# THE HARVESTING OF HIGH LIPID CONTENT MICROALGAE BIOMASS THROUGH A FLOCCULATION STRATEGY

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**ABSTRACT**

Different flocculants were evaluated for the flocculation of microalgae biomass of *Acutodesmus obliquus*. Flocculation was tested with $FeCl_3$ and NaOH at different concentrations and compared to a sample centrifuged at 7000 rpm. The evaluated parameters were absorbance (540 nm) in the clarified medium, and lipids concentration. For $FeCl_3$ (0.2 mmol $L^{-1}$) as flocculant, efficiency was 96.8%, and with NaOH (8 mmol $L^{-1}$) 93.5%. Centrifugation efficiency was lower than with either flocculants: 91.7%. However, NaOH flocculation reduced lipid content, which did not occur with $FeCl_3$. Flocculation efficiency was affected by salt concentration, reducing efficiency by 79% due to increased nutrient concentration (9 mL $L^{-1}$).

**Keywords:** biodiesel; microalgae; flocculation; lipids; *Acutodesmus obliquus*

## NOMENCLATURE

| | |
|---|---|
| $A_1$ | absorbance of the clarified medium after flocculation |
| $A_2$ | absorbance of the cultivation medium before flocculation |
| C | molar concentration, mmol $L^{-1}$ |
| S | standard deviation |
| Z | error propagation |

### Greek symbols

| | |
|---|---|
| $\alpha$ | absorbance |
| $\eta$ | efficiency of biomass recovery |
| $\lambda$ | lipid content |

## INTRODUCTION

The number of studies seeking the replacement of fossil fuels for biodiesel has increased due to the technical advantages biodiesel has over diesel, such as lower flash point, reduction of sulfur and carbon monoxide in the emissions, absence of aromatic hydrocarbons and other chemicals harmful to health and the environment, renewability, biodegradability, sustainability of the production process, etc (Beltrão and Oliveira, 2010; Huang et al., 2010). When vegetable oil is used in the biodiesel production process, the cost of raw material corresponds to approximately 70-85% of the total production cost (Meng et al., 2009). The use of grains for the production of biodiesel tends to increase the cost of the raw material, because of the competition with the food industry. However, higher plants oil could be replaced by microalgae oil (Mallick et al., 2012).

The main advantage of using microalgae as raw material for biodiesel production consists of the uniformity of the organism, differently from plants that have leaves, stems, fruits, seeds and roots that must be separated before the extraction of fatty acids. In addition to that, other microalgae positive aspects in comparison to crops could be cited: lower

investment cost for cultivation; usage of land unsuitable for agriculture; lower demand for land, higher amount of stored lipids compared to plant oilseeds; and possibility to capture carbon dioxide from a pollutant source. Likewise, other valuable products, such as biogas, could be obtained from the residual biomass after lipid extraction (Hundt and Reddy, 2011; Chisti et al., 2007).

Biomass separation from the culture medium is challenging due to the low concentration of microalgae in the medium, the cell having about the same density as that of water and the small cell diameter (3-30 μm). Therefore, it may represent a laborious, long and expensive step in the biodiesel production process from microalgae, according to the desired efficiency and volume involved. The separation cost may represent 20-30% of total biomass production cost (González-Fernández et al., 2012; Grima et al., 2003; Lourenço, 2006; Uduman et al., 2010).

Biomass separation may be achieved through centrifugation, filtration or sedimentation. Centrifugation may not be feasible to processes with high volumes of medium when the final products have low added value, due to the high energy cost (Lee et al., 2009; Pienkos et al., 2009; Schlesinger, 2012). In a large scale system, vacuum or pressure filters are satisfactory to recover microalgae biomass containing large or filamentous cells such as *Coelastrum proboscideum* and *Spirulina platensis*, but fail to recover small cells from *Acutodesmus*, *Scenedesmus*, *Dunaliella* and *Chlorella* genera, for example, which rapidly obstruct the membrane (Grima et al., 2003). Microalgae sedimentation is slow and, consequently, with reduced efficiency. Rapid sedimentation may occur during microalgae cultivation due to the grouping of cell colonies and changes in the medium characteristics allowing self-flocculation, however, the occurrence of these factors is unpredictable, thus, it is not possible to rely on them to increase the sedimentation rate in a process of biomass recovery. The rate of sedimentation, as well as filtration and centrifugation, may be increased by flocculation, which increases the effective size of the particles (Grima et al., 2003). Flocculation is an economically viable process, however, depending on the chemical characteristics of the flocculant agent, it may cause the rupture of the cells and, consequently, the loss of the metabolic product of interest (Lee et al., 2009). Also, the biomass recovery efficiency with flocculation may vary even when the same conditions are used.

