ENERGY ANALYSIS OF LIPID EXTRACTION OF *Scenedesmus* sp. PRODUCED IN PILOT SCALE

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ABSTRACT

The production of biodiesel from lipids extracted from microalgae biomass is a promising approach to biofuels. However, this approach is still not commercialized because of the high costs of processes associated with, for example, time consumption and / or biomass drying with intense energy usage. However, it was not possible to show extraction methods among the lipids existing in the literature, which could be applied specifically to the extraction of lipids from the microalgae *Scenedesmus* sp. from the large-scale wet biomass, which is the current challenge faced by the Center for Research and Development of Sustainable Energy Auto (NPDEAS). Therefore, in this study, the possibility of avoiding the drying process, and extracting lipids directly from humid biomass, using the saponification method, was tested and compared with conventional Bligh and Dyer extraction (B & D). This study introduced the cultivation of microalgae *Scenedesmus* sp. compact tubular photobioreactors 12 m3 in area 10 m2 (8 x 5 x 2 m). The classical method of lipid extraction from microalgae - B & D - brings many pigments and polar lipids that exist in the biomass and the conversion rate was only 65-66%, whereas the recovery of fatty material in the wet biomass by the saponification method showed high conversion rate (90-95%). Therefore, the saponification process showed a high recovery of fatty acids that can be easily converted into biodiesel by esterification, and it was shown that the stage of drying the biomass can be removed without losing the fatty acids. In relation to the energy usage in the process, it was shown that drying the biomass for extraction of fatty acids uses more energy than that produced in the final product, biodiesel, showing that the removal of fatty acids of the wet biomass is of strategic importance to the viability of microalgae biodiesel.

Keywords: fatty acid extraction, microalgae, saponification, energy analysis, photobioreactor

NOMENCLATURE

B&D	Bligh e Dyer method
BHT	butylhydroxytoluene
C12:0	lauric acid
C14:0	myristic acid
C16:0	palmitic acid
C16:1	palmitoleic acid
C18:0	stearic acid
C18:1ω9c	oleic acid
C18:2ω6c	linoleic acid
C18:3ω3	α-linolenic acid
C20:0	arachidic acid
GC	gas chromatography (GC)
C_{FARM}	conversion rate from fatty acid rich material to fatty acid methyl esters (%, w/w), Equation (4)
C_{TL}	conversion rate from total lipids to fatty acid methyl esters (%, w/w), Equation (3)
CVH	Calorific Value Higher (CVH) [(MJ.kg)]
EtOH	ethanol
FA	Fatty acid
FA_{FARM}	estimation of FA in fatty acid rich material (%, w/w), Equation (6)
FA_{TL}	estimation of FA in the total lipids (%, w/w), Equation (5)
$FARM$	fatty acid rich material (%, w/w), Equation (2)
$FAME$	fatty acid methyl ester
FFA	free fatty acid
HCV	high calorific value (kJ mol^{-1})
IV	iodine value [I_2 (g) sample (100 g^{-1})]
LCV	lower calorific value (kJ mol^{-1})

m_{dryB}	mass of dry microalgae (g)
m_{FARM}	mass of fatty acid rich material (g)
m_{FAME}	mass of fatty acid methyl ester (g)
$m_{dryFAME}$	mass of fatty acid methyl ester from dry microalgae biomass (g)
m_{wetB}	equivalent amount of dry biomass present in the wet biomass of microalgae (g)
$m_{wetFAME}$	mass of fatty acid methyl ester from wet microalgae biomass (g)
m_{TL}	mass of fatty material recovered by Bligh and Dyer method (g)
MMM	Molar mass media - fatty acids ($g.mol^{-1}$)
MMA	Molar mass average – FAME ($g.mol^{-1}$)
MUFA	monounsaturated fatty acid
PUFA	poly-unsaturated fatty acid
SAP	Saponification
SFA	saturated fatty acid
SN	saponification number [KOH (mg) sample (g^{-1})]
TL	total lipid (%, w/w), Equation (1)
TAG	triacilglicerideos

Superscripts

a C_{TL} (%, w/w) ± twice the standard deviation [Eq.(3)]

b C_{FARM} (%, w/w) ± twice the standard deviation [Eq.(4)]

c FA_{TL} (%, w/w) ± twice the standard deviation [Eq.(5)]

d FA_{FARM} (%, w/w) ± twice the standard deviation [Eq.(6)].

