A biorefinery from *Nannochloropsis* sp. microalga – Energy and CO₂ emission and economic analyses

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HIGHLIGHTS

- Oil obtained by SE has the lowest energy and CO₂ values per MJprod.
- Oil obtained by SFE proved to be more economically viable, with a cost of 365 €/kgoil.
- Biorefinery 2 (biodiesel SFE + bioH₂) has the best energy/CO₂/economy compromise.
- The bioH₂ as co-product may be advantageous in terms of product yield or profit.
- High-value pigments can be produced by SFE which is a clean technology.

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ABSTRACT

Are microalgae a potential energy source for biofuel production? This paper presents the laboratory results from a *Nannochloropsis* sp. microalgal biorefinery for the production of oil, high-value pigments, and biohydrogen (bioH₂). The energy consumption and CO₂ emissions involved in the whole process (microalgal cultivation, harvest, dewater, mill, extraction and leftover biomass fermentation) were evaluated. An economic evaluation was also performed. Oil was obtained by soxhlet (SE) and supercritical fluid extraction (SFE). The bioH₂ was produced by fermentation of the leftover biomass. The oil production pathway by SE shows the lowest value of energy consumption, 177-245 MJ/MJprod, and CO₂ emissions, 13–15 kgCO₂/MJprod. Despite consuming and emitting c.a. 20% more than the SE pathway, the oil obtained by SFE, proved to be more economically viable, with a cost of 365 €/kgoil produced and simultaneously extracting high-value pigments. The bioH₂ as co-product may be advantageous in terms of product yield or profit.

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1. Introduction

Microalgae biomass has the potential to provide renewable energy (e.g. biodiesel, bioethanol, biohydrogen and biogas) through the production of different energy vectors. In addition, they also have the capacity to synthesize bioactive molecules, such as carotenoids, fatty acids, antioxidants, anti-inflammatory and other valuable organic compounds, which can be used in food, feed, cosmetic, biomaterials, nanostructures and pharmaceutical industries (Marques et al., 2011). A systemic approach to the culture and refinery of microalgae is therefore essential and should integrate the production of biofuels and co-products. The biorefinery approach consists in the production of a wide range of biofuels and chemicals from biomass and it assists in making biofuel production economically feasible (Gouveia, 2011).

Without competing with food cultures, arable land, potable water and having a higher productivity and the possibility of daily harvesting, the microalgal potential for biofuel production is huge. However, the current implementation of microalgae-based systems has been economically constrained by their still poor volumetric efficiencies, which still lead to excessively high costs compared to petroleum prices. Moreover, technological drawbacks are also present, namely the maximum biomass concentration attainable in the available photobioreactors, the biomass productivity, the efficiency of harvesting and the relatively low microalgal intrinsic lipid content (Amaro et al., 2011). Therefore a careful assessment of the life cycle energy balances – including actual environmental
impacts and economic evaluation – should be addressed prior to the process being implemented on an industrial scale. Furthermore, when considering the microalgal biomass growth and lipid synthesis, it is essential to be aware of the impacts of downstream processing on the energy balance.

Exhaustive work has been done by other authors on the production of biofuels through various biological methods with different feedstocks, however most recently, studies of biofuel production by microalgae, such as biodiesel, are being widely developed in terms of energy and CO₂ assessment, including an assessment of their costs (Campbell et al., 2010; Soratana and Landis, 2011; Xu et al., 2011). Other studies covering the life cycle of biodiesel production (Stephenson et al., 2010; Lardon et al., 2009; Khoo et al., 2011) showing values in the range of 2.8–5.4 MJ/MJoil and 0.2–0.9 kgCO₂/MJoil. The values presented in these studies were obtained from the optimized processes with high productivities and used open ponds for algae production. An alternative solution to reduce the excessive energy that is required in microalgal growth is presented in a Lam et al. (2012) study, where the carbon capturing was carried out with CO₂ bio-fixation. Aiming to analyze the energy and environmental impacts in a photobioreactor pilot plant, Itoiz et al. (2012) achieved a total energy input for marine microalgal of 923 MJ/kg for indoor culture conditions. There are European and US databases for fuel life cycle inventories (LCIs), including energy. CO₂ emissions and cost evaluations which were produced by CONCAWE (CONCAWE, 2008) and Greet (Frank et al., 2011), respectively. A new version of the Greet model (Frank et al., 2011) includes new algae pathways but only to produce bio-oil, including the algae growth, dewatering and oil extraction stages.

The industrial viability of microalgal-based biofuels depends upon the economical aspects which are fundamental to the process. Furthermore, whatever advances might arise in terms of technological innovations, the market will not exhibit an enthusiasm for funding capital-intensive energy projects unless the risk–return ratio is acceptable (Singh and Gu, 2010).

The global cost of biodiesel production by microalgae may be split into the partial costs associated with the biomass growth, the harvesting (including the concentration of the biomass to a suitable level for further processing and dewatering), the oil extraction and the oil transesterification (conventional biodiesel).

