1-CIAB 1er. CONGRESO IBEROAMERICANO SOBRE BIORREFINERIAS

EXPLORING *Scenedesmus obliquus* AND *Nannochloropsis* sp. POTENTIAL AS A SUSTAINABLE RAW MATERIAL FOR BIOFUELS AND HIGH ADDED VALUE COMPOUNDS

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Abstract

In this work, the authors propose a microalga-based integrated system, where optimization of several energy vectors (biodiesel, bioethanol and bioH_2) is highlighted under the concept of biorefinery (Project PTDC/AAC-AMB/100354/2008). This involves the integration of different processes such as oil and sugar extraction from microalgae for biodiesel and bioethanol production respectively, and bioH_2 production from the whole and/or biomass leftovers. The extraction of high value added compounds, such as carotenoids, contributes to the economic viability of the overall process.

Keywords: Microalga, *Scenedesmus obliquus*, *Nannochloropsis* sp., Bioethanol, BioH_2, Pigments.

INTRODUCTION

Algal biofuels may offer great potential in contributing to a nation's renewable energy future, as well as helping to meet the 20% renewable biofuel energy in transport by 2020 (CEC, 2008) and the energy independence and security. In a carbon-smart society, it is imperative to produce both food and fuel in a sustainable way, as they are intricately interconnected. Moreover, microalgae never compete with the food crops in terms of land and water, with the advantages of e.g. higher productivities and daily biomass harvesting (Gouveia, 2011). Algae oils can be a suitable feedstock for high-energy density renewable biofuels to power both light- and heavy-duty vehicles, as well as jet and marine engines. On the other hand, algae sugars can be a source for bioethanol production through saccarification and fermentation (Miranda et al., 2012a). In addition, biohydrogen (bioH_2)

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can also be obtained by fermentation of the whole microalgal biomass, or after the extraction of its oils (Ferreira et al., 2012). Furthermore, algae produce many valuable bioactive compounds which can substantially reduce the production costs and can feed various industries, such as food, feed, pharmaceuticals and cosmetics.

The aim of this work was to evaluate the potential of a freshwater (*Scenedesmus obliquus*) and a marine (*Nannochloropsis* sp.) microalgae, as source of sugars and/or oils for the production of biofuels (biodiesel, bioethanol and bioH_2), as well as added value compounds (pigments). Supercritical fluid extraction (SFE) was used to extract and separate lipids from pigments. Bioethanol was produced by different yeasts from microalgae sugar extracts, and bioH_2, from the whole and leftovers (after oil extraction) biomass, by fermentation with *Enterobacter aerogenes* and *Clostridium butyricum.*

METHODOLOGY

Microalga production

Scenedesmus obliquus (*Sc*) and *Nannochloropsis* sp. (*Nanno*) were used in this study. *Sc* was cultured in Bristol medium (Vonshak, 1986), firstly indoors, in 1 L glass bubble column reactors, with bubbling air, at 25ºC, low light (150 $\mu E.m^{-2}.s^{-1}$). These cultures were scaled-up to open raceway ponds of 300 L (2 m^2) and then 4500 L capacity (48 m^2), agitated by paddle-wheels at ~5 m/min, under natural light/dark cycles and temperature conditions. *Nanno* was grown in GPM medium (75% filtered seawater:25% deionized water) (Vonshak, 1986), in polyethylene bags (10 L) indoors, under the same conditions described for indoor *Sc* cultivation. *Sc* and *Nanno* cultures were grown for 55 and 40 days, respectively, in order to guarantee high sugar and lipid accumulation during the stationary phase (Miranda el al., 2012a,b; Nobre et al., submit.). The growth of the cultures was monitored through $O.D._{540nm}$ and dry weight concentration (GF/C filter). The harvesting of the biomass was carried out by sedimentation followed by centrifugation (10000 rpm/10min) and drying in an oven at 80ºC. Microalgal biomass proximate composition was determined in terms of moisture (gravimetry), total ashes (gravimetry), crude protein (Kjeldhal), crude fat (Soxhlet), according to A.O.A.C. methods (2006). Total sugar content was evaluated by the phenol–sulphuric method (Dubois et al., 1956) after acid hydrolysis.

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Bioethanol production

Sugar extracts were prepared by the addition of H_2SO_4 2N (1L to 500 g alga dw) to *Sc* dried biomass, and autoclaving for 30 min at 120ºC (Miranda et al., 2012a), and further detoxified by sulfate precipitation with $Ca(OH)_2$ (107 g/L). Fermentation assays were performed in 1 L erlenmeyers containing 500 mL microalga hydrolysate which was inoculated with 300 mg/L of exponentially grown yeast. 3 different yeasts were tested: *Kluyveromyces marxianus* YPCC2671, *Saccharomyces carlsbergensis* ATCC6269 and *Saccharomyces bayanus*. The fermentation experiments were carried out at 30ºC in an orbital shaker (150 rpm). Ethanol concentration was determined by GC (Miranda et al., 2012b). Ethanol concentration was determined by GC (Miranda et al., 2012b).

