# METHODOLOGY FOR FATTY ACID EXTRACTION FROM SCENEDESMUS SPP BIOMASS FOR PRODUCTION OF BIODIESEL

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***Abstract.*** *Microalgae are considered a potential source of important fatty acids. Among them, we can mention the Linoleic Acid (C18:3), an unsaturated fatty acid that plays an important role in the prevention of human diseases as well as other polyunsaturated fatty acids that are very important in the synthesis of biodiesel. Numerous methodologies have been reported in the literature for fatty acids extraction from microalgae. A process for recovering fatty acids from microalgae biomass by saponification was developed and consists in three main steps: 1) direct saponification of lipids with potassium hydroxide (KOH) and ethanol; 2) extraction of unsaponifiable fatty acids with n-hexane and 3) purification of fatty acids by acidification of alcoholic solution followed by extraction of saponifiable fatty acid into n-hexane phase. Direct saponification was carried out using 10 mL/15ml of ethanol (96% v/v) per gram microalgae biomass mixed with 0,40/0,35 g of KOH per gram of biomass. With this methodology, the fatty acids yield was 6 % for wet biomass and 3 % for dry biomass.*

***Keywords****: microalgae, biodiesel, fatty acids extraction, Scenedesmus spp.*

## 1. INTRODUCTION

Microalgae have been studied in biotechnology research due to its nutritional importance, economic and ecological effects. Many Microalgae are used for food production by produce various substances, such as vitamins, minerals, pigments, lipids. The main applications of fatty acids in microalgae are enrichment of diets for fish, the possibility of use for biodiesel production and supply of fatty acids essential in the human diet (Costa *et al*., 2006).

The key processes involved in biodiesel production using microalgae are cultivation, harvest, lipid extraction (cell disruption), and the transesterification of the lipids. Although all these steps are essential, the cell disruption is particularly important, as the contents of the extracted lipids are determined according to the disruption method and device. Therefore, the appropriate cell disruption method and device are keys to increasing the lipid extraction efficiency (Lee *et al*., 2010).

The procedure of extracting lipids from microalgaes tissues requires the observation of some important steps: pre-treatment or sample preparation, homogenization of tissues in the presence of a solvent and separation of aqueous and organic phases; removal of non-lipid contaminants, removal of solvent and drying the extract (Shahidi and Wanasundara, 1998).

Several methods, such as mechanical pressing, centrifugation, and Soxhlet extraction have been tested for extraction of lipids. However, the most efficient method of extracting lipids from microalgae has not been achieved.

The costs of drying cultures of microalgae for lipid extraction is a major bottleneck that hinders and the production of algae-based fuels. As with many bioactive microbial processes for the production, secondary recovery of products from microalgae can be substantially more expensive than the cultivation of microalgae. Thus, this work suggests a methodology for the use of biomass with high humidity from the operation settling with NaOH by direct saponification of fatty acids. The process of saponification of fatty acids or a mixture of oils and fats is characterized by the use of an alcoholic alkaline solution.

The saponified fatty acids are separated by this method in the presence of solvent in two groups: saponifiable fatty acids and unsaponifiable fatty acids. The end of the process results in only the first group of purified, which is better for biodiesel production.

The proposed methodology is justified by the fact that process the alkaline hydrolysis esterification for biodiesel production was possible with humidy of the material above 80%, thus the drying step is not necessary and as consequence the costs are decreased. It also should be noticed that this hydrolysis step removed the undesirable glycerin.

The saponification method was adapted from a study by Molina Grima et al. (1998), who optimized a method for extraction of essential fatty acids from wet biomass of the microalgae *Phaeodactylum tricornutum*. It 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, safe, and low cost. The agent's basic hydrolysis (saponification) was NaOH (99%), because the low cost and it was the same agent used in the flocculation of the biomass.

The non-saponifiable material ~~is~~ was separated and analyzed for fatty acid search of high commercial value (usually EPA and DHA). The saponified material was treated with HCl to form fatty acids, and separated with n-hexane. After evaporation of the solvent, the fatty acids were analyzed for their composition by gas chromatography.

Figure 1 show the alkaline hydrolysis of triglycerides to produce glycerol (glycerin) and a mixture of salts of carboxylic acids (Solomons and Fryhle, 2002). Vegetable oils are mainly triglycerides (esters of glycerol with saturated and unsaturated fatty acids) and free fatty acids. During the saponification of the oils~~,~~ the step of breaking down fatty esters is followed by reaction of fatty acids synthesized in the first phase with the existing base, forming a mixture of salts of fatty acids.

