ARTIGO ORIGINAL
ISSN 1677-5090 impresso
ISSN 2236-5222 digital

DOI 10.9771/cmbio.v24i3.68441

Microalgae biomass recovery using organic and inorganic flocculants

Recuperação da biomassa de microalgas utilizando floculantes orgânicos e inorgânicos.

Ian da Silva Andrade[1], Gabriele Rodrigues Conceição[2], Carine Souza Silva[3], Alana Nogueira Silva[4], Rodrigo Miranda Pires Santos[5], Fabio Alexandre Chinalia[6*]

[1]Biotecnologista, Mestrando, Programa de Pós-graduação em Biotecnologia, Universidade Federal da Bahia – UFBA, Professor do Curso Técnico em Biotecnologia do SENAI CIMATEC;[2]Biotecnologista, Mestre, Doutoranda, Programa de Pós-graduação em Biotecnologia, Universidade Federal da Bahia – UFBA;[3]Biotecnologista, Mestre, Doutoranda, Programa de Pós-graduação em Biotecnologia, Universidade Federal da Bahia – UFBA; [4]Acadêmica do Curso de Biotecnologia, Universidade Federal da Bahia – UFBA; [5]Engenheiro Mecânico, Mestre, Doutorando, Programa de Pós-graduação em Biotecnologia, Universidade Federal da Bahia – UFBA; [6]Licenciado em Ciências Biológicas, Mestre em Ecologia e Recursos Naturais, Universidade Federal de São Carlos – UFSCAR, Doutor em Environmental Microbiology. University of Aberdeen – ABDN, [6]Licenciado em Ciências Biológicas, Mestre em Ecologia e Recursos Naturais, Universidade Federal de São Carlos – UFSCAR, Doutor em Environmental Microbiology. University of Aberdeen – ABDN, Escócia, Pos-doutor pela EESC/USP e NUI-Galway, Irlanda, Professor Associado 3 de Biotecnologia, Instituto de Ciências da Saúde, Universidade Federal da Bahia – UFBA

[1]Moacyr D. de Moura-Costa Biotechnology Laboratory, Institute of Health Sciences, Federal University of Bahia – UFBA, Salvador, Bahia, Brazil.
[2]Northeast Biotechnology Network, Federal University of Bahia – UFBA, Salvador, Bahia, Brazil.
[3]Laboratory of Bioprospecting and Biotechnology, Institute of Biology, Federal University of Bahia – UFBA, Salvador, Bahia, Brazil.

Abstract
Introduction: *Flocculation is an important step in the recovery of algal biomass, being associated with low cost and practical applicability at large volumes.* **Objective:** *This study aimed to select and evaluate the efficiency of different flocculating agents for recovering microalgal biomass after cultivation.* **Methodology:** *Three inorganic agents (ferrous sulfate, ferric chloride, and aluminium sulfate) and one tannin-based organic agent were tested. Flocculation assays were performed using Chlorella vulgaris and Nannochloropsis oceanica cultures under varying pH conditions and specific concentrations.* **Results:** *Aluminium sulfate (100 mg L^{-1}) showed the highest flocculation efficiency for C. vulgaris, while ferric chloride (50 mg L^{-1}) was more effective for N. oceanica, reaching 93% removal at pH 7 and 9, respectively. However, the tannin-based organic flocculant (75 mg L^{-1}) demonstrated satisfactory performance for both strains and stood out in the cost-benefit analysis.* **Conclusion:** *Although inorganic flocculants exhibited high efficiency, the cost-benefit analysis indicated the organic agent (75 mg L^{-1}) as the most advantageous flocculant for both strains. Furthermore, being of biological origin, this compound aligns with the principles of the circular economy, promoting the sustainable use of resources.*
Keywords: *Tannin-based; Tannic flocculant; Organic flocculation.*

