J Appl Biotechnol Rep. 2022 March;9(1):477-483

doi 10.30491/JABR.2021.291113.1405

Journal of
Applied Biotechnology Reports

Original Article

Evaluation of *Chlorella sorokiniana* Biomass Recovery by Using Different Chemical-based Flocculants

Mariana Lara Menegazzo [1], Dagon Manoel Ribeiro [1], Nathanya Nayla de Oliveira [1], Otavio Gonçalves Benites Marques [1], Gustavo Graciano Fonseca [2*]

[1] Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados, Dourados, MS, Brazil
[2] Faculty of Natural Resource Sciences, School of Business and Science, University of Akureyri, Akureyri, Iceland

Corresponding Author: Gustavo Graciano Fonseca, PhD, Associate Professor, Faculty of Natural Resource Sciences, University of Akureyri, Borgir v. Nordurslod, 600 Akureyri, Iceland. E-mail: gustavo@unak.is

Received June 17, 2021; **Accepted** September 17, 2021; **Online Published** March 5, 2022

Abstract

Introduction: The nature and the concentration of the chemical agents responsible for cell flocculation are the bottlenecks for microalgae recovery. The aim of the present study was to evaluate different chemical-based flocculants for *Chlorella sorokiniana* flocculation.

Materials and Methods: The biomass recovery efficiency was evaluated by comparing self-flocculation and flocculation with the ferric chloride, sodium hydroxide, aluminum sulfate, and zinc sulfate. After identifying the best flocculating agent, its concentration was varied to determine the optimal condition by rapid agitation followed by sedimentation (0.25 to 1 g/L).

Results: Zinc sulfate was unsuitable for this strain due to an efficiency lower than 40%. Self-flocculation and sodium hydroxide were fairly efficient (48.65% and 58.06%, respectively). Aluminum sulfate produced moderate results (56.27%), but flocculation took a long time to become efficient. Ferric chloride showed the best potential for flocculation, and in the analysis of different concentrations (0.25 to 1 g/L) showed to be fast and efficient (nearly 80% of biomass recovery in 10 min) at a concentration of 0.75 g/L.

Conclusions: All the flocculants tested in this study can be utilized for biomass recovery, except for the zinc sulfate. The procedure was efficient, inexpensive, and contaminant-free for the recovery of *C. sorokiniana* biomass.

Keywords: Microalgae, Flocculation, Sedimentation, Ferric Chloride
Citation: Menegazzo ML, Ribeiro DM, de Oliveira NN, Marques OGB, Fonseca GG. Evaluation of *Chlorella sorokiniana* Biomass Recovery by Using Different Chemical-based Flocculants. J Appl Biotechnol Rep. 2022;9(1):477-483. doi:10.30491/JABR.2021.291113.1405

Introduction

Microalgae are single-celled photosynthetic organisms with a great diversity of forms, traits, and ecological features. They are found in marine environment, freshwater, and soil, and can be exploited beneath several aspects, *e.g.* to obtain food and feed products, biofuels, bioplastics, pigments, cosmetics, medicinal drugs, organic fertilizer and other compounds.[1,2]

Their great diversity of species is reflected in their biochemical composition.[3] Microalgae such as *Chlorella* sp. are promising candidates for both food supplementation and biofuel production because of their total lipid content and rapid growth in nutrient minimal media at high temperatures.[4] However, the lipid yield of microalgae depends not only on the choice of the species to be cultivated and the culture parameters, but also on the biomass recovery system, and the lipid extraction system.[5]

After cultivation, biomass harvesting and drying are required in order to get the microalgae biomass. The difficulty in separating the microalgae biomass is aggravated by the low cell concentration, between 0.1 and 3.0 g/L, the microscopic size of microalgae, between 3 and 30 µm, the negative surface charge, which prevents or inhibits cell aggregation, and the density similar to water, which hinders its sedimentation.[4,6,7]

In the harvesting process, cells are separated from their environment by operations such as gravimetric sedimentation, flotation, and flocculation, followed by the dewatering methods of centrifugation or filtration, and then drying or lyophilization. The dry biomass is then suitable to produce bioproducts.[8-11]

The chosen operations directly have an influence on the cost and the quality of the final products.[4] Sedimentation and filtration steps occur slowly and, thus, may be not efficient for small cells, but can be applied to microalgae of greater volume and cell size.[12,13] Flotation is a gravity separation process promoted by air or gas bubbles that still lacks on information about its feasibility. Centrifugation is a fast separation step, but it requires high energy demand and possible cell disruption through gravitational and shear forces may occur. Flocculation is triggered out when smaller particles aggregate into larger particles through the interaction of coagulant or flocculating agents and, over time, decanting by sedimentation, and is one of the most widely used techniques for the separation of microalgal biomass.[5,6]

