

OIL EXTRACTION AND BIOMASS PRODUCTIVITY FROM MICROALGAE IN PILOT SCALE

Rui Miguel de Carvalho Júnior, ruiqmc@yahoo.com.br

José Viriato Coelho Vargas, jvargas@demec.ufpr.br

Universidade Federal do Paraná – Programa de Pós-Graduação em Engenharia (PIPE) – Centro Politécnico – Jardim das Américas, CEP - 81.531-990 Curitiba – PR, Brasil.

Luiz Pereira Ramos, lramos@quimica.ufpr.br

Universidade Federal do Paraná – Departamento de Química – Centro Politécnico – Jardim das Américas, CEP 81.531-990 Curitiba – PR, Brasil.

Roberto Bianchini Derner, robertoderner@lcm.ufsc.br

Universidade Federal de Santa Catarina, Departamento de Aqüicultura – Centro de Ciências Agrárias – Itacorubi, CEP 88.034-001, Florianópolis – SC, Brasil.

André Bellin Mariano, andrebmario@gmail.com

Universidade Federal do Paraná – Departamento de Engenharia Mecânica – Centro Politécnico – Jardim das Américas, CEP 81.531-990 Curitiba – PR, Brasil.

Abstract. *The expectation of decreasing oil reserves and the growing concern about the preservation of the environment, which has experienced an increased level of pollutants, especially those causing the greenhouse effect, mean that cleaner alternatives to generate energy are urgently need. A possible alternative for fossil fuels is the use of vegetable oils, which can be called biodiesel. Therefore this study aims to evaluate the potential performance of microalgae as a raw material for the production of biodiesel. For that, organic solvent extraction of lipids from microalgae that does not have dependency on petroleum is investigated. Therefore ethanol (ethyl alcohol) was studied as an alternative, and the oil was extracted by the so called Soxhlet extraction system during 24 hours. Using this method we could extract several forms of lipids and pigments (chlorophyll and carotenoids), but for the production of biodiesel what matters is the triacylglycerol which consists of three long chain fatty acids bound as esters to a molecule of glycerol. For that the technique of column chromatography using silica gel stationary phase second method AOCS Cd 11c-93 was used, thus separating only extracted material triacylglycerols. To confirm the results and to evaluate the potential of biodiesel production from microalgae, the acidity rate second method AOCS Ca 5a-040 was used to determine the amount of free fatty acids. To check the chemical structure of triacylglycerol, the Fourier Transform Infrared (FTIR) spectroscopy was used.*

Keywords: Photobioreactors, Extraction of Lipids, Microalgae.

1. INTRODUCTION

The expectation of decreasing oil reserves and the growing concern about preserving the environment which has experienced an increased level of pollutants, especially those causing the greenhouse effect, mainly coming from vehicle emissions that use fossil fuels, have encouraged the substitution of fossil oil in the Brazilian energetic matrix. The main feature of the Brazilian energetic matrix is the high percentage of renewable energy, as it is observed in Fig. 1. About 46% of the matrix comes from renewable sources (BEN, 2008). In Brazil, a large part of the energetic matrix is represented by bio-fuels, and the majority of the alcohol fuel is made from sugar cane.

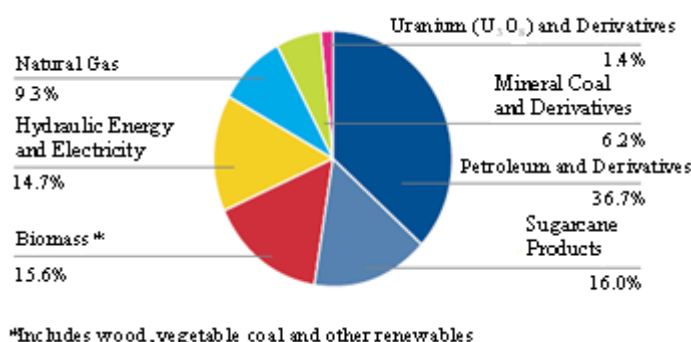


Figure 1. Brazilian national energy balance in 2008 (BEN, 2008)

A possible alternative to fossil fuel is the use of vegetable oils to produce esters, which are then called "biodiesel" (Meher et al., 2006). The vegetable oils and animal fats consist of triacylglycerol molecules, which are formed by three long chain fatty acids bound as esters to a glycerol molecule (Geris et al., 2007).

