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COMPARATIVE PERFORMANCE OF DIFFERENT FLOCCULANTS IN THE RECOVERY OF SCENEDESMUS OBLIQUUS BIOMASS WITH LIPID QUANTIFICATION

DESEMPENHO COMPARATIVO DE DIFERENTES FLOCULANTES NA RECUPERAÇÃO DE BIOMASSA DE SCENEDESMUS OBLIQUUS COM QUANTIFICAÇÃO DE LIPÍDIOS

RENDIMIENTO COMPARATIVO DE DIFERENTES FLOCULANTES EN LA RECUPERACIÓN DE BIOMASA DE SCENEDESMUS OBLIQUUS CON CUANTIFICACIÓN DE LÍPIDOS

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ABSTRACT

To evaluate the recovery of algal biomass in different harvesting methods of the microalgae Scenedesmus obliquus, using chemical and natural flocculating agents. The experiment was conducted in triplicate, evaluating five flocculating agents, three of which were chemical (sodium hydroxide, ferric chloride, and ferric sulfate) and two of which were natural (chitosan and Moringa oleifera). Flocculation efficiency, biomass recovery yield, and, subsequently, the lipid content (%) of the recovered biomass were analyzed in an integrated manner. In flocculation efficiency tests with 24 hours of interaction, all flocculants evaluated showed efficiency above 90%, demonstrating the effectiveness of the different agents. Biomass yield ranged from 0.157 g/L to 0.245 g/L, with the highest values observed in treatments with metallic salts. Regarding lipid content, chemical flocculants presented yields of 5.0% to 6.0%, while natural flocculants (Moringa oleifera and chitosan) provided higher levels, ranging from 7.6% to 11%. All flocculating agents evaluated demonstrated high efficiency in recovering

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algal biomass, with rates exceeding 90%, confirming the effectiveness of both chemical and natural flocculants. Among them, ferric sulfate stood out for presenting the highest biomass yield. On the other hand, the highest lipid content was observed in the biomass recovered by natural flocculants such as chitosan and M. oleifera, suggesting that these natural agents not only enable efficient recovery but also better preserve bioactive components.

Keywords: Algal Biomass. Bioenergy. Flocculant. Lipids.

RESUMO

Avaliar a recuperação de biomassa algal em diferentes métodos de colheita da microalga Scenedesmus obliquus, utilizando agentes floculantes químicos e naturais. O experimento foi conduzido em triplicata, avaliando cinco agentes floculantes, sendo três químicos (hidróxido de sódio, cloreto férrico e sulfato férrico) e dois naturais (quitosana e Moringa oleifera). A eficiência de floculação, o rendimento de recuperação de biomassa e, posteriormente, o teor de lipídios (%) da biomassa recuperada foram analisados de forma integrada. Nos testes de eficiência de floculação com 24 horas de interação, todos os floculantes avaliados apresentaram eficiência acima de 90%, demonstrando a eficácia dos diferentes agentes. O rendimento de biomassa variou de 0,157 g/L a 0,245 g/L, com os maiores valores observados nos tratamentos com sais metálicos. Em relação ao teor de lipídios, os floculantes químicos apresentaram rendimentos de 5,0% a 6,0%, enquanto os floculantes naturais (Moringa oleifera e quitosana) proporcionaram níveis mais elevados, variando de 7,6% a 11%. Todos os agentes floculantes avaliados demonstraram alta eficiência na recuperação da biomassa algal, com taxas superiores a 90%, confirmando a eficácia tanto dos floculantes químicos quanto dos naturais. Dentre eles, o sulfato férrico se destacou por apresentar o maior rendimento de biomassa. Por outro lado, o maior teor de lipídios foi observado na biomassa recuperada por floculantes naturais como quitosana e M. oleifera, sugerindo que esses agentes naturais não apenas permitem uma recuperação eficiente, mas também preservam melhor os componentes bioativos.

Palavras-chave: Biomassa de Algas. Bioenergia. Floculante. Lipídios.