Flocculation is a suitable harvesting technique because it allows the manipulation of large crop volumes, with low energy costs.[14] Cells concentrated by flocculation are in better physical condition than those recovered by centrifugation or filtration, since the cell integrity is preserved.[13,14] This process has been widely used in the industry to remove solids from suspensions, *e.g.*, water and effluent treatments.[7,12,13]

The choice of the flocculating agent must consider its degree of interference in the process, the application of the resulting biomass, and cost. Knowledge about flocculation mechanisms is necessary to ensure the efficient use of flocculants through the interactions that occur between flocculant and microalgae. The mechanism of microalgae flocculation evolves the electrostatic stabilization of the cells by negative surface charges at most pH levels. These charges must then be reduced or neutralized to enable cell agglutination and sedimentation.[7,14]

In flocculation induced by metal salts, a high dose of flocculant and an acidic pH may be required to achieve satisfactory results. Some studies have shown that high concentrations of flocculant may cause contamination of the biomass with aluminum or iron.[6,15] However, several authors have found that this contamination scarcely interferes in the extraction yields of lipid fraction and in fatty acids profile.[14,16]

Several authors have reported that there is no simple and low-cost method for microalgae dewatering at a large scale. Therefore, it is essential to develop more efficient separation processes in order to ensure the economic viability of the production of bioproducts.[14,17,18] Hence, the search for a recovery system with lower energy expenditure, which meets the requirements for biomass of acceptable quality, is highly challenging. Thus, the aim of this study was to evaluate the flocculating agents, which includes zinc sulfate, sodium hydroxide, ferric chloride, aluminum and self-flocculation in order to determine a fast, efficient and low-cost method for the recovery of *Chlorella sorokiniana* biomass.

Materials and Methods

Microalgal Species and Cultivation

Chlorella sorokiniana CTT 7727 microalgae from the André Tosello Foundation (Campinas, SP, Brazil) was kindly supplied by the Biodiversity Research Center of the State University of Mato Grosso do Sul (Dourados, MS, Brazil).

C. sorokiniana was grown in Bold's basal medium (BBM)[19] for 30 days in 9 L photobioreactors. All the components of the medium were sterilized by autoclaving (121 ºC, 15 min). The photoperiod was set to a simulated 12 h light/12 h dark cycle, and a vigorous and ascending aeration system was adjusted to 3 L/min. The final dry biomass concentration of the culture was 1.3 g/L with pH of 10.2.

Flocculation System

The flocculation experiment was carried out in cylindrical tubes with a 1 L volume. Ferric chloride (Fe_3Cl), aluminum sulfate ($Al_2 (SO_4)_3$, zinc sulfate ($ZnSO_4$) and sodium hydroxide (NaOH) at a concentration of 0.25 g/L were evaluated as flocculating agents. A cylindrical tube without added flocculant was used as a control (self-flocculation). After adding the flocculant, the cylindrical tubes were shaken rapidly by hand for 1 min before sedimentation occurred.[20]

A second study was carried out using the flocculant agent that exhibited the best flocculation efficiency in the shortest time, in order to determine the optimum flocculation concentration. Concentrations of 0.25, 0.5, 0.75 and 1 g/L were tested. All the experiments were conducted at the original pH level of the culture, which was at pH 10.2.

Degree of Flocculation

The degree of flocculation of the cell suspension was determined based on the relationship between the height of the sediment or thickness (He) and the height of the liquid phase (HI). This He/Hl ratio (dimensionless) indicates the sedimentation volume (V_S). The higher the V_S the greater the degree of flocculation of the suspension and the more easily it is redispersed.[21] To determine the He, labels were affixed onto each cylindrical tube at 10 min intervals up to 90 min and 24 h after the beginning of the experiment. A second study with the best flocculant was monitored for 90 min. These labels were made based on studies described elsewhere.[21,22].

Flocculation Efficiency

Flocculation efficiency was evaluated by comparing the optical density in the upper region of the cylindrical tube, 4 cm below the total height, at different stages of sedimentation. Optical density at 670 nm (OD_{670}) was measured uisng microplate reader (Biochrom Anthos Zenyth 200rt. OD_{670} was monitored at 10 min intervals up to 90 min and 24 h after the beginning of the experiment. The flocculation and bleaching efficiency of the culture was calculated according to Eq. 1:[23]

$$Efficiency\ (\%) = \frac{ODi - ODt}{ODi}\ x\ 100 \qquad \text{Eq. 1}$$

where ODi is the OD_{670} at time zero (initial) and ODt is the OD_{670} at time t.

Statistical Analysis

The results of the analyses are presented as mean ± standard deviation (SD). Data were subjected to analysis the variance (one-way ANOVA) by the Tukey test at 5% significance, using Statistica version 6.0 software (StatSoft, Tulsa, USA).

