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MICROALGAE FATTY ACID EXTRACTION FROM WET BIOMASS

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Abstract. *Microalgae are often reported as having great feedstock for biofuels potential due to their oil content and rapid growth. However,the high energy cost for drying the biomass and subsequent oil extraction with solvents or pressing are still hurdles to be overcome in order to make microalgae derived biodiesel economically and energetically competitive with fossil fuels. In this work, fatty acids were extracted directly from Scenedesmus sp. wet biomass through saponification followed by acidification. Autotrophic microalgae biomass was produced through cultivation in a pilot scale compact photobioreactor. From the dry biomass, with the Bligh and Dyer (1959) method it was recovered 11.4 - 15% (w/w) of fatty material with 65 - 66% of free fatty acids. The saponification method was capable of recovering 8.3 - 12.3% (w/w) of fatty material with 90 - 95% (w/w) of free fatty acids without the need to dry the biomass previously. The comparison of the results obtained with the two methodologies (Bligh and Dyer, and saponification) for autotrophic microalgae biomass shows that the process to obtain fatty material directly from wet biomass is more economically and energetically attractive than from dry biomass, and therefore expected to be more suitable for industrial application.*

Keywords: fatty acid extraction; wet biomass; saponification; fatty acids; microalgae;

1. INTRODUCTION

Products derived from microalgae are being marketed in recent decades by the pharmaceutical and food industry mainly due to its composition rich in proteins, carbohydrates, pigments and essential lipids. One of the uses for fatty acids of microalgae is the isolation to supplement children diets because of their recognized role on the brain development [1].

The number of papers investigating the bio–energetic potential of microalgae has been increasing because many studies describe these organisms as with rapid growth, homogeneous composition and high oil content [2]. Therefore, recent initiatives for biomass production from microalgae with focus on oil yield enable the production of a commercially attractive biodiesel for use in transportation, reducing the dependence on fossil fuels [3].

The production of liquid biofuel from microalgae biomass involves key processes such as cultivation, harvesting, oil extraction and the biodiesel synthesis itself. All these processes are important and demand investigative studies in order to increase productivity and reduce energy investment. Based on this, the cells disruption and the oil isolation are particularly interesting since the choice of inappropriate methods provides low–income, high energy expenditure and changes in the quality of the obtained material, resulting in lower quality of the biodiesel produced [4]. Indeed, an ideal lipid extraction process for microalgal biodiesel production needs to be not only lipid specific in order to minimize the co–extraction of non–lipid contaminants but also selective towards desirable lipid fractions (neutral lipids containing mono–, di– and trienoic fatty acids chains [5, 6].

Several studies describe processes using dry microalgae biomass to extract the lipids, although the energy cost of drying microalgae biomass is a major bottleneck in the production of algae–based fuels [7 – 9].

The classical Soxhlet [10] method is the most widely used for extracting lipids from solid material. Some advantages are the easy implementation and the fact that the sample remains in contact with the solvent, constantly being renewed by the extractor. Nevertheless, the time required for the extraction, and the large volume of solvent are disadvantages in the use of this type of process. Furthermore, the lipids and other compounds may suffer thermal degradation in the loading solvent phase [11].

Other widely used methods are the Folch [12] and Bligh and Dyer [13], which use a mixture of highly toxic organic solvents, chloroform and methanol, for oil extraction. They are known as cold extraction methods because there

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is no variation of temperature such as in the Soxhlet method. The disadvantages consist of the need of samples with low humidity, and the extraction of undesirable non–lipid contaminants from the organic phase. The literature describes the process of fatty acids isolation from wet microalgae biomass (70% moisture) using supercritical carbon dioxide extraction [14]. The total yield was 7% of the biomass, but the process has a high cost associated with infrastructure and operation.

The bibliographic review shows that a major bottleneck in oil extraction from microalgae biomass is the drying process; therefore, in order to reduce energy demand and cost, it would be attractive to obtain fatty acids directly from the wet biomass of microalgae, and in this way dropping the biomass drying costs off. Essentially such methodology has been applied previously with other objectives. For example, Gonzalez et al. [15] optimized the extraction of essential fatty acids from wet biomass of the microalgae Phaeodactylum tricornutum to obtain highly purified eicosapentaenoic acid (EPA). The methodology consisted of three steps: direct saponification of wet biomass, extraction of unsaponifiables, and subsequent extraction and purification of fatty acids with organic solvent. The solvent used for extraction was n–hexane, chosen for its low toxicity, easy handling, safety and low cost. The basic hydrolysis agent was KOH (99.0% w/w). The saponified material was treated with HCl to form fatty acids, and separated with n–hexane. After the solvent evaporation, all fatty acids had their composition analyzed by gas chromatography. Using wet biomass, the recovery of fatty acids was slightly lower than the recovery of fatty acids when using the freeze–dried biomass, but such fact is offset by the dramatic production cost reduction of highly purified fatty acids.