RESUMEN

Para evaluar la recuperación de biomasa algal mediante diferentes métodos de cosecha para la microalga Scenedesmus obliquus, utilizando agentes floculantes químicos y naturales. El experimento se realizó por triplicado, evaluándose cinco agentes floculantes: tres químicos (hidróxido de sodio, cloruro férrico y sulfato férrico) y dos naturales (quitosano y Moringa oleifera). La eficiencia de floculación, el rendimiento de recuperación de biomasa y, posteriormente, el contenido lipídico (%) de la biomasa recuperada se analizaron de forma integrada. En pruebas de eficiencia de floculación con 24 horas de interacción, todos los floculantes evaluados mostraron eficiencias superiores al 90%, demostrando la efectividad de los diferentes agentes. El rendimiento de biomasa osciló entre 0,157 g/L y 0,245 g/L, observándose los valores más altos en los tratamientos con sales metálicas. En cuanto al contenido lipídico, los floculantes químicos presentaron rendimientos de entre el 5,0% y el 6,0%, mientras que los floculantes naturales (Moringa oleifera y quitosano) presentaron niveles superiores, que oscilaron entre el 7,6% y el 11%. Todos los agentes floculantes evaluados demostraron una alta eficiencia en la recuperación de biomasa algal, con tasas superiores al 90%, lo que confirma la efectividad tanto de los floculantes químicos como de los naturales. Entre ellos, el sulfato férrico destacó por presentar el mayor rendimiento de biomasa. Por el contrario, el mayor contenido lipídico se observó en la biomasa recuperada con floculantes naturales como el quitosano y M. oleifera, lo que sugiere que estos agentes naturales no solo permiten una recuperación eficiente, sino que también conservan mejor los componentes bioactivos.

Palabras clave: Biomasa Algal. Bioenergía. Floculante. Lípidos.

1 INTRODUCTION

Microalgae are unicellular photosynthetic organisms, widely distributed in marine and freshwater environments, that perform essential ecological functions. In addition to actively participating in the biogeochemical cycling of nutrients and primary production, they constitute the trophic basis of aquatic ecosystems. Due to their high growth rate and photosynthetic efficiency, they have been explored in several biotechnological areas, including effluent bioremediation, the production of bioactive compounds with pharmaceutical potential, and the formulation of nutritional supplements for human consumption [1-3].

TheThe main problem in microalgae cultivation lies precisely in the process of harvesting the biomass produced, caused by its microscopic size and stability in the aqueous environment. Studies and techniques exist that achieve efficiency with viable production costs for obtaining biomass [4-5].

The main techniques used to recover microalgae biomass include centrifugation, flocculation, filtration, and gravitational sedimentation. While all are effective under certain conditions, many require high energy consumption and complex infrastructure, which significantly increases the cost of the process, especially on a commercial scale, where cell dispersion poses an additional challenge [6-9].

In this context, flocculation emerges as a promising alternative, standing out for its operational simplicity and relatively lower cost. The mechanism is based primarily on neutralizing the electrical charges present on the cell surface, which in microalgae are predominantly negative, a factor responsible for keeping them suspended in the aqueous medium. With the addition of flocculating agents, this electrostatic repulsion is reduced, promoting the agglomeration of cells into larger, denser flakes, which can be easily separated by subsequent processes such as decantation or filtration [10].

Many factors can influence this process, such as pH, time, concentration, and agitation to homogenize the flocculant. However, flocculation is classified as chemical because it is a simple and fast method, but it promotes biomass contamination, damages cells, and limits its application. Examples such as sodium hydroxide, iron chloride, and iron III sulfate are used in extensive research as sources for obtaining microalgae biomass. However, they are considered harmful due to the appearance of different colors in the biomass, concerns regarding biomass application, and environmental impacts [11-12].

On the other hand, biological flocculation occurs with the use of natural polymers, such as chitosan and moringa oleifera. They are used as flocculants because they do not cause secondary pollution, present less degradation in microalgae biomass, and at the same time allow the reuse of cultivation water [13].