The production and consumption of biofuels in Brazil gained remarkable progress with the consolidation of the National Program for Production and Use of Biodiesel (PNPB). In 2008 the Brazilian biodiesel production reached around 1.16 billion liters and thus Brazil became the third largest producer of biodiesel in the planet (Biodieselbr, 2009).

The biodiesel is being added to fossil diesel oil (Magossi, 2009) and fuel consumption continues to increase, therefore there is a need to develop new ways of obtaining oil. According to Chisti (2007), for deployment of B50% in the United States, it is necessary an area of 45 Mha, which corresponds to 24% of the agricultural territory of United States. In order to comply with this same demand, if 30% microalgae oil were used, only 4.5 Mha would be necessary, or 2.5% of the US agricultural territory, which means that food oriented agricultural land would not be threatened by that. For this reason, microalgae derived biodiesel is expected to be a viable alternative for biofuel production.

At this point, it is instructive to recall that the primary source of energy in our planet is the sun. What plants do is to intercept the solar radiation in order to build complex molecules (proteins, carbohydrates, lipids, etc.) from water and carbon dioxide (autotrophic). Hence, the production of biofuels from microalgae oil emerges as a very promising solution. The main advantage of microalgae to produce biofuels is that they require much less agricultural area than oil seeds such as corn, sunflower, soybean and others that are typically used for food production, i.e., they have much higher productivity than oil crops (Chisti, 2007) without significantly displacing food crops. Another advantage of microalgae is that, differently from all other cultures, they can be produced continuously at any time of the year and can therefore be harvested every day.

The production of biofuels from microalgae oil is regarded as a very promising solution when it is still urgent to find alternatives to oil, but without holding the world biofuels responsible for a possible future food crisis. In sum, microalgae do three important things simultaneously: they absorb CO₂ for their own growth, produce lipids for biodiesel production, and do not compete with food production.

The major difficulty in this process is the separation of the biomass of the culture medium and the efficient extraction of the produced oil, that is, in an economically viable way. For the oil extraction, several methods have been proposed (Wenk and Fernandes, 2007), but in general they require complex steps, and the use of solvents with high toxicity and oil dependencies in the matrix. Therefore, this work has the main scope of investigating alternative methods for extraction of lipids from microalgae.

2. MATERIALS AND METHODS

2.1. Extraction system

The microalgae utilized in this study were grown by the Integrated Aquaculture Group (GIA) of the Federal University of Parana, under specified conditions established by the team, and spray drying was performed by a team of the Center for Research and Development of Sustainable Energy – NPDEAS.

Handling the biomass and the extraction of non purified lipids in a Soxhlet system (Figs. 2a and 2b) was done by using ethyl alcohol 95% manufactured by BP Chemical Vetec Fine as the solvent. The extraction process involved boiling the solvent that was packaged in a round-bottomed flask. The boiling provided enough energy to the solvent to go through a road side in the extractor, capacitors and falling on the sample placed in a cartridge inside the Soxhlet extractor. This in turn received a maximum amount of matter to be determined by the siphon extractor, which underwent the phenomenon of solvent siphoning and extracting again with the balloon, and so on. The cycle was then repeated for 24 hours. Finally, the solvent was evaporated with the lipids in a system of brand rotavapor Switzerland Buchi R II as shown in Fig. 3, with a vacuum pump.

2.2. Separation of triglycerides

According to Vega and Voltolina (2007), the lipid is found within the tissues of microalgae connected by four types of bonds, Van der Waals, electrostatic associations, hydrogen bridges and covalent bonds from the binding of fatty acids and Oxican, which made us believe that it would be possible the separation of triglycerides with solvents with different polarities. After extraction, the total lipids were purified with a second method AOCs Cd 11c-93, in order to make possible the future production of biodiesel, which is really composed of monoalquil esters from the free fatty acids and triglycerides. For that, we used a column of chromatographic silica – Brand MERCK gel with particle size of 70 to 230 mesh ATSM, in stationary phase and mobile phase, as a mixture of 10% of diethyl ether manufactured by Biotec Analytical Reagents in petroleum ether, manufactured by PA Vetec Fine Chemistry. The sample was accommodated at the top of the column and subsequently eluted with 250 mL of the mixture with diethyl ether in

petroleum ether, followed by other elution with the same volume (250 mL) for modifying the elution fraction of 25% of the mixture and 100% of diethyl ether. The process is shown in Fig. 4.