INTRODUCTION

Several studies can be found in the literature on the cultivation of microalgae specifically for energy purposes (Antoni et al., 2007; Chisti, 2007; Li et al., 2007; Lardon et al., 2009; Clarens et al., 2010; Collet et al., 2011; Jorquera et al., 2010), moreover, the microalgae are considered a potential feedstock for the production of various bioactive compounds of high commercial value, such as pigments, food, chemical and pharmaceutical products (Molina Grima et al. 2003; Garcia Cerón et al., 2006; Owende and Brennan, 2010), and can also be applied for the treatment of waste effluents and/contaminated water (Abdel-Raouf et al., 2012; Mungo 2005; Guieysse and Munoz, 2006).

However, despite the fact that the microalgae can be used for various purposes and a wide spectrum of biofuels (Zhou et al., 2012c; Ellis et al., 2012), many obstacles have hindered the commercialization of the technology of microalgae for biofuels . These barriers include the need for large amounts of fresh water, nutrients such as nitrogen (N) and phosphorus (P), the lack of efficient methods for harvesting biomass of microalgae, the procedure for efficient extraction of lipid from the wet biomass (Lardon et al., 2009; Sander and Murthy, 2010; Molina Grima et al., 2013), as well as an integration of developed technologies for CO_2 mitigation via microalgae (Zhou et al., 2012a; Li et al., 2011; Zhou et al., 2012b; Van Beilen, 2010). Furthermore, for the production of biodiesel from algae in industrial scale become economically viable, it must be taken into account several factors, such as selecting the cultivation method (open or closed system) and species of microalgae with high oil content or genetically modified organisms (Smith et al., 2013). However overall, the current large-scale commercial production is only for high value products from microalgae (Rawat et al., 2013).

After the cultivation, either in open or closed systems, the microalgal biomass must be separated from the culture medium to be utilized. The cells of microalgae may contain 40 to 80% intercellular water (Cooney et al., 2009; Xu et al., 2011). The removal of that water is necessary for the triacylglycerides (TAG) to be extracted with high efficiency, according to Baliga and Powers (2010) and Xu et al. (2011), the extraction is more efficient for moisture contents from 5 to 15%. However, achieving these levels of moisture is one of the main bottlenecks of using microalgae as a feedstock for biodiesel (Lardon et al., 2009; Marsh, 2009; Stephenson et al., 2010).

This stage of recovery from biomass is considered high cost, and represents about 20-30% of the total cost of production (Davis et al., 2011; Grima et al., 2003; Ahmad et al., 2011), besides the technical and operational challenges for producing microalgal biomass on a large scale (Wang et al., 2008). Mainly, this is due to the low concentrations found in biomass production systems, which typically range from 0.3 to 0.5 g/L and in some cases may reach 5 g/l (Wang et al., 2008).

Therefore, the choice of the method of extraction of lipids will depend on the type of microalgae cells grown and the thickness of the cell walls, that prevent the release of intracellular lipids (Cooney et al., 2011). According to Lardon et al. (2009) due to the small diameter of this kind of biomass (2 to 20 microns) and its dispersion in the liquid medium, it requires the use of expensive technologies and high energy consumption.

In spite of the routine use of extraction protocols at the laboratory scale to determine the lipid content of microalgae (Sathish et al., 2014), the variables that affect the extraction of lipids from microalgae cells are not well understood, and no methods for the extraction on an industrial scale are established (U. S. Department of Energy, 2010;

Halim et al., 2011).

One of the methods reported in the literature for the extraction of algae lipids, is the classical Soxhlet method (Soxhet, 1879). However, the energy needed for heating and extracting the required large volume of solvent are disadvantages to using this type of process. Furthermore, the lipids and other compounds undergo thermal degradation during the loading solvent (Luke and Garcia de Castro-Ayuso, 1998).

Other methods used are Folch (Folch et al., 1957) and B&D (Bligh and Dyer, 1959), using a mixture of highly toxic organic solvents, namely chloroform and methanol, to obtain oil extraction. They are known as cold extraction methods because there is no temperature variation, as in the Soxhlet method. The disadvantages consist in the need of samples with low moisture content, because the B&D when used on a large scale generates significant amounts of residual solvents (Shin et al., 2014). However, many of these methods are not feasible on a large scale due to many input and energy requirements (Rawat et al., 2013).

Besides the classical methods of extraction of lipids, the literature describes the process of isolation of fatty acids from moist biomass of microalgae, for example, extraction of supercritical carbon dioxide (Halim, et al., 2011). However, the budget is low and the process has a high cost associated with the infrastructure and operational cost.

However, some authors (Uduman et al., 2010) suggest that the oil extraction may also be made by saponification, a method that tries to solve the bottleneck in the production of biodiesel from algae, which is the dewatering of the microalgae biomass since it uses moist for the extraction of lipids, which reach high extraction yields from wet biomass. This is one of the biggest challenges in the production of biodiesel from microalgae (Kim et al., 2013).