Thurmond (2009) highlighted the several strategic steps required for successful microalgal biofuel production and that they can be consolidated in the following five keywords: ‘fatter’, ‘faster’, ‘cheaper’, ‘easier’ and ‘fraction’. The first two, i.e. ‘fatter’ and ‘faster’, are the primary strategic needs for microalgal biodiesel trade and they mean that species which are richer in oil are easier to extract and therefore are to be preferentially sought. If producers can use fatter microalgae (e.g. with at least 60% oil content) that grow faster, then they can reduce both the size and footprint of the biofuel plant by as much as a half, which may lead to a significant reduction in capital and operating costs (Singh and Gu, 2010). In view of this, ‘cheaper’ and ‘easier’ processes are to be pursued. Based on several available reports (Thurmond, 2009), the current estimated costs for the production of microalgal biodiesel lie between 1.84€ and 5.12€ per liter in ponds and within the range of 3.07–8.19€ in photobioreactors. Since microalga production systems are a rather complex combination of several sub-processes (i.e. cultivation, harvesting, drying and extraction), reducing the associated number of steps is thus crucial to lower the costs. The company Algae to Energy LLC has accordingly been experimenting with a process patented by Missing Link Technology, to extract oil from microalgae at the price of 0.02–0.06€ per liter (depending on the species used) which compares with other methods that typically range from 0.41€ to 2.46€ per liter. Another example is the unique harvesting technology from Algae Venture Systems, which costs, less than 0.06€ per liter, and is thus much more profitable than traditional centrifugation that can cost up to 0.20e or more per liter (Singh and Gu, 2010). Finally, co-production of ‘fractions’ that possess an even higher added value, compared to bulk or fine chemicals, is also important towards overall commercial feasibility. Note that even when microalgal species possess 50% of oil, an additional 50% of biomass remains – which contains valuable proteins for livestock, poultry and fish feed, which are currently valued at 620€ to 1937€ per ton (Singh and Gu, 2010). Therefore the recycling of the spent biomass and unused nutrients, after downstream processing, for reuse in the installation of microalgae culture will help to reduce the costs incurred in providing nitrogen fertilizer (Scott et al., 2010). Moreover, the microalgal residues resulting from the lipid extraction have higher effective sugar concentration (fermentable compounds). The Nannochloropsis sp. used in this study contains approximately 17% w/w dry weight of total sugars (Nobre et al., 2013). Bellou and Aggelis (2012) reported a value of 17.5–20.5% w/w dry weight of total sugars for Nannochloropsis salina. Therefore, lipid extracted microalgal residues can further be converted into other energy vectors, such as bioethanol (fermentation), bioH₂ (fermentation) or biogas (anaerobic digestion), with potential energetic and economical advantages.

This work deals with the evaluation of the energy consumed, CO₂ emitted and the economic impact of a Nannochloropsis sp. microalga biorefinery that corresponds to a laboratory-scale study developed recently by Nobre et al. (2013). The combined lipid and high-value pigment extraction and fractionation were conducted, and from the leftover biomass, bioH₂ was produced by dark fermentation. The overall economic evaluation was performed including biodiesel and carotenoid production costs and benefits, as well as bioH₂ production from the leftover biomass. Despite being a laboratory experiment the authors aim to identify the processes that have high energy, emissions and cost requirements in order to optimize these items in futures works, not only small scale experiments but also pilot and industrial level biorefineries. Accordingly, the authors took into consideration the allocations for the amount/volume of samples used and not the maximum equipment capacities.

2. Methodology

2.1. Life cycle inventory of the biorefinery

The life cycle inventory (LCI) is a very important tool to quantify inputs and outputs of a system. At this stage, all emissions are reported on a volume or mass basis (e.g. kg of CO₂). The LCI is a phase of the life cycle assessment (LCA) which is a tool that analyzes a product during its lifetime from its production, to its utilization and end-of-life, including its recycling process. It is an important tool to estimate the energy balance and environmental impact of a system. It can also be used to compare different energy systems including vehicle technologies and production systems (e.g. biofuel production) (Ferreira et al., 2011). The LCA was performed according to the principles of ISO 14040 (ISO, 1997). This analysis is crucial to verify if the processes and technologies used are environmentally friendly and sufficiently efficient. If not, it is possible to identify the bottlenecks and apply improvements on the energy and CO₂ emission chain.