Moringa oleifera has proven to be an efficient alternative in the microalgae flocculation process, since its compounds act by neutralizing the charges present in the cell wall, which facilitates the formation of flakes and the subsequent sedimentation of the cells [14].

Chitosan is a polysaccharide derived from chitin that exhibits several bioactive characteristics, including being non-toxic, biodegradable, with antibacterial properties, and widely available in nature. Its use as a flocculant is due to its high cationic charge density and long polymer chain structure, which favor the neutralization of negative charges on the cell surface of microalgae, thus promoting the formation of stable aggregates [15].

The lipid content of microalgae can vary significantly, generally between 20% and 50% of the dry mass, depending on several factors, such as environmental cultivation conditions (light, temperature, nutrient availability) and the methods used to obtain and process the biomass [16]. Some microalgal species have a high capacity for lipid accumulation, especially under conditions of nutritional stress, and are therefore classified as oleaginous species. Among the most promising genera are Dunaliella, Nannochloropsis, Tetraselmis, Scenedesmus, and Chlorella, among others, which are widely studied for their potential in industrial applications, such as biofuel production, nutritional supplements, and cosmetics [17].

Microalgae of the genus Scenedesmus, belonging to the Chlorophyceae class, have significant potential due to their diverse functionalities. This microalgae stands out for its remarkable growth capacity, in addition to presenting different cell and colony formations, depending on the cultivation conditions[18].

Furthermore, it is worth noting that the microalgae in question are recognized for accumulating significant amounts of 12-14% lipids, 50-56% proteins, and 10-17% carbohydrates in their dry matter cells. This makes them of great importance due to their nutritional properties and potential application in various sectors, such as the production of biofuels, nutraceuticals, and food ingredients. They are also excellent assets in bioremediation processes, such as the purification of polluted water, and are particularly important in agriculture and even the cosmetics industry. Their versatility and ease of cultivation make them a promising option for various biotechnological applications[19-21].

Thus, the present work aimed to analyze five flocculants, three chemical and two biological, in the flocculation process of the microalgae Scenedesmus obliquus biomass and compare the flocculation efficiency, biomass yield and lipid production.

2 MATERIAL AND METHODS

2.1 MICROALGAE CULTIVATION

The experiment was carried out at the Planktology Laboratory of the Center for Biotechnology Applied to Aquaculture of the Federal University of Ceará, Fortaleza, CE, Brazil (3°44'S and 38°34'W).

The Scenedesmus obliquus strain from the Planktology Laboratory was propagated in modified Guillard f/2 medium, in which cultivation was carried out in a cylindrical reactor made of polyethylene terephthalate (PET), with constant aeration, with an air flow of 3 L min-1 and a photoperiod of 24 hours of light, provided by 18 W LED lamps and illuminance of 5.000 lux and temperature maintained at 27.2 °C, until reaching 40 L of culture for carrying out the experiment.

Microalgae population growth was monitored daily by cell counting (expressed in cells/mL) and determining optical density (OD 680 nm) by spectrophotometry, until reaching 0.649 nm. For cell counting, 1 mL samples were collected, fixed with Lugol's and transferred to a hemocytometer for counting under an optical microscope (Olympus cx 40) [22].

2.2 FLOCCULATION PROCESS

The experimental design was carried out using five flocculants, three chemical, sodium hydroxide (NaOH), iron III chloride (FeCl3) and iron sulfate (Fe2(SO4)3) and two biological (chitosan and M. oleifera seed).

Initially, preliminary tests were conducted to determine the optimal concentration of each flocculant, evaluating the efficiency of microalgal biomass recovery. The agents were tested at concentrations of 20, 40, and 60 mL/L, allowing for a comparative characterization of the performance of each treatment. The results indicated that the 60 mL/L concentration provided efficiency greater than 80% in biomass recovery for all flocculants evaluated, demonstrating that this dosage is adequate to maximize cell aggregation and sedimentation of microalgae, regardless of the chemical or biological nature of the flocculant. These results served as a basis for subsequent experiments, ensuring consistent and optimized conditions for evaluating biomass yield and lipid content in the following experiment.