Therefore, from the literature review, it is noticed that one of the main barriers to oil extraction from microalgae biomass is the drying process, which is responsible for most of the total energy consumption of the process, as well as the lack of commercial scale production of algae for biofuel production. Many recent studies have focused on increasing the efficiency of the extraction process, however these methods are only evaluated in laboratory scale (Santos et al., 2014; Shin et al., 2014; Taher et al., 2014; Callejón et al., 2014), and are directly related to the type of microalgae used, and most of them are not evaluated in the energy efficiency of the process. Thus it is not possible to state from the methodologies for extracting lipids existing in the literature, which could be specifically applied to the extraction of lipids from microalgae *Scenedesmus* sp. for large-scale production of biodiesel, which is the current challenge faced by NPDEAS. For the drying step is time consuming and, in many cases, a process of intensive use of energy and avoiding this step would significantly improve the process for production of biodiesel from algae. Thus, this study aims to compare the methods of lipid extraction by the classical method B&D and fatty acids from wet biomass of *Scenedesmus* sp. microalgae pilot scale by adapting the methodology of pre-existing saponification (Hartmann and Lago, 1973), focusing on the energy expenditure of each process.

EXPERIMENTS

Selection of wild mixed microalgae

Scenedesmus sp. isolated from water supply source (Curitiba - Brazil) was kept in 2 L Erlenmeyer flasks with in 1.8 L liquid medium (Chu, 1942), with the following composition (g L^{-1}): $NaNO_3$ (0.25), $CaCl_2.2H_2O$ (0.025), $MgSO_4.7H_2O$ (0.075), K_2HPO_4 (0.075), KH_2PO_4 (0.175), NaCl (0.025), EDTA (0.05), KOH (0.031), $FeSO_4.7H_2O$ (4.98 10^{-3}), H_3BO_3 (11.42 10^{-3}), $ZnSO4.7H_2O$ (8.82 10^{-6}), $MnCl_2.4H_2O$ (1.44 10^{-6}), $NaMoO_4.2H_2O$ (1.19 10^{-6}), $CuSO_4.5H_2O$ (1.57 10^{-6}), $Co(NO_3)_2.6H_2O$ (0.49 10^{-6}), at pH 7.0. Culture temperature was maintained at 17 ± 2 ºC under continuous illumination (cool-white fluorescent, 2500 lx) and mixing of the culture was provided by continuous bubbling of air (5 L min^{-1}).

Selecting these species has the advantage of pre-adaptation to growing conditions in the external environment, coupled with the low risk of generating environmental impact in case of a leak, since it is a natural kind in the region.

Biomass production

Wet biomass from mixed *Scenedesmus* was produced from autotrophic cultivation to evaluate the extraction of fatty acid by the saponification method. The production in a pilot scale photobioreactor contributed to the evaluation of all the difficulties of processing large-scale biomass and, therefore, allowing a complete evaluation of the whole process of obtaining fatty material for biodiesel production from microalgae.

An inoculum of 2 m^3 of *Scenedesmus* was produced in a rectangular tank (0.6 m height x 2.10 m length x 1.6 m wide) using the culture medium Chu (Chu, 1942) under constant aeration. After 7 days, the microalgae solution in the tank was used to inoculate a 12 m^3 compact tubular photobioreactor (10 m^2 area, 8 m x 5 m x 2 m), which has 3.5 km of transparent PVC pipes in its structure, located outdoors on a concrete base next to the laboratory (Fig. 1) (Vargas et al., 2011; Satyanarayana et al., 2011). The reactor was not illuminated during the night and the only carbon source provided was from CO_2 contained in the compressed air injected into the system through an 8 m tall gasser-degasser column and with a 0.11 m diameter (120 L min^{-1}). One week after inoculation, 5 m^3 of culture medium were harvested and the

microalgae biomass was separated by flocculation with sodium hydroxide (NaOH) (until pH 11) and 0.1 mol L^{-1} $Fe_2(SO_4)_3$. After decantation, the biomass was passed through a press filter providing 10 kg of biomass (20% dry weight).

Figure 1. Compact Photobioreactor from NPDEAS – UFPR (Curitiba, Paraná, Brazil).

Extraction of fatty material and esterification

In order to obtain reproducibility on the fatty acids recovery, all extraction experiments were performed in triplicate to calculate the experimental uncertainty associated with the methodology. All conditions of heating, agitation and reactants were standardized. The extractions and conversions performed with the microalgae biomass and the fatty material are shown in Fig. 2.

