RECOVERY OF MICROALGAL BIOMASS IN PHOTOBIOREACTORS FOR BIODIESEL PRODUCTION

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Abstract. *The interest in using biolipids of microalgae as feedstock for biodiesel production has increased currently, due to algae have nutritional needs relatively simple, its production does not compete with the food production, cultivation has no rotation, etc. Among the stages that involve the production of microalgae, recovery of the biomass from the medium, due to the small size of microalgae, can become tricky and expensive depending upon the desired efficiency and volumes involved. Although recovery by flocculation of the biomass be a viable procedure, depending the of flocculant and of the amount added to the cultivation, these can damage cells causing loss of cellular substances of interest, as lipids. In order to evaluate the efficiency of the biomass recovery the microalgae Scenedesmus sp. by flocculation an cultivation of microalgae was flocculated with ferric chloride ($FeCl_3$) and sodium hydroxide, (NaOH), compared with a process of cells recovering by centrifugation at 7000 rpm, in terms of cells residual present in the clarified medium and the amount of lipids present in the recovered biomass. The flocculation process with $FeCl_3$ with concentration in sample of 0.2 $mmol^{-1}$ showed an efficiency of 96.79% recovery of cells the medium, while the process of flocculation with NaOH with concentration in sample of 8 $mmol.L^{-1}$ allowed recovering 93.50% and the centrifugation 91.71% of the cells. To the comparing of lipid content present in the biomass recovered by flocculation with the recovered by centrifugation, the flocculation with NaOH 8 $mmol.L^{-1}$ showed significant variations unlike to the flocculation with $FeCl_3$ 0.2 $mmol.L^{-1}$.*

Keywords: *Biodiesel, microalgae, flocculation, lipids,* Scenedesmus sp..

1. INTRODUCTION

The search for substitute sources of energy from fossil fuels by biodiesel has increased, because biodiesel has technical advantages over diesel as lower flash point, reducing the release of sulfur and carbon monoxide, contains no aromatic hydrocarbons and other chemicals harmful to health and the environment, it is renewable, biodegradable, can be obtained in a sustainable manner, etc. (Beltrão, 2008; Huang, *et. al.*, 2010).

When used vegetable oil from oil plants in the biodiesel production, the cost of raw material varies around of 70-85% of the total production cost (Meng *et al.*, 2009). The use of grain for the production of biodiesel tends to increase the cost of the raw material always, due to generated competition with the food production.

However, the higher plants can be replaced by microalgae, which are microscopic organisms, unicellular autotrophic found in both water as in soil. Among the metabolic products of microalgae are proteins, carbohydrates, nucleic acids, vitamins and lipids (Derner *et al.*, 2006; Mallick *et al.*, 2011; Spolaore *et al.*, 2006). Algae are photosynthetic organisms that have cellular growth the faster, they can consume about two tons of CO_2 to produce one ton of biomass (Mallick *et al.*, 2011).

The advantages use of microalgae as raw material for the biodiesel production consisting in: uniformity of the organism, contrary to the plants that presents leaves, stems, fruits, seeds and roots that must be separated before the extraction of fatty acids. The cultivation requires less monetary investment for its production, use of land unsuitable for agriculture, when grown in photobioreactor compact, allows reduces the areas of crops and also participate in the capture of carbon dioxide from a pollutant source. As amount of stored lipids by microalgae is superior to plant oilseeds, reduces the area of investment and cost as a feedstock. Also the residual biomass of the lipids extraction of microalgae can be used in other productive processes for get products with high added value (pharmaceutical, cosmetics, food, etc.) well as, biogas generate in digester (Hundt; Reddy, 2011; Chisti, 2007).

Of the steps involving the production of microalgae, the cells separation of the medium, due that density of the cells is similar to water, the dilution of crops and the size of the microalgae (3-30 mm of diameter), may represent a step laborious, long or even expensive, depending upon the desired efficiency and volumes involved. And the cost of recovery of biomass can represent 20-30% of the total biomass production (González-Fernández, 2012; Lourenço, 2006; Grima *et al*, 2003).