Results and Discussion

Different Flocculating Agents

Mechanism of Action

The different flocculating agents produced varying heights of He and levels of efficiency, showing green coloration in the liquid phase during and even at the end of the flocculation process. The presence of staining indicates that some microalgae cells did not form flocs, *i.e.*, they did not participate in the reaction. The absence of floc formation was attributed mainly to the type, dose and concentration of the flocculating agent, decantation time and pH level. These factors interfere actively in the recovery of biomass.[12,21]

In a previous study carried out with freshwater microalgae, the medium was bleached using metal salts and chitosan as flocculants. However, a visual examination of the medium revealed that flocculation efficiency was influenced by the type and concentration of biomass.[12] It has also been reported that the flocculation of *Chlorella minutissima* with chlorinated and sulfate salts at concentrations varying from 0 to 5 g/L and 3 h of sedimentation presented a clear staining gradient, with floc formation and subsequent sedimentation.[20]

Cell density, pH, the type and concentration of the flocculating agent, and processing conditions are determining factors for flocculation.[4] In this study, flocs were formed with a moderate cell concentration (1.3 g/L), but reasonable flocculation yields were attained, indicating that biomass recovery was influenced by other factors besides cell density, particularly the initial pH. Several authors have considered that the cell concentration before flocculation should be as high as possible, as it increases the frequency of cells, favoring the formation of flocs and hence, the efficiency of the process. A minimum cell concentration of 0.5 g/L before separation is required, since efficiency decreases at lower concentrations.[12]

The flocculation agents $Al_2(SO_4)_3$ and $FeCl_3$ are widely used in water and waste treatment, because they are inexpensive, readily available in the market, relatively nontoxic and cause low environmental impacts.[17,20] However, at high concentrations, the reuse of the effluent would be inadequate.[15]

Degree of Flocculation

To determine the He, the labels of the cylindrical tubes were followed with the time. The label, at a given time, indicates the height that the sedimentation reached at that moment, until the total stagnation. The sedimentation volume does not represent flocculation efficiency but flocculation capacity through the thickening stage and the clear phase.[13,22]

In this study, the descending label stagnated at 2 min in self-flocculation. With $ZnSO_4$ and NaOH, stabilization occurred at 5 min. Stagnation with $FeCl_3$ occurred in 45 min, while with $Al_2(SO_4)$ it occurred in 24 h after beginning the experiment, albeit without changing significantly from 85 min up to 24 h. In the fast processes, a stagnation time of 60 min indicated reasonable yields (between 50 and 60%).

Based on Table 1, after 24 h, self-flocculation, $ZnSO_4$ and NaOH has the highest level of sedimentation volume. This indicates that the more flocs tend to redisperse in the medium, and the lower the sedimentation volume, the better the flocculation $FeCl_3$ and $Al_2(SO_4)_3$. $FeCl_3$ and $Al_2(SO_4)_3$ presented the best flocculation performance with sedimentation volume of 0.106 and 0.068. Moreover, the possibility of floc redispersion was low, as previously reported for *Chlorella* sp. (V_S = 8.5 to 11.0) and different concentrations of $CaCl_2$ (0.6 to 3.4 g/L), led to sedimentation volumes varying from 0.044 to 0.147, with optimal flocculation occurring at pH 10 and with 2.0 g/L of $CaCl_2$.[21]

Table 1. Sedimentation Volume

Experiment	Floculant Agent	Sedimentation Volume (Dimensionless)
First (24 h)	self-flocculation	13.889
	$ZnSO_4$ 0.25 g/L	11.407
	NaOH 0.25 g/L	9.469
	$FeCl_3$ 0.25 g/L	0.106
	$Al_2(SO_4)_3$ 0.25 g/L	0.068
Second (90 min)	$FeCl_3$ 0.25 g/L	0.095
	$FeCl_3$ 0.5 g/L	1.412
	$FeCl_3$ 0.75 g/L	0.165
	$FeCl_3$ 1.0 g/L	0.153

Biomass Recovery and Bleaching of the Medium

Monitoring optical density (OD_{670}) through the formation of a clear interface reveals the difference between the clarified and the thickened interface. The flocculation efficiency indicated that the flocculants behaved differently (Figure 1). In the first 10 min, the OD_{670} dropped dramatically with all the flocculants except in the self-flocculated material (control). However, the OD_{670} was less reduced between 90 min and 24 h, except in the self-flocculation. It should be noted that, when using $ZnSO_4$, floc redispersion showed higher OD_{670} values at 10 min when compared to that obtained at 45 and 60 min, which affected the flocculation efficiency (Figure 1). In the case of self-flocculation, there were significant differences between all the holding times, except for 45 and 60 min. However, the other flocculating agents showed no significant differences at sedimentation times of 10, 45, 60 and 90 min, except self-flocculation.

The differences in yield were significant in all the treatments when comparing the shortest (10 min) and longest sedimentation times (24 h). This indicated that the holding time influenced the sedimentation of the flocs and the non-aggregated cells in this system. Furthermore, the flocculation could be utilized as a fast step for dewatering microalgae cultivation.

