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# Using eco-friendly coagulants for biomass harvesting and water reuse in microalga cultivation

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## ABSTRACT

The cultivation of microalgae is gaining prominence, but one of its main challenges lies in effectively harvesting the biomass and recovering the water used in the cultivation process. Addressing these issues is critical to minimizing environmental impacts and enhancing the sustainability of microalgae production systems. This study evaluates the efficiency of polyaluminum chloride (PAC) and a tannin-derived coagulant for harvesting Nannochloropsis oculata biomass, while also analyzing the potential for reusing the treated water. Microalgae were grown in a 5 L culture until a cell density of $10^4$ cells $mL^{-1}$ was achieved. After cultivation, samples were subjected to coagulation, flocculation, sedimentation, and filtration through a granular activated carbon system. An acute toxicity assessment was conducted using Daphnia magna as a bioindicator to determine the safety of the recovered water. Both coagulants demonstrated over 98% removal efficiency for suspended solids, turbidity, chemical oxygen demand (COD), and biochemical oxygen demand (BOD). PAC, at a concentration of 50 mg $L^{-1}$, yielded higher water quality than the tannin-based coagulant, which required 150 mg $L^{-1}$ for optimal performance. Furthermore, the treated water met international standards for irrigation in non-food crops. Toxicity testing confirmed that the treated effluent was non-toxic, making both coagulants suitable for microalgae harvesting, with PAC offering a more efficient option for water quality enhancement and lower chemical consumption.


**Keywords:** biomass harvesting, *Nannochloropsis oculate.*, polyaluminum chloride, tannin-based coagulant, water reuse.

# Uso de coagulantes ecológicos para coleta de biomassa e a reutilização da água no cultivo de microalgas


## RESUMO

O cultivo de microalgas está ganhando destaque, mas um dos seus principais desafios está na colheita eficaz da biomassa e na recuperação da água usada no processo de cultivo. Abordar essas questões é fundamental para minimizar os impactos ambientais e aumentar a sustentabilidade dos sistemas de produção de microalgas. Este estudo avalia a eficiência do cloreto de polialumínio (PAC) e de um coagulante derivado de tanino para a colheita de biomassa de Nannochloropsis oculata, ao mesmo tempo em que analisa o potencial de reutilização da água tratada. As microalgas foram cultivadas em uma cultura de 5 L até que uma densidade celular de $10^4$ células $mL^{-1}$ fosse alcançada. Após o cultivo, as amostras foram submetidas à coagulação, floculação, sedimentação e filtração por meio de um sistema de carvão ativado granular. Uma avaliação de toxicidade aguda foi conduzida usando Daphnia magna como um bioindicador para determinar a segurança da água recuperada. Ambos os coagulantes demonstraram mais de 98% de eficiência de remoção de sólidos suspensos, turbidez, demanda química de oxigênio (DQO) e demanda bioquímica de oxigênio (DBO). O PAC, em uma concentração de 50 mg $L^{-1}$, produziu maior qualidade de água do que o coagulante à base de tanino, que exigiu 150 mg $L^{-1}$ para desempenho ideal. Além disso, a água tratada atendeu aos padrões internacionais para irrigação em culturas não alimentares. Os testes de toxicidade confirmaram que o efluente tratado não era tóxico, tornando ambos os coagulantes adequados para coleta de microalgas, com o PAC oferecendo uma opção mais eficiente para melhoria da qualidade da água e menor consumo de produtos químicos.

**Palavras-chave:** cloreto de polialumínio, coagulante à base de tanino, coleta de biomassa, *Nannochloropsis oculate*., reutilização de água.

## 1. INTRODUCTION

Over the past decade, the adverse impacts of climate change have accelerated, emphasizing the need to curb carbon emissions and prioritize renewable energy alternatives (Yuan *et al.*, 2022; Qi *et al.*, 2023). Microalgae cultivation presents a promising solution, given their ability to adapt to diverse environments, provided suitable parameters such as pH, light intensity, temperature, and nutrient availability are maintained (Chua *et al.*, 2020). Microalgae have been recognized for their rich nutritional profile, making them valuable sources of proteins and other dietary components for human and animal consumption (Liao *et al.*, 2022; Zanella *et al.*, 2020). Recent studies have further expanded their potential, focusing on applications such as carbon capture, biofuel production, biomaterial development, and the generation of nutraceuticals like omega-3 fatty acids, carotenoids, and specialized sugars (Yen *et al.*, 2013; Chua *et al.*, 2020).