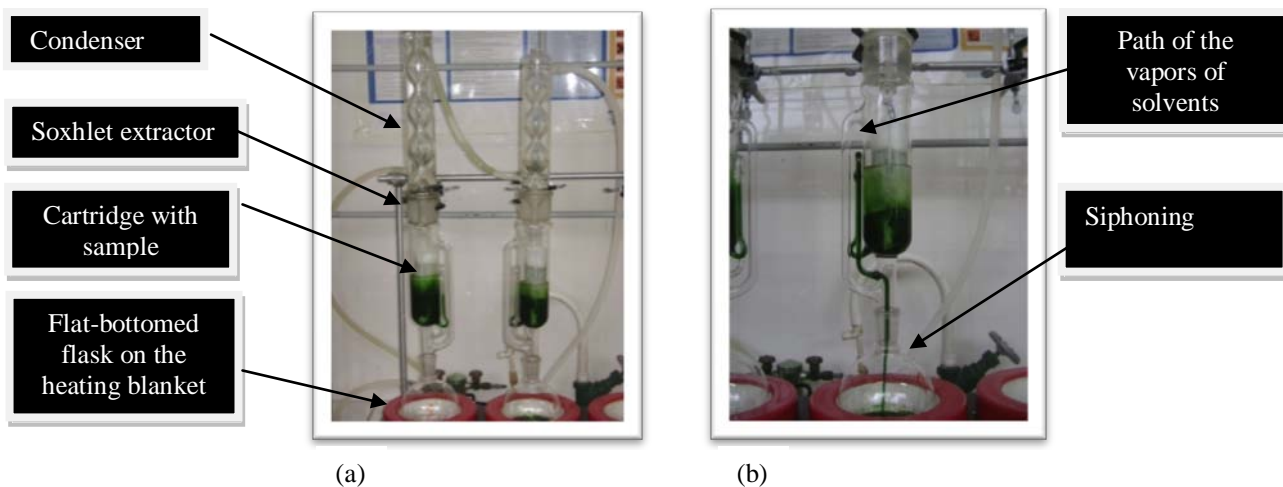


Figure 2. (a) System of Soxhlet extraction, (b) Siphoning system in Soxhlet



Figure 3. Rotavapor vacuum system

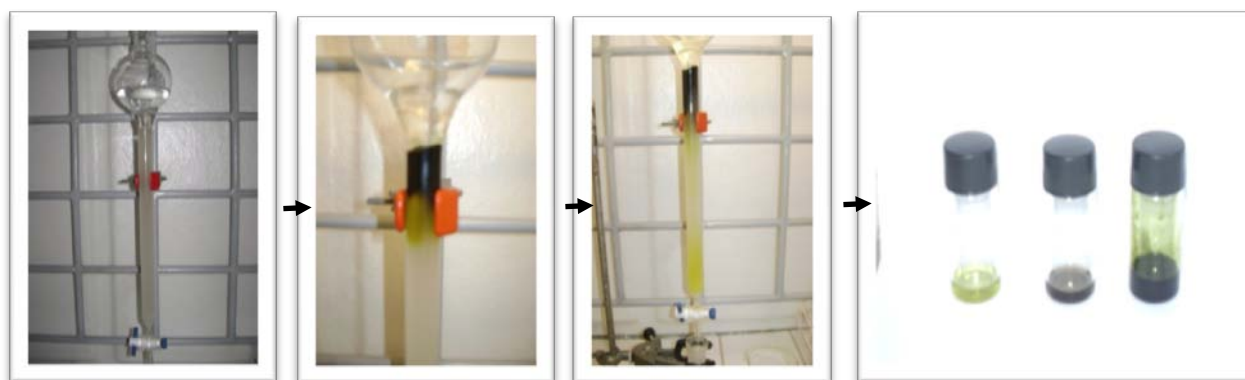


Figure 4. Separation of triglycerides process

2.3. Acidity index

In order to determine the index of acidity the AOCS method Ca 5a-040 was used. The sample was dissolved in ethanol: toluene (1:1), and then titrated with previously standardized NaOH. The sample weighed about 0.5 g in a 150 mL flask, which was then dissolved with a mixture of solvents and added approximately 4 drops of phenolphthalein, and subsequent titration with standardized NaOH. The acidity index, AI, was calculated as follows:

$$AI = \frac{MW \cdot [NaOH] \cdot V_{tit}}{W} \quad (1)$$

where MW is 1/10 of the molecular weight of oleic acid ($\text{g} \cdot \text{mol}^{-1}$), $[NaOH]$ is the concentration of the sodium hydroxide solution which was used to titrate the sample, and corresponds to the amount of titrant solution spent, V_{tit} (mL), and W is the sample weight (g).

2.4. Fourier Transform Infrared Spectroscopy (FTIR)

The analysis was conducted with a Fourier Transform Infrared Spectroscopy (FTIR) equipment and was performed in a BIO-RAD's trademark that used a Merlin 3.0 software, available in the Chemistry Department at UFPR. For the analysis, approximately 20 μ L of the sample supported in 100 mg KBr were used. The samples were expected to show the main structural features of triacylglycerols.

3. RESULTS AND DISCUSSION

3.1. Acidity index

The acidity index of vegetable oil directly affects the process of transesterification. When an homogeneous catalyst and alkali are used in the reaction, the oil must have low levels of free fatty acids for this route (Dorado et al. 2002, Lago et al., 1988), since during the transesterification catalyst they might react with the alkaline forming soap-like products (Kusdiana and Saka, 2001). For this reason the efficiency of conversion will be reduced, resulting in a complete reaction in the production of biodiesel in which the content of free fatty acids will be less than 3% (Dorado et al., 2002).

After the production of oil with the proposed process, it becomes feasible to obtain biodiesel through the transesterification route of vegetable oils using sodium hydroxide as catalyst. This is expected due to low acidity found in the sample that showed a mass fraction 0.5 g of oleic acid / 100g of sample, i.e., less than the maximum recommended amount for transesterification.

3.2 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR).

The application of the Fourier Transform Infrared (FTIR) spectroscopy technique allows for oil extracted from samples to be identified by characteristics of absorption bands. Figure 5 depicts the FTIR analysis performed in this work. Following the methodology found in Silverstein et al. (2006) it is observed that the spectrum below the bands were between 1750 cm^{-1} and 1715 cm^{-1} . These characteristics of the absorption band of C = O esters of saturated are called vibrations of axial deformation of C – O. The esters are in fact two asymmetrical coupled vibrations: C - C (= O) - O and O - C - C and the first is the most important being observed around 1300 cm^{-1} and 1000 cm^{-1} . Other bands may also be observed such as CH₃ (asymmetrical) in approximately 1450 cm^{-1} and CH₃ (symmetrical) at 1373 cm^{-1} . Those bands of axial deformation were identified there in 2936 cm^{-1} for all C - H and approximately 3300 cm^{-1} for large axial deformation of O - H. However, between 1440 cm^{-1} and 1395 cm^{-1} , respectively, it is identified the involvement of the interaction between the axial deformation of C - O and the deflection angle in terms of C - O - H. The analysis of the FTIR spectrum showed the bands corresponding to the bonds C = O and CO in the ester chemical function, which leads us to believe that the extraction of the desired material was indeed achieved.

With the analysis performed we can say that the extracted material is in fact triacylglycerol. Therefore it is possible to state that it can be converted into biodiesel via a transesterification reaction. Some of the results of the oil extractions from the microalgae used in this work are presented in the Table 1.

Table 1. Statistics of the oil extracted from microalgae produced in this work.