The flocculants showed a promising potential for use in the first step of microalgal biomass recovery, *i.e.*, a pretreatment, especially $FeCl_3$, since its efficiency in the first 10 min (53.21%) presented good levels of cell recovery and bleaching of the medium. At longer sedimentation times (24 h), the

flocculating agents NaOH and Al_2 $(SO_4)_3$ (58.06 and 56.27%, respectively) performed adequately, including from the standpoint of reducing operating costs. However, floc redispersion, as shown by the sedimentation volume, with NaOH should be considered when using these flocculants. The increments in the flocculation yields compared to the initial and final times indicate that self-flocculation and NaOH (40.84 and 21.18%) can also be potentially used as a pretreatment for biomass recovery. However, $FeCl_3$ presented a slight increase (0.3%) at initial and 60 min.

Considering the results obtained in this study, 10 and 60 min are suggested for the separation of microalgal biomass from water for large scale processes due to the satisfactory flocculation efficiencies obtained in the process of separating water and microalgal biomass. $FeCl_3$ and Al_2 $(SO_4)_3$ proved to be suitable for this separation, although NaOH and self-flocculation can potentially be used when there is enough time for sedimentation of the flocs. The flocculant concentration and pH of the medium used here proved to be suitable in the pretreatment for biomass recovery.

Reasonable results were achieved with self-flocculation (almost 50% efficiency after 24 h). Self-flocculation occurred, and although it was visualy imperceptible from the variation in the label, it was evident by the OD_{670} decrease. Due to the high volume of sedimentation, this self-flocculated material was easily redispersed. *Chlorella vulgaris* self-flocculation has reportedly achieved yields of 40-50% and considerable energy savings.[13] Self-flocculation can also occur due to low photosynthetic activity and reduction the of CO_2 supply, making this stage economically feasible.[4] However, studies are necessary for each species.[4,21]

The flocculating agent $ZnSO_4$ did not interact properly with the cells, forming few flocs and a high degree of redispersion in the medium. In the long flocculation period, it was observed that the cells remained in the medium, indicating that this compound partially inhibits flocculation and self-flocculation, achieving an efficiency rate of only 40% at 24 h. Using different concentrations of zinc chlorides and sulfates for the flocculation of *Chlorella minutissima*, another study found that flocs were formed, but that they adhered to the wall of the tube, making it difficult to monitor optical density and flocculation yields.[20]

Flocculation with NaOH yielded reasonable results (58.06%) only when the holding time was 24 h (Figure 1). A high flocculation efficiency (95%) was reported for *Chlorella vulgaris* upon increasing the pH to 11 and 12 in 60 min[24], unlike the efficiency found here. When the pH increased, followed by the addition of an organic flocculant, a microalgae harvest yield of 80% was successfully obtained.[25] These differences show that the use of NaOH may be a simple non-toxic method for cell flocculation, but it should be tested for each species of microalgae. Other authors have reported high performances, with recovery rates of more than 90%, using a slightly acidic pH.[26]

The use of Al_2 $(SO_4)_3$ produced satisfactory results (56.27%) after 24 h of sedimentation, with a significant difference from 60 min to 24 h. Flocculation with Al_2 $(SO_4)_3$ is highly efficient when it takes place in a pH ranging from 4 to 7.[27] Harvesting *Phaeodactylum tricornutum* with Al_2 $(SO_4)_3$ led to yields in excess of 80% when the pH was moderately basic at 9.[22] The higher the Al_2 $(SO_4)_3$ concentration, the greater the *Schizochytrium limacinum* flocculation efficiency with a moderately basic pH between 8 and 9.[15] In the case of *C. minutissima*, yields of less than 40% were obtained when using Al_2 $(SO_4)_3$ at 0.25 g/L and above 80% when the concentration ranged from 0.5 to 1 g/L.[20]