Since drying the biomass consumes about 84.9% of the total process energy [16], the main objective of this work was to adapt an existing microalgae wet biomass oil extraction methodology [15] for the purpose of providing a more economically competitive biodiesel production and possibly other more valuable products.

2. METODOLOGY

2.1 Selection of wild mixed microalgae

A mixture of microalgae was collected in a eutrophic lake in Public Promenade (Passeio Público – Downtown – Curitiba – Brazil) and kept in 2 L Erlenmeyer flasks with 1.6 L CHU [17] liquid medium, with the following composition (g L–1): NaNO3 (0.25), CaCl2.2H2O (0.025), MgSO4.7H2O (0.075), K2HPO4 (0.075), KH2PO4 (0.175), NaCl (0.025), EDTA (0.05), KOH (0.031), FeSO4.7H2O (4.98 10–3), H3BO3 (11.42 10–3), ZnSO4.7H2O (8.82 10–6), MnCl2.4H2O (1.44 10–6), NaMoO4.2H2O (1.19 10–6), CuSO4.5H2O (1.57 10–6), Co(NO3)2.6H2O (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}). After several cultivations was predominantly observed microalgae of the genus Scenedesmus and this material was referred to as "mixed Scenedesmus" and used as inoculum in further experiments.

2.2 Biomass production from autotrophic growth

An inoculum of 2 m3 of mixed 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 [17] at pH 7.0 under constant aeration. After 7 days, the microalgae solution in the tank was used to inoculate a 12 m3 compact tubular photobioreactor (10 m2 area, 8 m height, 5 m length and 2 m wide), which has 3.5 km transparent PVC pipe in its structure, located on the outside of the laboratory (Fig. 1) and exposed to the weather [3, 18]. The reactor was not illuminated during the night and the only carbon source provided were from the CO2 contained in the compressed air injected into the system through a gasser–degasser column with 10 meters high and 0.11 meters diameter. One week after inoculation, 5 m3 of culture medium were harvested and the microalgae biomass separated by flocculation with 3 mol L^{-1} NaOH and 0.1 mol L–1 FeSO4. After decantation, the biomass was passed through a filter press providing 10 kg of biomass (20% dry weight).

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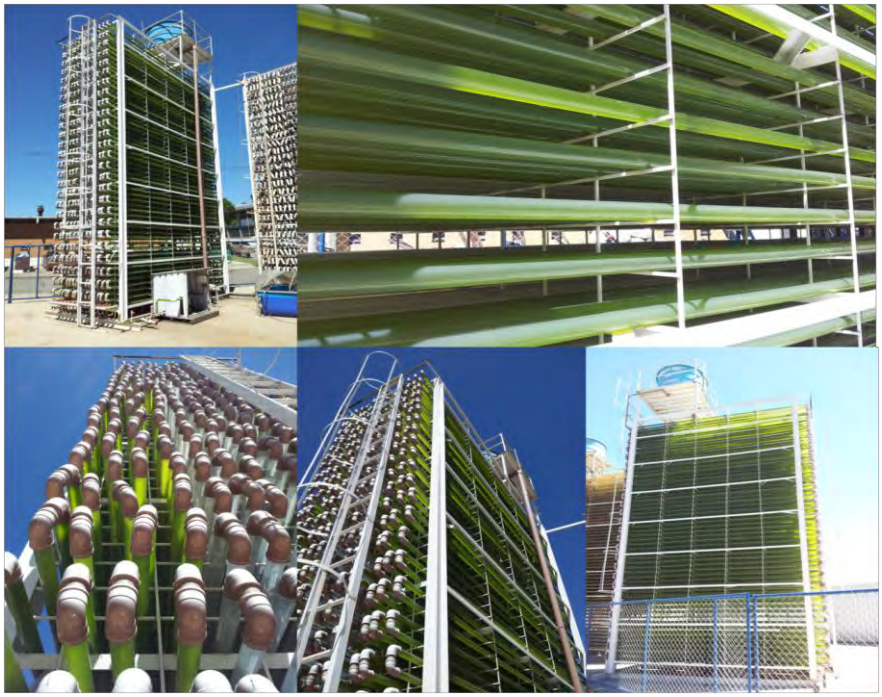

Figura 1. Photobioreactor

The species of microalgae used for this study was a Scenedesmus sp. isolated in NPDEAS, Federal University of Paraná (UFPR).