2.1.1. Units and characterization of the main processes, inputs and outputs

The LCI covers the fundamental processes concerning the biorefinery: microalgae cultivation, harvesting, dewatering, milling, lipid and pigment extraction and leftover biomass to bioH₂ production. The overall LCI system boundary and all the processes consid-
ered in this work are shown in Fig. 1. There are five pathways and three biorefineries that were analyzed: (Path #1) oil extraction by soxhlet (oil SE); (Path #2) oil and pigment extraction and fractionation by supercritical fluid extraction (oil and pigment SFE); (Path #3) hydrogen production by dark fermentation from the leftover biomass after Soxhlet extraction (bioH₂ via SE); (Path #4) hydrogen production by dark fermentation from the leftover biomass after supercritical fluid extraction (bioH₂ via SFE); (Path #5) hydrogen production from the whole biomass by dark fermentation (bioH₂ via whole biomass). Where Paths #1 and #3 are the biorefinery 1, Paths #2 and #4 are the biorefinery 2 and Path #5 is the direct bioH₂ production. The analysis of pathways #1, #2 and #5 considers a system boundary from the *Nannochloropsis* sp. microalgal culture to the final product output (oil, pigments, or bioH₂, respectively). For pathways #3 and #4, the bioH₂ production from the leftover biomass from SE and SFE, respectively is regarded. In these pathways only the system boundary downstream the SE and SFE processes is considered. This can be accepted since the algae leftovers used for #3 and #4 pathways are no longer usable in any other process in the biorefinery. Therefore, if it is admitted that only pathways #1, #2, and #5 are meant to be final product pathways (for instance oil and bioH₂), pathways #3 and #4 can be used as an energetic co-product of pathways #1 and #2, respectively.

The electricity consumed in all production processes is assumed to be generated from the 2011 Portuguese electricity production mix. The mix considered is composed of 54% non-renewable energy and 46% renewable energy with 8% energy losses in distribution (EDP, 2012; REN, 2012). The resulting average energy consumption and CO₂ emissions per 1 MJ of electricity produced are 1.17 MJ (0.94–1.31) and 76.32 g (68.18–81.70) respectively (Eqs. (1) and (2)). The electricity conversion factor to estimate energy consumption in each process is determined by Eq. (1) (Ferreira et al., 2013).

\[
E_{\text{electricity}} = \left( 1 - f \right) \times \left( \frac{1}{\text{eff}_{\text{grid}}} \times \sum \left( \frac{W_{e,i}}{\text{eff}_{e,i}} \right) \right)^{\text{national}} + f \\
\times \left( \frac{1}{\text{eff}_{\text{grid}}} \times \sum \left( \frac{W_{e,i}}{\text{eff}_{e,i}} \right) \right)^{\text{imported}} - (1 \text{ MJ/Output})
\]  

(1)

where \( E_{\text{electricity}} \) is the electricity energy (MJ), \( f \) is the import factor, \( i \) is the source (renewable, thermal and nuclear) \( W_{e,i} \) is the percentage of each source and \( \text{eff}_{e,i} \) is efficiency of each source (MJ). The estimation of the CO₂ factor in each process is represented by Eq. (2) (Ferreira et al., 2013).

\[
\text{CO}_2_{\text{electricity}} = \left( 1 - f \right) \times \left[ \left( \frac{1}{\text{eff}_{\text{grid}}} \times \sum \left( W_{e,i} \times c_i \right) \right)^{\text{national}} + f \\
\times \left( \frac{1}{\text{eff}_{\text{grid}}} \times \sum \left( W_{e,i} \times c_i \right) \right)^{\text{imported}} \right]
\]

(2)

where \( c_i \) is the CO₂ emission factor (g/MJ) which is 361 g/MJ in Portugal’s case. This value resulted from the sum of the CO₂ emission factor of each Portuguese electrical power plant (REN, 2012).

Portugal’s average electricity generation efficiency is 1/(1 + 1.17) = 46%. The uncertainty of the Portuguese electricity generation mix considered weighted minimum and maximum deviation values for each energy source, based on the CONCAWE study (CONCAWE, 2008). Energy consumption and CO₂ emission estimates were based on SimaPro 7.1 software (Goedkoop et al., 2008) adapted to the Portuguese electricity generation mix, for nutrients and deionized water. This software was used only as a

![Fig. 1. Scheme of energy inputs of the biorefinery for oil, pigment and bioH₂ production. The numbers in brackets correspond to the production pathways to analyze, mentioned in the text.](image-url)
database source. The remaining energy inputs, from the equipment/lighting used, were derived from the device specifications and working hours (Ferreira et al., 2012). The functional unit of energy consumption and CO2 emissions is defined as MJ/MJBD produced and g/MJBD produced, respectively. In this study, we used all the energy requirements that resulted from the experimental data. Only operational processes were accounted, i.e., equipment production and storage were not included. Following the methodology used in all pathways, the Portuguese electricity, \( E_e \), and CO2 emission factors, which have a resulting associated uncertainty, are regarded as mentioned below (Ferreira et al., 2013).

\[
E_{\text{nutrients, water, gases}} = E_{\text{Simapro}} \times E_e
\]  

(3)

\[
E_{\text{equip, measurement}} = P_{\text{equipment}} \times \Delta t \times cf \times E_e
\]  

(4)

where \( P \) is power (W), \( \Delta t \) is working time (h) and \( cf \) is the capacity factor (mL) of the equipment if \( \neq 0 \). Eq. (4) was used to estimate the energy consumption of centrifugation and sterilization equipment. All the other equipment’s energies were measured through Eq. (5).

\[
E_{\text{equip, measurement}} = \bar{A} \times V \times \Delta t \times (1 \times 10^{-06}) \times cf \times E_e
\]  

(5)

where \( \bar{A} \) is alternate electric current (A), \( V \) is electric tension (V) and \( \Delta t \) is working time (s).