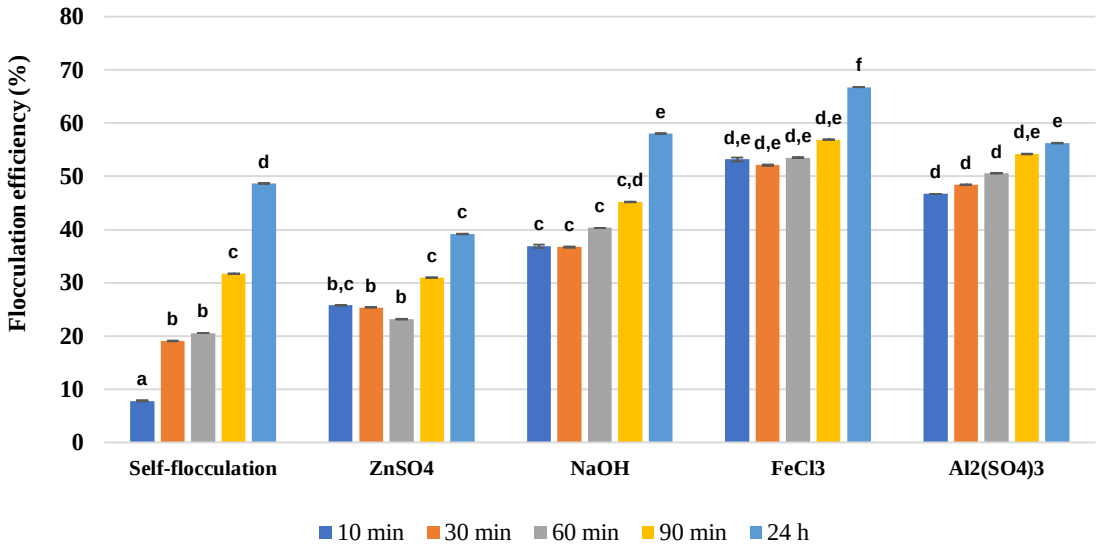

Figure 1. Flocculation Efficiency Variation in The Function of Different Chemical Agents Over Time. All chemical agents were used at the concentration of 0.25 g/L. Different letters within the same chemical agent indicate significant difference (p<0.05).

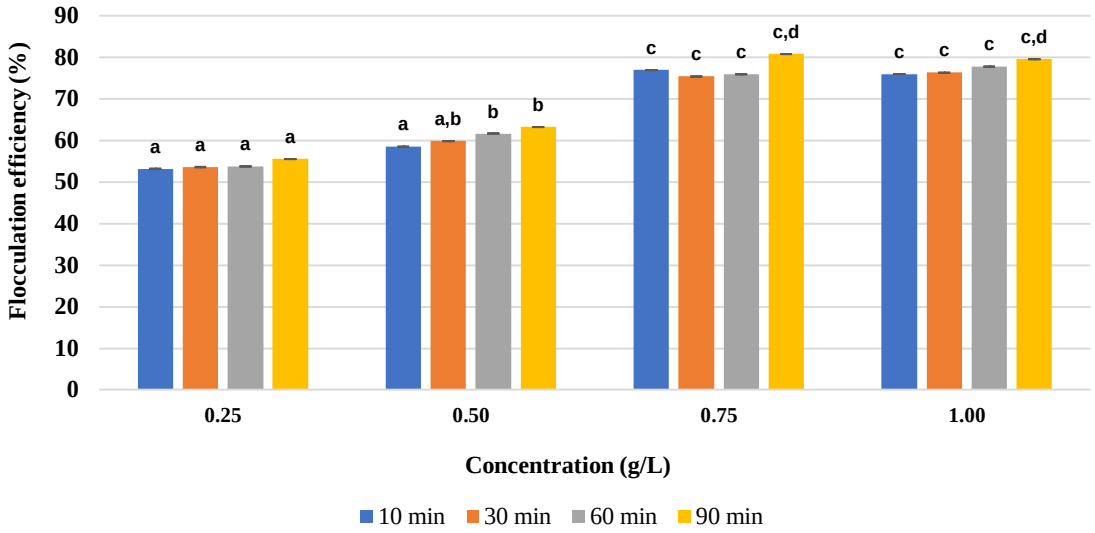

Figure 2. Flocculation Efficiency Variation in Function of Ferric Chloride ($FeCl_3$) Concentration Over Time. Different letters within the same concentration of ferric chloride indicate significant difference ($p<0.05$).

Flocculation with $FeCl_3$ presented satisfactory results in the initial minutes of the operation and with the holding time of 24 h. The significant difference between the yields indicates that the flocculant maintained its effect, *i.e.*, agglutinating the microalgae cells and causing them to settle, in the same way as $Al_2 (SO_4)_3$. Flocculation of *Chlorella zofingiensis* was affected by the microalgal concentration of the medium, $FeCl_3$ concentration and pH. The efficiency was greater than 90% when the parameters were 1.0 g/L of microalgae, 300 mg/L of $FeCl_3$, and pH 6.[28] The flocculation of *Chlorella* sp. exceeded 93% of efficiency with a $FeCl_3$ concentration of 122 mg/L and pH 6.[6] It was reported that the flocculant concentration for the removal of 95% of the cells increased linearly with the biomass concentration in the medium and that $Al_2 (SO_4)_3$ proved to be a better flocculant than $FeCl_3$ for most of the evaluated microalgae species.[17]

The non-interference of some agent for raising or lowering the pH, the moderate cell concentration of 1.3 g/L and the low flocculant concentration led to reasonable flocculation yields. Self-flocculation and flocculation performed with NaOH, $Al_2(SO_4)_3$ and $FeCl_3$ can affect the amount and quality of lipid extraction.[14,16,29,30] However, the choice of $FeCl_3$ at several concentrations does not affect the lipid extraction, with low possibility of lipid alterations.[16]

Based on the analyses performed in this study and the flocculation yields obtained, it can be stated that $FeCl_3$ is the best flocculating agent for the recovery of *Chlorella sorokiniana* cultivated in BBM, at a cell concentration of 1.3 g/L and pH of 10.2, with satisfactory yields (nearly 80%) and a short holding time (10 min).

