COBEM 2021 26th International Congress of Mechanical Engineering
November 22-26, 2021, Virtual Congress, Brazil

COB-2021-0302
COMPARISON OF LIPID EXTRACTION USING PURE HEXANE AND A MIXTURE OF HEXANE AND ETHANOL AS EXTRACTION SOLVENTS FOR MICROALGAE

Heloísa Roberto Pinheiro da Silva
Wellington Balmant
Federal University of Paraná (UFPR) 81531-980
helo.rps@gmail.com, wbalmant@gmail.com

José Viriato Coelho Vargas
Federal University of Paraná (UFPR) 81531-980
vargasjvcv@gmail.com

Abstract. *Due to the global need for cleaner and more renewable energy, the search for new sources is growing; among them are microalgae, single-celled microorganisms that have a high content of lipids and carbohydrates in the constitution of their biomass. These components can be used as raw material in biofuel production processes, thus the biodiesel is the most common currently used. The biodiesel is obtained through a transesterification process, a reaction that requires oil as a reagent, in addition to alcohol and a catalyst. As a result, the lipid content extraction of the microalgae is shown to be a decisive step for the analysis of the process viability. It depends on several factors to be analyzed, in the midst of them: the chosen method, extraction yield, the time required and the size of the sample to be extracted. Several techniques have been used for the oil extraction such as extraction with a supercritical fluid, osmotic shock and solvent extraction. Despite those different methodologies, the solvent extraction using the hot method is one of the most used industrially, due to its simplicity and relative low costs of solvents. In face of above, this study discusses an experimental method developed for the extraction of oil from microalgae using solvent in a hot process with agitation. It was evaluated two different solvents, one using pure hexane and other with a mixture of organic solvents (70%, hexane and 30% ethanol). The experimental results obtained proving the better extraction yield for the solvent mixture than that verified for the pure solvent. It showed that there is a difference in behavior of the separate components, in its pure form, showing a synergy with the extraction in mixture.*

Keywords: *microalgae, lipid extraction, extraction solvents.*

1. INTRODUCTION

The need to find new forms of energy to replace fossil fuels is increasing the world demand for cleaner and renewable options, such as biofuels. Thus, the search for different raw materials increases in the same proportion. Among them are microalgae, a green source option with less CO_2 generation, and their biochemical constitution can be improved according to the cultivation conditions. This characteristic directly affects the quantity and quality of biofuels, since there is the possibility of raising some constituents, such as the content of lipids and carbohydrates, which can be used in the production of biofuels. In addition, other components can be found in microalgae, some of them with high added value, such as pigments, antioxidants, vitamins, minerals and carotenoids (ANANTHI et al., 2021 and DERNER et al., 2006). Another advantage in the process is the sustainable production; the microalgae can be used in wastewater treatments to remove ammonia, phosphorus and heavy metals (HUSSAIN et al., 2021).

Regarding the biofuel's raw materials, the microalgae compared to crops have advantages such as the high cell division rate and enable a faster biomass production in a short period. Another attractive factor is the smaller area required for cultivation when compared to the square meter required for biodiesel production using soybeans. While the last need around 18 m^2 for producing 1 kg of biofuel, a microalgae with a low lipid content (30% of its biomass) requires 0.2 m^2 to produce the same fuel amount (MATA et al., 2010)

A major limiting factor for this clean source of energy is the cost of production, as it is still high when compared to fossil fuels (Sun et al., 2019). Therefore, it is essential to master all production processes and especially over the stage that requires a lot of energy, the extraction. Due to the complexity of the matrix, extraction is necessary to separate the lipid fraction, where the main challenge is to remove the highest lipid content from microalgae biomass. Several techniques have been used for the oil extraction such as mechanical pressing, enzymatic extractions, homogenization, solvent extraction, extraction with a supercritical fluid, osmotic shock, and ultrasound extraction (CHISTI, 2008).