Rough energy requirements may be summarized by Eq. (6).

\[
\frac{MJ_{\text{consumed}}}{MJ_{\text{produced}}} = \sum_{i=1}^{M} \left( \frac{MJ_{\text{Ecop}}}{LHV} \right)
\]  

(6)

\( LHV \) stands for the biodiesel a low heating value of 37.3 MJ/kg and for bioH2 a low heating value of 120 MJ/kg (Heywood, 1988). Biodiesel and hydrogen density is assumed to be 0.88 kg/L and 0.084 kg/m³, respectively (EEERE, 2001) and CO2 density is assumed to be 1.848 kg/m³ (Linde, 2012). Pigments do not have energetic value and therefore it is not possible to determine energy requirement in MJ/MJ unit.

2.1.3. Biomass culture

The microalgal biomass was produced in polyethylene bags having a 10 L capacity (PBR). Ten g of dried biomass was obtained in one PBR, but in this study 1 g was used as a calculation basis for the energy and CO2 balances for each pathway analyzed in the LCI. Therefore, for each process the calculations are made taking into account the energy/CO2 emitted and the economic impact of the SFE process, it was considered that the extraction ended at extract 2 (Nobre et al., 2013), since further extraction (extracts 3 and 4) would only slightly increase the amount of oil and pigment recovered. The SFE apparatus contained a CO2 pump, an ethanol (ETOH) pump, and bath heating and cooling systems. The energy consumed in all steps was determined by direct current measurements (Eq. (5)). This extraction was carried out using 1 g of milled microalgae with n-hexane for 6 h in a P-select Soxhlet with six mantles. The energy required was obtained by multiplying the direct current measurement (Eq. (5)) of one mantle of the Soxhlet equipment, because only one mantle was used, by the voltage, time work (seconds) and by the energy mix factor. The energy consumed was 0.76 MJ. The resulting oil extracts were evaporated in a Büch Vacuum controller V-800 for 30 min. The energy consumption was 0.25 MJ.

2.1.3.1. Soxhlet extraction (SE). The Soxhlet method was used to extract the oil from the alga. This extraction was carried out using 1 g of milled microalga with n-hexane for 6 h in a P-select Soxhlet with six mantles. The energy required was obtained by multiplying the direct current measurement (Eq. (5)) of one mantle of the Soxhlet equipment, because only one mantle was used, by the voltage, time work (seconds) and by the energy mix factor. The energy consumed was 0.76 MJ. The resulting oil extracts were evaporated in a Büch Vacuum controller V-800 for 30 min. The energy consumption was 0.25 MJ.

2.1.3.2. Supercritical fluid extraction (SFE). Using the supercritical fluid extraction process, two products were obtained, oil and pigments (Path #2) in two different fractions. The SFE apparatus used in this study was described by Mendes et al. (2003) in detail, and modified to include a co-solvent addition system (Nobre et al., 2013). The extraction was carried out using 1 g of milled microalga, firstly with pure supercritical CO2 as a solvent (extract 1), followed by extraction with supercritical CO2 modified with ethanol (20% wt.) (Extract 2). For the purpose of the energy consumed, the CO2 emitted and the economic impact of the SFE process, it was considered that the extraction ended at extract 2 (Nobre et al., 2013), since further extraction (extracts 3 and 4) would only slightly increase the amount of oil and pigment recovered. The SFE apparatus contained a CO2 pump, an ethanol (ETOH) pump, and bath heating and cooling systems. The energy consumed in all steps was determined by direct current measurements (Eq. (5)). This extraction was carried out using 1 g of biomass. The LDC/Milton Roy CO2 pump was used for 2.58 h requiring 0.95 MJ of energy. The Gilson 308 ETOH pump was used for 50 min to extract the pigment, requiring 0.28 MJ of energy.

The bath heating was performed by a Julabo thermostat at a maximum temperature of 40 °C and the three-way valve was heated at the same temperature by a rheostat Selecta model with 300 W of electrical power. The energy consumed in the thermostat was 0.48 MJ and was determined by direct current measurements in the thermostat during the heating process. The rheostat was used for about 20 min at 40 °C only to maintain the temperature, operating at 15–25% of the total electrical power consuming 0.088 MJ of energy. In the agitation procedure, the total electrical power used was 0.02 MJ. That is, in total the bath heating consumed 0.59 MJ of energy, including the electric generation mix.
The SFE system was cooled with ice from a Scotsman AF800 refrigerator for 10.8 min. The energy requirement for the refrigerator was 0.26 MJ. In summary, only the SFE extraction consumed 2.09 MJ.