Supercritical Fluid Extraction (SFE) of oil and pigments

SFE experiments were carried out in a flow-type apparatus (Mendes et al., 1995) modified to include a co-solvent addition system. A 5 cm^3 extraction vessel was used, which was filled with ~1.2 g *Nanno* biomass (dw) mixed with glass beads (3 mm Ø), put between two layers of glass wool. Extracts were collected with acetone. Quantification of total lipids was carried out gravimetrically, concentrating the collected solution under vacuum and drying the extract under nitrogen. Pigments (carotenoids) were identified and quantified by spectrophotometry and HPLC. The fatty acid composition of the extracted oil was accessed by GC (Lepage et al., 1986). SFE experiments were performed at 40ºC, 300 bar and CO_2 flow-rate of 0.62 g/min, using *Nanno* biomass ground in a bead mill. The separation of the oil from the pigments was carried out through a fractionation strategy extracting the oil with pure supercritical CO_2 (52.4 g/g alga dw) in a first step, and extracting the pigments with supercritical CO_2 modified with ethanol 20% (w/w) (61.8 g/g alga dw) in a second stage. *Nanno* biomass leftovers were used to produce bioH_2.

Biohydrogen (bioH_2) production

Batch fermentation assays were performed in sealed serum bottles containing fermentation medium (Ferreira et al., 2012), with a gas to liquid ratio of 6:1 (v/v), and *Sc* or *Nanno* biomass as substrate. The fermentation medium was aseptically purged with bubbling N_2 to eliminate O_2, before inoculation with exponentially grown *E. aerogenes* at 10% (v/v). The fermentation was carried out under orbital shaking (220 rpm), at 30°C, for 6 h. For *C. butyricum*, *Sc* biomass was added to the individual serum bottles before the

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anoxic distribution of the fermentation medium and sterilization. An overnight grown culture was inoculated at 1% (v/v) and the fermentation was conducted for 48 h at 37ºC, 150 rpm. $BioH_2$ production assays comparing the use of dried and wet (75% moisture paste obtained before drying) *Sc* biomass were also carried out, for the concentrations which previously led to the highest H_2 yields (2.5 g/L for *E. aerogenes* and 50 g/L or *C. butyricum*). $BioH_2$ volumetric production was analyzed in the headspace by GC (Ferreira et al., 2012).

RESULTS

Sc presented 30.7% of sugars (Table 1) which can be fermented to ethanol (Figure 1a) in less than 10 h, being the highest ethanol concentration (11.7 g/L) obtained with *K. marxianus* yeast. Figure 1b shows the results obtained for fermentative $bioH_2$ production by both bacteria from dried and wet *Sc* biomass. The highest $bioH_2$ production yield (90 mLH_2/g alga dw) was registered for *C. butyricum* as fermentative bacteria and an initial concentration of dry microalgal biomass of 50 g/L, after 48 h (equilibrium time). However, *E. aerogenes* yielded 57.7 mLH_2/g alga dw from wet *Sc* (2.5 g alga dw/L) after 6 h, which is advantageous from an energetic and economic point of view, taking into account a lower process time and the suppression of an intermediate drying step.

Nanno composition is shown in Table 1, being evident the high lipid content (40.7%) The SFE strategy in two steps (supercritical CO_2 for oils and CO_2 with ethanol (20% w/w), for pigments) allowed the fractionation showed in Figure 2a. The first extract collected 33 g_{oil}/100 g alga dw (78% oil recovery), and only 38 $mg_{pigments}$/100 g alga dw. The fatty acid composition of this oil was similar to that obtained with the Soxhlet extraction. In the second extract, 12 g_{oil}/100g alga dw, as well as 53 $mg_{pigments}$/100g alga dw were obtained, equivalent to the extraction of 50% of the total pigments remaining in the biomass. So, in the second extract it was possible to obtain an oil with a pigment concentration of 0.44 $g_{pigment}/100g_{oil}$, which contained 48.9% (w/w) of fatty acids, enriched in bioactive compounds (C20:5 $\cong$ 5%). Moreover, the pigment composition of this oil showed the presence of astaxanthin, zeaxanthin/lutein, canthaxanthin and β-carotene in a mixture corresponding to almost 50% of the total pigments, which would make it suitable for its use in the food/feed and/or nutraceutical industries. From the spent biomass after extraction the production of $bioH_2$ by dark fermentation with *E. aerogenes* was attained, resulting in a

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hydrogen production yield of 60.6 mL/g alga dw (Figure 2b), higher than the obtained with the whole biomass (48.0 mL/g alga dw), highlighting the benefits of a biorefinery concept.

Table 1. Microalgae biomass proximate composition.

