

Accepted Manuscript

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

Ana F. Ferreira, Lauro A. Ribeiro, Ana P. Batista, Paula A.S.S. Marques, Beatriz P. Nobre, António M.F. Palavra, Patrícia Pereira da Silva, Luísa Gouveia, Carla Silva

PII: S0960-8524(13)00561-0
DOI: <http://dx.doi.org/10.1016/j.biortech.2013.03.168>
Reference: BITE 11628

To appear in: *Bioresource Technology*

Received Date: 1 March 2013
Revised Date: 20 March 2013
Accepted Date: 24 March 2013

Please cite this article as: Ferreira, A.F., Ribeiro, L.A., Batista, A.P., Marques, P.A.S., Nobre, B.P., Palavra, A.M.F., Silva, c.P.d., Gouveia, L., Silva, C., A Biorefinery from *Nannochloropsis* sp. microalga – Energy and CO₂ emission and economic analyses, *Bioresource Technology* (2013), doi: <http://dx.doi.org/10.1016/j.biortech.2013.03.168>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



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

Ana F. Ferreira^{a,*}, Lauro A. Ribeiro^{b,c}, Ana P. Batista^d, Paula A. S. S. Marques^d,
Beatriz P. Nobre^{d,e}, António M. F. Palavra^e, Patrícia Pereira da Silva^b, Luísa
Gouveia^d, Carla Silva^a

^aIDMEC - Instituto Superior Técnico, Universidade Técnica de Lisboa, Av. Rovisco Pais, 1 - 1049-001 Lisbon – Portugal

^bINESCC, R. Antero de Quental, 199 - 3000-033 Coimbra – Portugal

^cSchool of Sciences and Technology, University of Coimbra, Rua Sílvio Lima – Pólo II 3030-790 Coimbra – Portugal

^dLNEG - Energy and Geology National Laboratory, Bioenergy Unit, Estrada do Paço do Lumiar 22 - 1649-038 – Lisbon - Portugal

^eIST – Centro Química Estrutural, DEQB, Av. Rovisco Pais, 1 - 1049-001 Lisbon – Portugal

* Corresponding author: Tel.: +351218419554; E-mail: filipa.ferreira@ist.utl.pt

Abstract

Are microalgae a potential energy source for biofuel production? This paper presents the laboratory results from a *Nannochloropsis* sp. microalga biorefinery for the production of oil, high-value pigments, and biohydrogen (bioH₂). The energy consumption and CO₂ emissions involved in the whole process (microalgae 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, 176-244 MJ/MJ_{prod}, and CO₂ emissions, 13-15 kgCO₂/MJ_{prod}. 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€/kg_{oil} produced and simultaneously extracting high-value pigments. The biohydrogen as co-product may be advantageous in terms of product yield or profit.

Keywords: Nannochloropsis sp., microalga biorefinery, CO₂, energy, economical evaluation, life cycle.

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 microalgae potential for biofuel production is huge. However, the current implementation of microalga-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 microalga 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/MJ_{BD} and 0.2-0.9 kgCO₂/MJ_{BD}. 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 microalgae 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 microalgae 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, 2011) includes new algae pathways but only to produce bio-oil, including the algae growth, dewatering and oil extraction stages.

The industrial viability of microalgae-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 consubstantiated 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 exploiting a process patented by Missing Link Technology, to extract oil from microalgae at the price of 0.02€ to 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 0.20€ 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 from 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., in press). 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, in press). 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., biofuels 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 considered in this work are shown in Figure 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 path #1 and path #3 are the biorefinery 1, path #2 and path #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 (equation 1 and 2). The electricity conversion factor to estimate energy consumption in each process is determined by equation 1 (Ferreira et al., 2013).

$$E_e = \left[(1 - f) \times \left(\frac{1}{eff_{e,avg}} \times \sum \left(\frac{W_{e,i}}{eff_{e,i}} \right) \right)^{renewable} + f \times \left(\frac{1}{eff_{e,avg}} \times \sum \left(\frac{W_{e,i}}{eff_{e,i}} \right) \right)^{imported} \right] - (LMF)^{output} \quad (Eq. 1)$$

where E_e 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 $eff_{e,i}$ is efficiency of each

source (MJ). The estimation of the CO₂ factor in each process is represented by equation 2 (Ferreira et al., 2012).

$$CO_{2e} = \left[(1 - f) \times \left(\frac{1}{eff_{grid}} \times \sum (W_{e,i} \times c_i) \right)^{National} + f \times \left(\frac{1}{eff_{grid}} \times \sum (W_{e,i} \times c_i) \right)^{Imported} \right] \quad (Eq. 2)$$

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 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 are defined as MJ/MJ_{BDproduced} and g/MJ_{BDproduced}, 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).

$$E_{nutrients,water,gases} (MJ) = E_{SimaPro} \times E_e \quad (Eq. 3)$$

$$E_{equip}^{estimated} (MJ) = P_{equipment} \times \Delta t \times cf \times E_e \quad (Eq. 4)$$

where P is power (W), Δt is working time (h) and cf is the capacity factor (mL) of the equipment if $\neq 0$. Equation 4 was used to estimate the energy consumption of centrifugation and sterilization equipment. All the other equipments' energies were measured through equation 5.

$$E_{\text{equip}}^{\text{MEASUREMENT}} (MJ) = \tilde{A} \times V \times \Delta t \times (1 \times 10^{-06}) \times cf \times E_e \quad (Eq. 5)$$

where \tilde{A} is alternate electric current (A), V is electric tension (V) and Δt is working time (s).