Resumo
Introdução: A floculação é uma etapa importante na recuperação de biomassa algal, sendo associada a baixo custo e aplicabilidade prática em volumes elevados. **Objetivo:** Este estudo teve como objetivo selecionar e avaliar a eficiência de diferentes agentes floculantes para a recuperação de biomassa de microalgas após o cultivo. **Metodologia:** Foram testados três agentes inorgânicos (sulfato ferroso, cloreto férrico e sulfato de alumínio) e um agente orgânico à base de tanino. As floculações foram conduzidas em culturas de *Chlorella vulgaris* e *Nannochloropsis oceanica* sob diferentes pHs e concentrações específicas. **Resultados:** O sulfato de alumínio (100 mg L^{-1}) apresentou a maior eficiência de floculação para *C. vulgaris*, enquanto o cloreto férrico (50 mg L^{-1}) foi mais eficaz para *N. oceanica*, atingindo 93% de remoção nos pHs 7 e 9, respectivamente. No entanto, o floculante orgânico à base de tanino (75 mg L^{-1}) demonstrou desempenho satisfatório para ambas as cepas e se destacou na análise de custo-benefício. **Conclusão:** Embora os floculantes inorgânicos tenham apresentado alta eficiência, a análise de custo-benefício indicou o agente orgânico (75 mg L^{-1}) como a melhor opção de floculante para ambas as cepas. Além disso, por ser de origem biológica, esse composto está alinhado aos princípios da economia circular, promovendo o uso sustentável de recursos.
Palavras-chave: Tanínico; Floculante tânico; Floculação orgânica.

Correspondente/Corresponding: *Fabio A. Chinalia. – Endereço: Av. Reitor Miguel Calmon, S/N, Salvador, BA, 40110-100, Brazil. – E-mail: chinalia@hotmail.com

INTRODUCTION

Microalgae biotechnologies hold promise in replacing fossil fuels with a significant impact on the control of climate instability. Growing microalgae for biofuels not only offers solutions to reduce carbon emissions[1-2], but also contributes to food, pharmaceuticals, and wastewater treatment industries[3].

The global market for algae-based products was valued at $4.9 billion in 2022 and $5.3 billion in 2023, with projections indicating it will surpass $7.3 billion by 2028. This corresponds to an annual growth rate (CAGR) of 6.4%[4]. Microalgal-driven systems are strategic tools endorsed by the United Nations for making the transition from fossil fuels to biofuels less disruptive[5-6]. Moreover, the recent COP26 meeting identified algal-based systems as crucial for such a goal in addition to carbon capture[7-8-9].

Despite advancements in microalgae culturing technologies, there are still significant challenges to cover. Harvesting and extracting oil from algae are significant limiting steps. They can contribute up to 60% of the total process costs. Harvesting methods and biomass dewatering for lipid extraction are critical stages[10-11-3]. Current techniques include gravity sedimentation, centrifugation, coagulation, flocculation, filtration, and flotation[11-12-13-14]. Hence, there is a need for low-energy, cost-effective, and ecologically sustainable harvesting techniques.

Flocculation remains the most efficient, economically viable, and technically suitable method for microalgae harvesting[15-14]. However, the choice of flocculant and its effectiveness vary depending on the algal species within any other factors. Common inorganic flocculants like ferrous sulfate, ferric chloride, and aluminium sulfate promote cell aggregation through cell wall electrical neutralization[16]. Tannic flocculant, an organic-cationic commercial polymer derived from plant sources, can also act in this manner[17]. However, determining the optimal dosage and effectiveness of each of these chemicals for specific algal species, remains case specific[14].

Chlorella vulgaris and Nannochloropsis oceanica are prominent microalgae in biotechnological applications. C. vulgaris is valued for its potential in the production of biofuels and various bioproducts, whereas N. oceanica is employed as a feed source in the cultivation of fish-larvae, fish, and crustaceans, in addition to being a promising candidate for biofuel production as well[18-19].

Thus, this study aims to identify the most efficient flocculation agent considering various factors such as microalgae species, medium type, flocculant concentration, pH, and sedimentation time. It compares the flocculation performance of a commercial tannin-based flocculant with three inorganics (ferrous sulfate, ferric chloride, and aluminium sulfate) using two distinct algae species, Chlorella vulgaris and Nannochloropsis oceanica (fresh and salt water, respectively).

MATERIAL AND METHODS

Strains and culturing media

The C. vulgaris and N. oceanica strains were generously provided by the Iracema Nascimento Microalgae Bank (BMIN) at the Bioprospection and Biotechnology Laboratory (LaBBiotec) of the Federal University of Bahia, under the supervision of Prof. Suzana Telles da Cunha Lima. For maintaining C. vulgaris, we utilised BG-11 medium[20], while Conway Medium[21] with a salinity of 35 g of Total Dissolved Solids (TDS) per litre was employed for cultivating N. oceanica. Cultures were triplicated in 1L Erlenmeyer flasks containing 600 mL of medium, following a photoperiod of 12 hours light and 12 hours dark, with a light intensity of 3500 lux. Agitation was maintained at 100 rpm, aeration was provided with atmospheric air, and the temperature was controlled at 25°C (±2°C) as used by Cabanelas et al, 2013[22].

