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Evaluating low-cost substrates for *Crypthecodinium cohnii* lipids and DHA production, by flow cytometry

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Abstract

Crypthecodinium cohnii growth was studied on pure carbon sources (glucose, acetate, glycerol) and low-cost complex carbon sources (sugarcane molasses, crude glycerol and vinegar effluent) for lipid and DHA production. Among the pure substrates, glucose induced the highest lipid content (14.75% w/w DCW) and DHA content (7.14 mg g^{-1} DCW). Among the low-cost substrates, the highest lipid and DHA content were observed for the crude glycerol assay (14.7% w/w DCW and 6.56 mg g^{-1}, respectively). Molasses induced the highest proportion of DHA of total fatty acids (49.58% w/w TFA) among all the substrates studied. Flow cytometric analysis revealed that the vinegar effluent induced the highest proportion of *C. cohnii* cells with injured membrane (92.8%). These results foresee the possibility of using these low-cost substrates at a larger scale for *C. cohnii* DHA and biodiesel production, aiming at zero wastes and process costs reduction.

Keywords *Crypthecodinium cohnii* · Pure substrates · Low-cost substrates · Lipids · DHA · Flow cytometry

Introduction

The marine microalga *Crypthecodinium cohnii*, a heterotrophic non-photosynthetic dinoflagellate, accumulates significant amounts of lipids (20–50% of its cell dry weight) with a high fraction of docosahexaenoic acid (DHA), a ω-3 polyunsaturated fatty acid which is a component of neural and retinal tissues, a key fatty acid component in breast milk, and it is necessary for brain development in infants. This compound has well known benefits on the human health, having currently several nutritional and pharmaceutical applications, and a growing market size (Diao et al. 2018).

DHA can be obtained from marine fish sources; however, its production from microalgal source shows benefits over DHA obtained from fish, since the pure microalgal oil is odourless, non-dependent on fish stocks, does not contain ocean-borne contaminants, and its vegetarian nature attracts young people (Lopes da Silva et al. 2019). Under certain cultivation conditions, *C. cohnii* cells accumulate less than 1% of the other type of PUFAs, which is a clear advantage for the downstream DHA purification process. On the other hand, the presence of more than 50–60% content of fatty acids with 16 and 18 carbon atoms (C16-C18) in *C. cohnii* total fatty acids makes this microalga a potential source for biodiesel production.

The carbon source is the most expensive component of fermentation media. In the late 1990s and early 2000s, several pure carbon sources were studied to grow *C. cohnii*, such as glucose (De Swaaaf et al. 1999), acetic acid (De Swaaf et al. 2003), ethanol (De Swaaf et al. 2003) and pure glycerol (Hosoglu and Elibol, 2017a). However, despite these carbon sources induce high lipid and DHA productivities, they are expensive to be used at large scale (glucose 16 € kg^{-1}; ethanol 1.82 € kg^{-1}; acetic acid 0.45 € kg^{-1}, www.alibaba.com). In addition, ethanol and acetic acid are dangerous compounds that are difficult to handle and transport. On the other hand, the increasing environmental awareness about the circular economy rules has boosted the necessity to use low-cost or zero-cost wastes/byproducts/effluents as nutrients in media formulations for microbial growth. In fact, in recent years, substrates as food waste such as carob pulp syrup (Mendes et al. 2007), rapeseed meal hydrolysate mixed with crude molasses (Gong et al. 2015) and cheese whey with corn steep liquor (Hosoglu and Elibol 2017b) have been used in media formulations for *C. cohnii* ω-3 compounds production. Nevertheless, despite the low cost of these substrates, they often contain inhibitory compounds that affect the cell

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