Rough energy requirements may be summarized by equation 6.

$$\frac{MJ_{\text{expended}}}{MJ_{\text{prod}}} = \frac{\sum_i \left[\left(\frac{MJ}{kg_{\text{prod}}} \right)_i \right]}{LHV} \quad (Eq.6)$$

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 (EERE, 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.

2.1.2 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 1g 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 required for handling this amount of biomass, which in some cases (bioH₂ production) means repeating operations due to the limited capacity of the laboratory equipment used. The GPM microalga culture medium composed of KNO₃ (0.200 g/L), K₂HPO₄ (0.038 g/L), H₃BO₃ (0.034 g/L), Na₂EDTA (0.030 g/L), MnCl₂.4H₂O (4.30 mg/L), FeCl₃.6H₂O (1.45

mg/L), ZnCl₂ (0.30 mg/L) and CoCl₂·6H₂O (0.13 mg/L) in 75% filtered seawater: 25% de-ionized water, was used for *Nannochloropsis* sp. growth.

The microalga was cultured under continuous light provided by fluorescence lamps (Philips TL-D 36 W/54-765) with a luminance average of 0.95 lux, for 40 days. The power requirement to produce 1 g of biomass was 2.17 MJ. This value was obtained by equation 4 multiplying the light energy allocated by the number of hours of illumination and energy mix factor according to equation 2. The light intensity was allocated, considering only the intensity of light focused on the bioreactor, i.e. only 15% of the total intensity of the lamp was regarded.

The PBR was agitated by bubbling air with a flow of 0.2 vvm (L/L.min). The air compressor had a capacity of 230 L/min while consuming 2.2 kW, but for the microalga culture the equipment was not at the maximum power and flow. The energy requirement in this case was 0.0019 W, which corresponds to 0.001 MJ.

After growth, the microalgal biomass was harvested by centrifugation of 0.75 L of culture in an Avanti J-25 series centrifuge for 10 min at 10,000 rpm (2000 W of motor power). The final estimated energy (equation 4) used in centrifugation was 0.11 MJ.

Subsequently, the biomass was dried in an oven at 70 °C for 12 h, calculated by direct measurement (equation 5), of 0.0028 MJ of energy and resulting in 1g of dried algal biomass.

2.1.3 Oil and pigment extraction

Milling of the dry alga was needed for the oil and pigment extraction (Path #1 and #2, respectively). For this purpose, two types of mill equipment were used: a commercial cutting mill and a ball mill Retsch® model MM400. To guarantee an efficient grinding of the algae, a maximum of a 1 g sample loading was used in the cutting and ball mills. In the

first sample, milling was needed for 1 min, requiring in total 0.01 MJ of energy. In the second one, the process lasted 3.5 min, using 8 stainless steel balls (10 mm \varnothing) and a speed of 25 s^{-1} , requiring a total of 0.02 MJ of energy. The alga oil was extracted by two methods: Soxhlet extraction (Path #1) and supercritical fluid extraction (Path #2).

Soxhlet extraction (SE)

The Soxhlet method was used to extract the oil from the algae. This extraction was carried out using 1 g of milled microalgae with n-hexane for 6 h in a P-select Soxhlet with 6 mantles. The energy required was obtained by multiplying the direct current measurement (equation 5) of 1 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.

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. (1995) in detail, and modified to include a co-solvent addition system (Nobre et al., 2013, in press). The extraction was carried out using 1 g of milled microalgae, firstly with pure supercritical CO_2 as a solvent (extract 1), followed by extraction with supercritical CO_2 modified with ethanol (20% wt.) (Extract 2). For the purpose of the energy consumed, the CO_2 emitted and the economic impact of the SFE process, it was considered that the extraction ended at extract 2 (Nobre et al., 2013, in press), since further extraction (extracts 3 and 4) would only slightly increase the amount of oil and pigment recovered. The SFE apparatus contained a CO_2 pump, an ethanol (EtOH)

pump, and bath heating and cooling systems. The energy consumed in all steps was determined by direct current measurements (equation 5). This extraction was carried out one time using a 5 cm² extraction vessel filled with 1g 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 0.008 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 microalga biomass, the remaining biomass was used in a fermentation process as a substrate to produce bioH₂ (Path #3 and #4).

*2.1.4 Biohydrogen (bioH₂) production from *Nannochloropsis* sp.*

Microorganism and culture conditions

The fermentative bioH₂ production (Path #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), C₆H₅NO₂ (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.

BioH₂ production from the leftover Nannochloropsis sp. (Path # 3 and Path #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 aluminium seals) were used to accommodate 53 mL of fermentation medium (volumetric gas to liquid ratio of 6:1) and the substrate (*Nannochloropsis* sp. leftover). The energy required, estimated by equation 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) (equation 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 equation 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, 2 serum bottles and some procedures were performed as described above (Path # 4).