Marine microalgae, in particular, are widely researched, with cultivation taking place in controlled systems like photobioreactors and open-pond setups (Kagan and Matulka, 2015; Esmaeili *et al.*, 2023). While photobioreactors allow precise management of environmental conditions, their high operational costs limit large-scale use (Kagan and Matulka, 2015; Narala *et al.*, 2016; Razzak *et al.*, 2024).

In aquaculture, marine microalgae serve as crucial feed for early-stage fish and shrimp, providing essential nutrients. Nannochloropsis oculata is a prominent species used for this purpose due to its rapid growth and resilience to varying environmental conditions (Mamdouh *et al.*, 2021; Hamzelou *et al.*, 2023). Belonging to the Eustigmatophyceae class, N. oculata can accumulate up to 21–28% of its dry mass as lipids, primarily in the form of triacylglycerols (TAG), with omega-3 fatty acids, particularly eicosapentaenoic acid (EPA), reaching up to 12% (Sultana *et al.*, 2022; Vítor *et al.*, 2023). This high EPA content makes N. oculata a potential candidate for vegan dietary supplements (Chua and Schenk, 2017; Gohara-Beirigo *et al.*, 2023). Moreover, EPA and docosahexaenoic acid (DHA) are associated with health benefits, including the prevention and management of chronic conditions such as cardiovascular diseases, neurodegenerative disorders, and certain cancers, as well as delaying aging-related diseases (Zanella *et al.*, 2020; Vítor *et al.*, 2022). Despite the growing interest in microalgae, effective biomass harvesting remains a major challenge, as the biomass must be fully recovered to avoid environmental contamination (Thiviyanathan *et al.*, 2024; Kurniawan *et al.*, 2022). Additionally, reclaiming water from microalgae systems can provide a sustainable source of freshwater, addressing global water scarcity driven by population growth and industrial expansion. Freshwater depletion, coupled with increased demands, highlights the need for innovative strategies to ensure sustainable resource management (Esmaeili *et al.*, 2023).

Various techniques are used for biomass separation, such as filtration and centrifugation, but these are often costly and energy-intensive, especially for small-celled species like Nannochloropsis (2 to 4 µm) (Kagan and Matulka, 2015). More practical alternatives include gravity sedimentation and flocculation, which are scalable and energy-efficient, maintaining cell integrity (Chua *et al.*, 2020; Vargas *et al.*, 2024). Flocculation, in particular, is a well-established practice for pollution management and water recirculation in aquaculture (Taghavijeloudar *et al.*, 2023; Zhang *et al.*, 2024; Shaikh *et al.*, 2024; Ren *et al.*, 2022).

Several flocculants have been tested for microalgae harvesting (Zhang *et al.*, 2014; Kujala *et al.*, 2020). However, some flocculants may introduce impurities that lower the quality of the harvested biomass, making it unsuitable for consumption (Gibson *et al.*, 2020). Natural polymers such as chitosan, cellulose, xanthan gum, lignin, and tannins, as well as synthetic inorganic polymers like polyaluminum chloride (PAC), have been suggested as alternatives (Ren *et al.*, 2022; Letelier-Gordo and Fernandes, 2021).

In this context, the present study aims to compare the effectiveness of TANFLOC SG, a natural tannin-based flocculant, and PAC in harvesting Nannochloropsis oculata biomass, as well as evaluate the quality of reclaimed water for reuse in cultivation.