Were performed in Erlenmeyer flask (borosilicate glass) with a capacity of 2 L, and the usable volume used in each Erlenmeyer flask was 1,8 L. The Erlenmeyer flask containing the culture medium CHU and effluent from cattle were inoculated with initial biomass of 0.1 $g.L^{-1}$, kept in Erlenmeyer flask until the tenth day

The cultures were kept in air-conditioned environment with controlled temperature at 22° ± 2 °C, the lighting of approximately 111.5 micromol photons.m-2.$^{s-1}$, photoperiod 12/12 obtained by means of lamps fluorecentes with constant aeration performed by air pumps, with flow regulated approximately 0,076 $l.s^{-1}$.

2.3 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.

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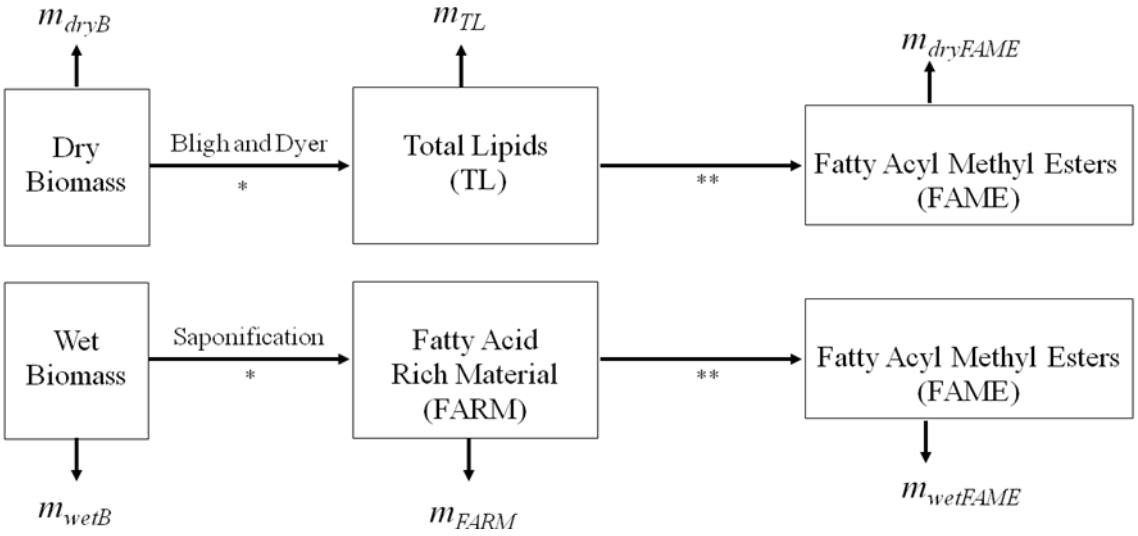

Figura 2 - – Extraction of fatty material and esterification performed with the microalgae biomass (dry and wet) grown in autotrophic conditions. * Fatty Material Extraction. ** Esterification.

2.3.1 Extraction of total lipid by modified Bligh and Dyer method

The extraction of total lipids ( ) was perfomed by Bligh and Dyer [13] adapted from Lourenço [19]. Samples of 50 mg of lyophilized biomass were extracted with 3 mL of chloroform:methanol (2:1 v/v) and 10 µL of a 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 final mass obtained, referred as fatty material recovered by Bligh and Dyer [13] method ( ), was quantified.

2.3.2 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 which consisted of three main steps: i) direct saponification of wet biomass; ii) acid hydrolysis and iii) subsequent extraction with hexane, schematically represented in Fig. 3.
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.