Preparation of flocculant agents and testing

Tannic flocculant, Ferrous Sulfate, Ferric Chloride and Aluminium Sulfate were purchased in powder form, and a stock solution of 10 g L^{-1} was prepared freshly before use. The company TANAC, located in the city of Montenegro, in the state of Rio Grande do Sul, is the producer of Tanfloc SG and kindly provided the chemical for the tests. Flocculation tests were carried out using algal biomass at the end of the exponential growth phase. C. vulgaris and N. oceanica reached the end of their exponential phases within 15 and 9 days, respectively. The flocculation recovery assay was carried out by adding the flocculant during 1 1-minute agitation period at 150 rpm, followed by a 10-minute slower agitation period (50 rpm). This procedure was adopted to promote a complete mixture as described elsewhere [23]. In sequence, such a mixture rested for 120 min (with a 30 min sampling strategy) to allow for the monitoring of the flocculation process[24]. Tannin-based flocculants and inorganic salts such as Iron Sulfate, Iron Chloride and Aluminium Sulfate were tested at concentrations of 25, 50, 75, 100, 150, 200, and 250 mg/L. These experiments were also carried out at distinct pH values of 7 and 9, respectively (using NaOH or hydrochloric acid for pH correction). Chlorella vulgaris and Nannochloropsis oceanica biomass were sampled at the end of the exponential growth phase for the tests.

Flocculation efficiency was assessed using a PRO-TOOLS V-1100D spectrophotometer (680 nm) before (OD_i) and after (OD_f) the addition of the flocculant. Evaluating changes in optical density is the most practical and straightforward method for measuring algal flocculation, particularly when compared to alterna-

Rev. Ciênc. Méd. Biol., Salvador, v. 24, n. 3, p. 739-745, set./dez. 2025

tives such as direct biomass weight determination or chlorophyll extraction[16]. Each flocculant was tested in triplicate, and flocculation efficiency (FE%) was then calculated using Eq. (01):

Flocculation Efficiency (%) = *x 100 (01)*

Statistical analysis

Flocculation efficiency was measured using Equation 1 at different reaction times and with distinct flocculants. Means were calculated from triplicates of each experiment, and ANOVA was used to compare potential differences among treatments (Minitab v23).

RESULTS AND DISCUSSION

As described previously, flocculation tests were conducted using algal biomass harvested at the end of the exponential growth phase. C. vulgaris and N. oceanica reached this stage in 15 and 9 days, with maximum biomass production rates of 0.095 and 0.032 g L^{-1} d^{-1} and specific growth coefficients (μ) of 0.13 and 0.10 d^{-1}, respectively. Although C. vulgaris demonstrates faster growth and accumulates more biomass than N. oceanica, both algae were sampled in the same physiological stage.

Flocculation tests carried out with periods shorter than 60 min showed results below 80% efficiencies and were not discussed or compared with other treatments. Thus, the ideal treatment is identified as the one with the best balance between lower dose, lower cost, and higher flocculation efficiency (>80%)[16]. Figure 1 shows the flocculation results using a tannin-based flocculant from 25 to 250 mg L^{-1} with a one-hour incubation. For C. vulgaris, the highest efficiency (89%) was obtained at 75 mg L^{-1} under pH 7 (Figure 1A). For N. oceanica, the highest efficiency (83%) was achieved at 50 mg L^{-1} at pH 7 (Figure 1B). Increasing tannin-based flocculant concentrations negatively affected N. oceanica flocculation, and alkaline pH produced significant changes in C. vulgaris at concentrations above 100 mg L^{-1}.

The flocculation efficiency using ferrous sulfate is shown in Figure 2. For C. vulgaris, maximum efficiency (87%) occurred at 200 mg L^{-1} at pH 9; the second highest (84%) was obtained using 150 mg L^{-1} at pH 9 (Figure 2A). For N. oceanica, distinct pH values did not cause significant interference across treatments with concentrations higher than 75 mg L-1 (Figure 2B). Above such concentration, distinct pH values were responsible for only approximately 5% difference between the averages of flocculation efficiencies. Nonetheless, the best efficiency for N. oceanica was achieved using 25 mg L^{-1} (84%) at pH 9.