After extraction of oil and pigments from the microalgal biomass, the remaining biomass was used in a fermentation process as a substrate to produce bioH2 (Paths #3 and #4).

2.1.4. Biohydrogen (bioH2) production from Nannochloropsis sp.

2.1.4.1. Microorganism and culture conditions. The fermentative bioH2 production (Paths #3, #4 and #5) was performed by the bacteria Enterobacter aerogenes ATCC 13048 harvested from exponentially grown cultures. The original culture was kept at 4 °C in solid CASO Agar. The Nannochloropsis sp. biomass was used as a substrate, at a concentration of 10 g/L.

Bacteria synthetic growth media was a 20 g/L peptone solution (in 5 g/L NaCl), while the fermentation medium for the bioH2 production assays contained K2HPO4 (7.0 g/L), KH2PO4 (5.5 g/L), tryptone (5 g/L), yeast extract (5 g/L). (NH4)2SO4 (1.0 g/L), MgSO4·7H2O (0.25 g/L), CaCl2·2H2O (0.021 g/L), Na2MoO4·2H2O (0.12 g/L), C6H7NO6 (0.02 g/L), Na2SeO3 (0.172 mg/L), NiCl2 (0.02 g/L). The fermentative process occurred using the Nannochloropsis sp. biomass (substrate) in three different statuses – leftover biomass after oil extraction by SE (Path #3); leftover biomass after oil and pigment extraction by SFE (Path #4) and whole alga biomass (Path #5), for comparison.

2.1.4.2. BioH2 production from the leftover of Nannochloropsis sp. (Paths #3 and #4). After the oil extraction by Soxhlet, 0.593 g of microalgal biomass was recovered and used for bioH2 production. For this purpose, two 159 mL serum bottles (closed with butyl rubber stoppers and crimped with aluminum seals) were used to accommodate 53 mL of fermentation medium (volumetric gas to liquid ratio of 5:1) and the substrate (Nannochloropsis sp. leftover).

The energy required, estimated by Eq. (3), for the fermentation medium was 0.002 MJ. Before the fermentation process, the medium solution containing the algal biomass was sterilized for 15 min, which required 0.002 MJ of energy (which included volume allocation) (Eq. (4)). After sterilization the serum bottles were incubated anaerobically in an orbital shaker (220 rpm) for 6 h at 30 °C and this meant an energy requirement of 0.05 MJ (Path #3), where the energy was measured and determined by Eq. (5).

After the oil and pigment extraction by the supercritical fluids, 0.450 g of microalgal biomass was recovered and used for bioH2 production. For that, two serum bottles and the same procedures were performed as described above (Path #4).

2.1.4.3. BioH2 production from the whole biomass of Nannochloropsis sp. (Path #5). In order to compare the results obtained by the different bioH2 production pathways and verify their viability from an economic viewpoint, trials were conducted using the whole dry Nannochloropsis sp. biomass without the extraction processes, as a substrate. So, batch fermentation assays were performed in four 159 mL serum bottles, using 106 mL of fermentation medium, which required 0.0045 MJ of energy. The sterilization required 0.004 MJ of energy (Eq. (4)) and the incubation 0.10 MJ of energy (Eq. (5)) (as described above).

2.1.5. Analytical methods

Quantification of the total lipids was carried out gravimetrically concentrating the collected solution under vacuum and drying the extract under nitrogen. The identification and quantification of the extracted carotenoids was carried out by spectrophotometry (spectra were run between 380 and 700 nm), TLC and HPLC. The concentrations of H2 and CO2 after fermentation in the serum bottles were determined by gas chromatography.

All analytical methods used to quantify lipids, pigments and bioH2 were described with more detail in Nobre et al. (2013). The analytical methods were not considered in the life cycle study nor in the economic analysis.

2.1.6. Total energy requirements and respective CO2 emissions in the Nannochloropsis sp. biorefinery

The biorefinery energy requirement values and respective CO2 emissions values are summarized in Tables 1 and 2. The authors assumed that all obtained algal oil was used for biodiesel and that the conversion of algal oil into biodiesel resulted in a 1:1 mass conversion ratio (Rosenberg et al., 2011).

2.1.7. Co-product credits

Many processes have more than one energy product. In the present biorefinery, the oil (used to produce biodiesel) is the main product but it is possible to obtain co-products, such as pigments and bioH2. The co-product generates an energy and emission credit equal to the energy and emissions saved by not producing the material that the co-product is most likely to displace (Concawe, 2008). The energy credits were calculated by energy costs, because the pigments do not have an energy value, as referred to in Section 2.1.1.