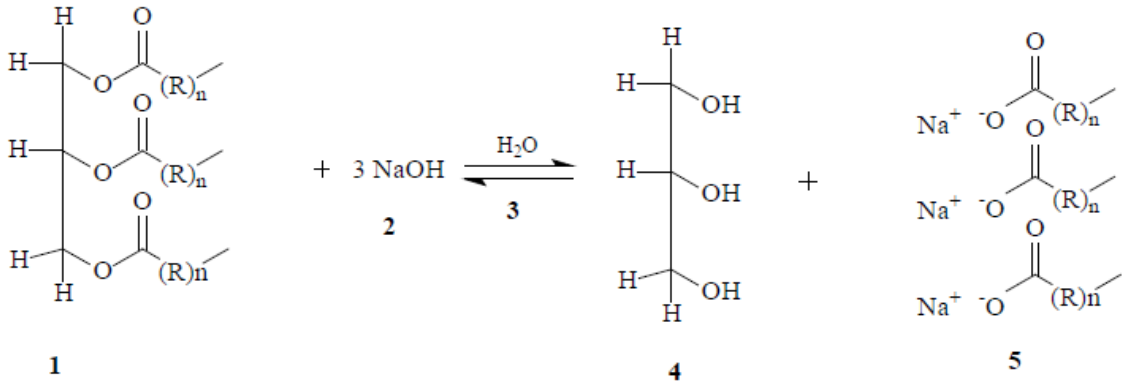


Figure 1. Alkaline hydrolysis reaction (saponification). (R)n: chain carbonic where n is the number of carbons. (1-triglycerides, 2-base, 3-solvent, 4-glycerol, 5-salt of fat acid "soap").

## 2. MATERIALS AND METHODS

### 2.1. Biomass of microalgae

The biomass used for the experiments was grown by Integrated Group for Aquaculture and Environmental Studies - GIA - Federal University of Parana, and belongs to the genus *Scenedesmus*. The samples arrived at Núcleo de Pesquisa e Desenvolvimento em Energia Autossustentável (NPDEAS) in gallons of 20 L, which were flakes with NaOH, and the biomass was filtered and then frozen for the experiments. For the tests with dry biomass, it was dried at 60 °C for 24 h.

### 2.2. Extraction of lipid by saponification

The experiment was performed by saponification in three stages, according to the "Fig.2": direct saponification of biomass wet, extraction of unsaponifiables, subsequent extraction and purification of fatty acids. To evaluate the effect of moisture on the extraction of lipids, four samples of microalgae *Scenedesmus* were used, two of them with more than 50% moisture (80%) and rest with under 50% of humidity. The moisture level was measured in infrared model.