Item	Unit	*Scenedesmus obliquus**	*Nannochloropsis* sp.*
Total Minerals	% dry weight	20.2 ± 0.5	13.2 ± 1.2
Crude Protein	% dry weight	20.4 ± 0.02	*n.d.*
Crude Fat	% dry weight	17.1 ± 0.2	40.7 ± 2.3
Total Sugars	% dry weight	30.7 ± 0.8	17.0 ± 0.2

*average ± standard deviation; *n.d.* not determined

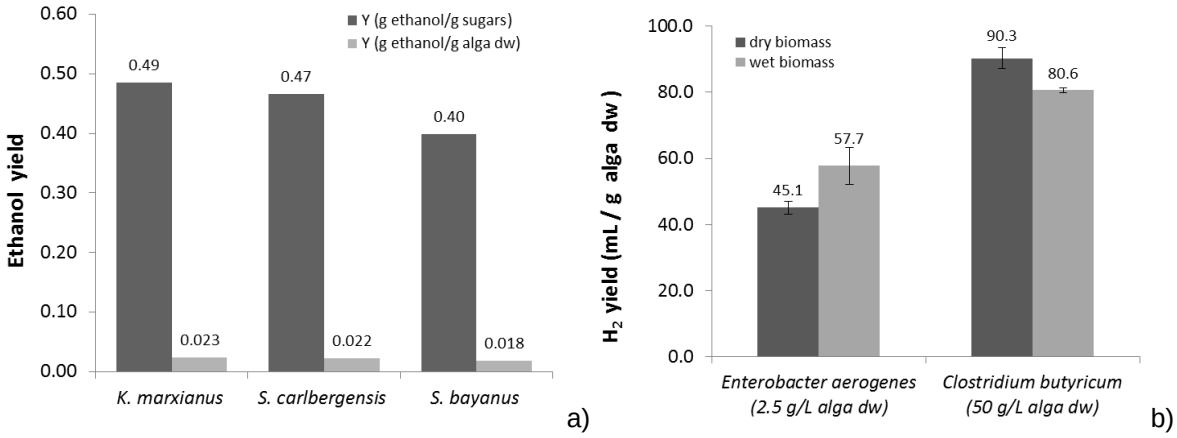

Figure 1. Bioethanol (a) and BioH_2 (b) production yields from *Sc* sugar extracts (500 g/L) and biomass fermentation (2.5 and 50 g/L), respectively.

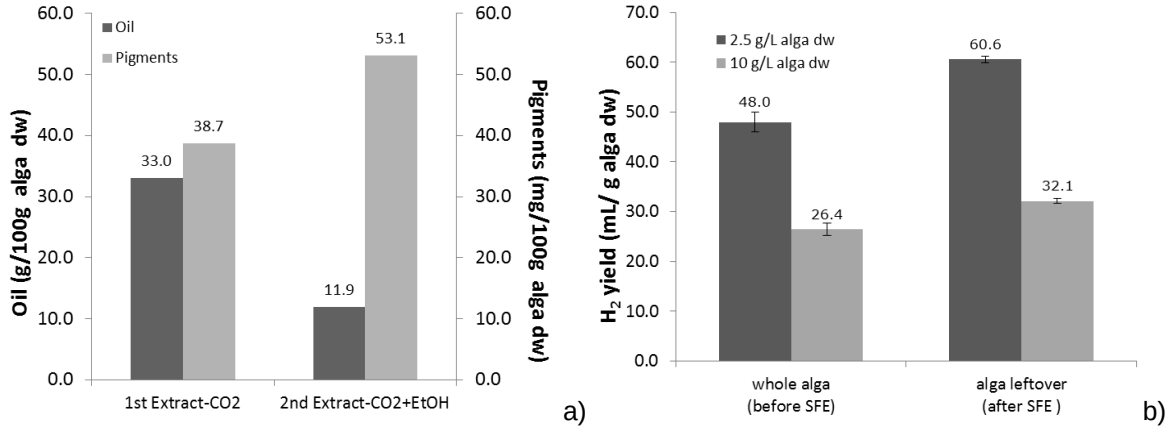

Figure 2. Oil and pigment extraction yield from SFE (a) and fermentative BioH_2 production yields (with *E. aerogenes*) (b) from *Nanno* biomass.

CONCLUSIONS

In this work, *Sc* and *Nanno* sp. shown to be effective as a sustainable raw material for ethanol, oils, pigments and bioH_2 production in a biorefinery context. Also, SFE proved to

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be a green alternative method for microalgal lipids extraction (for biodiesel), allowing the fractionation of the pigments to valuable applications. Finally, the potential of *Sc* and spent *Nanno* biomass as feedstock for bioH_2 production was also demonstrated. The proposed integrated system brought about important technological and economic improvements to the process of biofuels and high-added value compounds production from microalgae.

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