Table 1

<table>
<thead>
<tr>
<th>Inputs</th>
<th>Value (MJ)</th>
<th>Energy (g)</th>
<th>CO2 (g)</th>
</tr>
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<tr>
<td>Nutrients (kg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2.08E−05</td>
<td>2.72E−04</td>
<td>2.90E−04</td>
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<tr>
<td>K</td>
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<tr>
<td>P</td>
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<td>4.46E−05</td>
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</tr>
<tr>
<td>EDTA</td>
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<tr>
<td>Na</td>
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<td>B</td>
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<td>Mn</td>
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<td>Co</td>
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<td>Deionized water (L)</td>
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<tr>
<td>Light intensity (W)</td>
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<tr>
<td>Centrifugation (W)</td>
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<td>0.12</td>
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<tr>
<td>Drying (W)</td>
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<td>0.0023</td>
<td>0.0032</td>
</tr>
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<td>Cutting mill</td>
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Table 2

<table>
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<tr>
<th>Inputs</th>
<th>Value (MJ)</th>
<th>Energy (g)</th>
<th>CO2 (g)</th>
</tr>
</thead>
</table>
Eq. (7) was used to calculate energy costs:

$$\sum \frac{\text{MJ}_i}{3.6} \times \frac{\varepsilon}{\text{€/gprod}} = \frac{\varepsilon}{\text{€/kWh}}$$

(7)

The MJ can be seen in Tables 1 and 2 and €/kWh can be seen in Table 3.

Credits were determined by Eq. (8).

$$\frac{\varepsilon}{\text{€oil}} - \frac{\varepsilon}{\text{€eco-product}} = \text{total cost in biorefinery}$$

(8)

Tables 3 and 4 show the energy prices considered in Eqs. (7) and (8).

2.2. Economic assumptions

Before analyzing the production costs of the microalgae oil of this study, it is important to take into account the following aspects:

- all the laboratory procedures were made aiming to fulfill the biological efficiency of the system; saving money or electricity were never the main goals;
- the calculations were made based on a laboratory production scale;
- only the costs of electricity and inputs were taken into account. Labor, equipment, land investments or indirect costs were not considered in this study.

All production costs were estimated based on the current market prices (see Table 4). For the electricity costs, the unit used was euro per kWh, while for CO2, H2, and nutrients, the unit was euro per kg. The nutrients required and their costs are listed in Table 4. The salt water used in the processes was considered free of charge. The price of CO2 used in the process was based on retail prices. No other source of CO2 was considered (i.e. a power station, flue gas). The Portugal Electricity fare in very high tension and long utilization mode without additional taxes was used (0.0535 €/kWh).

An oil fraction containing different pigments (e.g. 50% of pigments were a mixture of astaxanthin, canthaxanthin, lutein and beta-carotene) was extracted from Nannochloropsis sp. Since not all pigments have established market prices, an average hypothetical value of 450 €/kg was considered from market prices found of known pigments (Oilgae, 2013). With all input data, electricity used, volume of oil and H2 obtained and prices, it was possible to calculate the cost of the different processes and evaluate diverse possibilities of production. Finally, a retail value of the algal oil was established aiming to achieve a financial break even, where all the costs studied were covered if the oil could be sold at this price.

The possibility to use the bioH2 generated to provide all the electricity of the system was also assessed from an economical point of view. For this purpose, how much it would be necessary to spend on Paths #3, #4 or #5 was calculated in order to produce the bioH2 needed. Electricity costs for all processes were
produced to provide a solvent, where 0.33 goil \(\times\) pigment SFE outputs, respectively, resulting in biorefineries that showed the lower energy consumption values (Fig. 2). In fact, among the two pathways of oil extraction (SE, #1 and SFE, #2), the Soxhlet was more energetically efficient and emitted less CO2 than the supercritical fluid extraction which resulted in 210–291 MJ/MJBDproduced and 15,167–18,258 g/MJBDproduced of CO2 emissions. However, with SFE it was possible to remove high-value pigments (Fig. 1), as explained in subsection 2.1.7. Furthermore, SFE is considered a clean technology because the compounds can be obtained without contamination by toxic organic solvents.

Fig. 2 shows the energy consumption (total and partial) and CO2 emissions, for each of the biorefinery process. Note that the H2 production (Fig. 2) only takes into account the analysis of SE and SFE downstream processes and does not consider energy and emissions from the microalga production nor the SE and SFE processes. These H2 pathways can in some way be considered as a possible additional product from pathways #1 and #2 (oil SE and oil plus pigment SFE outputs, respectively), resulting in biorefineries 1 and 2, respectively.

Considering all stages of oil extraction (Paths #1 and #2), the microalga culture was the stage that consumed the most energy and emitted the most CO2. 105–161 MJ/MJBDproduced and 7,577–10,089 gCO2/MJBDproduced. The illumination with artificial light and drying steps are eliminated, it would correspond to a 2.17 MJ reduction in the energy input and 142 g in CO2 emissions (8195 MJ/MJBDproduced and 534,503 gCO2/MJBDproduced). Additionally there are no significant differences in the resulting H2 yields, regarding these improvements.