## 2. MATERIAL AND METHODS

### 2.1. Microalgae cultivation

*Nannochloropsis oculata* cultures were obtained from the microalgae culture collection of the Algae Cultivation and Biotechnology Laboratory, Santa Catarina State University (UDESC), Brazil. The microalgae were initially grown in a 250 mL Erlenmeyer flask and gradually scaled up to a 5 L culture volume. The cultivation medium was prepared using 20 g $L^{-1}$ of sea salt supplemented with f/2 nutrients, consisting of: 75 mg $NaNO_3$, 5 mg $NaH_2PO_4 \cdot H_2O$, 3.15 mg $FeCl_3 \cdot 6H_2O$, 4.36 mg $Na_2EDTA \cdot 2H_2O$, 9.8 µg $CuSO_4 \cdot 5H_2O$, 6.3 µg $Na_2MoO_4 \cdot 2H_2O$, 22 µg $ZnSO_4 \cdot 7H_2O$, 10 µg $CoCl_2 \cdot 6H_2O$, 180 µg $MnCl_2 \cdot 4H_2O$, 200 mg thiamine HCl, 1 µg biotin, and 1 µg cyanocobalamin (Ma *et al.*, 2018; Chua *et al.*, 2019). Continuous lighting was provided using fluorescent lamps, and aeration was maintained with an air pump. The initial pH was adjusted to 8.2, and the microalgae were grown until reaching a density of $10^4$ cells $mL^{-1}$. For optimal growth, the only carbon source used was high-purity $CO_2$ (99.99%).

### 2.2. Water clarification tests

Water samples were taken from *Nannochloropsis oculta*, and the clarification cycle, which includes coagulation, flocculation, sedimentation, and gravity filtration, was employed to recover the biomass and treat the water. Two different coagulants were evaluated: a tannin-based commercial product (Tanfloc SG, Tanac, Brazil) and polyaluminum chloride (PAC). The optimal dosages were determined through JAR TEST experiments. Each test was performed using 400 mL of Nannochloropsis oculata culture in a 600 mL beaker. Coagulant concentrations ranged from 50 to 200 mg $L^{-1}$ for Tanfloc SG and 10 to 100 mg $L^{-1}$ for PAC. The samples were stirred at 120 rpm for 20 seconds (rapid mixing) and then at 20 rpm for 10 minutes (slow mixing). After the flocculation stage, samples were allowed to settle for 15 minutes to ensure complete sedimentation of the biomass. The clarified supernatant was analyzed for pH, turbidity, total suspended solids (TSS), chemical oxygen demand (COD), and biochemical oxygen demand ($BOD_5$). Removal efficiency (RE%) was calculated according to Equation 1 by comparing cell density (CD) before and after the clarification cycle using a spectrophotometer (KASVI) at 682 nm.

$$RE(\%) = \left(1 - \frac{CD}{CD_0}\right).100 \quad (1)$$

Where $CD_0$ and *CD* represent the initial and final cell densities, respectively. After sedimentation, samples underwent gravity filtration through a column packed with 6 cm of activated carbon (Carbonífera Criciúma SA), set within a glass column (1.5 cm internal diameter, 20 cm height). The filtration rate was maintained at 40 $m^3/m^2$ per day (Emerick *et al.*, 2020).

### 2.3. Analytical procedures

The physicochemical parameters of the treated water were determined according to the Standard Methods for Examination of Water and Wastewater, 22nd Edition (APHA *et al.*, 2012). Turbidity, TSS, and COD were assessed using a UV/Vis spectrophotometer (PROVE 300, Merck®, Germany) operating within a range of 190–1100 nm. Cell density was measured using a UV/Vis spectrophotometer (model K37, Kasvi, Brazil) at 682 nm, which corresponds to the peak absorption for chlorophyll. $BOD_5$ measurements were carried out using an Oxitop system (WTW®, Germany), with samples incubated at 20°C for five days. pH was measured using a DEL LAB digital pH meter. All measurements were performed in triplicate.

### 2.4. Toxicity tests

Toxicity assessments were conducted using clarified water samples. Each test involved placing four dilutions of the treated water, along with a control, into 150 mL Erlenmeyer flasks, which were then shaken and incubated for two hours. Temperature, pH, and dissolved oxygen (DO) were measured prior to starting the tests (US-EPA, 2002). Twenty Daphnia magna neonates were exposed to each concentration for 48 hours at 20 ± 2.0°C under a 16:8 hour light-dark cycle and light intensity between 500 and 1000 Lux. After exposure, the immobile organisms were counted, and pH and DO were measured in both test and control samples (Foudhaili *et al.*, 2020). The 50% effective concentration (EC50) was determined using a 95% confidence interval, calculated using the Trimmed Spearman-Karber method (Hamilton *et al.*, 1977).

