Comparing PAC and Tannin-Based Coagulants for *Nannochloropsis oculata* Biomass Harvesting and Water Reuse

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ABSTRACT

Microalgae have been considered a sustainable source of proteins and food supplements for animal and human nutrition. This study compared the performance of polyaluminum chloride (PAC) and a tannin-based coagulant on *Nannochloropsis oculata* microalgae biomass harvesting and discussed the potential of the culture medium water reclamation. The microalgae were cultivated until a volume of 5L and a cell density of 10^4 cells mL^{-1} was reached. Samples underwent coagulation/flocculation/sedimentation and gravity filtration. An acute toxicity test using *Daphnia magna* was performed to assess the reclaimed water's safety. Both coagulants achieved over 98% reduction in total suspended solids, turbidity, COD, and BOD, with PAC demonstrating superior performance at a lower dosage (50 mg L^{-1}) compared to the tannin-based coagulant (150 mg L^{-1}). The treated effluent was non-toxic. Both coagulants have the potential for *N. oculata* biomass harvesting and PAC produces higher quality reclaimed water with a lower coagulant demand.

Keywords: Microalgae. Flocculation. Sustainable. Biorefinery. Water reuse.

1 INTRODUCTION

Since global warming has become increasingly severe in the last decade, there is an urgent need to reduce carbon emissions and invest in renewable energy sources[1]. Cultivating microalgae, photosynthetic microorganisms that thrive under favorable conditions like pH, temperature, light, and nutrients[2], offers a solution. Microalgae, with their high nutritional value, have been alternatives for protein sources and food supplements for animal and human nutrition[3,4]. Recent research focuses on their role in biofuel production, biomaterials, chemical commodities[5], and nutraceuticals, such as omega-3 fatty acids, carotenoid antioxidants, unique sugars, and proteins[2]. Marine microalgae, particularly *Nannochloropsis oculata*, cultivated in photobioreactors or open tanks like raceways, are popular in aquaculture for feeding larvae[6,7]. *N. oculata* is notable for its high growth rates, lipid productivity, and environmental resilience, storing lipids (21–28% dry matter) as triacylglycerols with EPA up to 12% of dry mass[8, 9]. Omega-3 fatty acids, especially EPA, are vital for preventing chronic diseases[4,9]. Harvesting microalgae biomass and reusing water is challenging, with conventional methods like filtration and centrifugation being costly and energy-intensive[10, 11]. Flocculation offers a more scalable solution, using flocculants such as chitosan, cellulose, xanthan gum, lignin, and tannins, or synthetic polymers like PAC[12,13]. This study evaluates the efficiency of TANFLOC SG and PAC in harvesting N. oculata biomass and the quality of reclaimed water for reuse.

2 MATERIAL & METHODS

Nannochloropsis oculata was obtained from the microalgae culture collection of the Algae Cultivation and Biotechnology Laboratory, Santa Catarina State University (UDESC), Brazil. The species was initially cultivated in a 250 mL Erlenmeyer flask and expanded to 5 L. The culture medium consisted of 20 g L^{-1} sea salt enriched with f/2 medium: 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[14,15]. The culture was continuously illuminated with fluorescent light and aerated with an air compressor pump. The pH was maintained at 8.2 until 10^4 cells mL^{-1} were reached, using CO_2 (food grade, 99.99%) as the carbon source.

Water samples from *Nannochloropsis oculata* cultivation underwent coagulation, flocculation, sedimentation, and gravity filtration to clarify the water and recover the microalgae biomass. Two coagulants were tested: tannin-based Tanfloc SG (Tanac, Brazil) and polyaluminum chloride (PAC). JAR TESTS optimized the coagulant dosages, with concentrations of 50 to 200 mg L^{-1} for Tanfloc SG and 10 to 100 mg L^{-1} for PAC. Samples were stirred at 120 rpm for 20 s (rapid mixing) and then at 20 rpm for 10 minutes (slow mixing). After flocculation, samples were left to settle for 15 minutes for complete biomass sedimentation, followed by turbidity, pH, total suspended solids, COD, and BOD_5 analysis of the supernatant. Removal efficiency (RE%) was determined by cell density (CD) of treated samples using a spectrophotometer (KASVI) at 682 nm.

Samples were gravity-filtered through a commercial activated carbon filter (Carbonífera Criciúma SA) in a glass column (1.5 cm internal diameter, 20 cm height) filled with 6 cm of activated carbon, with a filtration rate of 40 m3/m2.day.

Physicochemical analyses followed the Standard Methods for Examination of Water and Wastewater, 22nd edition [28]. Turbidity, total suspended solids, and COD were evaluated using a UV/Vis spectrophotometer 190-1100 nm, PROVE 300 (Merck®, Germany). Cell density was analyzed using a UV/Vis spectrophotometer 190-1100 nm, model K37 (Kasvi, Brazil) at 682 nm. BOD5 was determined with an Oxitop (WTW®, Germany), incubated at 20 °C for five days. pH was measured with a DEL LAB digital pH meter. All determinations were performed in triplicate.