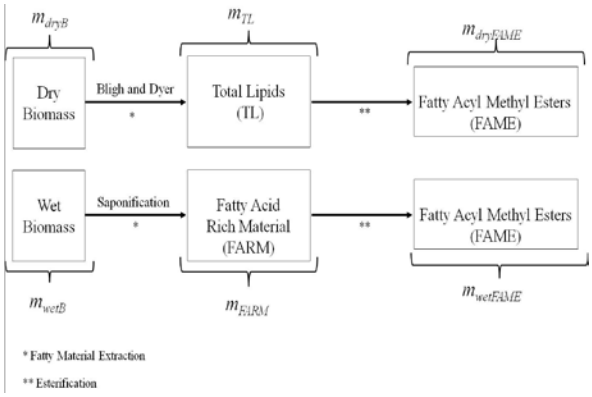

Figure 2. Extraction of fatty material and sterification performed with the microalgae biomass (dry and wet). * Fatty Material Extraction. ** Esterification.

Extraction of total lipid by modified Bligh and Dyer method

The extraction of total lipids (TL) was performed by B&D (Bligh e Dyer, 1959) adapted from Lourenço (2006). Samples of 50 mg of lyophilized biomass were extracted with 3 mL of chloroform: methanol (2:1 v/v) and 10 µL of an aqueous solution of BHT (10% w/v). The extraction was performed in an ultrasonic cleaner for 3 cycles of 15 minutes, and then kept at 4 ºC. After 24 hours, the samples were sonicated again for 3 cycles of 15 minutes and then centrifuged at 5000 rpm at 5 ºC for 20 minutes. The liquid phase was recovered and reserved. Another extraction was performed in the solid phase as described above and the resulting liquid phase was recovered. The liquid phases of the two previous extractions were merged and were added 2 mL of distilled water and 1 mL of chloroform. The samples were shaken and centrifuged at 5000 rpm for 10 minutes at 5 ºC. The lower phase was recovered and reserved in a vial tube, with previously measured mass. The aqueous phase was washed with 1 mL of chloroform and centrifuged, and the lower phase was transferred to the vial. The chloroform: lipid phase was dried with nitrogen gas, and the final mass obtained, referred as fatty material recovered by B&D (Bligh e Dyer, 1959) method (m_{TL}), was quantified.

Extraction of fatty acid rich material by saponification followed by acidification

For the extraction by saponification followed by acid hydrolysis, the cells were previously disrupted in a mixer. The experiments were performed in a process that consisted of three main steps: i) direct saponification of wet biomass; ii) acid hydrolysis; iii) subsequent extraction with hexane, schematically represented in Fig. 3.

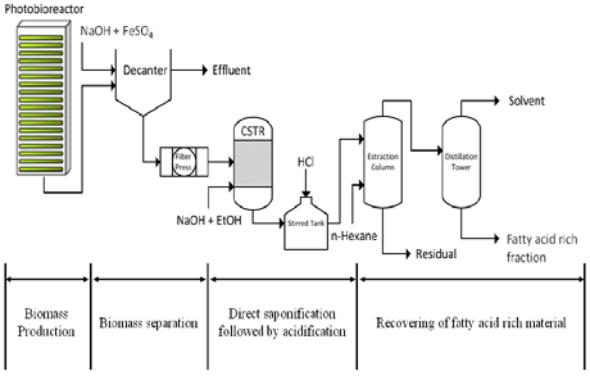

Figure 3. Fatty acid rich fraction recovery performed in pilot scale by saponification methodology from wet microalgal biomass.

i) Direct saponification of wet biomass: the saponification of triglycerides and free fatty acids occurred in the presence of NaOH (99% w/w) and ethanol (96.5% v/v). For each 1 gram of wet biomass was added 0.25 g of NaOH and 10 mL of ethanol. The reaction was performed in a 100 L jacketed

reactor under constant agitation at 60 ºC;

ii) Acidification: salts of fatty acids (soaps) obtained in the previous step were changed to fatty acids by the addition of HCl (36.5% v/v) to pH 1.0;

iii) Extraction by solvent: a liquid-liquid extraction using n-hexane was performed. Two extractions with 60 L of n-hexane (98.5% v/v) were performed at room temperature (25 ± 2 ºC). The phases were separated. The n-hexane was recovered by distillation and the fatty acid rich fraction was separated for analysis.

Esterification

Aiming to determine the conversion rate in $FAME$ from fatty material isolated by B&D (Bligh e Dyer, 1959) or saponification methods, an acid esterification of the material was performed. The fatty acid present in the samples were esterified by the modified Hartman and Lago (1973) method, on account of: a) using a solution of sodium methoxide 0.5 mol L^{-1} and b) esterification with a solution of sulfuric acid in methanol. The $FAME$ formed were extracted with n-heptane. After evaporation of the organic solvent, the mass of products were determined and expressed as m_{FAME}.