Path #1 seems to be the most energetically efficient one, showing the lowest energy consumption values, 177–245 MJ/MJBDproduced and CO2 emissions, 12,774–15,357 g/MJBDproduced, between all biorefineries. It should be noted that due to the equipment dimensioning, Soxhlet extraction of the whole biomass available was completed in only one batch of 6 h, which clearly contributes to this favorable result as compared to the processes used in other paths (e.g. SFE, #2). In fact, among the two pathways of oil extraction (SE, #1 and SFE, #2), the Soxhlet was more energetically efficient and emitted less CO2 than the supercritical fluid extraction which resulted in 210–291 MJ/MJBDproduced and 15,167–18,258 g/MJBDproduced of CO2 emissions. However, with SFE it was possible to recover high-value pigments (Fig. 1), as explained in subsection 2.1.7. Furthermore, SFE is considered a clean technology because the compounds can be obtained without contamination by toxic organic solvents.

Table 4

<table>
<thead>
<tr>
<th>Nutrient prices.</th>
<th>Price per g (€)</th>
<th>Price per g (€)</th>
<th>Price per g (€)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KNO3</td>
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<td>ZnCl2</td>
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</tr>
<tr>
<td>K2HPO4</td>
<td>0.0896</td>
<td>CoCl2</td>
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<td>KH2PO4</td>
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<td>MgSO4</td>
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</tr>
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<td>CaCl2</td>
<td>0.0360</td>
</tr>
<tr>
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<td>0.1148</td>
<td>(NH4)2SO4 (N)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>NiCl2 (Cl)</td>
<td>0.6680</td>
</tr>
</tbody>
</table>


Table 5

| Total energy consumption and CO2 emissions for each pathway and biorefineries and respective uncertainty. (Paths #3 and #4 are co-products of Paths #1 and #2, respectively (see Fig. 1).) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| E (MJ/MJBD)                     | Min             | Max             | CO2 (g/MJBD)    | Min             | Max             |
| Path #1                         | 220             | 177             | 245             | 14,320          | 12,774          | 15,357          |
| Path #2                         | 262             | 210             | 291             | 17,123          | 15,167          | 18,258          |
| Path #3                         | 147             | 119             | 164             | 9665           | 8645            | 10,369          |
| Path #4                         | 168             | 136             | 187             | 11,020          | 9858            | 11,820          |
| Path #5                         | 9058            | 7285            | 10,123          | 591,112         | 527,022         | 634,402         |
| Biorefinery 1 (Path #1 + Path #3) | 214             | 172             | 239             | 13,982          | 12,471          | 14,994          |
| Biorefinery 2 (Path #2 + Path #4) | 258             | 206             | 286             | 16,800          | 14,881          | 17,913          |
emits more CO2 because it requires the highest amount of nutrients and water.

In the case of bioH2 production from the whole biomass, the microalga culture was the stage that consumed the most energy due to the same reason referred to earlier, following the harvest and fermentation medium stages. The microalga drying stage showed the lower values of energy consumption.

In order to be possible to compare all the products and co-products, such as oil and pigments, the unit used was euro per mass of product/co-product. Energy and CO2 credits were also considered in this study.

3.3. Energy and CO2 emissions balance calculation

The biorefinery energy consumption and CO2 emission balance were based and the results are presented in Table 5. Since pathways 3 and 4 (Fig. 1, Table 5) are co-products from pathways #1 and #2 respectively, it is possible to perform both pathways #1 and #3 (biorefinery 1), or both #2 and #4 (biorefinery 2), producing oil (and pigments from SFE) and bioH2. Therefore, if this option is chosen, new energy balances must be performed.

In the case of biorefinery 1, the energy requirement was 2.67–3.70 MJ. The product energy produced was 0.015 MJ of biodiesel and 0.0004 MJ of bioH2. These results in an energy consumption of 172–239 MJ/MJproduced and 12,471–14,994 gCO2/MJproduced. In the case of biorefinery 2, a total energy consumption of 206–286 MJ/MJproduced, and 14,881–17,913 gCO2/MJproduced of emissions are achieved. This coupling of pathways (products and co-products) bring a small energy efficiency improvement of 2.7% to biorefinery 1 and 1.9% to biorefinery 2 and it may be further advantageous in terms of product yield or profit. For example, the bioH2 produced could be used in an industrial hydrogenation process. In case of Path #5, there were no byproducts because it considered that only bioH2 was produced from the whole biomass.

The obtained values are higher than those for conventional industrial biodiesel and bioH2 production which are referred by other literature (Frank et al., 2011 and CONCAWE, 2008). In order to achieve the viability of the whole process, energy requirements should go down to about 2 orders of magnitude. With this study, it was possible to identify the most critical steps of microalga and fuel production in terms of energy consumption and which therefore require mandatory optimisation. However, there are no similar biorefinery studies that have been made to be able to make a fair comparison.

