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Using agro-industrial wastes for mixotrophic growth and lipids production by the green microalga *Chlorella sorokiniana*

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ABSTRACT

There has been growing interest in the use of microalgae for the production of biofuels, but production costs continue to be too high to compete with fossil fuel prices. One of the main limitations for photobioreactor productivity is light shielding, especially at high cell densities. The growth of the green microalga *Chlorella sorokiniana*, a robust industrial species, has been evaluated under different trophic conditions with traditional carbon sources, such as glucose and sucrose, and alternative low cost carbon sources, such as carob pod extract, industrial glycerol and acetate-rich oxidized wine waste lees. The mixotrophic cultivation of this microalga with wine waste lees alleviated the problems of light shielding observed in photoautotrophic cultures, improving specific growth rate ($0.052\ h^{-1}$) compared with the other organic sources. The fed-batch mixotrophic culture of *Chlorella sorokiniana* in a 2 L stirred tank reactor, with optimized nutritional conditions, 100 mM of acetate coming from the oxidized wine waste lees and 30 mM of ammonium, produced an algal biomass concentration of $11\ g\ L^{-1}$ with a lipid content of 38 % (w/w). This fed-batch strategy has been found to be a very effective means to enhance the biomass and neutral lipid productivity.

Introduction

Microalgae are a heterogeneous group of photosynthetic microorganisms with unique properties, widely used as high nutritional value additives in aquaculture, human food, animal feed and cosmetics [1]. Per year, over 5000 tons of dry microalgal biomass are produced globally and marketed with an average value of more than 1 billion euros [2]. Microalgae are the main natural source of carotenoids [3,4], a potential resource for long-chain polyunsaturated essential fatty acids [5] and, given the diversity of microalgal species that exist and the many that remain to be identified, it is estimated that they can be a potential source of new bioactive compounds still unexplored with importance in the food and pharmaceutical industries [6,7]. Moreover, there has been an increasing interest in microalgae for the production of carbon-neutral biofuels [8–10] and for their ability to mitigate greenhouse gas emissions, which has stimulated research into the design of cheaper and more efficient photobioreactors [11,12]. However, the cost of microalgae production, harvesting and processing is still very far from being competitive with that of fossil fuels [13].

The importance of bioreactor design is more evident in high-cell density cultures [14], due to the difficulties of dealing with cellular self-shading within the photobioreactor. While the input of carbon, nitrogen or other nutrients in a reactor can be controlled by operational factors (dilution rate, initial concentration), light input is controlled by the design of the photobioreactor and the avaibility of external light, which changes through the day and the year. In addition to the standard photoautotrophic growth, many microalgal species can be cultured under heterotrophic conditions with an organic source of carbon and/or energy [15], or under mixotrophic conditions, where they are grown with an organic carbon source in the presence of light [16]. Mixotrophic growth is a mixed approach that combines aspects of both photo- and heterotrophic technologies and a promising alternative for microalgae, capitalizing on the simultaneous assimilation of carbon dioxide and organic carbon sources such as sugars [17,18], acetate [19] or glycerol [20]. However, to achieve economically feasible mixotrophic production it is necessary to choose a cheap organic carbon

Abbreviations: A. acetii, *Acetobacter acetii*; *C. sorokiniana*, *Chlorella sorokiniana*; DMSO, Dimethylsulfoxide; DW, Dry Weight; FI, Fluorescence Intensity; HPLC, High Performance Liquid Chromatography; ISA, Ionic Strength Adjusted; SRT, Stirred Tank

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