### 2.5. Data analysis

All tests, including clarification, microalgae cultivation, and analytical procedures, were performed in triplicate. Data were analyzed using ANOVA, with a significance level of 5% and a confidence interval of 95%.

## 3. RESULTS AND DISCUSSION

The microalgae *Nannochloropsis oculata* is known for its remarkable ability to accumulate high concentrations of valuable biomolecules, including lipids, proteins, and antioxidants, making it an excellent candidate for human and animal nutrition applications. In this study, *N. oculata* was cultivated for a period of 15 days in a 5 L bioreactor, reaching a cell density of approximately $10^4$ cells $mL^{-1}$.

### 3.1. Water clarification tests

To identify the optimal coagulant concentrations for *Nannochloropsis oculata* biomass harvesting, specific functional parameters were selected, including turbidity, total suspended solids (TSS), chemical oxygen demand (COD), and biochemical oxygen demand ($BOD_5$). These are key indicators commonly used to evaluate the effectiveness of water treatment processes. Samples underwent a clarification process to determine the most efficient concentration for biomass recovery. Tanfloc SG, a cationic, tannin-based coagulant derived from the bark of Acacia mearnsii (black wattle), has a molecular structure that allows it to interact effectively with suspended particles, and it has a solid content purity of 30-31%. As a natural and biodegradable flocculant, Tanfloc SG minimizes the risk of adverse effects on the nutritional quality of the microalgal biomass. Conversely, PAC (poly aluminum chloride) is an inorganic, pre-hydrolyzed polymer that forms stable and large flocs over a wide pH range, eliminating the need for additional pH adjustment typically required with conventional coagulants, making it highly efficient for turbidity removal.

Physicochemical analyses were initially performed on the cultivation medium to establish baseline values for pH, turbidity, TSS, COD, and $BOD_5$. After 15 days of cultivation in closed tanks, the water became increasingly alkaline, reaching a pH of 9.1. The average values for the water samples were as follows: turbidity of 667 mg $L^{-1}$, TSS of 1,745 mg $L^{-1}$, COD of 1,580 mg $L^{-1}$, and $BOD_5$ of 1,280 mg $L^{-1}$ (Table 1). Such elevated levels indicate that, without proper biomass removal, the water could contribute significantly to environmental pollution.

As demonstrated in Table 1, the flocculation/coagulation pH was 7.1, and the greatest reduction in turbidity, total suspended solids (TSS), COD, and $BOD_5$ was achieved at a Tanfloc SG dosage of 125 mg $L^{-1}$. At this concentration, average removal efficiencies were as follows: 98.5% for TSS, 99.2% for turbidity, 97.6% for COD, and 98% for $BOD_5$, based on samples collected after the sedimentation stage, as shown in Figure 1.

These results indicate that this concentration provides the optimal conditions for sweep flocculation, which facilitates the interaction between the coagulant and the suspended colloidal particles. The TSS consisted mainly of microalgal cells, residual organic compounds, and secondary metabolites from the cultivation process. Lower doses were insufficient to neutralize the negatively charged colloids, while higher doses (above 125 mg $L^{-1}$) resulted in an excess of positively charged particles due to the overdosage of the tannin-based coagulant. Following the sedimentation step, the clarified water was analyzed using UV/VIS spectrophotometry and compared to the original *Nannochloropsis oculata* culture.

The UV/VIS spectra in Figure 2 confirmed that the clarified water did not contain any detectable microalgal residues, indicating that Tanfloc SG effectively removed the microalgal biomass from the medium.