The recovery of the biomass consists of removal of the microalgae of the culture medium, can be performed by physical systems. The physical systems traditional are the centrifugation, filtration and sedimentation, in which there is a considerable time or energy expenditure. These systems may be associated with a system physic – chemical, traditionally coagulation / flocculation, in order to increase the rate of removal of the solid particles, reduce the time and energy expenditure.

The flocculation is performed to increase the effective size of the particles and increase the rate of sedimentation, well as, centrifugation and filtration (Grima *et al*, 2003). Is a process viable for recovering biomass, however, depending on the chemical characteristics of the flocculant agent, this can damage the cells, causing loss of cellular substances of interest (Lee *et al*, 2009).

The cultivation of microalgae in photobioreactor of high rate, aimed at producing biodiesel, requires a biomass retrieval system that enables fast and efficient recovery of cells without occurs loss of lipid content of the same. Thus, this study sought evaluates the efficiency of the flocculation with ferric chloride and sodium hydroxide as recovery process of biomass, aimed obtaining lipids.

2. METHODOLOGY

The cultivation of microalgae *Scenedesmus* sp. used in flocculation tests, was carried on the NPDEAS in culture medium *Guillard "F/2"* modified Lourenço (2006), without photoperiod in *Elenmeyer* balloon of 2 liters, with aeration of 0.17 $L.s^{-1}$ per liter of culture and temperature of 17 ± 0.2 °C by nine days.

The recovery process of the culture medium by flocculation / sedimentation was set from tests, in duplicates, with two different flocculants, ferric chloride ($FeCl_3$) and sodium hydroxide (NaOH), keeping fixed the time and the stirring conditions in the following ranges: rapid stirring of 500 rpm for 5 seconds, slow stirring of 250 rpm for 5 minutes and 10 minutes of sedimentation. The amount of flocculant added to the samples of cultures was changed, as shown the Tab. 1, in order to define the most efficient concentration.

Table 1. Volume of flocculant and concentration in sample

Flocculant	Concentração – $mmol.L^{-1}$
NaOH	4
	6
	8
$FeCl_3$	0.1
	0.2
	0.3

Centrifugation was used as reference biomass recovery process, in order to compare the efficiency of the flocculant analyzed. Therefore, samples of the same cultivation of microalgae were centrifuged for 15 minutes at 7000 rpm and maintaining the temperature fixed at 4 °C (Soares, 2010).

To define the concentration of flocculant optimal for recovering biomass, were compared absorbance values, reading held in wavelength of 540 nm, of the clarified of the samples flocculated with the value obtained in the clarified of the sample centrifuged. The efficiency of each process was defined, based on the absorbance of the culture to be flocculated, according to the equation:

$$E(\%) = \left(1 - \frac{A_1}{A_2}\right).100 \quad (1)$$

where:
A_1 – absorbance of clarified medium;
A_2 – absorbance of cultivation;

The most effective process (has capacity to recover the biomass and does not damage the cells) was determined from the lipid content found in the recovered biomass, in each of the flocculation processes, compared with results obtained in the process of centrifugation. The lipid content was determined using the method of *Bligh & Dyer* adapted from Rodriguez *et al*. (2007).

3. RESULTS AND DISCUSSION

To set the process of recovering the biomass microalgae *Scenedesmus* sp. by flocculation / sedimentation with high efficiency, one culture was used to conduct the tests of centrifugation, flocculation with $FeCl_3$ and NaOH.

Figure 1 shows the absorbance value and pH of the medium clarified with NaOH for each concentration of flocculant utilized, as flocculation process with NaOH occurs by means of increasing the pH of the solution, to obtain a high removal of cell culture was necessary increasing the pH of the sample in 3.43, due to the initial pH of the sample be alkaline (8.37). It is observed that the flocculant concentration in the sample equal to 8 $mmol.L^{-1}$ (Fig. 1 and 2) showed significant recovery of the cells when compared with sample of 4 and 6 mmol L^{-1}.