For the chemical reagents, solutions of sodium hydroxide (0.5 M), iron (III) chloride (0.006 M), and iron sulfate (0.025 M) were prepared and stirred until the mixture was dissolved. On the other hand, biological reagents, such as chitosan, were dissolved in 1 g L-1 of acetic acid (1%) until the mixture was completely dissolved[23]. In this case, the moringa seeds were collected and taken to the laboratory, where they were shelled, crushed, and dehydrated in ovens with recirculating air at 100 °C for 6 hours. To prepare the solution, 10 g

of moringa powder were weighed, then 1 L of distilled water was added, and the solution was homogenized by magnetic stirring until completely dissolved[24].

All flocculants were used at a ratio of 6:100 (flocculants:culture medium). After adding the flocculant solution, stirring was performed slowly for 1 min, and the periods analyzed to verify efficiency were 30 minutes, 3 hours, and 24 hours.

To calculate the Flocculation Efficiency (E) [10], the difference between the initial optical density before the flocculant addition process (Di) and the optical density of the supernatant at the end of the process (Df) was used, as demonstrated in the following equation:

$$E = \frac{(D_i - D_f)}{D_i} * 100 \quad (1)$$

After the flocculation process using the different agents, the liquid phase of the supernatant was carefully discarded, preserving only the precipitated material. The biomass was transferred to appropriate vials and dehydrated in an oven with recirculating air at 60°C for 24 hours.

2.3 LIPID EXTRACTION

Lipid extraction was adapted from Martins25, who used the Bligh & Dyer method26, with the combination of chloroform and methanol in a 1:2 ratio.

The dried biomass (0.5 g) was hydrated with 2 mL of distilled water and then 7.5 mL of chloroform:methanol (1:2), followed by manual stirring for 3 min. Subsequently, 10 mL of 1.5% anhydrous sodium sulfate solution and 2.5 mL of distilled water were added, and the mixture was again manually stirred for 30 s. To promote separation of the solid phase, the extract was centrifuged for 10 min at 4.500 rpm and the supernatant transferred to a separation hopper, in which the organic (lower) phase was collected in a previously dried oven at 60 °C until constant weight.

To calculate the final lipid content, the values of the oven-dried glassware, the weight of the biomass used in the process and the value of the glassware with the oven-dried sample after 24 h were used, according to the equation below:

$$L\,(\%) = 100 \times \frac{Dry\ glassware\ weight - Weight\ of\ glassware\ with\ dry\ sample}{Weight\ of\ dry\ biomass} \quad (2)$$

2.4 STATISTICAL ANALYSIS

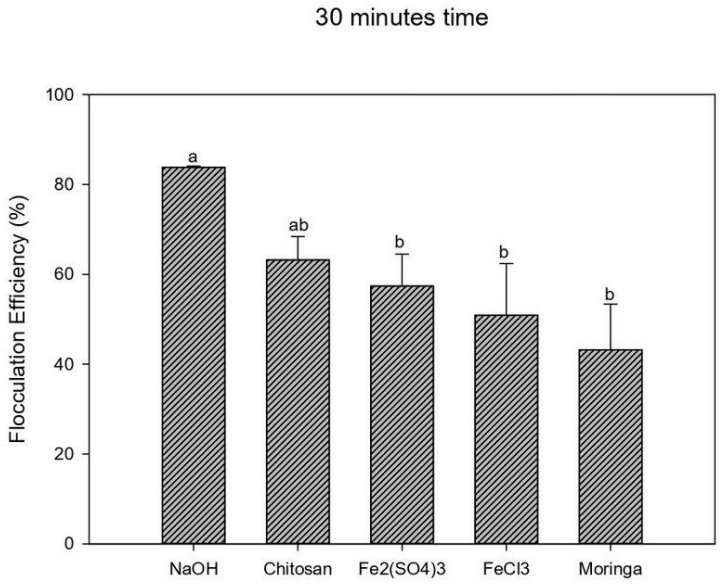
The data obtained were initially subjected to normality tests to verify the distribution of residuals, using the Shapiro-Wilk and Kolmogorov-Smirnov tests. Homogeneity of variances between groups was assessed using Levene's test. For data sets that met the assumptions of normality and homoscedasticity, analysis of variance (ANOVA) was applied with a significance level of p (≤ 0.05).

