Mixotrophic cultivation of *Chlorella vulgaris* using industrial dairy waste as organic carbon source

Ana P. Abreu, Bruno Fernandes, António A. Vicente, José Teixeira, Giuliano Dragone *

IBB – Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

**HIGHLIGHTS**

- Cheese whey was used as carbon source for *Chlorella vulgaris* growth.
- Mixotrophic microalgae grew faster than photoautotrophic cells.
- Maximum starch productivity was achieved under mixotrophic conditions.
- Highest pigment content (0.74%) was obtained in the photoautotrophic culture.

**ABSTRACT**

Growth parameters and biochemical composition of the green microalga *Chlorella vulgaris* cultivated under different mixotrophic conditions were determined and compared to those obtained from a photoautotrophic control culture. Mixotrophic microalgae showed higher specific growth rate, final biomass concentration and productivities of lipids, starch and proteins than microalgae cultivated under photoautotrophic conditions. Moreover, supplementation of the inorganic culture medium with hydrolyzed cheese whey powder solution led to a significant improvement in microalgal biomass production and carbohydrate utilization when compared with the culture enriched with a mixture of pure glucose and galactose, due to the presence of growth promoting nutrients in cheese whey. Mixotrophic cultivation of *C. vulgaris* using the main dairy industry by-product could be considered a feasible alternative to reduce the costs of microalgal biomass production, since it does not require the addition of expensive carbohydrates to the culture medium.

1. Introduction

Microalgae cultivation has been carried out throughout the world in order to produce animal feed or high-value added products, such as cosmetics, pharmaceuticals and health supplements (Das et al., 2011). More recently, microalgae have also been used for wastewater treatment, carbon dioxide (CO₂) mitigation, or as a feedstock for biofuel production (Brennan and Owende, 2010). These photosynthetic microorganisms can be cultivated either in open ponds or closed photobioreactors (PBR) using CO₂ and light as carbon and energy sources, respectively (Chen et al., 2011). Nonetheless, this culture mode, known as photoautotrophic, presents several disadvantages including low cell densities and long cultivation periods. Hence, heterotrophic and mixotrophic growth regimes have been proposed as feasible alternatives for the production of microalgal biomass (Yu et al., 2009).

Heterotrophic cultivation of microalgae involves the utilization of organic compounds as sole carbon source, while mixotrophic cultivation use simultaneously inorganic (for example CO₂) and organic compounds as carbon source (Dragone et al., 2010). Therefore, microorganisms cultivated under mixotrophic conditions synthesize compounds characteristic of both photosynthetic and heterotrophic metabolisms at high production rates. Additionally, lower energy costs have been associated with mixotrophic cultivation in comparison with photoautotrophic cultures, due to its relatively lower requirements for light intensities (Cerón García et al., 2005).

Despite mixotrophic cultivation of microalgae provides higher biomass and lipid productivities than cultivation under photoautotrophic conditions, the cost of the organic carbon substrate is estimated to be about 80% of the total cost of the cultivation medium (Bhatnagar et al., 2011). As a result, less costly organic sources have to be found in order to overcome the high carbon cost resulting from mixotrophic culture conditions (Liang et al., 2009). Cost reduction of growth media preparation with minimal undesired effects is crucial for a potential commercial application (Abad and