Based on the state-of-the-art on the subject, it is reasonable to state that effective alternative flocculation strategies are still needed. Therefore, this study aimed to experimentally establish a flocculation process for the recovery of microalgae biomass of *Acutodesmus obliquus* with efficiency similar to centrifugation and without loss of intracellular lipids. The relationship between salt concentration in the cultivation medium and the flocculation efficiency was also investigated.

## MATERIALS AND METHODS

The experiments were performed with microalgae *Acutodesmus obliquus*, courtesy of the Integrated Group of Aquaculture of the Federal University of Paraná (GIA - UFPR). The microalgae was cultivated in Guillard "f/2" modified medium (Lourenço, 2006) in 2 L Erlenmeyer flasks, with aeration rate of 0.05 $L.s^{-1}$ of atmospheric air per liter of culture and without photoperiod. Two incubation conditions were used in order to have samples with different biomass concentrations: in condition A, microalgae was cultivated at 17 ± 0.2°C for nine days resulting in final biomass concentration of 0.28 ± 0.002 g $L^{-1}$, and in condition B, microalgae was cultivated at 22 ± 2ºC for four days, achieving 0.12 ± 0.002 g $L^{-1}$ of biomass.

### Microorganism and Cultivation Conditions

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### Flocculation Tests

The flocculation tests were performed with different flocculants, ferric chloride ($FeCl_3.6H_2O$) and sodium hydroxide (NaOH). For both flocculants, the same agitation conditions were used: rapid stirring of 500 rpm for 5 seconds, slow agitation of 250 rpm for 5 min and settling for 10 min. The absorbance ($\alpha$) of the samples was measured before and after flocculation. Absorbances were measured using a Shimadzu UV -1800 spectrophotometer, with operating wavelength ranging from 190 to 1100 nm with 1 nm resolution. The measurements were taken for a wavelength of 540.0 nm using a 1 cm-spectrophotometer cuvette. The efficiency of biomass recovery ($\eta$) was determined as follows:

$$\eta\ (\%) = \left(1 - \frac{A_1}{A_2}\right) \times 100 \qquad (1)$$

where $A_1$ is the absorbance of the clarified medium after flocculation and $A_2$ is the absorbance of the cultivation medium before flocculation. For comparison, a sample of the medium culture was centrifuged for 15 min at 7000 rpm and at 4°C and the resulting efficiency was compared to the efficiencies of the flocculation tests (Soares, 2010). The optimum concentration (C) for each flocculant was selected based on biomass recovery efficiencies measured when flocculating samples obtained in cultivation condition A.

### Flocculation Mechanism

The flocculation mechanism was investigating using samples obtained in cultivation condition B, in which biomass concentration is lower than in samples obtained in condition A. The samples were flocculated using NaOH and $FeCl_3$ at their optimum concentration of flocculant (previously determined for samples in condition A) and at five times their optimum.

### Effect of Salt Concentration in the Cultivation Medium on Flocculation Efficiency

In order to verify the influence of salt concentration in the medium on the efficiency of flocculation, three samples containing different salt concentrations were flocculated with the same concentration of $FeCl_3$. The following samples were used:

- Sample 1: obtained in cultivation condition B;
- Sample 2: obtained in cultivation condition B, added 5 mL $L^{-1}$ of the nutrients solutions of the modified Guillard "f/2" medium in sample after cultivation but before flocculation, and
- Sample 3: obtained in cultivation condition B, added 9 mL $L^{-1}$ of the nutrients solutions of the modified Guillard "f/2" medium in sample after cultivation but before flocculation.

### Analysis of Lipid Content

The lipid content (or mass fraction, w/w), λ (%), was analyzed in the recovered biomass of the centrifugation and the flocculation processes with the optimum concentration of each flocculant, using the adapted Bligh & Dyer method (Rodríguez et al., 2007).

### Statistical Analysis

All measurements results are expressed as mean values ± 2S (standard deviation), while the calculated values are expressed as mean values ± Z (error propagation). Statistical differences between experimental groups were assessed by analysis of variance (Student's t-test), with a significance level of 95%.

## RESULTS

### Efficiency of Flocculation

In order to identify an efficient process of flocculation for *A. obliquus*, the microalgae were cultivated for nine days (condition A) and samples of the homogeneous culture were used to conduct tests using centrifugation and flocculation with $FeCl_3$ and NaOH at different concentrations. After flocculation, the absorbance value and pH of the clarified medium were determined, for NaOH and $FeCl_3$, as shown in Figs. 1 and 2, respectively.