3 RESULTS

After 30 minutes of application of the different flocculating agents, it was observed that the treatment with 0.5 M sodium hydroxide (NaOH) presented the highest flocculation efficiency, reaching 83.80%. This value was significantly higher than those obtained with the metallic salts $Fe_2(SO_4)_3$ (57.40%) and $FeCl_3$ (50.92%), as well as with the natural agent moringa (43.20%), evidencing clear statistical differences between these treatments. On the other hand, the efficiency of NaOH showed statistical similarity in relation to the natural polymer chitosan (63.22%), suggesting that, although both promote effective interactions in cell aggregation, NaOH presented more expressive performance in a short period of time, as demonstrated in Figure 1.

Figure 1

Flocculation efficiency after 30 minutes of exposure to flocculants

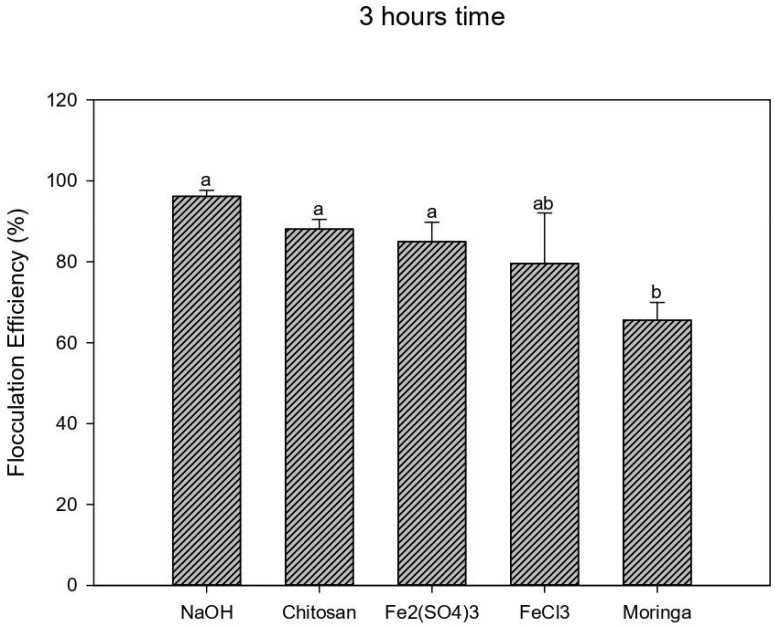


During the 3-hour decantation period (Figure 2), a significant increase in flocculation efficiency was observed for all agents evaluated, indicating that the extended time favored cell sedimentation. Among the treatments, Moringa oleifera presented significantly lower

performance, with 65.5% efficacy, evidencing a statistical difference in relation to the other flocculants tested. In contrast, the agents of chemical origin maintained the highest efficiency rates, with emphasis on NaOH (96.1%), followed by chitosan (88.07%), ferric sulfate (84.9%) and ferric chloride (79.5%).