Solvent extraction techniques can be established with or without heating. In cold extraction techniques, the lipids are extracted without heating, whereas in hot extraction it is obtained by contacting the organic material with a boiling solvent. Despite the different methodologies in the literature, the hot extraction is among the most used due to its great efficiency to vegetable and animal matrix (LUQUE and GARCÍA, 1998). In this methodology is essential to evaluate the process variables, such as the nature and polarity of the solvent (CECCHI, 2003) to improve the efficiency of the process using different biomass.

Regarding the efficiency of extractions using solvents, Mubarak et al., (2015) in a bibliographic study compared the influences using different solvents for Soxhlet extraction of lipids where a higher lipid yield was performed using ethanol, dichloromethane, and n-hexane as a solvent, respectively. The influence of the lipid extraction solvent mix was evaluated by Escorsim et al. (2018) where the lipids yield with different solvents and their mixtures was used for oil extraction from the microalgae *Tetradesmus obliquus* through different ethanol and hexane mixtures containing ratios 1:1, 2:1 and 1:2 (vol/vol). The solvent mixtures resulted in a better yield than the pure solvents being ethanol and hexane 1:2 ratio showed the best efficiency.

The research sought to verify the difference between the extraction behavior for microalgae oil using a pure hexane, compared to the extraction using a mixture of solvents, 70% hexane and 30% ethanol (v/v).

2. METHODOLOGY

The Self-sustainable Energy Research and Development Center (NPDEAS), located at the Federal University of Paraná, is responsible for cultivating microalgae. The activities involve the steps of cultivation, harvesting, biomass processing, lipid extraction and its transesterification. It all starts with the cultivation of microorganisms on a laboratory scale for pre-inoculum production. Gradually, production is scaled up until cultivation is established in photobioreactors (FBR). The microalgae used in the extraction obtained after 15 days of cultivation in photobioreactors. After cultivation, the microalgae biomass was pre-flocculated, eliminating more than 90% of the water present in the culture medium. Then, the pre-flocculated material is subjected to centrifugation, obtaining a mass with a moisture content of approximately 70%. This material is dried in a temperature-controlled oven with running air to a moisture content of less than 10%. Dry biomass was extracted to obtain the necessary experimental data.

To obtain the experimental data of extraction, the steps described in the flowchart of Figure 1 were followed.