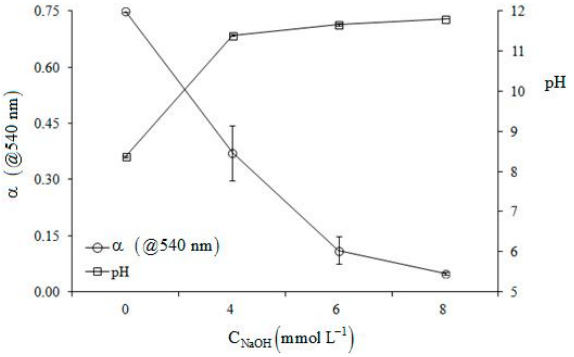


Figure 1. The pH and absorbance of clarified medium variation after flocculation of sample 1 with respect to NaOH concentration.

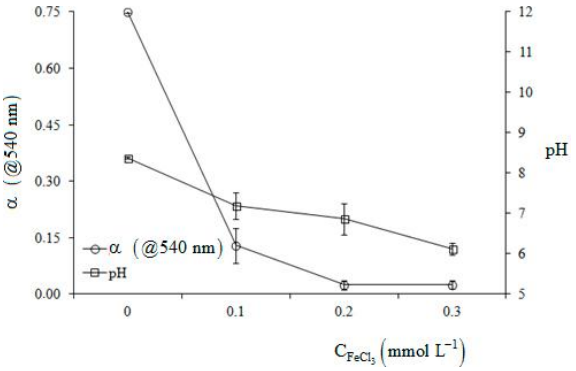


Figure 2. The pH and absorbance of clarified medium variation after flocculation of sample 1 with respect to $FeCl_3$ concentration.

The samples with NaOH concentration of 8 mmol $L^{-1}$ showed a significantly higher recovery when compared to the sample with 4 and 6 mmol $L^{-1}$. In order to obtain a high biomass removal from the cultivation medium, the pH of the samples varied from 8.38 to 11.81. When using ferric chloride, the highest cell recovery was obtained in the samples with $C_{FeCl_3} = 0.2$ mmol $L^{-1}$, while the sample with 0.3 mmol $L^{-1}$ a similar value for absorbance, indicating loss of ability to recover biomass or excess of iron in solution. In the flocculation tests with ferric chloride the pH of the clarified medium was reduced from 8.38 to 6.87, for 0.2 mmol $L^{-1}$, because the

solubilization of $FeCl_3$ in the sample formed hydrochloric acid.

Table 1 shows the biomass recovery efficiency (η) for the flocculation tests and for centrifugation, calculated with Eq. (1). The recovery efficiencies at $C_{NaOH} = 8$ mmol $L^{-1}$ and $C_{FeCl_3} = 0.2$ mmol $L^{-1}$, highlighted in Tab. 1, were higher than the efficiency with centrifugation.

Table 1. Efficiency of biomass recovery with centrifugation and flocculation at different concentrations of NaOH and $FeCl_3$.

| Process | Biomass Recovery Efficiency | |
|---|---|---|
| | Flocculant concentration, C (mmol L-1) | Efficiency, η |
| Centrifugation | - | 91.7% |
| Flocculation with NaOH | 4 | 50.6% |
| | 6 | 85.3% |
| | 8 | 93.5% |
| Flocculation with $FeCl_3$ | 0.1 | 82.8% |
| | 0.2 | 96.8% |
| | 0.3 | 96.6% |

## Flocculation Mechanism

For samples obtained in cultivation conditions B, which resulted in approximately half of the biomass concentration from condition A, the amount of flocculant necessary for maximum biomass recovery would be $C_{NaOH} = 4$ mmol $L^{-1}$ and $C_{FeCl_3} = 0.1$ mmol $L^{-1}$, based on the stoichiometric relation between cell concentration in the medium and the amount of flocculant necessary to remove it (Wyatt et al., 2012; Chen et al., 2013) and the optimum flocculant concentration determined for samples from condition A. However, the samples from condition B were flocculated with flocculant in excess, 2 and 12 times the concentration necessary, in order to identify the flocculation mechanism of NaOH and $FeCl_3$. The efficiency of cell recovery for flocculation with NaOH increased with the concentration of the flocculant in the sample, as shown in Fig. 3, unlike in the flocculation with $FeCl_3$, as shown in Fig. 4, in which the ability to recover the biomass decreases with a higher flocculant concentration.