FeCl3 at Different Concentrations

Mechanism of Action

The different concentrations of $FeCl_3$ acted in flocculation with good yields of thickness. However, the concentrations of 0.25 and 0.5 g/L showed a green color at the end of the flocculation, unlike the concentrations of 0.75 and 1.0 g/L. This green staining indicates that there were microalgae cells that did not self-flocculate and did not participate in the reaction with the flocculating agent, even after the set time of flocculation and the failure to increase in thickness. Bleaching of the medium at the concentrations of 0.75 and 1.0 g/L produced remarkable results. The higher concentration made the transparent phase slightly yellowish and brown-yellowish in color, which is typical when using high concentrations of $FeCl_3$.[12]

Degree of Flocculation

An observation of the descending flocs indicated that there was an abrupt drop in the descending labels of the sedimented volume in the first 10 min. The descending labels stagnated happened at 35 min at the concentration of 1 g/L, at 45 min at the concentration of 0.5 g/L and at 55 min at the other concentrations. The sedimentation volumes did not indicate that a certain concentration was the most efficient, given the green color at the end, but a lower possibility of dispersion of the sedimented flocs.

The lowest concentrations of $FeCl_3$ (0.25 and 0.5 g/L) produced sedimentation volumes of 0.095 and 1.412, while at the concentrations of 0.75 and 1.0 g/L the attained values were at 0.165 and 0.153, respectively, at 90 min (Table 1). The concentration of 0.75 g/L, even with the highest sedimentation volume, showed significant bleaching of the medium by visual observation, and lower possibility of redispersion of the flocs, making this concentration the most suitable one for flocculation.

Biomass Recovery and Bleaching of the Medium

The flocculation eficiency showed that the flocculants

behaved similarly and that there was an exponential drop in OD_{670} at all the concentrations in the first 10 min (Figure 2). This drop is explained by the higher concentrations of the flocculant agent $FeCl_3$ to interact with the microalgae driven to cell agglutination and sedimentation, and consequently to higher flocculation yields.[14]

The labels (height of the sediment and height of the liquid) indicated that all the concentrations were already stagnant at 60 min, as visual observed. This is same as the biomass recovery efficiency. Furthermore, there was no significant variation from 10 to 90 min at all the flocculant concentrations (Figure 2). The significant differences occurred in three distinct blocks, with concentrations of 0.25, 0.5 g/L and of 0.75 and 1.0 g/L.

The evaluation of yield in 10 min demonstrated that this holding time favored regular and good yields in the water and microalgal biomass separation process and prevented possible losses through floc redispersion. The yields achieved during the flocculation processes indicate that at 10 min and concentrations of 0.75 and 1.0 g/L, the biomass recovery is as efficient as at 60 and 90 min. At a concentration of 0.75 g/L at 90 min, the increase in yield was negligible, and flocculation may have ended at 10 min. A reasonable flocculation potential was achieved with the other concentrations. The yields achieved in this study were slightly lower than those reported by other authors, mainly due to the pH of the medium, which was higher than 10.[12,23]

The literature reports concentration gradients of various sulfated and chlorinated salts between 0.1 and 1.5 g/L for the flocculation of *Chlorella* sp. For example, at 0.50 g/L $FeCl_3$ a flocculation efficiency of about 80% was obtained after 300 min of holding time for *Chlorella minutissima*.[25] In another study, a yield of more than 90% was achieved in the flocculation of *Chlorella* sp. at 0.122 g/L of $FeCl_3$, which colored the medium from light green to brown.[6] Here it was observed that when the concentration of $FeCl_3$ was 1.0 g/L, the transparent phase became slightly yellowish.

In a previous study which aimed at correlating the $FeCl_3$ concentration with the cell concentration (0.05 to 1.5 g/L) at a slightly low pH, it was shown that there are three different flocculation behaviors: ineffective at low $FeCl_3$ concentrations, high flocculation efficiency (> 90%) at moderate concentrations of $FeCl_3$, and again inefficient flocculation at higher $FeCl_3$ concentrations.[28] Those findings corroborate the results found in our work, in which low efficiency was obtained with low flocculant concentrations (0.25 and 0.5 g/L), and satisfactory efficiency was achieved at 0.75 and 1.0 g/L.

Comparing different concentrations of $Al_2(SO_4)_3$ and $FeCl_3$ for flocculation of five microalgal species, other authors found that these agents behaved differently with each species, and that the best flocculant for *Chlorella vulgaris* was $FeCl_3$. However, a brown-yellowish coloration tends to occur[17], like it did when flocculation with 1.0 g/L of $FeCl_3$ was performed in the present study.