Fatty material quantification and conversion rates

To quantify the fatty material, vial bottles with samples were taken to a laminar flow chamber under constant introduction of nitrogen gas. After evaporation of n-hexane, the vials were weighed. The results were expressed in terms of fatty material / dry biomass ratio (%, w/w).

The percentage of total lipids (TL) in microalgae biomass was determined as follows:

$$TL(\%, w/w) = \frac{m_{TL}}{m_{dryB}} \times 100 \quad (1)$$

where m_{LT} is the mass of fatty material gravimetrically recovered by the B&D method (Bligh e Dyer, 1959) and m_{dryB} the mass of dry microalgae biomass.

The percentage of ag rich material ($FARM$) in microalgae biomass was calculated as follows:

$$FARM(\%, w/w) = \frac{m_{FARM}}{m_{wetB}} \times 100 \quad (2)$$

where m_{FARM} is the mass of fatty material gravimetrically recovered by the saponification method and m_{wetB} the equivalent amount of dry biomass present in the wet biomass of microalgae used in saponification extractions.

The conversion rates of the materials used in $FAME$ were determined as functions of microalgae biomass. The conversion rates of microalgae biomass in $FAME$ (%, w/w) were determined as follows:

The fatty acid in fatty material were estimated as follows:

$$FA_{TL}\ (\%, w/w) = \frac{m_{dryFAME}}{m_{TL}} \times 95 \quad (3)$$

$$FA_{FARM}\ (\%, w/w) = \frac{m_{wetFAME}}{m_{FARM}} \times 95 \quad (4)$$

where FA_{TL} is the estimation of fatty acids presents in fatty material isolated from dry biomass by B&D (Bligh e Dyer, 1959) method and FA_{FARM} the estimation of fatty acids presents in fatty material isolated from wet biomass by saponification. It is important to remember that the molecules of $FAME$ have a methyl radical per molecule, which increases the mass by about 5%. Thus, this fact was taken into account and incorporated into Eq. (4).

Fatty acid characterization by gas chromatography (GC)

The fatty acids contained in the vial bottles were analyzed by gas chromatography, preceded by esterification according to Metcalfe et al. (1966). The GC analyses were performed with an Varian model CP® 3900 gas chromatograph, equipped with a 4000 MS detector (ion trap), equipped with a CpSil for FAMES capillary column 100 m (length) × 0.25 mm (internal diameter) × 0.39 μm (film thickness). The temperature ramp was: injector 210 ºC, then to 280 ºC (5 ºC min^{-1}, then held for 2 min). An electron ionization (EI) spectrum was obtained at 70 eV and 260 ºC. The injection volume was 1 μL, with a split ratio of 1:10. A post-run analysis was performed with a Starwork Station 5.0. The retention times of the fatty acids of the sample were compared with the retention times of standards, standardized and results expressed in g.100g-1. For detection of fatty acid profiles in the samples of the Standard FAME's Sigma Supelco® with 37 components was used.

Properties of the fatty acid methyl esters from microalgae

The properties of biodiesel are dependent on the profile of alkyl esters of fatty acids. Changes in the composition of the feedstock used in biodiesel production will change the properties of the fuel. The properties: iodine value (IV), Higher Heating Value (HHV) and Lower Heating Value (LHV) were calculated according to current norms (AOCS International, 1997; Krisnangkura, 1991) as described in detail in a previous work (Carvalho Junior et al.,

2011).

Uncertainty analysis

All measurements were taken in triplicate. Steady-state conditions were reached after 30 min before starting all the experiments. The accuracy limit was calculated as being twice the standard deviation of these measurements with a confidence level of 95%. The criteria for error propagation in the following experimental measurements consist of the standards recommended by the editorial board of the ASME J. Heat Transfer (1993). The properties and lengths bias limits were found negligible in comparison with the precision limit of the measured quantities.

Energy analysis

For energy analysis was stipulated, for calculation basis, 10 kg of wet biomass with 80% humidity. In calculating the saponification, was considered the energy expenditure for heating the reactor from 20° C to 60° C. With this amount of wet biomass are needed 79 kg of ethanol as reaction medium. It was found that the reaction mixture is compound of ethanol and water in the biomass. The dried biomass and sodium hydroxide for they are in very small amounts compared to ethanol and water were disregarded for the calculation of energy balance.

To calculate the drying was found that a reduction of biomass humidity from 80% to 10% is required. It was considered that the energy expended in drying taking into account the enthalpy of evaporation of water at 60 ° C.