Figure 2

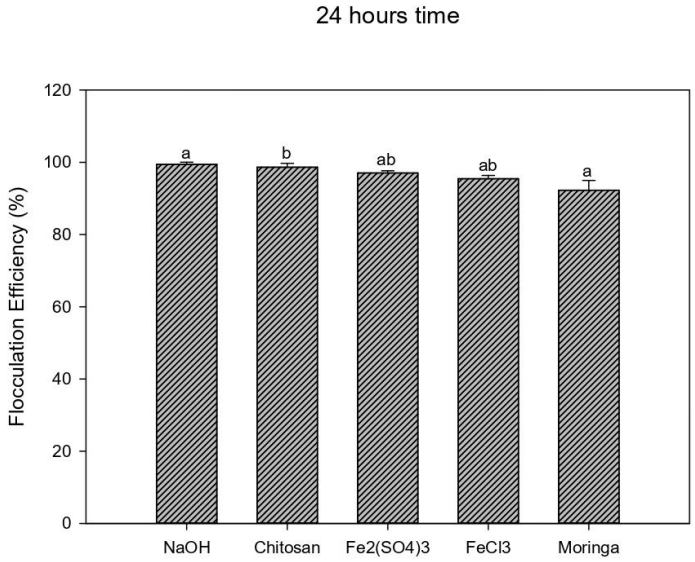
Flocculation efficiency after 3 minutes of exposure to flocculants



After 24 hours of interaction between the different flocculating agents (Figure 3), NaOH performed better, achieving flocculation efficiency values above 99%, consolidating its position as the most prominent treatment. However, statistical analysis revealed no significant differences between this treatment and the other agents evaluated, demonstrating that all presented a high capacity to promote cell aggregation over time. Chitosan presented an efficiency of 98.6%, followed by ferric sulfate (97.0%), ferric chloride (95.4%), and Moringa oleifera (92.2%).

Figure 3

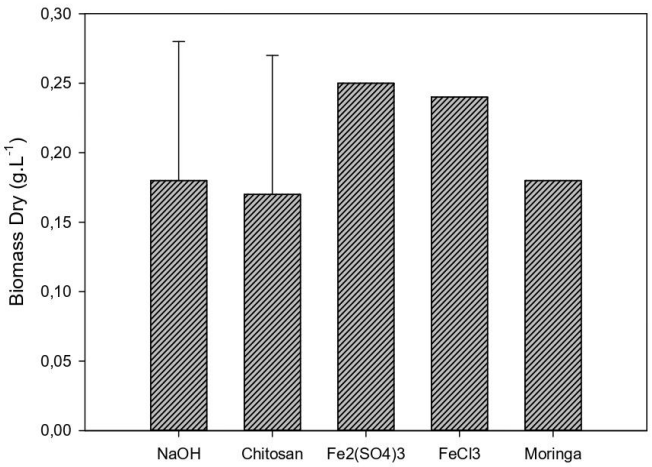
Flocculation efficiency after 24 minutes of exposure to flocculants



The biomass yield, presented in Figure 4, demonstrated variations among the different flocculation methods used. The observed values were 0.18 g/L for the sodium hydroxide treatment, 0.242 g/L for ferric chloride, 0.245 g/L for ferric sulfate, 0.170 g/L for chitosan, and 0.177 g/L for Moringa oleifera. Although numerical differences were observed among the treatments, statistical analysis revealed no significant differences, indicating that, under the experimental conditions adopted, all flocculating agents and separation methods promoted comparable biomass recovery.

Figure 4

Biomass yield of the flocculating agents in question



After efficient recovery of microalgal biomass, lipids constitute the main raw material for the production of biofuels, edible oils, and various high-value-added products. The data presented in Table 1 indicate the lipid yield (%), demonstrating that natural flocculants, such as chitosan and Moringa oleifera, resulted in higher lipid contents compared to the chemical agents used, including NaOH, $FeCl_3$, and $FeSO_4$. These results suggest that natural flocculants can better preserve cellular integrity and lipid content, offering a significant advantage for applications requiring biomass with high energy and nutritional value.

Table 1

Lipid extraction results found

	Unit	NaOH	Chitosan	$Fe_2(SO_4)_3$	$FeCl_3$	Moringa
		Media/Desv	Media/Desv	Media/Desv	Media/Desv	Media/Desv
Lipid	(%)	5,27 ± 2,0	7,60 ± 0,6	6,00 ± 0,01	5,40 ± 0,07	10,80 ± 0,07

4 DISCUSSION

In a study with Scenedesmus sp., Chen et al. (2013) [8], obtained 96% flocculation efficiency with $FeCl_3$ at 150 mg/L. In the present work, using 60 mg/L, the efficiency ranged from 50% to 95%. However, the use of $FeCl_3$ and $Fe_2(SO_4)_3$ caused an orange coloration of the biomass, possibly due to the presence of iron, which may compromise its application in pigment products.