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 (equation 4) and the incubation 0.10 MJ of energy (equation 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, in press). 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 emission 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.

Equation 7 was used to calculate energy costs:

$$\sum \frac{MJ_i / \text{kg}_i}{\text{Spread}} \times \frac{\text{€}}{\text{kWh}} = \frac{\text{€}}{\text{Spread}} \quad (\text{Eq.7})$$

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

Credits were determined by equation 8.

$$\frac{\text{€}}{\text{MJ}_i} - \frac{\text{€}}{\text{MJ}_{\text{main-product}}} = \text{total cost in biorefinery} \quad (\text{Eq.8})$$

Tables 3 and 4 show the energy prices considered in equations 7 and 8.

2.2 Economic assumptions

Before analyzing the production costs of the microalgal 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 website).

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 hydrogen 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 H₂ needed. Electricity costs for all processes were subtracted from the final cost, as the H₂ produced to provide this entire electrical power system.

3. Results and Discussion

3.1 Oil, pigment and hydrogen (bioH₂) yield

The amount of the total oil obtained from Soxhlet extraction of *Nannochloropsis* sp. using n-hexane was 40.7% of the alga mass, corresponding to 0.41 g_{oil}/g_{dry alga} (Path #1).

In the SFE (Path #2) a total of 0.42 $\text{g}_{\text{oil}}/\text{g}_{\text{dry alga}}$ was produced. This value was obtained from two fractions: in the 1st fraction, pure CO_2 was used as a solvent, where 0.33 $\text{g}_{\text{oil}}/\text{g}_{\text{dry alga}}$ was reached, corresponding to a recovery of 78% of lipids. In the 2nd fraction (obtained using CO_2+EtOH 20% wt.) it was possible to attain 0.09 $\text{g}_{\text{oil}}/\text{g}_{\text{dry alga}}$, as well as 0.0049 $\text{g}_{\text{pigments}}/\text{g}_{\text{dry alga}}$, (Nobre et al., 2013, in press).

After the extraction of the oils and pigments, under different conditions, the remaining biomass was used as a substrate in a dark fermentation process to produce bioH_2 , by the *E. aerogenes* bacteria (Paths #3 and #4). The results obtained showed that the microalgal residues can be efficiently used as a substrate to produce bioH_2 , leading to specific H_2 yields of 36.6 and 32.1 $\text{mL}_{\text{H}_2}/\text{g}_{\text{alga}}$ for Paths #3 and 4, respectively. However, when using the whole *Nannochloropsis* sp. dried biomass, a lower bioH_2 yield of 26.4 $\text{mL}_{\text{H}_2}/\text{g}_{\text{alga}}$ was attained. From Nobre et al. (2013, in press), it was observed that using 2.5 g/L of alga concentration leads to higher specific hydrogen yields when compared to using 10 g/L alga (48-60 vs. 26-37 $\text{mL H}_2/\text{g alga}$, respectively). However, the volumetric H_2 production is much higher in the latter case.

Concerning oil Soxhlet extraction (SE, Path #3), 0.593 g of microalgal biomass was recovered and used in the fermentative process, producing 21.7 mL of H_2 , while 0.450 g of microalgal biomass after oil and pigment supercritical fluid extraction (SFE, Path #4) was recovered and yielded 14.4 mL of H_2 . Finally, it was observed when using 1 g of the whole dried biomass (Path #5) as a substrate that this allowed the production of 26.4 mL of H_2 .

3.2 Life cycle inventory

The total energy consumption and CO_2 emissions for each process of the biorefineries and respective uncertainties are summarized in Table 5. As can be seen in Table 5, the process that shows higher energy consumption and CO_2 emissions is Path #5. However, it should

be noted that for this pathway, if the artificial illumination 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/MJ_{H₂produced} and 534503 gCO₂/MJ_{H₂produced}). 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, 176-244 MJ/MJ_{BDproduced}, and CO₂ emissions, 12,737-1,569 grams/MJ_{BDproduced}, 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 209-290 MJ/MJ_{BDproduced}, and 15,134-18,178 grams/MJ_{BDproduced} of CO₂ emissions. However, with SFE it was possible to recover high-value pigments (Figure 1), as explained in subsection *Co-products credits* (3.3). Furthermore, SFE is considered a clean technology because the compounds can be obtained without contamination by toxic organic solvents.

Figure 2 shows the energy consumption (total and partial) and CO₂ emissions, for each of the biorefinery process. Note that the H₂ production (Figure 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 biorefinery 1 and 2, respectively.

Considering all stages of oil extraction (Paths #1 and #2), the microalgae culture was the stage that consumed the most energy and emitted the most CO₂ with 104-160

$\text{MJ/MJ}_{\text{BDproduced}}$ and $7544\text{-}10001 \text{ gCO}_2\text{CO}_2/\text{MJ}_{\text{BDproduced}}$. The illumination with artificial light is the process which most contributes to the high value of energy consumption in microalga culture, with 99% of the total energy and emissions in that considered stage. Drying was the process that showed the lower energy consumption values (Figure 2). The use of alternative lighting such as LEDs in PBRs (indoors) and the use of natural light in PBRs (outdoor) are to be studied in a future work covering the reduction of this energy consumption fraction.