The findings for PAC are illustrated in Figure 3, showing that the best removal efficiencies were achieved at a concentration of 50 mg $L^{-1}$ and pH 7.1, as shown in Table 1. At this dosage, reductions of over 98% were observed for all analyzed parameters in the samples collected after sedimentation. These values were higher than the 57.5% of chemical oxygen demand (COD), 96.8% total suspended solids (TSS), and 90.5% color removal were obtained found by Guvenc *et al.* (2023); using sequential treatment of real wastewater from the chemical industry with chemical coagulation in the first stage and Electro-Fenton (EF) in the second stage. Similarly, to the results obtained with Tanfloc SG, the performance declined when PAC concentrations were either lower or higher than 50 mg $L^{-1}$, following a similar trend due to the imbalance in charge neutralization. The increased concentration of PAC probably causes excess aluminum ions to form complexes with organic compounds present in the water, reducing the efficiency of coagulation. Furthermore, in high concentrations, PAC can alter the pH of the water, affecting the coagulation process (Cargnin and João, 2023).

**Table 1.** Physicochemical parameters of water samples analyzed. Treated samples were prepared using the optimum dosage of Tanfloc SG (125 mg $L^{-1}$) or PAC (50 mg $L^{-1}$). Reference values for water quality in non-food crop irrigation are also provided for comparison.

| Sample | | Parameters | | | | |
|---|---|---|---|---|---|---|
| | | **pH** | **Turbidity (NTU)** | **TSS (mg $L^{-1}$)** | **BOD (mg $L^{-1}$)** | **COD (mg $L^{-1}$)** |
| Cultivation water | | 9.1 ± 0.2 | 667 ± 42.5 | 1745 ± 22.7 | 1280 ± 22.0 | 1460 ± 28.0 |
| Tanfloc SG | After settling | 7.1 ± 0.1 | 4.9 ± 0.8 | 10.4 ± 3.3 | 22 ± 3.0 | 16 ± 1.0 |
| | After filtration | 7.1 ± 0.2 | 0.7 ± 0.5 | 0.7 ± 0.5 | 16 ± 0.5 | 12 ± 2.0 |
| PAC | After settling | 7.1 ± 0,1 | 2.3 ± 0,5 | 1.0 ± 0.0 | 6 ± 1.0 | 38 ± 5.0 |
| | After filtration | 7.0 ± 0.1 | 0.4 ± 0.6 | 0.4 ± 0.6 | 3.0 ± 1.0 | 22 ± 6.0 |
| Water reuse guideline [29] | | 6.0 - 9.0 | <2[a] | <30 | <30 | N/A |

[a] Recommended turbidity value for agricultural reuse – food crops to be met before disinfection. N/A – not available.