Flocculation efficiency should be assessed not only by the cell aggregation rate but also by the viability of the biomass for later use. Previous studies, such as that by Hesse, reported efficiencies of approximately 85% for $FeCl_3$ and $FeSO_4$ after 30 minutes, values about 10% lower than those obtained in this study. For chitosan, values close to 100% were observed, similar to those observed in this study after 3 hours of interaction. Comparatively, Scherer et al. (2016) [27], reported recoveries of 50.4% and 52.5% with NaOH and $FeCl_3$, respectively, while the results obtained here ranged from 83% to 99% for NaOH and from 50% to 95% for $FeCl_3$, demonstrating superior performance and greater consistency compared to the literature.

Ruiz-Marin [24], obtained a flocculation efficiency with M. oleifera of 71 to 78% with pH variation, and the present work obtained an efficiency of 43.20% in 30 min, 65.56% in 3 hours and 92.29% in 24 hours of rest after the addition of the flocculant, without pH variation.

In this context, they indicate that, in addition to the type of flocculant, the interaction time and experimental conditions play a critical role in maximizing efficiency. The superior

performance of NaOH suggests that alkaline agents can promote a rapid change in the surface charge of cells, favoring aggregation and sedimentation. Chitosan, acting as a natural polymer, provides stability and allows for high recovery even over prolonged periods, while Moringa oleifera, although less efficient, still shows promise as a sustainable alternative.

Silva et al.,[5], evaluated the biomass yield of Chlorella vulgaris using NaOH (0.136 g/L) and $FeCl_3$ (0.124 g/L), both at 0.5 M and a dosage of 1 mL/L. In the present study, the application of 0.5 M NaOH at a higher dosage (60 mL/L) resulted in an increase in biomass yield compared to the values reported by Silva et al. However, despite this increase, in this study, the treatment with $FeCl_3$ demonstrated superior performance, reaching approximately 0.242 g/L, about twice as high as NaOH, evidencing that, even with an increase in the amount of alkaline flocculant, metallic salts continue to be more efficient in recovering microalgae biomass.

WU et al.,[28], investigated the influence of flocculants chitosan and NaOH on the lipid yield of the microalgae Scenedesmus obliquus and quantified 26% lipids in the biomass recovered with chitosan and 27% when using NaOH, values higher than those found in the present work, however the lipid extraction methods used in both studies were different, which may have influenced the yield.

A study conducted by Zhu et al.,[29], with the microalgae Chlorella vulgaris comparing the effect of flocculants on lipid yield, obtained an optimal result when the biomass was recovered with chitosan, making this flocculant a potential for biomass recovery and not harming lipid yield.

Ferreira [30], worked with the lipid extraction of the dry biomass of S. obliquus subjected to three different extraction methods, with ethanol the result was 1.30 and 4.40%, with ethyl acetate it was 5.50 to 11.1% and with hexane the results were 3.50 and 1.60% in lipid yield, when compared with the present work that used the Bligh & Dyer method 18 the result varied from 5.0 to 11.0% in yield.

5 CONCLUSION

Therefore, with the different flocculating agents, after 24 hours of exposure, all treatments showed algal biomass recovery efficiencies exceeding 90%, demonstrating the effectiveness of these methods in separating Scenedesmus obliquus cells. Among the agents evaluated, iron sulfate stood out, presenting the highest biomass yield, indicating its high capacity to promote cell aggregation and sedimentation. However, among the natural flocculants, Moringa oleifera and chitosan stood out, presenting higher lipid contents than chemical agents. This indicates that the use of biological flocculants not only allows for

efficient biomass recovery but also better preserves bioactive components, such as lipids, making them promising for applications in biofuels, nutraceuticals, and high-value oils.

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