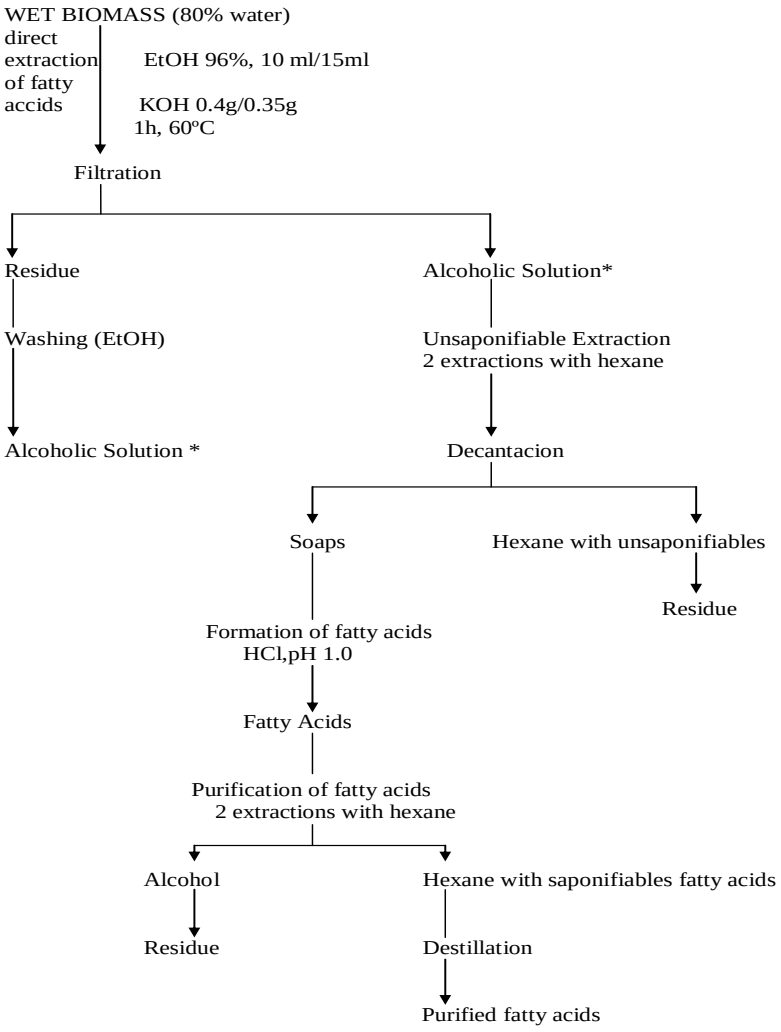


Figure 2. Process to extract and purify fatty acids from the microalgae *Scenedesmus spp*. (*The two alcoholic solutions are mixed). (Adapted for González *et al*., 1998)

To evaluate the optimum amount of reagent to be used for treatment of biomass it was performed two experiments on each type of biomass. Thus, biomass treatment was carried out with excess of KOH (0.4 g KOH / g of biomass) and ethanol limitation (EtOH 10 ml / g biomass), and another with excess ethanol (15 ml of ethanol / g biomass) and limiting KOH (0.35 g KOH / g biomass). These data are shown in "Tab. 1".

Table 1. Experimental design saponification.

| Test | Microalgae biomass (g) | Relative humidity (%) | Dry weight (g) | KOH mass (g) | EtOH volume (mL) |
|---|---|---|---|---|---|
| I - Dry biomass - EtOH excess | 4 | 13.8 | 3.45 | 1.15 | 51.75 |
| II - Dry biomass - KOH excess | 4 | 13.8 | 3.45 | 1.38 | 34.5 |
| III - Wet biomass - EtOH excess | 20 | 80.5 | 3.9 | 1.30 | 58.5 |
| IV - Wet biomass - KOH excess | 20 | 80.5 | 3.9 | 1.56 | 39.0 |

The reaction was carried out in beckers of 1 L with constant stirring and at temperature of 60 °C on a magnetic stirrer for 1 hour; the beckers were closed to preserve the reaction of oxygen in the oxidation of fatty

acids. The saponified mixture was filtered in Büchner funnel in a qualitative band filter and residual biomass was washed with a small volume of ethanol (96% vol / vol). Figure 3 shows the reaction in the laboratory.

Figure 3. Saponification reaction.

### 2.3. Extraction of unsaponifiables

The unsaponifiables were extracted by adding n-hexane (98.5% vol / vol) to the filtrate in a separating funnel, the volume of n-hexane used was approximately the same volume of the sample in two consecutive extractions (variable volume due to equilibrium phase). The solvent phase containing unsaponifiable fatty acids was stored for further studies, as in the "Fig. 4".


Figure 4. Unsaponifiable fatty acids in n-hexane phase.

### 2.4. Extraction of purified fatty acids

The pH of the remaining solution was adjusted to 1.0 with HCl (35.5-38% vol / vol). The balance of the distribution of fatty acids was determined by adding volumes of n-hexane (98.5 % vol / vol) until phase separation.

### 2.5. Solvent distillation for determination of fatty acids

The n-hexane phase containing fatty acids was purified distilled ("Fig.- 5") and placed in previously weighed *vial* bottles for gravimetric determination of fatty acids. The fatty acids contained in *vial* bottles were sent for analysis by gas chromatography. Fatty acids were identified by comparing the retention times with the standard.

Figure 5. Solvent distillation.

## 3. RESULTS AND DISCUSSION

Table 2 shows the tests performed with different mass of lipids recovered. The best efficiency was with extraction of lipids in samples of high moisture. The mechanical agitation was more intense in samples with high water content, because the formation of a mixture less viscous. Thus, the cell disruption may have resulted in higher recovery of lipids, reaching about 6%, almost the double of the percentage recovered as dry biomass.