Comparing the stages of bioH_2 production from the leftover biomass, the fermentation presented the highest values for both energy consumption and emissions. The fermentation medium is the second stage that consumes more energy and consequently emits more CO_2 because it requires the highest amount of nutrients and water.

In the case of bioH_2 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 CO_2 credits were also considered in this study.

3.3 Energy and CO_2 emissions balance calculation

The biorefinery energy consumption and CO_2 emission balance were based and the results are presented in Table 5. Since pathways 3 and 4 (Figure 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 bioH_2 . 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/MJ_{produced} and 12,471-14,994 gCO₂/MJ_{produced}. In the case of biorefinery 2, a total energy consumption of 206-286 MJ/MJ_{produced}, and 14,881-17,913 gCO₂/MJ_{produced} 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 the 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 cited in 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 algae 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 outdoors 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 2 times more expensive than Path #2 to produce algal oil. Comparing the different dark fermentation pathways studied (Path #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 Path #3 and #4, as stated before, is the higher production of H₂ of Path #3, a figure 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; Ferreira et al., 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 breakeven 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, pigments and H₂ 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.

Acknowledgements

The authors would like to acknowledge the Fundação para a Ciência e Tecnologia for the financial support of Ana Ferreira PhD grant (SFRH/BD/60373/2009), Beatriz P. Nobre Post-Doc grant (SFRH/BPD/42004/2007) and for the following national projects: “Power demand estimation and power system impacts resulting of fleet penetration of electric/plug-in vehicles” (MIT-Pt/SES-GI/0008/2008) and “Microalgae as a sustainable raw material for biofuels production (biodiesel, bioethanol, bio-H₂ and biogas)” (PTDC/AAC-AMB/100354/2008); the Brazilian National Council for the Improvement of Higher Education (CAPES) for the financial support of Lauro Ribeiro PhD grant. This work has been partially supported by the FCT under the project grant PEst-C/EEI/UI0308/2011 and has been framed under the Energy for Sustainability Initiative of the University of Coimbra and supported by the R&D Project EMSURE Energy and Mobility for Sustainable Regions (CENTRO 07 0224 FEDER 002004). The authors also thank Stephanie Seddon-Brown for English proof reading of this manuscript.

References

1. Amaro, H.M., Guedes, A.C., Malcata, F.X., 2011. Advances and perspectives in using microalgae to produce biodiesel. *Applied Energy* 88, 3402–3410.
2. Bellou, S., Aggelis, G., 2012. Biochemical activities in *Chlorella* sp. and *Nannochloropsis salina* during lipid and sugar synthesis in a lab-scale open pond simulating reactor. *Journal of Biotechnology* 164, 318-329.
3. Campbell, P., Beer, T., Batten, D., 2010. Life cycle assessment of biodiesel production from microalgae in ponds. *Bioresource Technology* 102, 50-56.
4. Concawe, 2008. Well-to-wheels analysis of future automotive fuels and powertrains in the European context. Well-to-wheels Report. Web: <http://ies.jrc.ec.europa.eu> (last access 18.06.2012).
5. EDP, Energia de Portugal S.A., 2012. Resultados 2010. Web: www.edp.pt (last access 18.06.2012).
6. EERE, Energy Efficiency & Renewable Energy, 2001. Hydrogen Fuel Cell engines and related Technologies Course Manual, 2001. U.S department of Energy. Web: <http://www1.eere.energy.gov/hydrogenandfuelcells>.
7. ERSE, Entidade Reguladora dos Serviços Energéticos, 2012. Web: http://www.erse.pt/pt/gasnatural/tarifaseprecos/0711_0612/Documents/Despacho%20ERSE%2008-2011%20_Tarifas%20GN%202011-2012.pdf (last access 6/07/2012).
8. Ferreira, A.F., Marques, A.C., Batista, A.P., Marques, P.A.S.S., Gouveia, L., Silva, C.M., 2012. Biological hydrogen production by *Anabaena* sp. – yield, energy and CO₂ analysis including fermentative biomass recovery. *International Journal of Hydrogen Energy* 37, 179-190.
9. Ferreira, A.F., Ortigueira J., Alves, L., Gouveia, L., Moura, P., Silva, C.M., 2013. Energy requirement and CO₂ emissions of BioH₂ production from microalgal biomass. *Biomass and Bioenergy* 49, 249-259.

