to the control medium. The microalgae were grown in bubble columns photobioreactors containing 400 ml of medium. Cultures were maintained at 30°C under continuous, cool white, fluorescent light. Light intensity was approximately  $70 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the surface of the photobioreactors.  $\text{CO}_2$ -enriched air (2% v/v  $\text{CO}_2$ ) was supplied at an aeration rate of 0.400 vvm.

**Biomass concentration:** Microalgal cell density was measured microscopically using an improved Neubauer hemocytometer. The growth rate of microalgae was also measured by cell dry weight.

### RESULTS & CONCLUSIONS

The use of CW and CWH as substrate for mixotrophic growth, stimulates the growth of *Chlorella vulgaris*, leading to the achievement of high biomass productivities ( $0.316$  and  $0.739 \text{ g L}^{-1} \text{ d}^{-1}$ , respectively) representing an increase in order of 3 and 7 times, respectively, compared with the photoautotrophic growth ( $0.103 \text{ g L}^{-1} \text{ d}^{-1}$ ). The biomass productivities found when using CW and CWH were higher in comparison with the obtained with the synthetic mediums that mimic them in terms of sugars, which means that the composition of CW and CWH is well suited for the mixotrophic growth of *Chlorella vulgaris*. In terms of biochemical composition, the results indicate that mixotrophic growth influence the pigment content, but does not have a significant influence on lipid, starch and protein content, thus mixotrophy it is only responsible for a considerable increase in the productivity in terms of these constituents, but not for an

increase of its content.

Biomass productivity and, inherently, the productivity in terms of lipids, starch and protein was stimulated by CW and CWH, suggesting that this industrial by-product could be used as a low-cost supplement for the mixotrophic growth of *Chlorella vulgaris*.

### ACKNOWLEDGEMENTS

The financial support from Fundação para a Ciência e Tecnologia (FCT) through the PhD grant SFRH / BD / 44724 / 2008 given to Fernandes, B., and G. Dragone grant SFRH/BPD/44935/2008 is gratefully acknowledged.

### REFERENCES

- Andrade, M., and Costa, J. (2007) Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate, *Aquaculture* 264, 130-134.
- Carvalho, A.P., Meireles, L.A., Malcata, F.X., 2006. Microalgal reactors: a review of enclosed system designs and performances. *Biotechnol. Prog.* 22, 1490e1506.
- Perez-Garcia 2005; Mixotrophic growth of the microalga *Phaeodactylum tricornutum* Influence of different nitrogen and organic carbon sources on productivity and biomass composition

### AUTHOR BIOGRAPHY

**BRUNO FERNANDES** was born in Guimarães and went to the University of Minho, where he studied Biological Engineering and obtained his Integrated Master Degree in Biological Engineering in 2007. During 2007 and 2008 he worked as a researcher in the Laboratory of Pilot Plants, University of



Minho, on two different projects (OVOALOR and Flowcaps – Hovione). Since 2009, he is doing his Ph.D. project in University of Minho. His e-mail address is: [brunofernandes@deb.uminho.pt](mailto:brunofernandes@deb.uminho.pt) His webpage can be found at: <http://www.ceb.uminho.pt/pessoas/pid.aspx?id=236>.