Some authors have observed a brown coloration when $FeCl_3$ is added at high concentrations[6,17,25] and the Fe^{3+} is absorbed by the cell up to its flocculation. However, if all the metal adsorption sites on the biomass are occupied by Fe^{3+}, the ferric chloride in excess led to an increase in the brown coloration in the supernatant. This can be a problem if the microalgae are to be utilized for pigment extraction.[25]

In fact, the choice of flocculant directly depends on what the microalgae biomass is intended for.[4] If the aim is to produce pigment, iron-based flocculating agents are not recommended.[25] However, for the extraction of oils and biodiesel, flocculants can be chosen based on parameters of efficiency (higher than 80%), costs and toxicity.[6,12,14,17,24] If the effluent need to be reused, high concentrations of $FeCl_3$ are not recommended because of the iron in excess.

Conclusion

Chlorella sorokiniana biomass was recovered with satisfactory efficiency using a simple low-cost flocculation method that does not have biomass contamination potential and that can be employed on a larger scale. These factors are critical to ensure the feasibility of the method on an industrial scale, especially when the focus is on biofuel production. The addition of a flocculating agent rendered the flocculation process faster than in self-flocculation. Among the flocculants analyzed here, NaOH, $FeCl_3$ and $Al_2(SO_4)_3$ presented promising results. $FeCl_3$ was chosen for further analysis of the flocculant concentration due to its higher flocculation yield in the first 10 min. The microalgal biomass recovery using different concentrations of $FeCl_3$ proved to be fairly efficient at moderate concentrations of microalgae (1.3 g/L), particularly at the concentration of 0.75 g/L of $FeCl_3$. Satisfactory flocculation performance in the first 10 min indicates that these conditions can be used as a pretreatment for microalgae harvesting.

Authors' Contributions

MLM designed the study. DMR, NNO, and OGBM carried out the laboratory work. MLM and GGF analyzed the data and wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

Acknowledgment

The authors gratefully acknowledge the Brazilian research funding agencies CNPq (National Council for Scientific and Technological Development), FUNDECT (Mato Grosso do Sul State Foundation for the Support and Development of Education, Science and Technology) and CAPES (Federal Agency for the Support and Improvement of Higher Education) for their financial support.