10. Ferreira, A.F., Ribau, J.P., Silva, C.M., 2011. Energy consumption and CO₂ emissions of potato peel and sugarcane biohydrogen production pathways, applied to Portuguese road transportation. *International Journal of Hydrogen Energy* 36, 13547-58.
11. Frank, E.D., Han, J., Palou-Rivera, I., Elgowainy, A., Wang, M.Q., 2011. Life Cycle Analysis of Algal Lipid Fuels with the GREET Model. Energy System Division, Argonne National Laboratory.
12. Goedkoop, M., De Schryver, A., Oele, M., 2008. Introduction to LCA with SimaPro 7. Pre-Consultants.
13. Gouveia, L., 2011. Microalgae as a feedstock for biofuels. *Springer Briefs in Microbiology*. Springer. Heidelberg, Germany.
14. Heywood, J.B., 1988. *Internal Combustion Engine Fundamentals*. McGraw-Hill.
15. ISO 14040, 1997. Environmental management—life cycle assessment—principles and framework. International Organisation for Standardisation, Geneva.
16. Itoiz, E.S., Fuentes-Grünwald, C., Gasol, C.M., Garcés, E., Alacid, E., Rossi, S., Rieradevall, J., 2012. Energy balance and environmental impact analysis of marine microalgal biomass production for biodiesel generation in a photobioreactor pilot plant. *Biomass and Bioenergy* 39, 324-335.
17. Khoo, H.H., Sharratt, P.N., Das, P., Balasubramanian, R.K., Narahariseti, P.K., Shaik, S., 2011. Life cycle energy and CO₂ analysis of microalgae-to-biodiesel: Preliminary results and comparisons. *Bioresource Technology* 102, 5800–5807.
18. Lakaniemi, A.M., Hulatt, C.J., Thomas, D.N., Tuovinen, O.H., Puhakka, J.A., 2011. Biogenic hydrogen and methane production from *Chlorella vulgaris* and *Dunaliella tertiolecta*. *Biotechnology for Biofuels* 4, 34.
19. Lam, M.L., Lee, K.T., Mohamed, A.R., 2012. Current status and challenges on microalgae-based carbon capture. *International Journal of Greenhouse Gas Control* 10, 456-469.
20. Lardon, L., Helias, A., Sialve, B., Steyer, J.P., Bernard, O., 2009. Life-cycle assessment of biodiesel production from microalgae. *Environmental Science and Technology* 43, 6475-6481.

21. Linde Sogás, Lda. Web: <http://www.linde-gas.com/en/index.html> (last access 18.06.2012)
22. Marques, A., Miranda, J., Batista, A.P., Gouveia, L., 2011. Microalgae Biotechnological Applications: Nutrition, Health and Environment. In *Microalgae: Biotechnology, Microbiology and Energy*. Ed. M. N. Johansen. Nova Science Publishers Inc. pp. 1-60.
23. Mendes, R.L., Nobre, B.P., Cardoso, M.T., Pereira, A.P., Palavra, A.F., 2003. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. *Inorganica Chimica Acta* 356, 328-334.
24. Nobre, B.P., Villalobos, F., Barragán, B.E., Oliveira, A.C., Batista, A.P., Marques, P.A.S.S., Mendes, R.L., Sovová, H., Palavra, A.F., Gouveia, L., 2012. Biorefinery from *Nannochloropsis* sp. microalga - Extraction of microalgal oils and pigments and biohydrogen production from biomass leftover. *Bioresource Technology*, *In press* (<http://dx.doi.org/10.1016/j.biortech.2012.11.084>)
25. Oilgae. Web: <http://www.oilgae.com> (last access 10/01/2013)
26. REN. Rede Eléctrica Nacional. Sistema informação mensal, sistema electroprodutor, <http://www.ren.pt>; 2012. Web: (last access 18.06.2012).
27. Rosenberg, J.N., Mathias, A., Korth, K., Betenbaugh, M.J., Oyler, G.A., 2011. Microalgal biomass production and carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: A technical appraisal and economic feasibility evaluation. *Biomass and Bioenergy* 35, 3865-3876.
28. Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., 2010. Biodiesel from algae: challenges and prospects. *Current Opinion in Biotechnology* 21, 277-286.
29. Sigma. Web: <http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do>.
30. Silva, T.L., Reis, A., Medeiros, R., Oliveira, A.C., Gouveia, L., 2009. Oil production towards biofuel from autotrophic microalgae semicontinuous cultivations monitored by flow cytometry. *Applied Biochemistry and Biotechnology* 159: 568-578.
31. Singh, J., Gu, S., 2010. Commercialization potential of microalgae for biofuels production. *Renewable and Sustainable Energy Reviews* 14, 2596-2610.

32. Soratana, K., Landis, A.E., 2011. Evaluating industrial symbiosis and algae cultivation from a life cycle perspective. *Bioresource Technology* 102, 6892-6901.
33. Stephenson, A.L., Kazamia, E., Dennis, J.S., Howe, C.J., Scott, S.A., Smith, A.G., 2010. Life cycle assessment of potential algal biodiesel production in the United Kingdom: a comparison of raceways and air-lift tubular bioreactors. *Energy Fuels* 24, 4062–4077.
34. Thurmond, W., 2009. *Algae 2020: advanced biofuel markets and commercialization outlook*. 1st ed.; p. 460.
35. Xu, L., Brilman, D.W.F., Withag, J.A.M., Brem, G., Kersten, S., 2011. Assessment of a dry and a wet route for the production of biodiesel from microalgae: energy balance analysis. *Bioresource Technology* 102, 5113-5122.