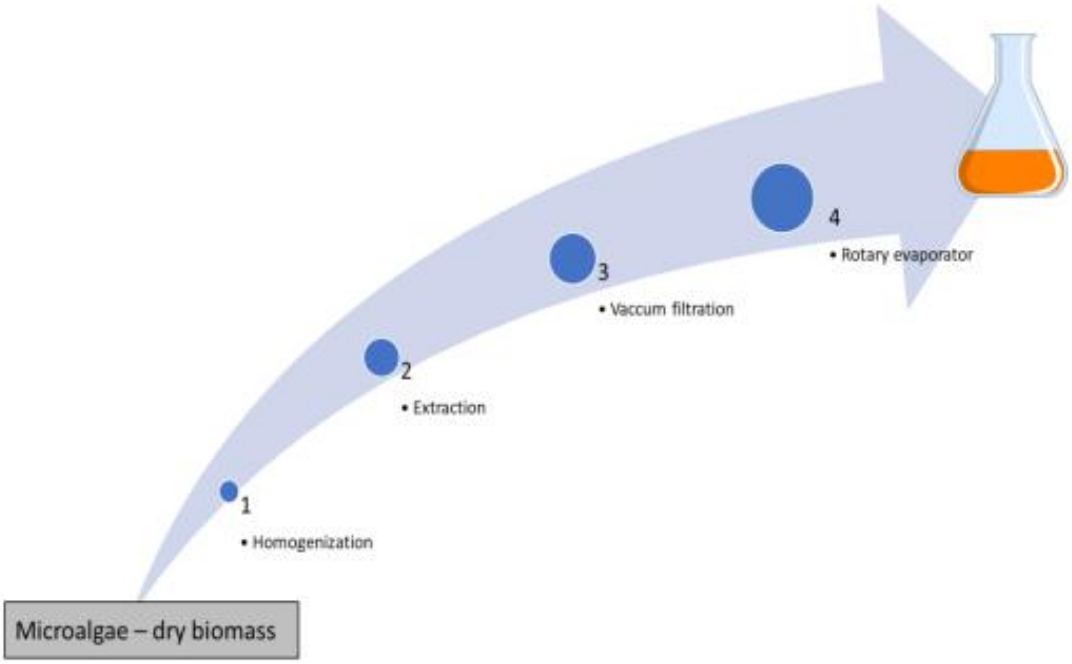

Figure 1. Flow chart of the experimental procedure.

As we can see with Figure 1, the experimental procedure starts with step 1, which consists of passing the biomass through a coffee mill, later in step 2 the microalgae oil extraction process occurs through hot extraction. In step 3 a vacuum filtration is carried out, in which the collected material is then separated from its extraction solvents through step 4 of rotary evaporator. In this way, the desired lipid content is obtained. All these unit operations for the experimental procedure will be further detailed below.

2.1 Experimental procedure

The biomass was processed in coffee mill and then passes through a sieve. This step consists of a pre-treatment, generating a more homogeneous sample, as can be seen from the Figure 2. This procedure enables an improvement in the extraction yield, as it increases the contact surface between the sample and the extraction solvent, through the greater porosity obtained.

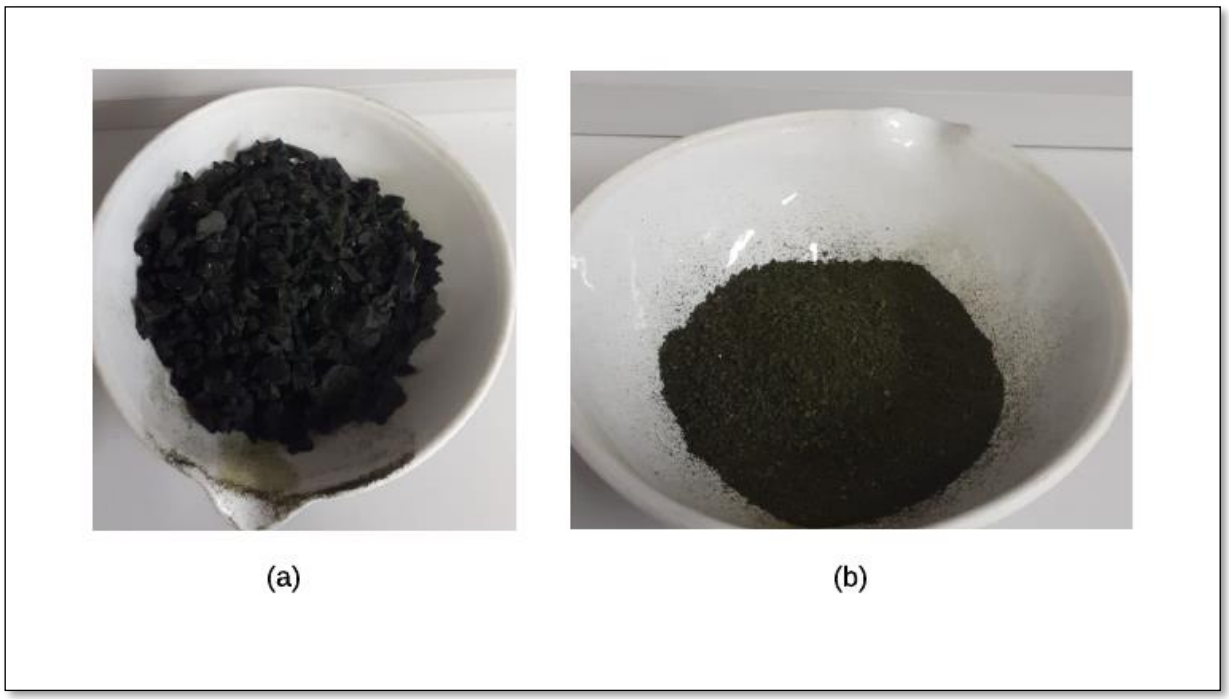

Figure 2. Aspect of dry microalgae (a) before the homogenization process and (b) after the homogenization stage.