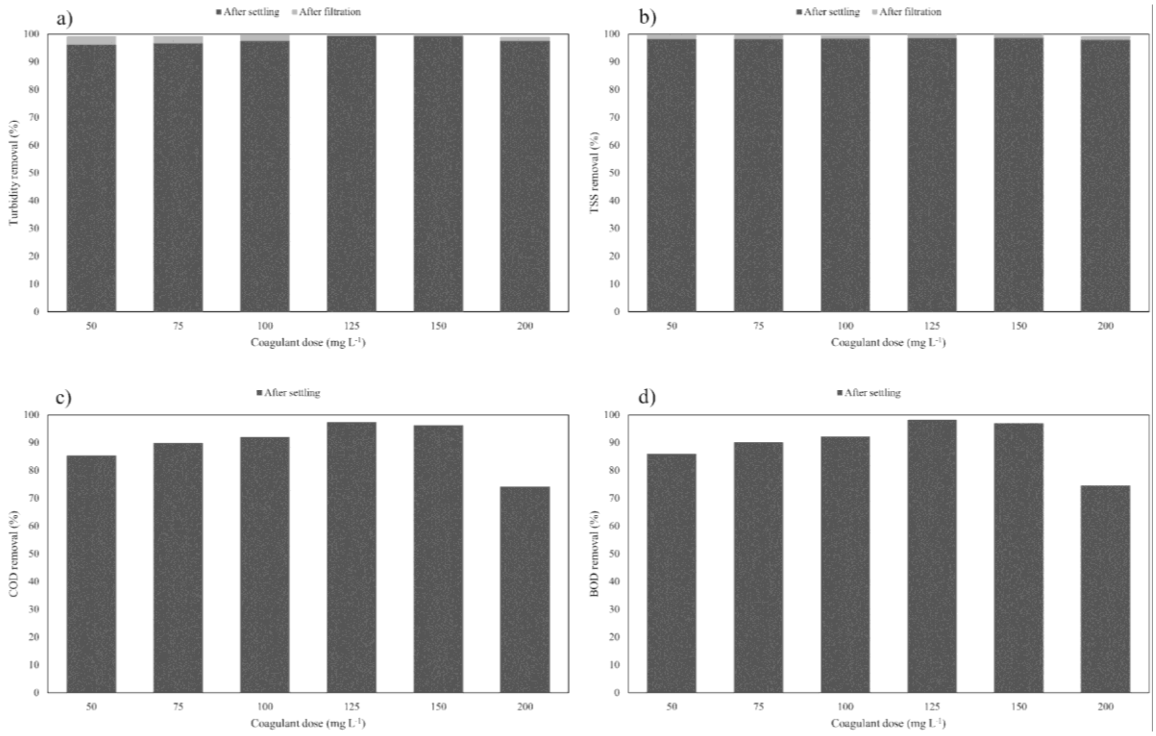


**Figure 1.** Removal efficiency of Tanfloc SG for a) turbidity, b) total suspended solids (TSS), c) chemical oxygen demand (COD), and d) biochemical oxygen demand ($BOD_5$).

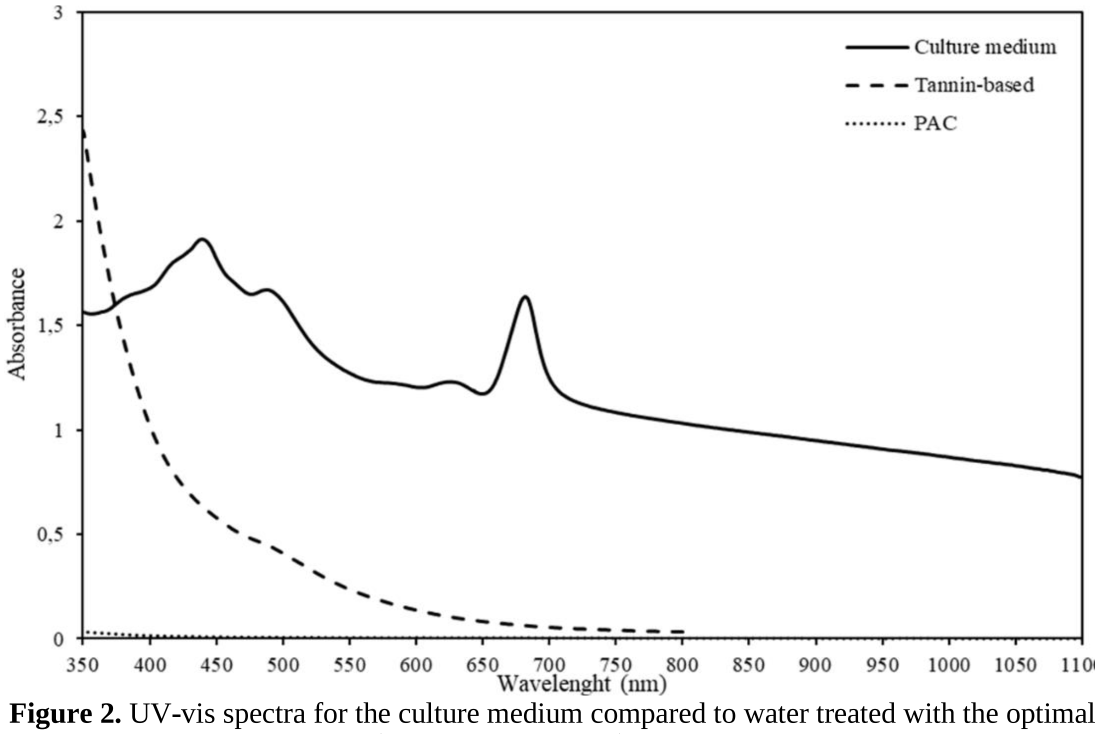


**Figure 2.** UV-vis spectra for the culture medium compared to water treated with the optimal dose of Tanfloc SG (125 mg $L^{-1}$) or PAC (50 mg $L^{-1}$).