Glossary

BioH ₂	Biohydrogen
BD	Biodiesel
CASO	Casein-peptone SOymeal-peptone
EDTA	Ethylenediaminetetraacetic acid
EtOH	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 _{expended}	Energy expended in a process excluding fuel final energy
PBR	PhotoBioReactor
TCD	Thermal Conductivity Detector
TLC	Thin Layer Chromatography

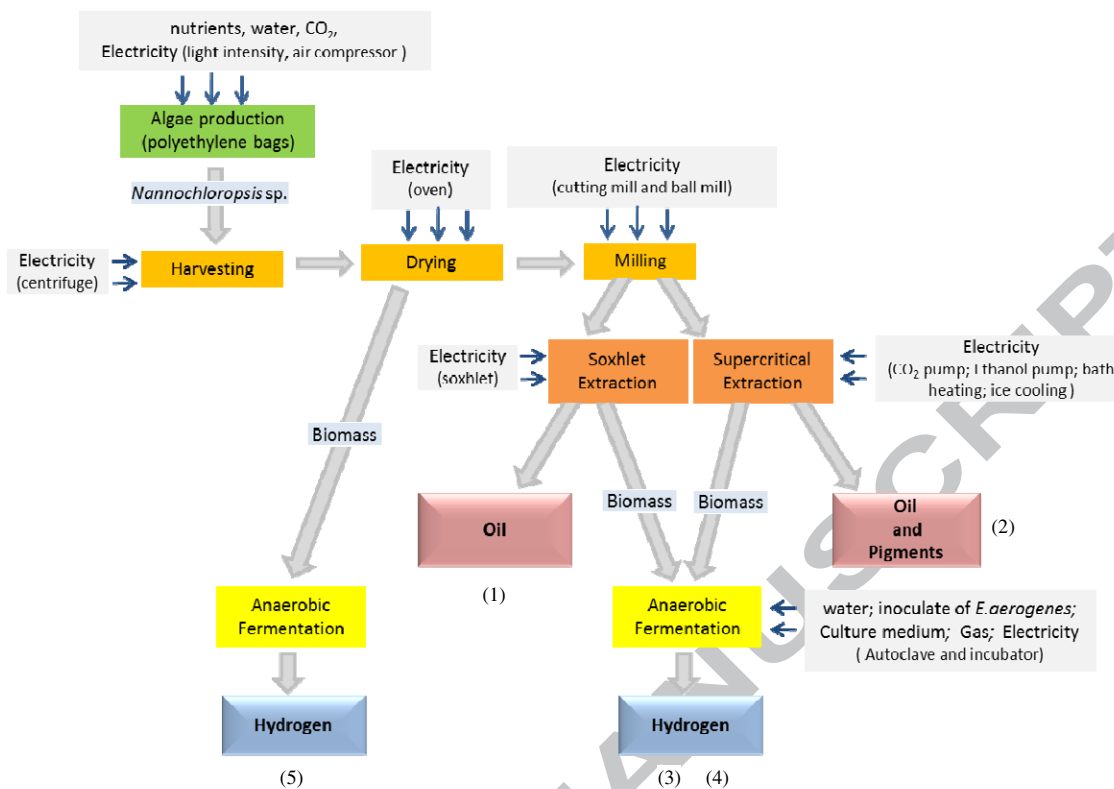
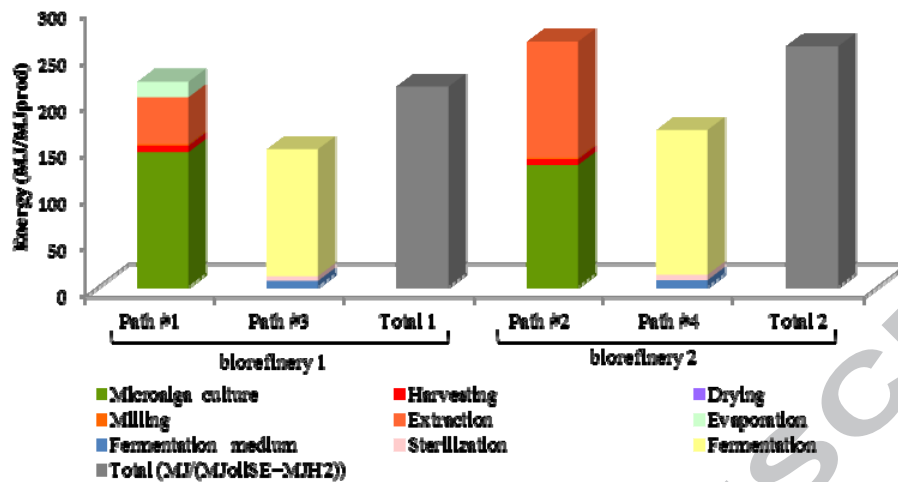
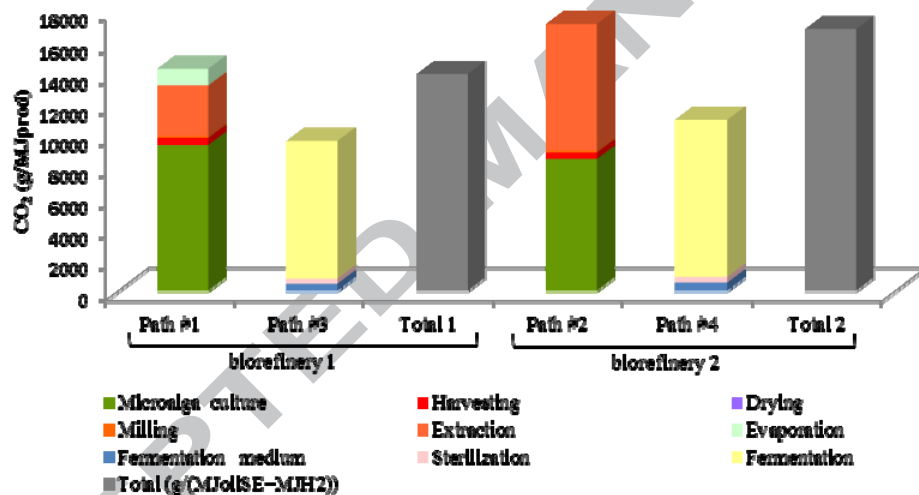