Figure 1 – *Tannin-based (flocculation efficiencies (%) at concentrations ranging from 25 to 250 mg L^{-1} during a one-hour incubation period under pH 7 ( ) and 9 ( ) for Chlorella vulgaris (A) and Nannochloropsis oceanica (B), respectively.*

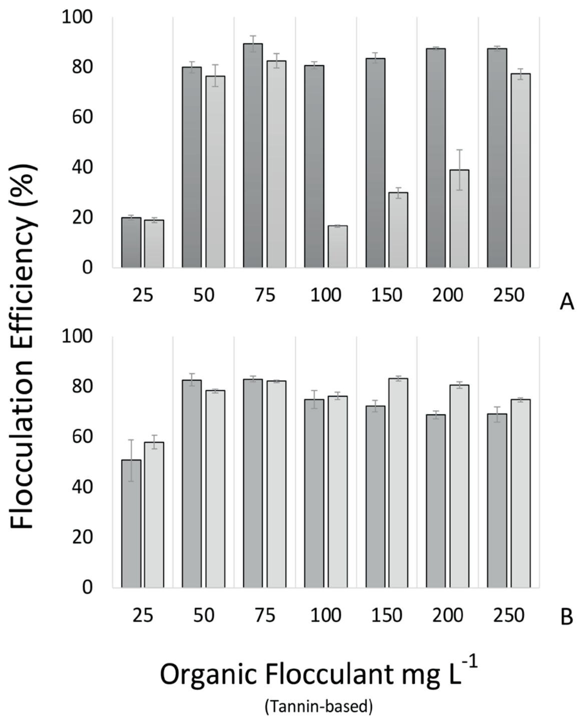
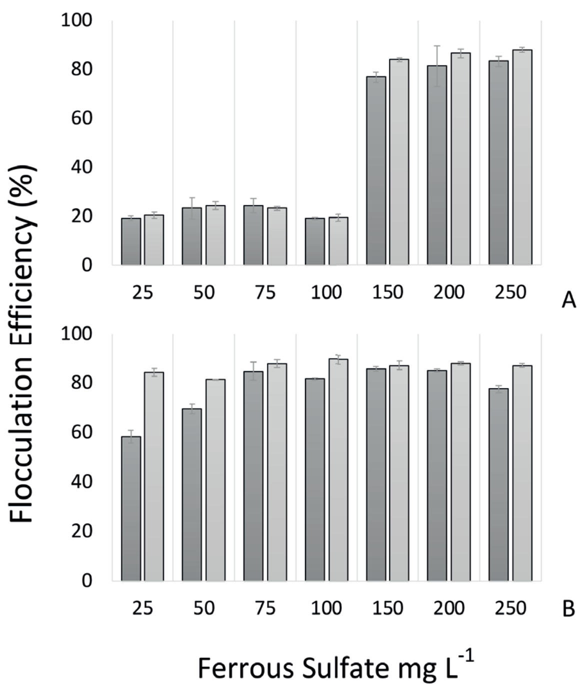
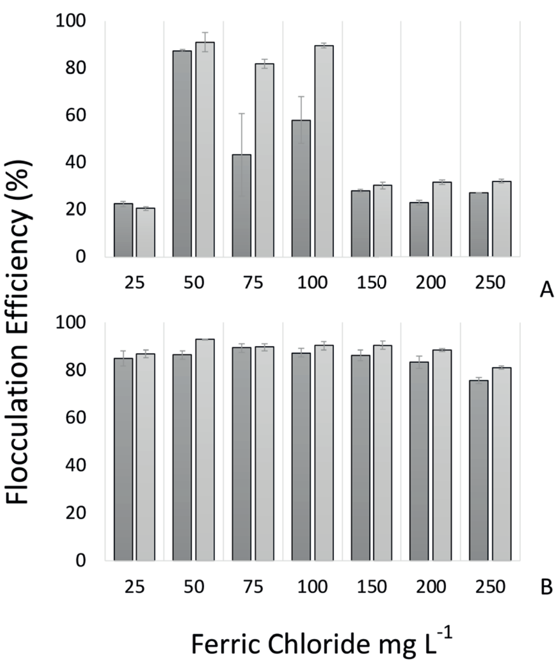