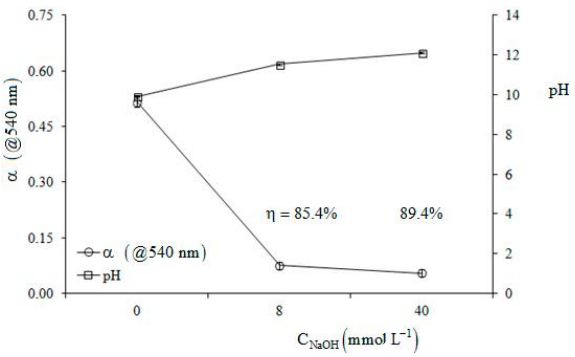


Figure 3. The pH and absorbance of clarified medium variation after flocculation of sample 2 with respect to NaOH concentration. Biomass recovery efficiencies are also shown in selected points.

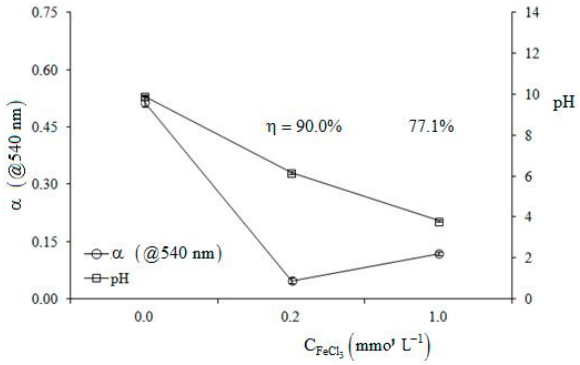


Figure 4. The pH and absorbance of clarified medium variation after flocculation of sample 2 with respect to $FeCl_3$ concentration. Biomass recovery efficiencies are also shown in selected points.

When comparing flocculation of condition A and B with the same amount of flocculant, $C_{NaOH} = 8$ mmol $L^{-1}$ and $C_{FeCl_3} = 0.2$ mmol $L^{-1}$, there was a reduction in efficiency from condition A to B for both flocculants (labels of Figs. 3 and 4), although biomass concentration is lower in condition B. This behavior cannot be attributed only to flocculant overdose, because efficiency is increased with overdose when NaOH is used as flocculant, as shown in Fig. 3. The reduction in efficiency from condition A to B may be caused by the higher concentration of salts present in sample B, since the cultivation time was lower, thus, microalgae consumed less nutrients that in condition A.

The flocculation process of colloidal substances in suspension due to the addition of electrolyte salts in solution can occur by three mechanisms: double-layer compression, adsorption and charge neutralization or enmeshment (Alcantara, 2010; Pavanelli, 2001; Letterman et al., 1999). In biomass recovery with ferric chloride the mechanism of flocculation is of adsorption and charge neutralization, in which charges are reversed with flocculant overdose, as shown in Fig. 4, and the flocs formed are smaller than in the enmeshment process, however, the amount of flocculant used is lower. When a trivalent cation is used as flocculant, its ability to neutralize the charges is approximately 100 times greater than that of a monovalent cation and 10 times greater than that of a bivalent cation, thus, the amount of $FeCl_3$ necessary is always less than the required for an electrolyte such as $FeSO_4$ (Schulze-Hardy rule) to recover cells of cultures with the same biomass concentration.

Based on the characteristics of the sodium hydroxide, hydrolysis, adsorption, high solubility in water (about 27 mol $L^{-1}$) and high alkalinity, the flocculation process with NaOH does not follow any of the three mechanisms described above. The

separation of cells from the medium occurs due to changes in the isoelectric point of the solution (Chatelier law) by increasing pH of the solution, which allows for the destabilization of cells and their precipitation. Thus, when the pH of the cultivation medium is alkaline, the use of alkaline flocculants with high solubility is not viable for biomass recovery, because the amount of flocculant required to destabilize the cells is high.

### Effect of Salt Concentration in the Cultivation Medium on Flocculation Efficienc

In order to verify the influence of salt concentration in the sample on the efficiency of flocculation, a defined amount of nutrients was added to the samples after cultivation in condition B but before flocculation, thus, each sample had the same biomass concentration, but different salinities. Sample 1 corresponds to the original sample obtained in cultivation condition B, meanwhile, 5 mL $L^{-1}$ and 9 mL $L^{-1}$ of the nutrients solution of the modified Guillard "f/2" medium were added to samples 2 and 3, respectively. All samples were flocculated with $FeCl_3$ at 0.2 mmol $L^{-1}$. Results show that, as the concentration of salts (nutrients) in the cultivation medium increased from samples 1 to 3, biomass recovery efficiency decreased, as shown in Fig. 5.