Figure 1: Scheme of energy inputs of the biorefinery for oil, pigment and biohydrogen production. The numbers in brackets correspond to the production pathways to analyze, mentioned in the text.



a)



b)

Figure 2: a) Energy consumption and b) CO₂ emissions (total and partial) for biorefinery 1 and 2 processes: oil SE (Path #1) and co-product bioH₂ after SE (Path #3) and oil plus pigment SFE (Path #2) after SFE (Path #4) outputs.

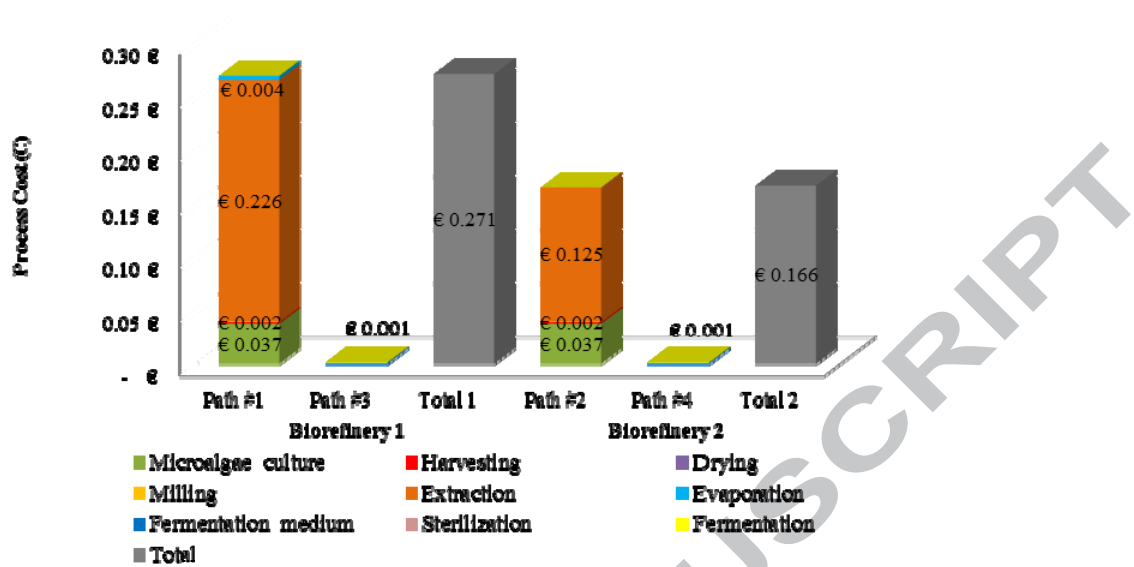


Figure 3: Cost of different pathways per 1g of initial algal biomass for biorefinery 1 and 2 processes: oil SE (Path #1) and co-product bioH₂ after SE (Path #3) and oil plus pigment SFE (Path #2) after SFE (Path #4) outputs.

Table 1: Energy inputs and respective CO₂ emissions for the microalga culture and downstream processing

Inputs	Value	Energy (MJ)	Energy		CO ₂ (g)	CO ₂	
			Min	Max		Min	Max
Nutrients (kg)							
N	2.08E-05	2.72E-04	2.53E-04	2.90E-04	2.06E-02	1.97E-02	2.17E-02
K	7.08E-05	2.01E-04	1.76E-04	2.23E-04	1.64E-02	1.49E-02	1.80E-02
P	5.07E-06	4.46E-05	3.89E-05	5.00E-05	3.57E-03	3.22E-03	3.93E-03
EDTA	1.95E-05	3.93E-05	1.75E-05	8.22E-05	2.55E-03	1.23E-03	5.19E-03
Fe	2.25E-07	2.27E-09	1.43E-09	3.66E-09	1.69E-07	1.10E-07	2.83E-07
Na	1.95E-05	3.81E-05	2.48E-05	5.45E-05	2.48E-03	1.74E-03	3.44E-03
B	3.75E-04	7.59E-04	3.38E-04	1.59E-03	4.93E-02	2.36E-02	1.00E-01
Mn	8.96E-07	9.92E-06	7.27E-06	1.22E-05	6.47E-04	5.26E-04	7.62E-04
Zn	1.08E-08	1.25E-08	6.99E-09	2.23E-08	8.04E-07	4.58E-07	1.44E-06
Co	2.42E-08	5.39E-07	2.60E-07	1.06E-06	3.62E-05	1.90E-05	7.07E-05
Deionized Water (L)	0.19	6.66E-04	1.55E-05	7.33E-05	4.35E-02	1.12E-03	4.59E-03
Light intensity (W)	0.54	2.17	1.75	2.42	1.42E+02	1.27E+02	1.52E+02
Centrifugation (W)	2000	0.11	0.08	0.12	6.87	6.14	7.35
Drying (W)	-	0.0028	0.0023	0.0032	0.18	0.17	0.20
Cutting mill	-	0.01	0.01	0.01	0.48	0.43	0.52
Ball mill	-	0.02	0.01	0.02	1.16	1.04	1.25