It is possible to verify through the Figure 2 the aspect of the biomass granulometry before the homogenization step (a) and after (b). From there, the biomass is ready for the next phase.

With the microalgae prepared, the first step consists of the extraction step, which was performed using an experimental apparatus. It is characterized by an extraction with stirring of the mixture, obtained through a magnetic stirrer with heating adjusted to a temperature of 60 ºC, a Graham glass condenser and a system of water cooling. Each extraction uses 50 grams of dry microalgae biomass for 250 ml of solvent.

Two different extraction conditions were used. For the first, the extraction solvent was in the proportion of 30:70 (% v/v) using ethanol 99.5% (Neon®) and hexane P.A. (Synth®), while the second condition was performed using pure hexane. The retained time for each extraction with the solvent mixture varied between 15, 30, 45, 60, 75, 120 and 180 minutes. For the extraction points with pure hexane the times were 30, 45, 60 and 120 minutes. All experimental tests were executed in triplicate.

After the extractions are carried out at the pre-determined times, the mixture is filtered in an apparatus consisting of a Kitasato coupled to a vacuum pump. The filtered liquid phase is sent to a rotary evaporator. The process involves evaporation and condensation, separating the solvent from the lipid of interest and using water as a refrigerant. The mixture from the filtration step is retained in a 25 ml glass flask and remains contained in the heating bath between temperatures of 30 and 40 ºC for the separation of hexane. For the separation of ethanol, the temperature of the heating bath varies between 60 and 70 ºC. The centrifugal pump of the ejector is activated providing a pressure of approximately 400 mmHg and the glass flask operates at rotations of 120 rpm.

2.2 Determination of extracted lipid mass

The glass flask coupled to the rotary evaporator was previously weighed. At the end of the operations, the bottles containing the extracted oil were sent to an air-circulating oven at 60°C to ensure that all the remaining solvent was vaporized. Then, the flask containing the lipid content is weighed. The extracted lipid mass for each extraction time is obtained through Equation 1:

$$\boldsymbol{Mf = Ma - Mb} \tag{1}$$

$\boldsymbol{Mf}$ - is the final mass of extracted lipid content (g);
$\boldsymbol{Ma}$ - is mass of the flask after it leaves the air-circulating oven (g);
$\boldsymbol{Mb}$ - is the mass of the empty flask (g).

2.3 Results and discussions

The values of extracted oil were collected as described above and through these experimental data it was possible to obtain the results in the graph in the Figure 3:

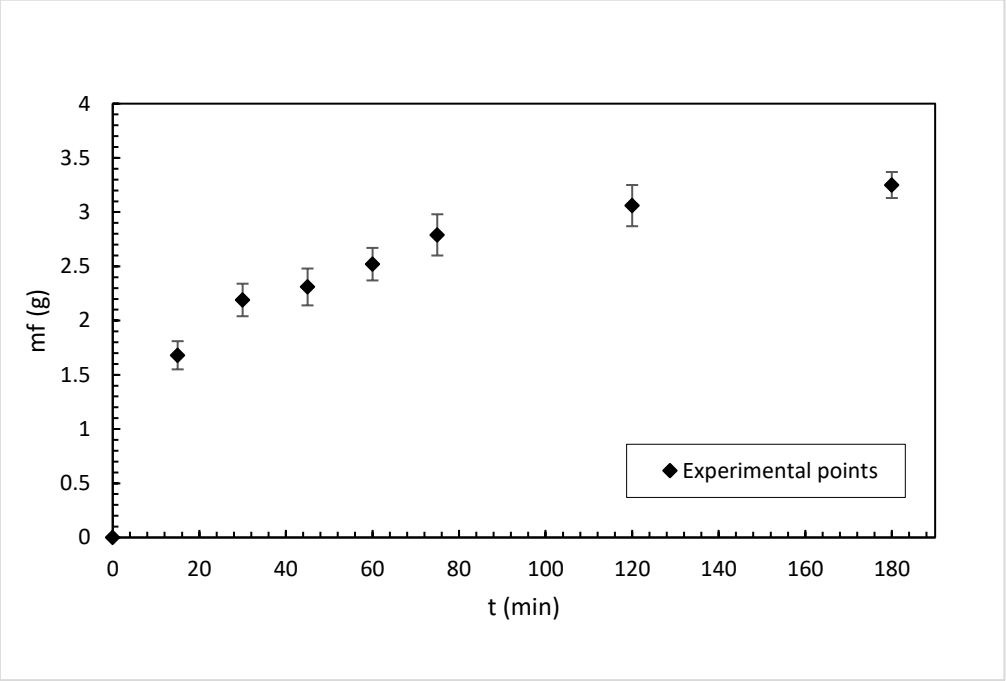

Figure 3. Experimental results of the extracted lipids mass using the hexane-ethanol solvents mixture.

It was possible to verify through the results presented in Figure 3 that the mass of oil extracted in the period of 180 minutes was 3.25 g. Comparing with the previous extraction points, it is noticed that the mass variation is decreasing for the last extraction points. As can be seen between points of 60 minutes and 75 minutes, in which there is a 10% increase in the extracted mass, which was also observed between points of 75 minutes and 120 minutes. As for the last two points (120 e 180 minutes) there is an increase of only 6%. Based on the above, it is possible to verify that it approaches the equilibrium state, since the increase in extracted mass decreased over a longer period of time, as the previous interval was 45 minutes against 60 minutes between the last two extractions.

The values of extracted oil were collected using pure hexane as solvent. Through these experimental data it was possible to obtain the results presented in the graph in the Figure 4.