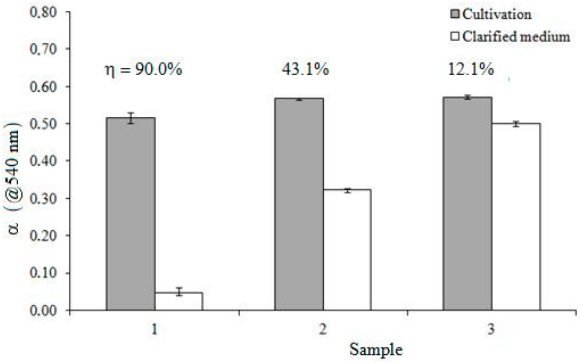


Figure 5. Variation of absorbance of samples 1 to 3 before and after flocculation with 0.2 mmol $L^{-1}$ of $FeCl_3$. Biomass recovery efficiencies are also shown.

### Lipid Content of Microalgae *A. obliquus* Recovered by Flocculation and Centrifugation

Lipid content of recovered biomass was analyzed in order to verify if the selected flocculants caused cell damage leading to loss of intracellular lipid. Lipid concentration in samples recovered with the optimum concentrations of flocculants were compared to lipid content in the sample recovered by centrifugation, which is assumed not to have affected the lipid concentration, as shown in Fig. 6. Flocculation with $FeCl_3$ did not alter lipid content in biomass ($p > 0.05$). On the other hand, there was a reduction in cellular lipid content of 19.70% for flocculation with NaOH, indicating that the use of NaOH as flocculant may rupture cell membrane, leading to loss of lipid content in microalga *A. obliquus* biomass. Therefore, the use of NaOH as flocculant would not be viable for biomass recovery of microalga *A. obliquus* in processes designed for lipid production.

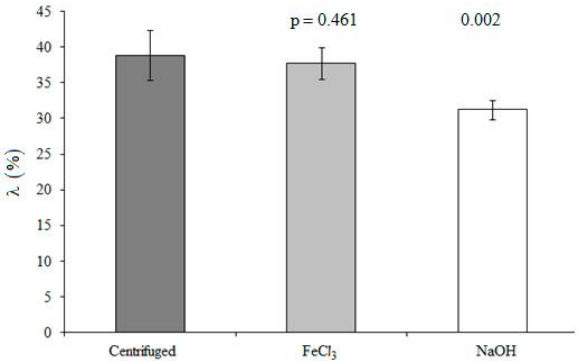


Figure 6. Lipid content (λ) in biomass recovered by centrifugation and flocculation with 0.2 mmol $L^{-1}$ of $FeCl_3$, and with 8 mmol $L^{-1}$ NaOH.

## DISCUSSION

During a flocculation process, as a flocculant is dispersed in the sample, its ions bind to the extracellular charges of the cells as well as to other ions in solution, thus, the amount of flocculant required for biomass recovery increases with medium salinity and biomass concentration. Consequently, it is difficult to compare the concentrations of flocculant used and to reproduce the flocculation efficiency with the same concentration of flocculant with different samples, even with the same biomass concentration, since cultivation conditions may alter both final biomass concentration and salinity.

Sodium hydroxide is described as an option for flocculation of marine microalgae biomass that does not damage cells (Soares, 2010; Horiuchi et al., 2003; Wu et al., 2012). However, marine microalgae are subject to different environmental conditions than freshwater microalgae, such as higher salinity and pressure, so that their cell membrane is more resistant. Thus, pH variations that do not damage cells of marine microalgae may disrupt cells of microalga *A. obliquus*.

Flocculation with ferric chloride, on the other hand, does not affect cellular integrity and presents a higher biomass recovery efficiency. In addition, it does not generate a significant change in pH and ferric chloride is also a component of the microalgae culture medium, thus, if the clarified medium were to be reused as a new growth media (Kim et al., 2011; Rodolfi et al., 2003) it would not be necessary to correct the pH and the increment in salinity at each recycling step would be small, unlike what would occur if the cultivation had been flocculated with NaOH.

## CONCLUSIONS

Flocculation is an economically viable process for the recovery of biomass for the production of low valued products from microalgal oil, such as biodiesel. In comparison with centrifugation, the energy consumption in a flocculation process is considerably lower. The evaluation of flocculation followed by sedimentation presented in this study showed that this method is efficient as a process for biomass recovery from microalga *A. obliquus*, resulting in a higher removal efficiency than that of centrifugation. Since biomass recovered with ferric chloride contains the same concentration of lipids than biomass recovered with centrifugation, it is reasonable to state that ferric chloride has potential to recover biomass of microalga *A. obliquus* without any loss of cellular lipid content.

## ACKNOWLEDGEMENTS

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