Figure 2 – *Ferrous Sulfate flocculation efficiencies (%) at concentrations ranging from 25 to 250 mg L^{-1} during a one-hour incubation period under pH 7 ( ) and 9 ( ) for Chlorella vulgaris (A) and Nannochloropsis oceanica (B), respectively.*



C. vulgaris flocculation efficiency using ferric chloride was highest at a concentration of 50 mg L^{-1} in both pH values tested (Figure 3A). Higher ferric chloride concentrations resulted in a reduction in the flocculation efficiency of C. vulgaris biomass. For N. oceanica, neither ferric chloride concentration nor pH altered flocculation efficiency significantly (Figure 3B). The highest average of 93% was obtained using 50 mg L^{-1}, but, although statistically different, the concentration of 25 mg L^{-1} (half of the previously cited) also produced a significant result (87%).

Figure 3 – *Ferric Chloride flocculation efficiencies (%) at concentrations ranging from 25 to 250 mg L^{-1} during a one-hour incubation period under pH 7 (■) and 9 (■) for Chlorella vulgaris (A) and Nannochloropsis oceanica (B), respectively.*



The highest flocculation efficiency using aluminium sulfate is shown in Figure 4. The results indicate that, for C. vulgaris, the best concentration was 100 mg L^{-1} under pH 7 (Figure 4A). For N. oceanica, the best result was observed when using 50 mg L^{-1} under pH 9 (Figure 4B). It is important to highlight that at lower aluminium sulfate concentrations, pH was a critical variable affecting flocculation efficiency.

Figure 4 – *Aluminium Sulfate flocculation efficiencies (%) at concentrations ranging from 25 to 250 mg L^{-1} during a one-hour incubation period under pH 7 (■) and 9 (■) for Chlorella vulgaris (A) and Nannochloropsis oceanica (B), respectively.*

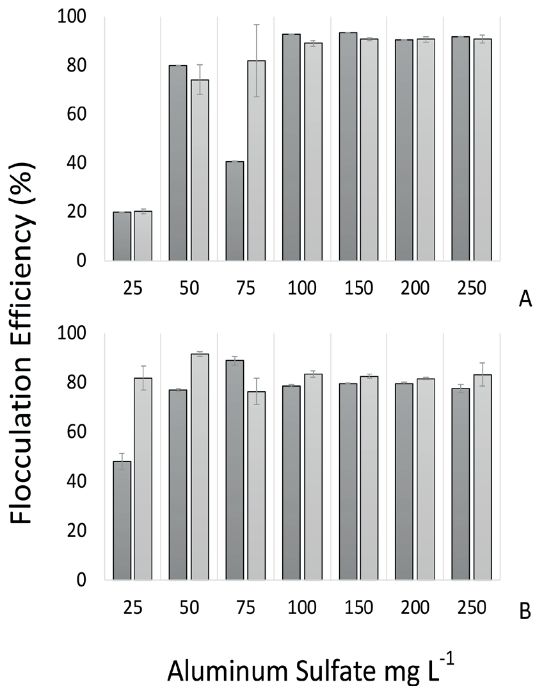


Table 1 summarises dose–efficiency ratios across all agents. For C. vulgaris, 50 mg L^{-1} ferric chloride provided the best flocculation performance, and for N. oceanica, 25 mg L^{-1} ferrous sulfate was the best treatment. Statistical comparison also identified that a tannin-based flocculant at 75 mg L^{-1} produced an effect equivalent efficiency to ferric chloride for C. vulgaris. This is important for cost-analysis considerations.