Table 2: Energy input and CO₂ emission in different processes in the biorefinery

	Oil SE (Path #1)		Oil and Pigment SFE (Path #2)				BioH ₂ via SE (Path #3)			BioH ₂ via SFE (Path #4)			BioH ₂ via direct biomass (Path #5)		
	Soxhlet	Evaporation	CO ₂ pump	EtOH pump	Bath heating	Cooling	Fermentation medium	Sterilization	Fermentation (incubation)	Fermentation medium	Sterilization	Fermentation (incubation)	Fermentation medium	Sterilization	Fermentation (incubation)
Energy (MJ)	0.760	0.252	0.947	0.285	0.594	0.259	0.002	0.002	0.050	0.002	0.002	0.050	0.004	0.004	0.100
Min	0.612	0.203	0.763	0.230	0.453	0.209	0.0019	0.001	0.040	0.0019	0.001	0.040	0.0040	0.003	0.081
Max	0.847	0.281	1.056	0.318	0.636	0.289	0.0023	0.002	0.056	0.0023	0.002	0.056	0.0051	0.004	0.112
CO ₂ (g)	49.6	16.4	61.8	18.6	38.8	16.9	0.16	0.12	3.26	0.16	0.12	3.26	0.33	0.24	6.53
Min	44.3	14.7	55.2	16.6	32.7	15.1	0.15	0.11	2.92	0.15	0.11	2.92	0.31	0.22	5.83
Max	53.1	17.6	66.1	19.9	39.9	18.1	0.18	0.13	3.49	0.18	0.13	3.49	0.35	0.26	6.99

ACCEPTED MANUSCRIPT

Table 3: Electricity and fuel commercial cost in Portugal (Euro per kWh of fuel)

	Cost	Refs.
	€/kWh	
Electricity	0.0535	EDP (2012)
Diesel	0.0083	ERSE (2012)
Heavy Oil	0.0033	ERSE (2012)
Coal	0.0006	ERSE (2012)
Natural Gas	0.0290	ERSE (2012)

ACCEPTED MANUSCRIPT

Table 4 - Nutrient prices

Element	Price per g (€)	Element	Price per g (€)	Element	Price per g (€)
KNO ₃	0.0884	ZnCl ₂	0.1110	Tryptone	0.3010
K ₂ HPO ₄	0.0896	CoCl ₂ ·6H ₂ O	1.1700	Yeast extract	0.0704
H ₃ BO ₃	0.0515	KH ₂ PO ₄	0.1090	Na ₂ MoO ₄ ·2H ₂ O (Mo)	0.2900
Na ₂ EDTA	0.4580	MgSO ₄ ·7H ₂ O	0.0579	C ₆ H ₅ NO ₂ (C)	0.1035
MnCl ₂ ·4H ₂ O	0.1510	CaCl ₂ ·2H ₂ O (Ca)	0.0360	Na ₂ SeO ₃ (Na)	0.2900
FeCl ₃ ·6H ₂ O	0.1148	(NH ₄) ₂ SO ₄ (N)	0.0891	NiCl ₂ (Cl)	0.6680

Source: <http://www.sigmaaldrich.com/catalog/AdvancedSearchPage.do>

ACCEPTED MANUSCRIPT

Table 5: Total energy consumption and CO₂ emissions for each pathway and biorefinaries and respective uncertainty. (*Paths #3 and #4* are co-products of paths #1 and #2, respectively (see Figure 1))

	E (MJ/MJprod)	min	max	CO₂ (g/MJprod)	min	max
Path #1	220	177	245	14320	12774	15357
Path #2	262	210	291	17123	15167	18258
<i>Path #3</i>	<i>147</i>	<i>119</i>	<i>164</i>	<i>9665</i>	<i>8645</i>	<i>10369</i>
<i>Path #4</i>	<i>168</i>	<i>136</i>	<i>187</i>	<i>11020</i>	<i>9858</i>	<i>11820</i>
Path #5	9058	7285	10123	591112	527022	634402
Biorefinery 1 (Path #1+ <i>Path #3</i>)	214	172	239	13982	124712	214
Biorefinery 2 (Path #2+ <i>Path #4</i>)	258	206	286	16800	14881	17913

ACCEPTED MANUSCRIPT

Research highlights of the paper "*A Biorefinery from Nannochloropsis sp. microalga – Energy and CO₂ emission and economic analyses*”:

- Oil obtained by SE has the lowest energy and CO₂ values per MJ_{prod}.
- Oil obtained by SFE proved to be more economically viable, with a cost of 365€/kg_{oil}.
- Biorefinery 2 (biodiesel SFE + hydrogen) has the best energy/CO₂/economy compromise.
- The hydrogen 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.

ACCEPTED MANUSCRIPT