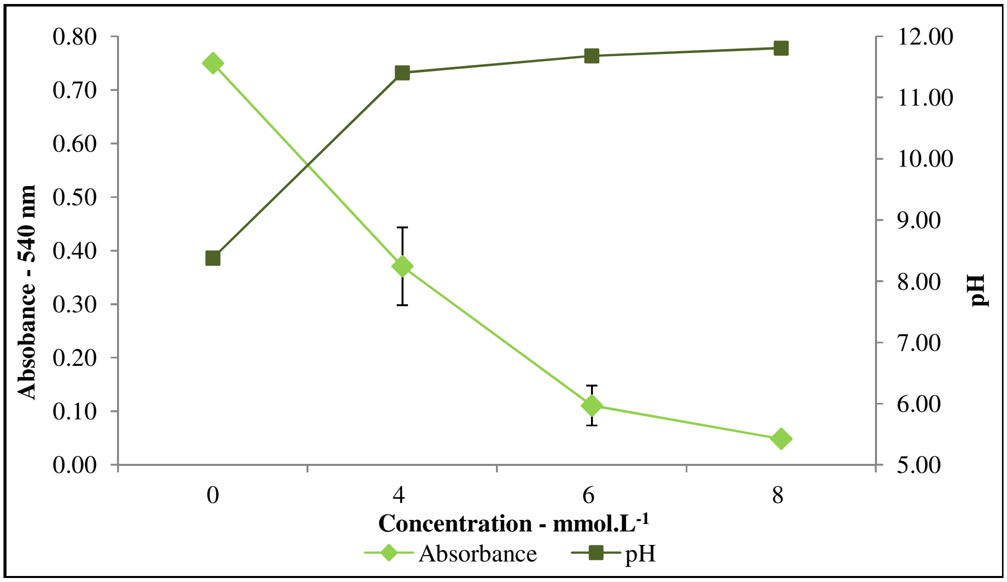

Figure 1. Absorbance variation of clarified medium in function of concentration increase of NaOH in solution.

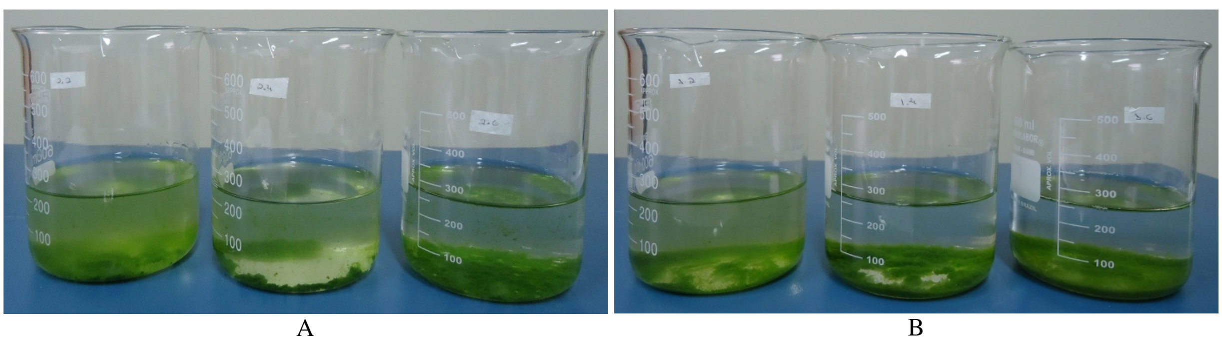

Figure 2. Recovery of cells by flocculation – A: NaOH – 4; 6 and 8 $mmol.L^{-1}$; B: $FeCl_3$ – 0.1; 0.2 and 0.3 $mmol.L^{-1}$.

As the process of recovery with $FeCl_3$ cells by flocculation occurs by charge neutralization, the pH variation in the clarified medium (Fig. 3) is small, ± 1.51, and in function of the $FeCl_3$ be an acid salt. The greatest recovery of cells was observed in the sample with a concentration of flocculant equal to 0.2 $mmol.L^{-1}$ of $FeCl_3$ (Fig. 2 and 3), because, the sample with 0.3 $mmol.L^{-1}$ showed an increase in absorbance value, indicating the loss of ability to recover biomass or excess iron in solution.

Figure 4 shows the effectiveness, of each evaluated process, to clarify the cultivation of microalgae and Table 2 shows the percentage recovery biomass, calculated from the absorbance of the clarified medium, both processes show efficiency greater than 90%, but with FeCl3 flocculation efficiency was 96.8%. According to Kim et al. (2011) the best recovery of cells from cultures of *Scenedesmus* sp. occurs with combination of cationic coagulant and the achieved maximum efficiency was of 91.3% for flocculation with calcium chloride and ferric chloride at a concentration of 9.6 mmol L^{-1} and 0.22 mmol L^{-1}, respectively.