3.4. Economic feasibility analysis

The costs of microalga culture and downstream processes are mostly due to lighting (82%), water (13%) and nutrient consumption (4%). Together harvesting, drying and milling represent roughly 1% of the related costs. The higher lighting costs are associated to the relatively long (40 days) period of alga growth. It may
be advantageous to grow the alga for a shorter period of time, even if the oil and pigment yields are lower. The cellular accumulation of this compound should be monitored (e.g. by flow cytometry (Silva et al., 2009) along the algal growth in order to determine the most favorable harvesting date, regarding a cost/benefit evaluation in terms of product yield, energy consumption, CO₂ emissions and economic feasibility. For bioH₂ production Path #5, it is necessary to monitor fermentable sugar accumulation rather than oils. Culturing the microalgae outdoor under natural light is also a more economical alternative, although lower growth rates and higher contamination risks are possible drawbacks to be considered.

For producing the oil Soxhlet extraction (Path #1), and the oil and pigment supercritical extraction (Path #2), inputs and electricity use were calculated and added up for a final cost, taking into account the amount of oil and pigments produced, as shown in Section 3.1.

All costs for the culture and downstream processes were added to the expenses of Path #1 and therefore a cost of 660.56 €/kg of algal oil was found. The same was done with Path #2 with a result of 365.42 €/kg of algal oil. Path #2 proved to be more economically feasible than Path #1 and it produced pigments as co-products. Although Path #2 was energetically more intensive (see Section 3.2), the inputs, such as hexane, on the Soxhlet process were substantially more expensive and affected the costs of Path #1. The revenue from the pigments which could be sold is minimal due to the low volume produced. Considering a mean pigment market price of 450 €/kg, the current income of selling pigments resulting from Path 2 per 1 g of initial algal biomass would be 0.00024 €.

Therefore, cost analysis showed that Path #1 is roughly two times more expensive than Path #2 to produce algal oil.

Comparing the different dark fermentation pathways studied (Paths #3, #4 and #5), the fermentation via direct biomass (Path #5) is by far the most expensive. The processes via supercritical extraction (Path #4) and Soxhlet extraction (Path #3) presented similar costs.

The main difference between Paths #3 and #4, as stated before, is the higher production of H₂ of Path #3. Fig. 3 that could represent an increased importance when selling the bioH₂ produced to the market. However, even though Path #3 would be the most recommended for producing H₂, when comparing the overall process costs, Paths #2 and #4 (biorefinery 2) are the most economically feasible.

When assessing the possibility of selling the H₂ produced, all studied Pathways produce modest amounts of H₂ that are unlikely to be sold with the actual production costs and do not represent a feasible source of financial return. Considering a market price of 80 €/kg of H₂, the current income resulting from Paths #3, #4 and #5 per 1 g of initial algal biomass would be 0.00025, 0.00022 and 0.00018 €, respectively. However it must be considered that this is an innovative process with emerging potential, and that the yields attained in this study are significantly higher than the ones recently published by other studies (e.g. Lakaniemi et al., 2011; Ferreira et al., 2012, 2013).

As stated before, all cost calculations of this study were based on a laboratory scale of production. Therefore, the high price per kilogram of algal oil which was found in this study to be able to break even is perfectly understandable and there is plenty of room to improve the feasibility and efficiency of all processes. The use of Soxhlet extraction for oil production did not result in an economically feasible option.

In order to decrease the energy consumption (and associated CO₂ emissions) and costs, the experimental procedure must be optimised aiming to process a larger amount of biomass, to be able to achieve production at an industrial scale. Special attention should be paid on the use of sunlight for culturing the microalgae, as also using equipment with higher capacity in order to process all the biomass in one step, and the optimization of the SFE methodology in terms of heating and cooling systems. Additionally the use of a marginal energy mix of 100% renewables in the whole process should be taken into account.

4. Conclusions

Economically the most favorable _Nannochloropsis_ sp. biorefinery was oil, pigment and bioH₂ production via supercritical fluid extraction (SFE). From net energy balance and CO₂ emissions analysis, biorefinery 1 (biodiesel SE + bioH₂) presented better results. Biorefinery 2 (biodiesel SFE + bioH₂) showed results in the same range of those in biorefinery 1. However, in SFE it’s possible to produce high-value pigments and additionally being a clean technology not
using toxic organic solvents. Biorefinery 2 is the best energy/CO2 economy compromise. Pilot studies should complement this work in order to achieve an economically beneficial and feasible process at an industrial scale.

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References


Glossary

BioH2: biohydrogen
BD: biodiesel
CASS: CAsein-peptone STOmeal-peptone
ETHA: ethylenediaminetetraacetic acid
E10H: ethanol
GHG: greenhouse gas
GPM: general purpose media
HPLC: high performance liquid chromatography
LCA: life cycle assessment
LCI: life cycle inventory
LHV: low heating value
Mj_response: energy expended in a process excluding fuel final energy
PBR: PhotoBioReactor
TCD: thermal conductivity detector
TLC: thin layer chromatography