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A biorefinery from *Nannochloropsis* sp. microalga – Extraction of oils and pigments. Production of biohydrogen from the leftover biomass

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HIGHLIGHTS

- *Nannochloropsis* sp. was studied as a biorefinery context.
- Production of fatty acids for biodiesel, high added-value compounds and biohydrogen.
- Extracts fractionation by SFE (oils for biodiesel and pigments for food).
- Fermentation of biomass leftover by *E. aerogenes* yielded maximum 60.6 mL $H_2/g_{dry\ biomass}$ alga.
- Best SFE conditions extracted 45 $g_{lipids}/100\ g_{dry\ biomass}$ (40 °C, 300 bar, CO_2 + ethanol).

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ABSTRACT

The microalga *Nannochloropsis* sp. was used in this study, in a biorefinery context, as biomass feedstock for the production of fatty acids for biodiesel, biohydrogen and high added-value compounds. The microalgal biomass, which has a high lipid and pigment content (mainly carotenoids), was submitted to supercritical CO_2 extraction. The temperature, pressure and solvent flow-rate were evaluated to check their effect on the extraction yield. The best operational conditions to extract 33 $g_{lipids}/100\ g_{dry\ biomass}$ were found to be at 40 °C, 300 bar and a CO_2 flow-rate of 0.62 g/min. The effect of adding a co-solvent (ethanol) was also studied. When supercritical CO_2 doped with 20% (w/w) ethanol was used, it was possible to extract 45 $g_{lipids}/100\ g_{dry\ biomass}$ of lipids and recover 70% of the pigments. Furthermore, the remaining biomass after extraction was effectively used as feedstock to produce biohydrogen through dark fermentation by *Enterobacter aerogenes* resulting in a hydrogen production yield of 60.6 $mL/g_{dry\ biomass}$.

1. Introduction

Renewable carbon-neutral liquid biofuels are needed to replace petroleum-derived transport fuels in the near future as they contribute to global warming and are of a limited availability. A promising alternative is microalgae, because of their much higher photosynthetic efficiency, areal productivity and oil content and also that they do not compete with food cultures, arable land, and potable water, and they have the possibility of being harvested on a daily basis (Gouveia and Oliveira, 2009; Gouveia, 2011). However, the current implementation of microalga-based systems has been economically constrained and still has some technological drawbacks. The main constraints include the limited amount of biomass which can be obtained with currently available photobioreactors, the low biomass productivity, the low harvesting efficiency and the relatively low microalga intrinsic lipid content (Amaro et al., 2011).

In terms of microalgal lipids, the total amount and the type of fatty acids are specific for each species of microalgae and this is linked to environmental factors (e.g. light intensity, pH, salinity, temperature, concentration of nitrogen, and other nutrients; Gouveia and Oliveira, 2009; Gouveia et al., 2009). The lipid content of microalgae is approximately 20–50% of its dry weight with a possibility to attain up to 80% (Spolaore et al., 2006).

In addition to the energy content of the microalgal biomass, these microorganisms have the capacity to synthesize bioactive molecules, such as carotenoids, antioxidants, anti-inflammatory

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