Toxicity tests used clarified cultivation water, with *Daphnia magna* neonates exposed for 48 hours at 20 ± 2.0 °C, under a 16-hour light and 8-hour dark photoperiod. The 50% effective concentration (EC_{50}) was determined with a 95% confidence interval using the Trimmed Spearman-Karber method[16].

Data were processed (ANOVA) with a significance level of 5% and a confidence level of 95%.

3 RESULTS & DISCUSSION

The biomass of the microalgae *Nannochloropsis oculata* has a unique capacity to store large amounts of lipids, proteins, and antioxidants, making it a potential source of biomolecules for human and animal food. This study involved cultivating *Nannochloropsis oculata* in a 5 L bioreactor for 15 days, with cell density reaching 10^4 cells mL^{-1}.

Some functional parameters were selected to determine the optimal coagulant concentrations for harvesting *Nannochloropsis oculata* biomass. We chose turbidity, total suspended solids, COD, and BOD as indicators for water clarification assessment. Tanfloc SG, a natural and biodegradable polymer, and PAC, an effective coagulant over a wide pH range, were tested. Physical-chemical analyses of the cultivation water showed pH 9.1, turbidity 667 mg L^{-1}, total suspended solids 1,745 mg L^{-1}, COD 1,580 mg L^{-1}, and BOD_5 1,280 mg L^{-1} (Table 1). The highest removals of turbidity, total suspended solids, COD, and BOD_5 were observed at 125 mg L^{-1} for Tanfloc SG and 50 mg L^{-1} for PAC. Tanfloc SG removed 98.5% total suspended solids, 99.2% turbidity, 97.6% COD, and 98% BOD, while PAC achieved similar results.

Table 1 Water quality parameters for diverse samples studied. Treated samples were obtained using the optimal dose of Tanfloc SG (125 mg L^{-1}) or PAC (50 mg L-1), Guideline water quality for agricultural reuse on non-food crop irrigation.

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.

UV/VIS analysis confirmed the effective removal of microalgae biomass with both coagulants (Figure 1). PAC had lower optimal concentrations compared to Tanfloc SG. Filtration of settled water further improved water quality, achieving over 99% removal for all parameters. Clarified water met guidelines for non-food crop irrigation, with turbidity below 2 NTU and TSS below 30 mg L^{-1}.

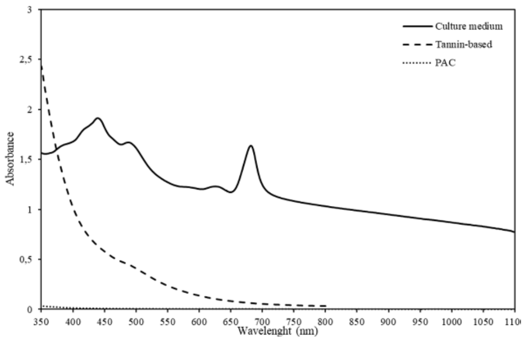

Figure 1 UV-vis spectra for the culture medium and water treated using the optimal dose of Tanfloc SG (125 mg L^{-1}) or PAC (50 mg L^{-1}).

The filtration process using activated carbon effectively reduced COD, BOD, turbidity, and suspended solids. Acute toxicity tests with *Daphnia magna* revealed a toxicity factor of 2, indicating non-toxicity. Immobility was 20% with 100% water samples and reduced to 0% when diluted four times, showing the reclaimed water can be reused or released without environmental harm.

Overall, the results indicate that the clarification cycle, involving coagulation, flocculation, sedimentation, and filtration, effectively recovers microalgae biomass and produces high-quality water suitable for irrigation and potentially other uses.

4 CONCLUSION

Proposed clarification process for *Nannochloropsis oculata* biomass harvesting was feasible, making microalgae cultivation sustainable. Additionally, reclaimed water with potential for non-food crop irrigation was obtained. The best removals in the microalgae biomass harvest were observed with 125 mg L^{-1} of TANFLOC SG and 50 mg L^{-1} of PAC. Specifically, TANFLOC SG achieved an average efficiency of 98.3% for color, 98.5% for suspended solids, 99.2% for turbidity, 97.6% for COD, and 98% for BOD. Similarly, PAC showed an average efficiency above 98% for all analyzed parameters in harvesting the microalgae biomass. The assessment of acute toxicity of the water after coagulation and filtration indicated a toxicity factor of 2, considered non-toxic and within the maximum limits established by local legislation. Finally, the coagulation and flocculation treatment with PAC is a viable alternative for harvesting microalgae biomass and conditioning water for reuse, while TANFLOC SG is a reliable and efficient organic alternative for the proposed aim.

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