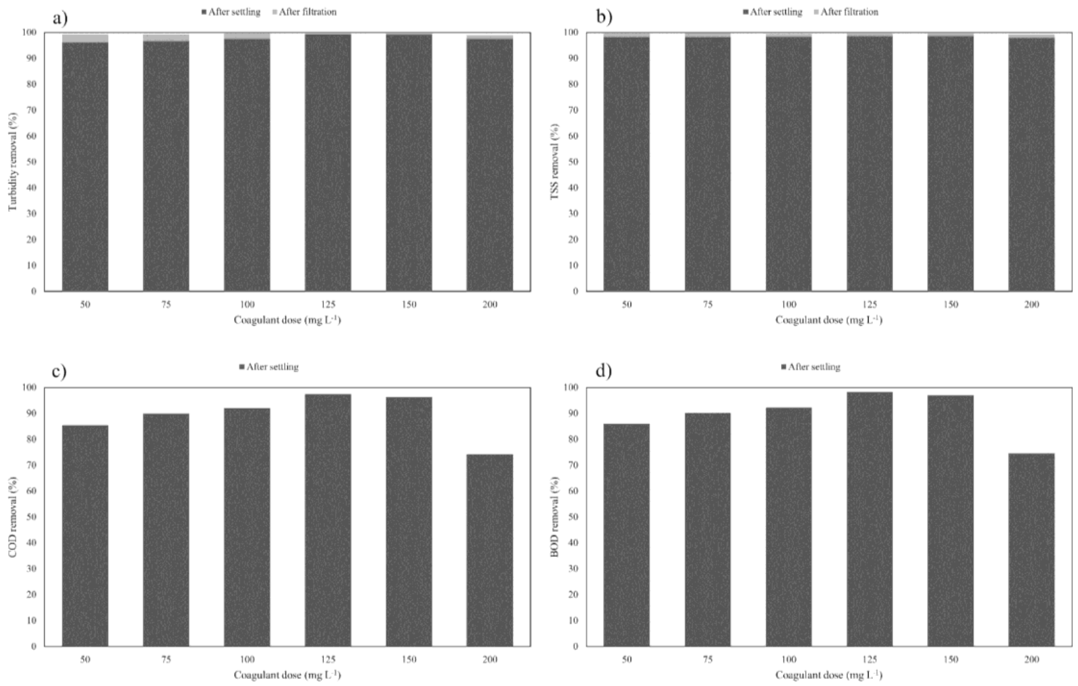


**Figure 3.** Performance of PAC on the removal of a) turbidity, b) total suspended solids (TSS), c) chemical oxygen demand (COD) and d) biochemical oxygen demand ($BOD_5$).

Additionally, a comparison of the UV/VIS spectra for the PAC-treated water and the untreated microalgae culture demonstrated that the biomass was almost entirely removed (Figure 2), confirming that PAC is also highly effective in harvesting *Nannochloropsis oculata* biomass.

In terms of the overall performance, PAC outperformed Tanfloc SG, as indicated by the lower dosages required for achieving similar or superior removal efficiencies. The optimal concentration of PAC was significantly lower than that of the tannin-based coagulant, which emphasizes its cost-effectiveness and lower chemical input requirements.

Moreover, Figures 1 and 2 show that an additional polishing step was achieved after the filtered supernatant passed through the activated carbon column. This step enhanced the removal of all parameters to above 99%, irrespective of the coagulant used. Such high-quality clarification is essential for water reclamation and reuse, making the treated water suitable for applications like irrigation. The final values of turbidity for the clarified water were 0.7 NTU for Tanfloc SG and 0.4 NTU for PAC, both well below the 2 NTU limit recommended for crop irrigation, even when considering food crops (US-EPA, 2002). Similarly, TSS concentrations were measured at 22 mg $L^{-1}$ for Tanfloc SG and 6 mg $L^{-1}$ for PAC, both of which are below the guideline threshold of 30 mg $L^{-1}$ for non-food crop irrigation. Additionally, BOD levels were substantially lower than 1 mg $L^{-1}$, far below the maximum allowable limit of 30 mg $L^{-1}$, and the pH values for both treated samples ranged between 6.0 and 9.0, meeting the criteria for safe water reuse. The data obtained corroborate with studies in the literature, where PAC and TANFLOC were used in the physical-chemical treatment of wastewater generated in shrimp farming tanks (Cargnin and João, 2023).