RESULTS AND DISCUSSION

Biomass production

The biomass production was conducted on a pilot scale through cultivation of microalgae in a 12 m^3 tubular compact photobioreactor. One week after inoculation 5 m^3 of medium were collected. From this material 10 kg of wet biomass was recovered by flocculation. The wet biomass produced presented a humidity of 80% and thus the amount of dry biomass present in this material was 2 kg. Therefore, the final concentration of dry biomass in the collected material was 0.4 g L^{-1} and the productivity was 0.06 g L^{-1} day^{-1}.

Recovery of Lipids
Total Lipids and Fatty Acid rich material

The total lipid (TL) recovered by the B&D methodology (Bligh e Dyer, 1959) from microalgae varied from 11.4 to 15 % (Table 1). The difference between the conversion values, which for the extracts of FARM (saponification) is around 90% and for the TL extracts, ranging 60 - 80%, can be perceived. It doesn't matter if it is achieved relatively high values in the recovery of TL, it is difficult to obtain efficient conversion into FAME, because there is, between TL, unsaponifiable material, as well as chlorophyll pigment, which is strongly apolar, and is extracted together when using B&D method. Moreover, the modest recovery of FARM using the method of saponification can ensure higher conversions and less separation phase problems at the end of the esterification methodology.

Table 1. Recovery of fatty material from microalgal biomass through saponification and Bligh and Dyer methodologies.

Extraction Method	Lot 1 FA - TL	Lot 2 FA - TL
Saponification	11.92 ± 0.96	12.17 ± 0.39
B&D	14.96 ± 1.31	16.62 ± 0.20

The amount of lipid recovered is below what is expected for projects whose aim is the production of biofuels from microalgae. Nevertheless, it is important to mention that lipid yield of this microalgae when cultivated longer than 15 days ranges from 20 - 30%. Though, the quantities of lipid recovered in this case are consistent with results found in the literature from researchers who, while working with microalgae of the genus *Scenedesmus*, reported amounts of oil ranging from 3 to 39 % (Table 2). In the works without optimization in the conditions (light, CO_2 supply, temperature) or changing composition of the culture medium, the amount of lipid reported correspond to 4 - 14% (Sánchez et al., 2008; Kim et al., 2007), showing that this microalgae genus has usually low fat content.

Table 2. Total lipid in *Scenedesmus* reported in literature.

Microalgae	TL (%, w/w)	Publication
Scenedesmus almeriensis	12	Sánchez et al. (2008)
Scenedesmus sp.	7 – 14	Kim et al. (2007)
Scenedesmus obliqus	12 – 39	Ho et al. (2010)
Scenedesmus sp. LX1	3 – 35	Li et al. (2010) Li et al. (2011)
Scendedesmus obliquus (different strains)	8.32 – 11.71	Ho et al. (2012)
Scenedesmus obliquus	4.21 – 33.14	Baky et al. (2012)
Scenedesmus quadricauda	14 – 28 33.1	Zhao et al. (2012)
Scenedesmus sp.	20 – 25	Yin–Hu et al. (2012)

The saponification methodology used in this work was able to recover fatty acids from wet biomass microalgae grown. It is very common that researchers represent the total lipids present in microalgae as indicative of its potential as feedstock for biodiesel production. The methods more usually employed are Folch (Folch et al., 1957), B&D (Bligh e Dyer, 1959) and Soxhlet extractor (Soxhlet, 1879). However, because these analytical methods for lipids determination don't extract only esterifiable lipids, only the results presented as lipid content (% dry biomass) are not sufficient to express the potential of microalgae for biodiesel production.

Fatty acid estimation

The fatty materials isolated by the B&D (TL) and saponification ($FARM$) methodologies were esterified to determine the efficiencies of $FAME$ production from the microalgae biomass (FA_{TL} and FA_{FARM}). The results are shown in Table 3.

Table 3. Efficiency of the process for obtaining fatty acids from biomass of microalgae as a function of the $FAME$ conversion and amount of fatty acids in fatty materials recovered.

Biomass Condition		Method	$FAME$ (%, w/w)	FA (%, w/w))
Dry	TL	B&D	9.2 ± 1.5^{a}	65 ± 11^{c}
Wet	FARM	Sap	10.6 ± 3.2^{b}	90 ± 12^{d}

In Table 3, it is also observed that the saponification method performed in this study provides a material with 90 % of fatty acids from wet microalgae biomasses grown. Moreover, since the extraction of fatty acids by saponification was conducted in a pilot scale, a high concentration of fatty acids in the material obtained without prior drying of the biomass suggest that this method has potential for large scale industrial applications.

The B&D method is widely used to estimate the amount of lipids in microalgae for assessing the potential as raw material for biofuels was able to provide a material with 65 - 66 % of fatty acids in its composition. Since the method is unspecific and removes all neutral and polar lipids besides the pigments present in the material analyzed, these data indicates that the B&D method should be used with caution in assessing the production of lipids in microalgae.