It was also noted that the lower recovery of lipids was presented at test with biomass less moisture. This could be due to the drying method used, which was incubating at 60 °C. The biomass formed small granules that were crushed in an agate mortar with a pestle; probably the breaking of the cell wall was not effective.

Table 2. Recovery of lipids by saponification mass

| Test | Microalgae biomass (g) | Relative humidity (%) | Dry weight (g) | Lipid mass (g) | Oil on dry biomass (%) |
|---|---|---|---|---|---|
| I - Dry biomass - EtOH excess | 4 | 13.8 | 3.45 | 0.1026 | 2.98 |
| II - Dry biomass - KOH excess | 4 | 13.8 | 3.45 | 0.0934 | 2.71 |
| III - Wet biomass - EtOH excess | 20 | 80.5 | 3.9 | 0.2183 | 5.60 |
| IV - Wet biomass - KOH excess | 20 | 80.5 | 3.9 | 0.1932 | 4.95 |

The reactions of I and III tests with an excess of ethanol showed an appreciable increase in the recovery of lipids, however, more tests are needed for a statistical analysis.

There were differences between to carry out the saponification reactions in dry and wet samples in the method for extraction of lipids.

Parallel to the tests with wet and dry biomass, a sample of 25 g of biomass with 80% humidity was saponified with excess KOH in order to evaluate the saponified lipid extract. This extract was analyzed by ~~to~~ gas chromatography and the fatty acids are shown in the "Fig. 6", which compares the results obtained by saponification to results from tests generic Soxhlet extraction with dry biomass.

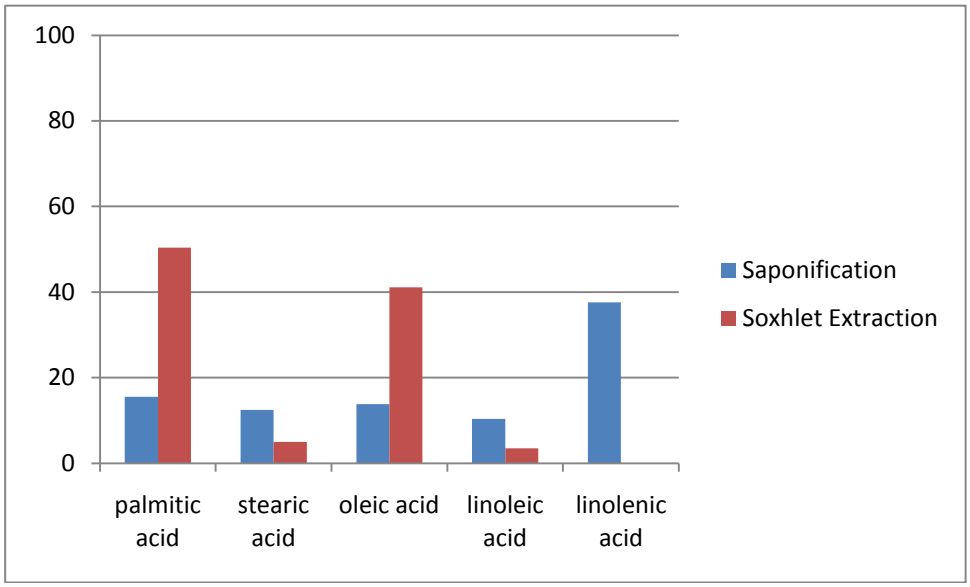


Figure 6. Fatty acid composition of microalgae

The saponification test produced (on total fatty acids) linolenic acid ("Fig. 6"), which contains three double bonds (C18: 3). The linolenic acid is an ω-3 fatty acid, a polyunsaturated that it is not synthesized by the cells of our body, so it must be acquired through diet.

Making a qualitative assessment of fatty acids obtained, the higher hydrocarbon chain of the molecule, the higher cetane number and better lubricity of the fuel. However, a higher point of fog increases the point of blockage. Thus, overly large molecules (alkyl esters of erucic acid, arachidonic acid or eicosanoic) due to the process of pre-heating the fuel make them difficult to use in regions with low temperatures (Harrison *et al*, 2007).

Chromatographic analysis was used for the four tests; the fatty acids analyzed were grouped into three main classes in order to facilitate interpretation of quality of lipids recovered. The percentage of each group is represented by percentage as in "Fig. 7".