Table 1 – *Ranking the best dose/flocculation efficiency ratio for Chlorella vulgaris and Nannochloropsis oceanica grown in BG-11 and Conway synthetic media, respectively (pH 7 and 9, separately).*

Microalgae culture	*Flocculant*	*Ideal dose (mg L^{-1})*	*Efficiency (%)*	*Ideal pH*
Chlorella vulgaris	*Iron (III) chloride[a]*	50	91 (±4,5)	9
Chlorella vulgaris	*Tanfloc SG[a]*	75	89 (±3,1)	7
Chlorella vulgaris	*Aluminum sulfate[a]*	100	93 (±1,4)	7
Chlorella vulgaris	*Iron (II) sulfate*	150	84 (±0,8)	9
Nannochloropsis oceanica	*Iron (II) sulfate*	25	84 (±1,6)	9
Nannochloropsis oceanica	*Tanfloc SG*	50	83 (±2,4)	7
Nannochloropsis oceanica	*Aluminum sulfate*	50	92 (±1,0)	9
Nannochloropsis oceanica	*Iron (III) chloride*	50	93 (±1,4)	9

[a]*Statistically equivalent treatments ($p > 0,05$).*

The common prices of the flocculants are shown in Table 2. The tannin-based flocculant shows a commercial value of USD$1,500.00 per ton, which is substantially lower than the prices for iron sulfate and iron chloride (USD$10,447.66 and USD$29,529.66 per ton, respectively).

Table 2. *Dollar value of one (01) ton of flocculant.*

Flocculant	*US$/ton*	*References*
Tannic flocculant	*1.500*	*(ROSELET et al., 2016)[31]*
Iron (II) sulfate	*10.448*	*(BUTLER et al., 2021)[20]*
Iron (III) chloride	*29.530*	*(LI et al., 2020)[22]*
Aluminum sulfate	*2.074*	*(BUTLER et al., 2021)[20]*

DISCUSSION

A tannin-based agent is an organic flocculant often recommended for the treatment of water supplies and industrial effluents, such as those from mining, refineries, metallurgies, and pulp mills[21]. It is basically composed of vegetable tannins that can be applied sole or in combination with other flocculant agents such as aluminium sulfate or ferric chloride[21]. It is often effective in a wide pH range and does not interfere with medium alkalinity[16-24]. The highest flocculation efficiency for C. vulgaris biomass (89%) was obtained when using 75 mg L^{-1} under pH 7 (Figure 1A). The optimal flocculation efficiency for N. oceanica biomass (83%) was achieved when using 50 mg L^{-1} of tannin-based flocculant at pH 7 (Figure 1B). It is important to highlight that an increase in tannin-based flocculant concentrations had a detrimental effect on the flocculation of N. oceanica biomass. In addition, an alkaline pH showed considerable changes in C. vulgaris flocculation efficiencies at concentrations above 100 mg L^{-1}. Algal biomass responses to tannin-based flocculants vary considerably[17]." This organic substance can successfully harvest C. vulgaris and Scenedesmus obliquus biomass, but flocculation efficiency may significantly decrease at alkaline pH levels[17-24].

Polyvalent metallic and polymetallic salts represented by iron salts and aluminium salts, such as $FeCl_3$, $Al_2(SO_4)_3$ and $Fe_2(SO_4)_3$, are the most common flocculants in traditional water treatment, and they were the first agents applied for microalgae flocculation purposes. Metal salt flocculants mainly promote the aggregation of algal cells through electrical neutralisation. Free cations like Al^{3+}, Fe^{3+}, Mg^{2+}, Zn^{2+}, and their various hydrolysates can neutralise negative charges on the surface of algal cells, promoting the formation of cell clusters [15-25].

Ferrous Sulfate is an inorganic salt with a greenish colour and is available in liquid or solid forms. It is often used as an acidifier for adjusting pH levels, but also as an organic and phosphorus flocculant[16-26-27]. Figure 2 shows that the flocculation of C vulgaris biomass reached its maximum efficiency (87%) when using 200 mg L^{-1} of ferrous sulfate at pH 9. However, a lower concentration of ferrous sulfate (150 mg L^{-1}) may produce a similar flocculation efficiency (84%) but only at pH 9 (Figure 2A). Previous research suggested that applying ferrous sulfate at concentrations above 2500 mg L^{-1} resulted in effective C. vulgaris biomass flocculation (>85%)[28]. Nevertheless, the authors did not report on pH value adjustments before the flocculation procedure. Thus, it is possible that correcting the pH value may significantly improve flocculation efficiency for C vulgaris when using this agent. However, pH adjustment tested in this research does not indeed interfere with any of the treatments with N oceanica (Figure 2B). In fact, it is observed that pH adjustments accounted for just 5% of variations in flocculation efficiencies with this alga. Therefore, the best efficiency was defined as achievable with 25 mg L^{-1} (84%) using either pH value.