The final water quality for non-food crop irrigation also requires that faecal coliform levels be kept below 200 coli/100 mL and a minimum free residual chlorine concentration of 1 mg $L^{-1}$. Given that the initial cultivation water was free from faecal contamination, only a minor chlorination step would be necessary to meet these standards, making the reclaimed water fit for various non-potable uses.

Although the filtration of water and wastewater using various filter media has been extensively studied (Tusiime *et al.*, 2022; Song *et al.*, 2023), most of these studies focus on conventional water matrices and do not consider the unique characteristics of microalgal culture media. Granular filtration typically employs materials such as sand, anthracite coal, gravel, crushed stone, and activated carbon (Kramer *et al.*, 2021). Among these, activated carbon is particularly versatile and widely utilized due to its high efficacy in removing a variety of contaminants (Matilainen *et al.*, 2006; Rigobello *et al.*, 2013). However, the composition of the water used for microalgae cultivation poses unique challenges, as it contains specific micro and macronutrients required for algal growth, which can complicate the filtration process. There is a noticeable gap in the literature regarding the impact of activated carbon on water quality when filtering microalgae biomass effluent, making this study a valuable contribution to the field (Monnot *et al.*, 2016; Chen *et al.*, 2023). The results from this study demonstrate that, following a combination of coagulation, flocculation, sedimentation, and subsequent filtration using granular activated carbon, the clarified water exhibited significant reductions in COD, BOD, turbidity, and suspended solids. This suggests that granular activated carbon is effective in further polishing the effluent post-biomass harvesting, resulting in a high-quality water (Emerick *et al.*, 2020; Ebrahimzadeh *et al.*, 2022).

Additionally, the water obtained after the clarification cycle was subjected to an acute toxicity test using Daphnia magna as the bioindicator. According to the data presented in Table 2, the toxicity factor (TF) for the treated water was 2, classifying it as non-toxic for Daphnia magna and falling well within the permissible limits established by local environmental regulations (Consema, 2021). Table 2 provides a detailed account of the immobility rates of Daphnia magna following exposure to the treated effluent, confirming that the treatment did not produce any harmful effects on the tested organisms.

**Table 2.** Results for the acute toxicity tests using *Daphnia magna*.

| **Toxicity factor (TF)** | **Concentration (%)** | **Immobile *Daphnias magna*** | **Immobility (%)** |
|---|---|---|---|
| C | 0 | 0 | 0 |
| 1 | 100 | 4 | 20 |
| 2 | 50 | 1 | 5 |
| 4 | 25 | 0 | 0 |
| 8 | 12.5 | 0 | 0 |
| 16 | 6.25 | 0 | 0 |

C = Control.

The EC50-48 h value could not be calculated, as the immobility rate observed for the undiluted treated water was only 20% (Table 2). When the sample was diluted to 50% of its original concentration, the immobility rate decreased to 5%, and no immobilization was recorded when the sample was diluted to 25% or lower concentrations. These findings suggest that the clarified water has low toxicity and, therefore, can either be safely reused or discharged into natural water bodies without posing a threat to aquatic ecosystems.

## 4. CONCLUSION

The findings of this study allow us to draw the following conclusions:

● The proposed clarification method proved effective for harvesting *Nannochloropsis oculata* biomass, contributing to a more sustainable microalgae production process.

● The treated water achieved a quality suitable for irrigation purposes in non-food crops, demonstrating its potential for water reuse.

● Toxicity evaluations of the reclaimed water following coagulation and filtration showed a toxicity factor of 2, which classifies it as non-toxic and within the acceptable limits set by local regulations.

● PAC demonstrated high efficiency as a coagulant, making it a viable option for both biomass harvesting and water conditioning. Additionally, TANFLOC SG, as a natural coagulant, presented itself as an effective and environmentally friendly alternative for the same purpose.

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