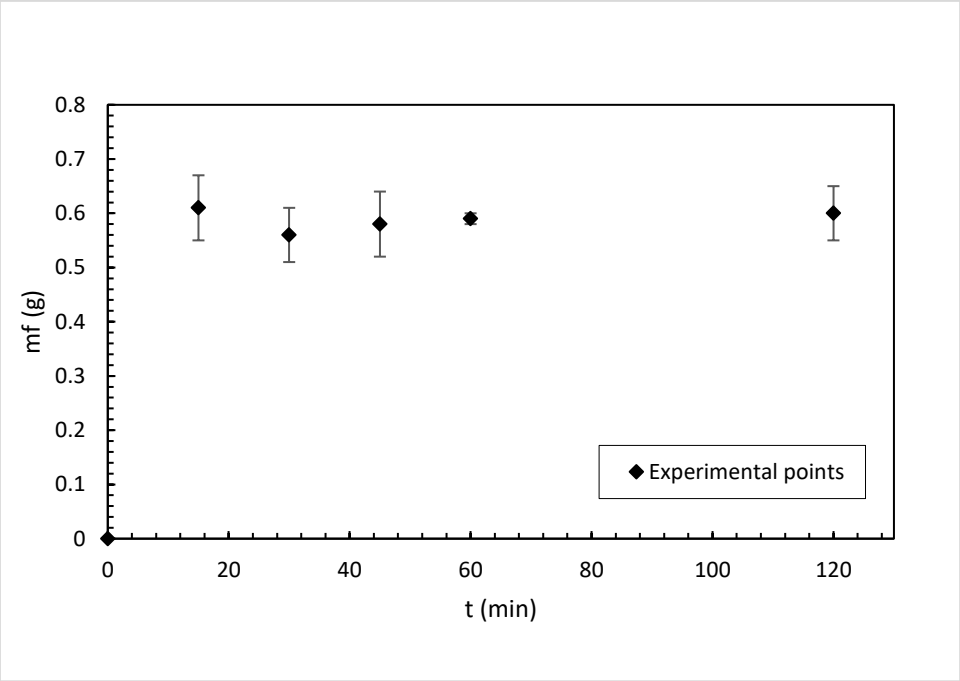

Figure 4. Experimental results of extracted lipids mass using pure hexane

As can be seen from the Figure 4, this experimental condition was carried out up to a time of 120 minutes. It was possible to reach an oil mass of 0.62 grams, 80% smaller than the mass extracted at the same time for the solvent mixture. This behavior was already expected and was also verified in the work by Escorsim et al. (2018). For the extraction with pure hexane, it can be noted that the values stabilized in the first minutes, since if the average standard deviations are considered, the difference between the points is practically nil.

As it was possible to revise with the experimental results, proving that the best extraction yield occurs for the solvent mixture than the one verified for the pure solvent. Comparing the final extracted masses, it was reached a 5.4 times greater extraction for the mixture. Proving that there is a difference in the behavior of the separate components, in their pure form, showing synergy with the mixed extraction.

3. CONCLUSIONS

From the objectives proposed in the work, this research carried out the collection of experimental data in the laboratory for extraction with pure hexane and a mixture of organic solvents (70%, hexane and 30% ethanol). The experimental system set up proved to be reliable, considering the standard deviations of the data collected (not exceeding 18% for the mixture of solvates and 12% for pure hexane) and considering the agreement with the values presented in the literature.

4. ACKNOWLEDGEMENTS

To the Brazilian National Council of Scientific and Technological Development, CNPq (projects 407198/2013-0, 403560/2013-6, 407204/2013-0, 430986/2016-5, 443823/2018-9, 313646/2020-1, 310708/2017-6, 308460/2020-0 and 446787/2020-5), CAPES, Ministry of Education, Brazil (projects 062/14 and CAPES-PRINT-UFPR-88881.311981/2018-01), and Araucaria Foundation of Parana, Brazil (project 115/2018, no. 50.579 – PRONEX).

5. REFERENCES

Ananthi, V., Brindhadevi, K., Pugazhendhi, A., & Arun, A. (2021). Impact of abiotic factors on biodiesel production by microalgae. *Fuel*, *284*, 118962.

Chisti, Yusuf. "Biodiesel from microalgae beats bioethanol." *Trends in biotechnology* 26.3 (2008): 126-131.

Cecchi, Heloísa Máscia. *Fundamentos teóricos e práticos em análise de alimentos*. Editora da UNICAMP, 2003.

De Castro, MD Luque, and L. E. Garcıa-Ayuso. "Soxhlet extraction of solid materials: an outdated technique with a promising innovative future." *Analytica chimica acta* 369.1-2 (1998): 1-10.

Derner, R. B., Ohse, S., Villela, M., Carvalho, S. M. D., & Fett, R. (2006). Microalgas, produtos e aplicações. *Ciência Rural*, *36*(6), 1959-196.

Escorsim, Alexis M., et al. "Extraction of Acutodesmus obliquus lipids using a mixture of ethanol and hexane as solvent." *Biomass and Bioenergy* 108 (2018): 470-478.

Hussain, Fida, et al. "Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and bio-fertilizer production. A review." *Renewable and Sustainable Energy Reviews* 137 (2021): 110603.

Mubarak, M., A. Shaija, and T. V. Suchithra. "A review on the extraction of lipid from microalgae for biodiesel production." *Algal Research* 7 (2015): 117-123.

Soxhlet, Franz von. Die gewichtsanalytische bestimmung des milchfettes. Polytechnisches J, v. 232, p. 461-465, 1879.

Sun, Jun, et al. "Microalgae biodiesel production in China: a preliminary economic analysis." *Renewable and Sustainable Energy Reviews* 104 (2019): 296-306.

6. RESPONSIBILITY NOTICE

The authors are the only responsible for the printed material included in this paper.