Biomass	Solvent	Extraction yield (%)	Purification yield (%)	Total mass purified oil (g)
Nannochloropsis salina	Ethanol	41.33	43.68	5.415
Nannochloropsis salina	Ethanol	35.09	14.21	1.626
Nannochloropsis salina	Ethanol	36.17	23.45	2.333
Nannochloropsis salina	Ethanol	28.74	29.73	2.607
Nannochloropsis salina	Ethanol	22.22	37.44	3.436

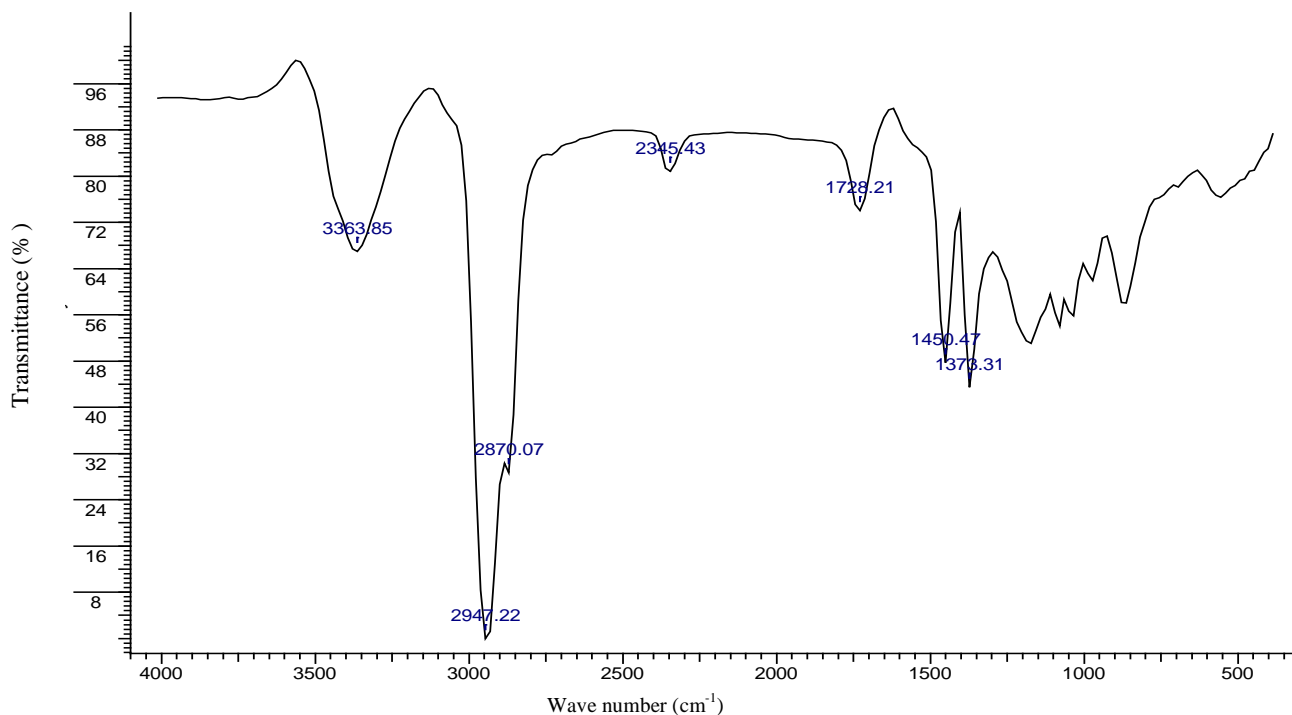


Figure 5. FTIR spectrum of the sample

4. CONCLUSION

An evaluation of the potential performance of microalgae as a raw material for the production of biodiesel was conducted experimentally. For that, organic solvent extraction of lipids from microalgae that does not have dependency on petroleum was investigated as an alternative technique. The microalgae extracted oil had its chemical structure checked via the Fourier Transform Infrared (FTIR) spectroscopy method, confirming that indeed triacylglycerol was obtained.

The main conclusion is that the production of biodiesel through the proposed system, in view of the high production of ethanol in Brazil, has the potential to be independent of exports such as solvents of fossil origin, therefore allowing for a sustainable microalgae derived biodiesel production. As a sequence of this study, the *in situ* transesterification process is currently being investigated, i.e., the direct conversion of microalgae biomass into biodiesel, ie without having to convert or extract oil from biomass, to avoid unnecessary steps in the process, therefore increasing energetic and productivity efficiency.

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7. RESPONSIBILITY NOTICE

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