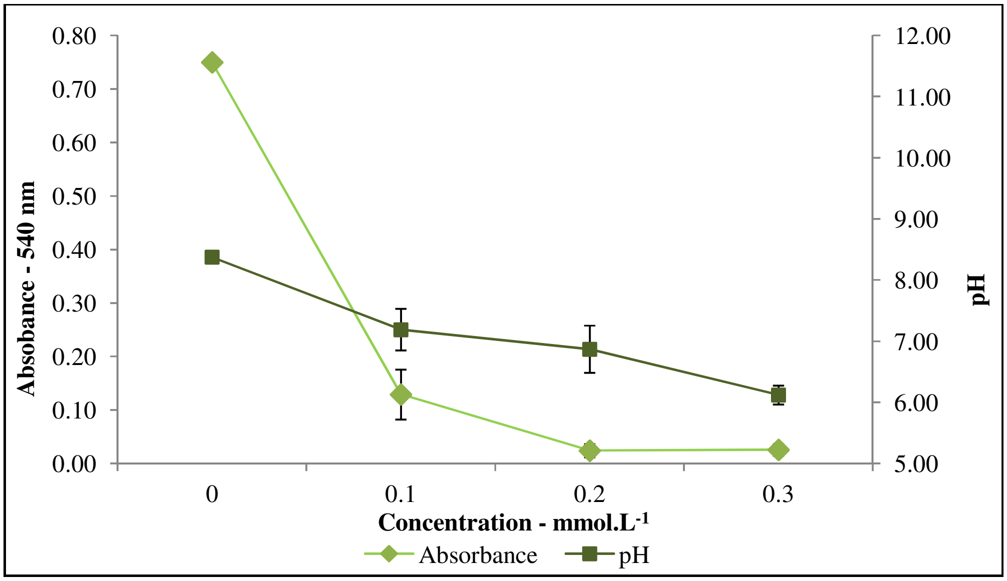

Figure 3. Absorbance variation of clarified medium in function of concentration increase of $Fecl_3$ in solution.

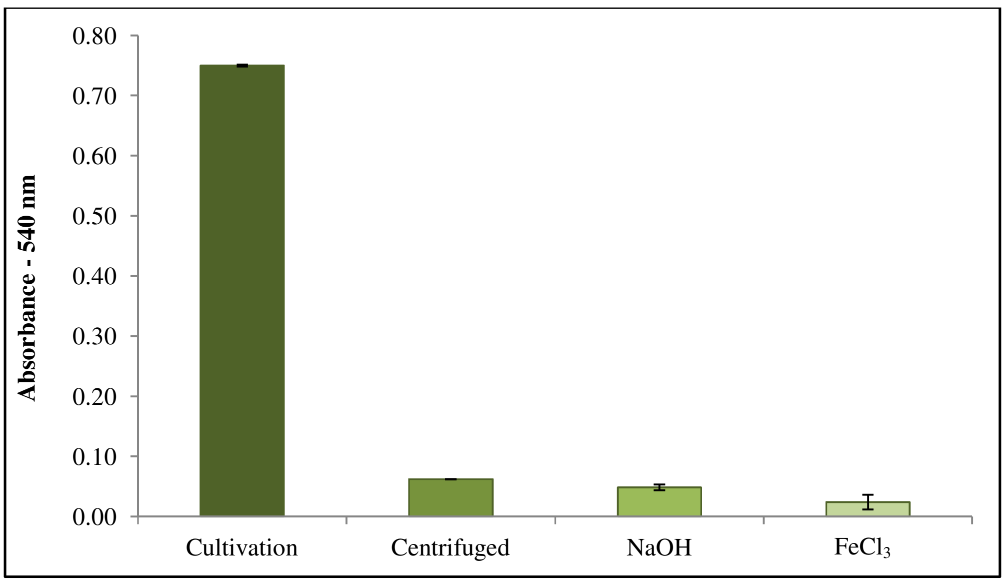

Figure 4. Efficiency in clarification of the cultivation medium by centrifugation, flocculation with $FeCl_3$ – 0.2 $mmol.L^{-1}$ and with NaOH – 8 $mmol.L^{-1}$.