References

1. Getachew D, Mulugeta K, Gemechu G, Murugesan K. Values and drawbacks of biofuel production from microalgae. J Appl Biotechnol Rep. 2020;7(1):1-6. doi:10.30491/JABR.2020.105917
2. Oliveira NN, Menegazzo ML, Fonseca GG. Determination of kinetic parameters for biotechnological applications with microalgae. Apple Academic Press: Phycobiotechnology: Biodiversity and Biotechnology of Algae and Algal Products for Food, Feed and Fuel, chapter 11; 2021.
3. Nascimento VM, Nascimento KM, Fonseca GG. Biotechnological potential of *Pseudokirchneriella subcapitata*, *Scenedesmus spinosus* and *Scenedesmus acuminatus*. Acta Alim. 2020;49:154-62. doi:10.1556/066.2020.49.2.4
4. Menegazzo ML, Fonseca GG. Biomass recovery and lipid extraction processes for microalgae biofuels production: a review. Renew Sustain Energy Rev. 2019;107:87-107. doi:10.1016/j.rser.2019.01.064
5. Menegazzo ML, Nascimento VM, Hestekin C, Hestekin J, Fonseca GG. Evaluation of *Chlorella sorokiniana* cultivated in outdoor photobioreactors for biodiesel production. Biofuels. 2020. doi:10.1080/17597269.2020.1763094
6. Udom I, Zaribaf BH, Halfhide T, Gillie B, Dalrymple O, Zhang Q, et al. Harvesting microalgae grown on wastewater. Bioresour Technol. 2013;139:101-6. doi:10.1016/j.biortech.2013.04.002
7. Banerjee C, Ghosh S, Sem G, Mishra S, Shukla P, Bandopadhyay R. Study of algal biomass harvesting using cationic guar gum from the natural plant source as flocculant. Carbohydr Polym. 2013;92(1):675-81. doi:10.1016/j.carbpol.2012.09.022
8. Choix FJ, De-Bashan LE, Bashan Y. Enhanced accumulation of starch and total carbohydrates in alginate-immobilized *Chlorella* spp. induced by *Azospirillum brasilense*: I. Autotrophic conditions. Enz Microb Technol. 2012;51(5):294-9. doi:10.1016/j.enzmictec.2012.07.013
9. S Benemann J. Microalgae for biofuels and animal feeds. Energies. 2013;6(11):5869-86. doi:10.3390/en6115869
10. Rawat I, Kumar RR, Bux TMF. Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. Appl Energy. 2013;103:444-67. doi:10.1016/j.apenergy.2012.10.004
11. Rodrigues DB, Menezes CR, Mercadante AZ, Jacob-Lopes E, Zepka LQ. Bioactive pigments from microalgae *Phormidium autumnale*. Food Res Int. 2015;77(part 2):273-9. doi:10.1016/j.foodres.2015.04.027
12. Granados MR, Acien FG, Gymez C, Fernandez-Sevilla JM, Grima EM. Evaluation of flocculants for the recovery of freshwater microalgae. Bioresour Technol. 2012;118:102-10. doi:10.1016/j.biortech.2012.05.018
13. Salim S, Vermue MH, Wijffels RH. Ratio between autoflocculating and target microalgae affects the energy-efficient harvesting by bio-flocculation. Bioresour Technol. 2012;118:49-55. doi:10.1016/j.biortech.2012.0 5.007
14. Borges L, Caldas S, D'Oca MGM, Abreu PC. Effect of harvesting processes on the lipid yield and fatty acid profile of the marine microalga *Nannochloropsis oculata*. Aquacul Rep. 2016;4:164-8. doi:10.1016/j.aqrep.2016.10.004
15. Gerde JA, Yao L, Lio JY, Wen Z, Wang T. Microalgae flocculation: Impact of flocculant type, algae species and cell concentration. Algal Res. 2014;3:30-5. doi:10.1016/j.algal.2013.11.015
16. Chatsungnoen T, Chisti Y. Oil production by six microalgae: impact of flocculants and drying on oil recovery from the biomass. J Appl Phycol. 2016;28(5):2697-2705. doi:10.1007/s10811-016-0823-6
17. Chatsungnoen T, Chisti Y. Harvesting microalgae by flocculation–sedimentation. Algal Res. 2016;13:271-83. doi:10.1016/j.algal.2015.12.009
18. Liu J, Zhu Y, Tao Y, Zhang Y, Li A, Li T, Sang M, Zhang C. Freshwater microalgae harvested via flocculation induced by pH decrease. Biotechnol Biofuels. 2013;6(1):98. doi:10.1186/1754-6834-6-98
19. Bischoff HC. Some soil algae from enchanted rock and related algal species. Phycological Studies IV. University of Texas Publications. No. 6318. 1963;6318:1-95.
20. Papazi A, Makridis P, Divanach P. Harvesting *Chlorella minutissima* using cell coagulants. J Appl Phycol. 2010;22(3):349-55. doi:10.1007/s10811-009-9465-2
21. Morioka LRI, Matos BP, Olivo G, Sant'Anna ES. Evaluation of flocculation and lipid extraction of *Chlorella* sp. cultivated in concentrated desalination. Quнм Nova. 2014;37(1):44-9. doi:10.1590/S0100-40422014000100009
22. Şirin S, Trobajo R, Ibanez C, Salvady J. Harvesting the microalgae *Phaeodactylum tricornutum* with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. J Appl Phycol. 2012;24(5):1067-80. doi:10.1007/s10811-011-9736-6
23. Rashid N, Rehman, SU, Han J-I. Rapid harvesting of freshwater microalgae using chitosan. Proc Biochem. 2013;48(7):1107-10. doi:10.1016/j.procbio.2013.04.018
24. Ras M, Lardon L, Bruno S, Bernet N, Steyer J-P. Experimental study on a coupled process of production and anaerobic digestion of *Chlorella vulgaris*. Bioresour Technol. 2011;102(1):200-6. doi:10.1016/j.biortech.2010.06.146
25. Knuckey RM, Brown MR, Robert R, Frampton DMF. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacul Eng. 2006;35(3):300-13. doi:10.1016/j.aquaeng.2006.04.001
26. Chen CY, Yeh KL, Aisyah R, Lee DJ, Chang JS. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol. 2011;102(1):71-81. doi:10.1016/j.biortech.2010.06.159
27. Wang LK, Hung YT, Shammas NK, editors. Physicochemical Treatment Processes. London: Springer: Handbook of Environmental Engineering. Humana Press; 2005. doi:10.1385/159259820x
28. Wyatt NB, Gloe LM, Brady PV, Hewson JC, Grillet AM, Hankins MG, et al. Critical conditions for ferric chloride-induced flocculation of freshwater algae. Biotechnol Bioeng. 2012;109(2):493-501. doi:10.1002/bit.23319
29. Ahmad AL, Mat Yasin NH, Derek CJ, Lim JK. Comparison of harvesting methods for microalgae *Chlorella* sp, its potential use as a biodiesel feedstock. Environ Technol. 2014;35(17):2244-53. doi:10.1080/09593330.2014.900117
30. Taher H, Al-Zuhair S, Al-Marzouqi AH, Haik Y, Farid M. Effective extraction of microalgae lipids from wet biomass for biodiesel production. Biomass Bioenerg. 2014;66:159-67. doi:10.1016/j.biombioe.2014.02.034