Ferric chloride solution displays a reddish-brown colour with a pungent odour. It is corrosive to ferrous chemical structures but does not alter non-metallic materials such as plastics or paint covers. It is used as a coagulant agent in the treatment of drinking water, domestic sewage, industrial liquid effluents and sludge dewatering[16-27]. Figure 3A shows that 50 mg L^{-1} of ferric chloride resulted in a higher efficiency of C vulgaris flocculation at both tested pH values. It is important to note that higher ferric chloride concentrations negatively affected C vulgaris flocculation efficiencies. This is conflicting with the literature, as some studies have shown C vulgaris high flocculation efficiencies only at concentrations above 160 mg L^{-1} [16]. Neither concentration nor pH values altered N oceanica flocculation efficiencies (Figure 3B). The highest average of 93% flocculation ratio was obtained with 50 mg L^{-1}. However, the obtained average of 87% flocculation efficiency with 25 mg L^{-1} showed a close result. Despite being statistically different, it is half of the amount cited previously.

Aluminium sulfate is a well-established industrial flocculating agent. It is frequently used in water treatment plants and swimming pools. The application and dosages vary significantly according to the nature of the water to be treated[16-26-27]. Figure 4A shows that the highest C. vulgaris biomass flocculation efficiency occurred when applying 100 mg L^{-1}, pH 7. Previous studies recommend an aluminium sulfate concentration above 160-200 mg L^{-1} for C. vulgaris[16]. However, reports are suggesting that the dosage of 2500 mg L^{-1} can promote flocculation in a short period of 10 min incubation[29]. Thus, a balance between costs and incubation time may be an important issue for some industries. The best concentration of aluminium sulfate for recovering N oceanica biomass was observed with 50 mg L^{-1} at pH 9 (Figure 4B). The results show that pH values are a critical variable at lower aluminium sulfate concentrations.

The dose/flocculation efficiency ratios were summarised in Table 1. The results suggest that the best flocculant agents for C vulgaris may be iron chloride (50 mg L^{-1}) and iron sulfate (25 mg L^{-1}) for N. oceanica. On the other hand, the statistical comparison of flocculation efficiencies identified that tannic flocculant at 75 mg L^{-1} produced an equivalent flocculation effect as iron chloride *for C vulgaris. This is a significant observation due to the costs of each compound. Flocculant costs that are above USD$0.10 per kg of recovered algal dry weight restrict commercial implementation[30]. The ideal or industrially viable cost has been identified as USD$0.04 per kg of algal dry weight[24]. Table 2 shows that the tannic flocculant commercial value of USD$1,500.00 per ton is significantly lower than iron sulfate or iron chloride (USD$10,447.66 and 29,529.66 per ton, respectively). This comparison identifies tannic flocculant as close to USD$0.04 per kg of algal dry weight. Thus, tannin-based agents are more cost-effective than chemical flocculants and are effective at concentrations of 75 and 50 mg L^{-1} for C vulgaris and N oceanica, respectively. In addition, tannin-based agents can achieve flocculation without compromising cell integrity and without affecting cell viability[17]. Thus, it reduces the losses of lipids, carbohydrates, or proteins during the process of algal biomass flocculation. In addition, metal-based substances may not only interfere with cell viability but may also contaminate algal biomass. After the extraction of lipids, carbohydrates or pigments, the final residues may be toxic and require further downstream treatment before use or discharge[24].*

CONCLUSIONS

In the common understanding of a linear economy, industrial processes are based on the “take, produce, use and discard” principle. The circular economy, however, introduces a different perspective. To diminish environmental impact, residual biomass should be targeted as a useful resource and contribute to further production and treatment before being discarded. Therefore, renewable organic substances such as tannin-based substances are preferable to non-renewable chemicals that, in addition, may add downstream problems to the industrial process[8-7]. This research shows that the choice of an ideal flocculant depends on the microalgae species, pH, concentration, costs, interaction with the biomass and potential environmental impacts. This research identified tannin-based agents as the best flocculants when compared to other common inorganic agents. However, chemical agents such as iron chloride and sulfate may be useful depending on availability and costs. The economic limiting factor is the price of USD$0.10 per kg of recovered algal dry weight.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Brazilian National Councils CAPES and CNPq. This research received funding from CNPq 315170/2023-9.

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Sub: 05/07/2025
Aceite: 29/08/2025