Table 2. Efficiency of the recovery of biomass.

Process	%
Centrifugation	91.7
Flocculation with NaOH – 8 $mmol.L^{-1}$ / sedimentation	93.5
Flocculation with $FeCl_3$. – 0.2 $mmol.L^{-1}$ / sedimentation	96.8

Efficacy the evaluation of the flocculation processes (Fig. 5) with NaOH and $FeCl_3$ allowed identified a loss of lipids in the recovered biomass of 20 and 3%, respectively, compared to the lipid content found in the biomass recovered by centrifugation.

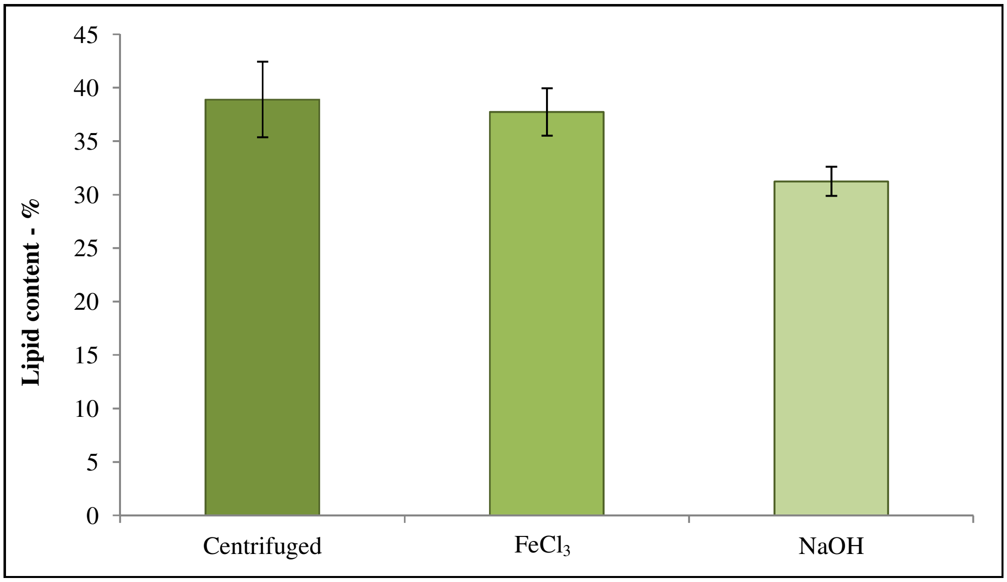

Figure 5. Lipid content in biomass recovered by centrifugation, flocculation with $FeCl_3$ 0.2 mmol.L^{-1} and with NaOH 8 mmol.L^{-1}

Based on the outcome of the Student's t-test (Tab. 3) observed that there were significant variations between the results of total lipids of the biomass samples recovered by flocculation with NaOH and centrifuged, indicating that sodium hydroxide is aggressive to cells, unlike the ferric chloride, whose results of total lipids did not show significant variations when compared with the results of the samples centrifuged. Allowing said that the use of NaOH as flocculant agent is not feasible to recovery cells from of the microalgae *Scenedesmus* sp. to obtain lipids.

Table 3 – Comparison between centrifugation and flocculation

	Student's t - test
Centrifugation – $FeCl_3$	0,461 ($p > 0,05$)
Centrifugation – NaOH	0,002 ($p < 0,05$)

4. CONCLUSION

When the lipids from microalgae are used to produce biodiesel, the process of flocculation becomes more interesting than the centrifugation, because the expenditure of energy to this process will be considerably greater than that spent by flocculation. The evaluation of the system flocculation / sedimentation, conducted in this research, showed to be effective as a process of recovering biomass crops microalgae *Scenedesmus* sp., allowing remove more cells than the centrifugation. As the biomass recovered with ferric chloride not presents significant variation when compared to the centrifugation in terms of lipid, it can be stated that the ferric chloride has the potential to recover the biomass from the microalgae *Scenedesmus* sp. without losses of the lipid content of the cells.

5. ACKNOWLEDGEMENTS

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7. RESPONSIBILITY NOTICE

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