The non-selectivity of the B&D method becomes apparent when observing the aspect of fatty material isolated. Biomass of microalgae generates a dark green tone material.

Fatty Acid composition from fatty acid rich material

The fatty acid profile of $FARM$ isolated by saponification were determined by GC (Table 4) in which some properties of $FAME$ were calculated according to current norms (AOCS International, 1997; Krisnangkura, 1991) and included.

Table 4. Fatty acid profile of mix *Scenedesmus* (mass fraction in %) from biomass produced in industrial scale photobioreactor.

Fatty Acids	Lot 1		Lot 2	
	B&D	SAP	B&D	SAP
C12:0	-	-	-	-
C13:0	-	-	-	-
C14:0	3.08	-	1.01	1.43
C14:1	-	-	-	-
C15:0	-	-	2.57	2.52
C16:0	43.97	60.09	24.25	38.75
C16:1	4.34	6.01	2.42	4.49
C17:0	-	-	1.42	1.17
C17:1	3.40	-	-	-
C18:0	-	-	0.46	0.96
C18:1	20.57	22.46	14.74	21.46
C18:2	10.61	3.93	21.02	11.85
C18:3	14.05	7.52	27.57	15.78
C20:1	-	-	-	1.32
C20:2	-	-	-	0.29
SFA	47.05	60.09	29.72	44.79
MUFA	28.31	28.46	21.73	27.26
PUFA	24.65	11.45	48.57	27.93

According to Makulla (2000), the pattern of fatty acids in microalgae is known to be highly variable, primarily depending on the group to which the species belongs (Lee et al., 1971). Another factor is the internal and external conditions that microalgae are subjected, such as temperature (Mortensen et al., 1988), irradiation (Thompson et al., 1990), nutrient limitation (Muller-Navarra, 1995) and finally the stage of growth of the population and its rate of microalgae growth (Cohen et al., 1988), which may influence the chemical composition of the microalgae.

Thus, a comparison of the profiles fatty acids is simply to determine whether the proposed method obtains a profile similar to a conventional method such as Bligh and Dyer, because they are intrinsically different methods, which could result in the removal of the majority of fatty acid. It can be seen that the two methods showed similar profiles for all batches, either minority fatty acids was extracted by a methodology and the other not.

The fatty acid C16:0 which is stable to oxidation, is in nearly all profiles. However, there is also the presence of unsaturated: C18:1, C18:2 and C18:3, which unsaturation's presence have poor

stability to oxidative processes. However, the estimate properties of fatty acids obtained from these profiles assists in defining some characteristics of biodiesel from microalgae from a technical standpoint.

In all samples worked, there was considerable recovery of polyunsaturated fatty acids, highlight the C18:2 and C18:3. Therefore, cultivation of microalgae can be seen as an alternative for production of these polyunsaturated fatty acids for nutrition of animals and humans (Medina et al., 1998).

In short, the method of saponification obtained in the extraction of saturated fatty acids, as for polyunsaturated fatty acids by saponification extraction was slightly lower.

In general, the idea is to obtain biodiesel with predominance in combined monounsaturated fatty acids, which make the results from saponification very interesting, if biodiesel production is the main objective. Considering the existence of pure fatty acids by the saponification method, the esterification reaction may be used to produce biodiesel. The greater advantage in this case is the previous removal of glycerin before the reaction itself, helping the biofuel purification process.

Once the environmental conditions are changed, an adjustment in the structure of the membrane lipids of chloroplasts might occur so that an efficient lipid synthesis results (Sakthivel et al., 2012). However, numerous other biochemical factors may cause such a difference and could be the focus of follow up investigations.

Table 5. Properties of FAME from microalgae and soyben.

	Lot 1		Lot 2		Soybean
Estimates of the biodiesel properties	B&D	SAP	B&D	SAP	-
MMM	257.59	264.51	247.81	251.60	-
MMA	271.11	278.52	260.65	264.72	-
IV	80.42	53.85	129.06	88.36	120 – 141
CVH	44.10	41.67	41.25	41.39	39.5

The iodine calculated values were higher for the method of B&D extraction of the photobioreactor in two lots, and smaller for saponification, since this is an index that reflects the degree of unsaturation of the fatty material, as the B&D resulted in a composition of fatty acids with higher percentage of unsaturated hence its iodine value would be higher. For comparison, soybean biodiesel usually presents values between 120-141 I_2 (g). sample (100 g^{-1}) (Cecchi, 2003 cited by Costa, 2006), European Standard (EN 14214) establishes the maximum Iodine value of 120 g I_2 / 100 g (Lobo et al., 2009), thus the values obtained for the profile extracted by saponification, have better rates than the classical B&D methodology, for two lots within the limits of the rules.