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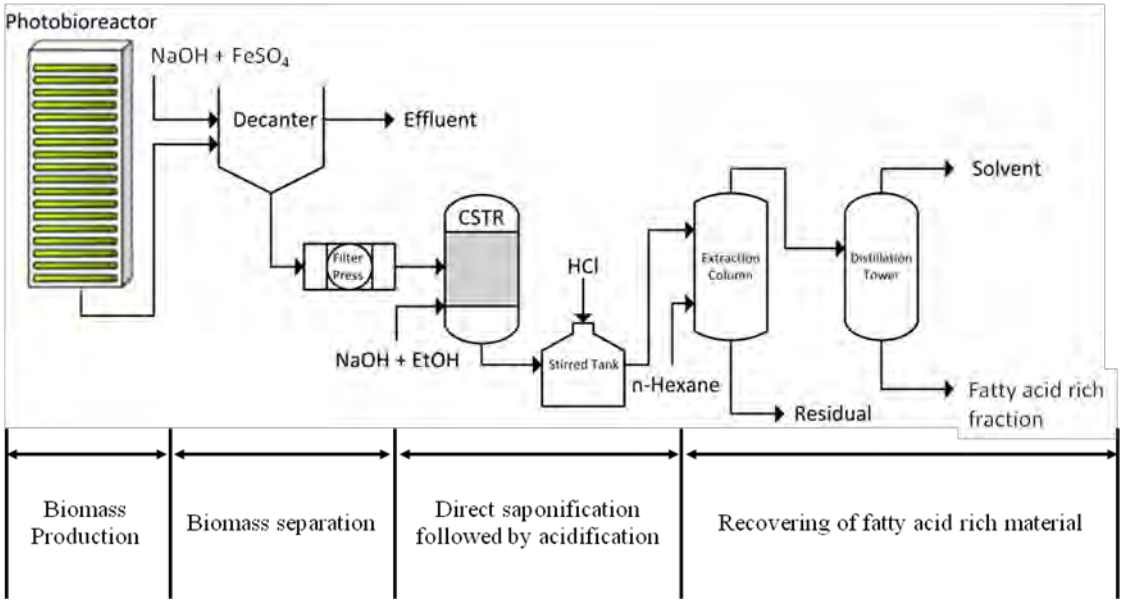

Figura 3. Fatty acid rich fraction recovery performed in pilot scale by saponification methodology from wet microalgal biomass.

2.3.3 Esterification

Aiming to determine the conversion rate in from fatty material isolated by Bligh and Dyer [13] or saponification methods, an acid esterification of the material was performed. The fatty acids present in the samples were esterified by Hartman and Lago [20] method modified, 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 formed were extracted with n–heptane. After evaporation of the organic solvent, the mass of products were determined and expressed as .

2.4 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 ( ) in microalgae biomass was determined as follows:

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

where is the mass of fatty material gravimetric recovered by Bligh and Dyer [13] method and the mass of dry microalgae biomass.

The percentage of fatty acids rich material ( ) in microalgae biomass was calculated as follows:

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

where is the mass of fatty material gravimetric recovered by saponification method and 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 were determined as a function of microalgae biomass. The conversion rates of microalgae biomass in (%, w/w) were determined as follows:

$$C_{TL}\ (\%, w/w) = \frac{m_{dryFAME}}{m_{dryB}} \times 100 \quad (3)$$

$$C_{FARM}\ (\%, w/w) = \frac{m_{wetFAME}}{m_{wetB}} \times 100 \quad (4)$$

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where is the conversion rate in for fatty material isolated from dry biomass through Bligh and Dyer [13] method and the conversion rate in for fatty material isolated from wet biomass by saponification method.

The estimation of fatty acids in fatty material was calculated as follows:

$$FA_{TL}\ (\%, w/w) = \frac{m_{dryFAME}}{m_{TL}} \times 95 \quad (5)$$

$$FA_{FARM}\ (\%, w/w) = \frac{m_{wetFAME}}{m_{FARM}} \times 95 \quad (6)$$

where is the estimation of fatty acids presents in fatty material isolated from dry biomass by Bligh and Dyer [13] method and the estimation of fatty acids presents in fatty material isolated from wet biomass by saponification. It is important to remember that the molecules of have a methyl radical per molecule, which increases the mass by about 5%. Thus, this fact was taken into account and incorporated into the Eq. 6.