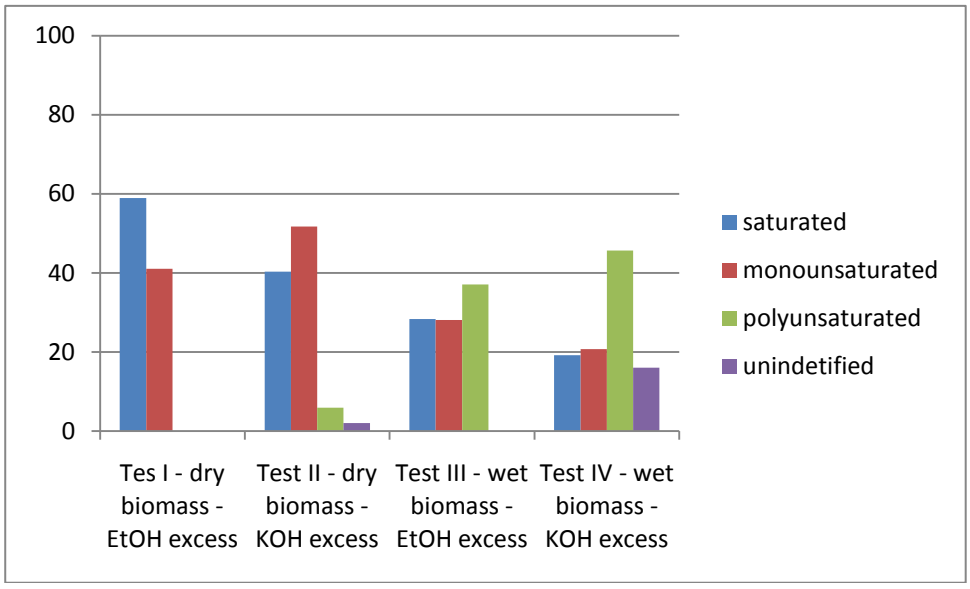


Figure 7. Fatty acids profile.

As for the unsaturated, the smaller number of unsaturations (double bonds) in molecules, the higher cetane number fuel, leads to a better quality for combustion. Moreover, an increase in cetane number also causes an increase in the point of fog and blinding (increased sensitivity to cold climates). Moreover, a large number of unsaturated molecules become chemically less stable. This can cause difficulties due to oxidation, degradation

and polymerization of the fuel (causing a lower cetane number or formation of solid waste), if improperly stored or transported. Thus, both alkyl esters of saturated fatty acids (lauric, palmitic, stearic) such as poly-unsaturated (linoleic, linolenic) have some drawbacks (Harrison *et al*, 2007).

In this case, the same tests with wet biomass showed better recovery of lipids, with a large recovery of unsaturated fatty acids. Although, in the tests with biomass, the recovery of saturated and monounsaturated fatty acids were complete.

## 4. CONCLUSIONS

There are several options for recovery and conversion of microalgae biomass to obtain intracellular metabolites produced by microalgae. It is better to use the moist biomass because a prior step of drying increases significantly production costs. A chromatographic method should be used for recovering when the high value-added products are added to the method.

There were significant differences in the tests with wet and dry biomass. The wet biomass demonstrated potential of use up to 80.5% moisture, having more advantage than dry biomass.

In general, the best results were obtained when a predominant biodiesel is a mixture of oleic and ricinoleic (mono-unsaturated) fatty acids.

The recovery of lipids from the dry biomass was qualitatively better, because fatty acids recovered are very good for biodiesel production. The recovery of lipids of wet biomass was not as good as the recovery of dry biomass for biofuel, however, the large proportion of poly unsaturated obtained is of the great interest for to biotechnology. However, the drying of microalgae biomass is very expensive for the production of biodiesel. With the manipulation of the extraction by saponification of wet biomass most of the mono-unsaturated fatty acids are obtained.

The saponification method used in ~~of~~ this methodology is relatively simple, and showed effectiveness in the extraction of fatty acids as well as specificyness of fatty acids. This methodology also avoid the problem of the undesirable residues (such as glycerin) in the production of biodiesel.

Other studies in the NPDEAS are being conducted in a larger scale, such as reaction of saponification with NaOH, the same as used for flocculation in order to reduce costs.

## 5.ACKNOWLEDGMENT

This work was supported by the CNPq and NPDEAS.

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## 7. RESPONSIBILITY NOTICE

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