As the calculated calorific value extracted by saponification profiles showed slightly higher values, it is considered that the higher the value of this parameter is a bigger amount of energy is generated. In MJ, however, the values are of the same order, 41-44 MJ per kg of biodiesel. In his work, Carvalho Junior et al. (2011) gave HHV approximately 41.6 MJ per mole of biodiesel from algae methanolysis in situ, thus the values found are consistent with the reality of microalgae for biodiesel, since the soybean has around 39.5 MJ (Cerbio, 2007) per kg.

Energy analysis

In the reaction medium, there is 79 kg of ethanol and 8 kg of water (10 kg of biomass with 80% humidity equals to 8 kg of adsorbed water in the biomass). The amount of heat for heating the reaction medium from 20 ° C to 60 ° C can be estimated by using the sensible heat of the equations:

$$Q_{Eth} = M_{Eth}.cp_{Eth}.(60-20) \quad (5)$$

$$Q_{Wat} = M_{Wat}.cp_{wat}.(60-20) \quad (6)$$

The specific heat of ethanol and water are 2443.47 $J.kg^{-1}$ and 4180 $J.kg^{-1}$ respectively. Thereby it is obtained that:

$$Q_{Eth} = 7721\,\mathrm{k}J \quad (7)$$

$$Q_{Wat} = 1337\ \mathrm{k}J \quad (8)$$

$$Q_{Tot} = 9058\ \mathrm{k}J \quad (9)$$

The enthalpy of the reaction was disregarded in this calculation, considering that in 10 kg of wet biomass there is approximately 250 grams of fatty acid, equivalent to 1 mole of fatty acid about (based on palmitic acid). The enthalpy for saponification of 1 mol of fatty acid release only 11 heat kJ, negligible as compared to total 9058 kJ spent on heating the reactor.

With respect to drying the biomass, for the moisture reduction from 80% to 10% in 10 kg of wet biomass, is necessary to evaporate 7.7 kg of water. Therefore the final amount of water adsorbed onto the biomass is 0.3 kilograms. The enthalpy of vaporization of water is 2360.47 $kJ.kg^{-1}$, there has then:

$$Q_{evap} = M_{Wat}.H_{Wat} \quad (10)$$

$$Q_{evap} = 18172\ kJ \tag{11}$$

The heat released in the combustion of 1 mol of hexadecanoate methyl (based on the calculation that all fatty acid sample was palmitic acid) is 10107 kJ. The energy efficiency of the two processes can be calculated as follows:

$$Ef_{sap} = \frac{Q_{gen}}{Q_{con}} \tag{12}$$

$$Ef_{sap} = 1.124 \tag{13}$$

$$Ef_{dry} = \frac{Q_{gen}}{Q_{con}} \tag{14}$$

$$Ef_{dry} = 0.5587 \tag{15}$$

Therefore, the traditional oil extraction process that requires biomass drying demands more energy than it is contained in the biodiesel.

CONCLUSIONS

The global energy crisis makes necessary the search for alternative and environmentally friendly energy. In this context biodiesel from microalgae is shown as a potential biofuel to replace fossil fuels. However, laboratory scale to industrial scale processes still seems to be an obstacle due to the few credible reports of successes. Therefore, this work contributes to advancing the state of the art, featuring cultivation of microalgae *Scenedesmus* sp. pilot-scale tubular compact photobioreactors for cultivation of 12 m^3 in 10 m^2 area (8 m x 5 m x 2 m), and presents the extraction of oil from microalgae biomass wet pilot scale through saponification. The main Conclusions are summarized as follows:

i) The amount of fatty materials extracted by the B&D method and saponification from *Scenedesmus* sp. are consistent with literature data;

ii) The classic method of extracting lipids from microalgae - B&D method - carries many pigments and polar lipids that exist in the biomass and the conversion rates was only of 65 - 66%;

iii) The recovery of fatty acids of wet biomass, by the saponification method, showed high conversion rates 90 - 95%;

iv) The biodiesel produced by esterification of the extracted materials by saponification has high oxidative stability due to the presence of saturated and monounsaturated fatty acids;

v) The saponification process showed a high recovery rate of fatty acids, wich can be easily converted into biodiesel by esterification, and showed that cost of drying the biomass can be dropped without loss of fatty material;

vi) When compared to the total energy of the produced oil, the results show that the traditional oil extraction process consumes more energy than it is contained in the biodiesel. Hence, saponification is expected to be more advantageous than the traditional oil extraction process for scaling up microalgae derived biodiesel production.

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