3. RESULTS

3.1 Recovery of Lipids

3.1.1 Total Lipid

The total lipid (TL) recovered by Bligh and Dyer [13] methodology from microalgae cultivated in different scales and conditions varied from 11.4 to 15 % (Table 2). The amount of lipid recovered in both cases is below what is expected for projects whose aim is the production of biofuels from microalgae. However, the quantities of lipid recovered in this case are consistent with results found in the literature of researchers who, while working with microalgae of the genus Scenedesmus, reported amounts of oil ranging from 3 to 39 % (Table 1). 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% [26, 27], showing that this microalgae has usually low fat content.

Table 1 – Recovery of fatty material from microalgal biomass through saponification and Bligh and Dyer[13] methodologies.

Biomass Condition	Material recovered	Method	Fatty material
			Autotrophic
			(%, w/w)*
Dry	*TL*	Bligh and Dyer	15.0 ± 2.6
Wet	*FARM*	Saponification	12.3 ± 4.1

* Total Lipids / Dry biomass ratio (%, w/w) ± twice the standard deviation

*** Fatty acids / Dry biomass ratio (%, w/w) ± twice the standard deviation*

3.1.2 Fatty Acid rich material

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The saponification methodology discussed in this article was able to recover fatty acids from wet biomass microalgae grown in autotrophic conditions. The fatty acid rich material (FARM) recovered from autotrophic microalgae was 12.3 % (w/w).

3.2 Fatty acid estimation

The fatty materials isolated by Bligh and Dyer [13] (TL) and saponification (FARM) methodologies were esterified to determine the efficiencies of production from the microalgae biomass (C_{TL} and C_{FARM}) and also to estimate the amount of fatty acids present in those materials (FA_{TL} and FA_{FARM}). The results are shown in Table 2.

Table 2 – 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	**Fatty Material Recovered**	**Method**	***FAME* CONVERSION* (%, w/w)**	**FATTY ACID ESTIMATION** (%, w/w)**
Dry	*TL*	**Bligh and Dyer**	**9.2 ± 1.5**	**65 ± 11**
Wet	*FARM*	**Saponification**	**10.6 ± 3.2**	**90 ± 12**

* C_{TL} and C_{FARM} (%, w/w) ± twice the standard deviation. ** FA_{TL} and FA_{FARM} (%, w/w) ± twice the standard deviation

It can be observed by the results in Table 4 that the conversion rates for autotrophic biomass were similar for two different fatty materials used in the acid esterifications. The yield varied from 9.2 to 10.6 % (w/w).

In Table 4, it can be also observed that the saponification method assessed in this article provides a material with 90 to 95 % of fatty acids from wet microalgae biomass. Moreover, as 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 method of Bligh and Dyer [13] 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. This data indicates that the method of Bligh and Dyer [13] should be used with caution in assessing the production of lipids in microalgae, as the method is unspecific, removes all neutral and polar lipids besides the pigments present in the material analyzed. Meanwhile, only the fatty acids are converted to (biodiesel).

The non–selectivity of Bligh and Dyer [13] method becomes apparent when observing the aspect of fatty material isolated, dark green color. The material obtained by saponification has the yellow color, indicating selectivity for fatty acids.

4. CONCLUSIONS

In this study, fatty acids from autotrophic biomass from Scenedesmus sp. were obtained by the process of saponification and were converted to for to obtain properties of microalgae biodiesel. The conclusions may be summarized be as follows:

[1] The biomass production on an pilot scale is relatively smaller than one carried out in laboratory scale, but was produced using only the CO2 presents in the air;

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[2] The amounts of fatty material extracted by Bligh and Dyer method [13] and saponification from Scenedesmus sp. are consistent with literature data;

[3] The classic method of extracting lipids from microalgae – Bligh and Dyer [13] – draws many pigments and polar lipids presents in the biomass and the conversion to was only 65–66 %;

[4] The recovery of fatty material of wet biomass by saponification method showed high conversion (90–95%) to FAME.

[5] The saponification process performed on an pilot scale showed a high recovery of fatty acids that can be easily converted into biodiesel by esterification and showed that the cost of drying the biomass can be dispensed without loss fatty material.

5. ACKNOWLEDGEMENTS

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7. RESPONSIBILITY NOTICE

The authors: Luiza Schroeder, Marisa Daniele Scherer, André Bellin Mariano e José Viriato Coelho Vargas are the only responsible for the printed material included in this paper.