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$CaCl_2$ applied to the extraction of *Moringa oleifera* seeds and the use for *Microcystis aeruginosa* removal

M.S. Carvalho [a,*], B.R.R. Alves [a], M.F. Silva [b], R. Bergamasco [b], L.A. Coral [a], F.J. Bassetti [a]

[a] Academic Department of Chemistry and Biology, Universidade Tecnológica Federal do Paraná (UTFPR), Curitiba, PR, Brazil
[b] Department of Chemical Engineering, Universidade Estadual de Maringá (UEM), Maringa, PR, Brazil

HIGHLIGHTS

- Different salts resulted in diverse coagulating capability.
- Calcium chloride coagulant had higher capability of treating a 25 NTU water.
- It is expected calcium ions assists the flocs formation.

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ABSTRACT

The *Moringa oleifera* seed coagulant provides high efficiency for water treatment, especially for bacteria removal and potentially cyanobacteria. The present study evaluates the difference between two saline extracts of *M. oleifera* Lam seeds in the removal of *Microcystis aeruginosa* cells, using dissolved air flotation (DAF). Samples were generated by synthetic water spiked with humic acid and *M. aeruginosa* cells to obtain 25 NTU. The saline extracts were obtained by seed extraction using 1 M NaCl or $CaCl_2$, and dosages from 25 to 200 mg L^{-1} were applied from each of the extractions. The $CaCl_2$ coagulant extract showed a better efficiency, achieving 78.9% cell removal when applying a dosage of 50 mg L^{-1}. Comparison of saline extractions and aqueous extraction suggested that calcium ions participate in coagulation/flocculation process, as a response of its double-valency. The $CaCl_2$ extract of the *M. oleifera* seeds is indicated for the treatment of water with the presence of cyanobacteria, even in water with low turbidity, a condition not commonly evaluated in other studies.

1. Introduction

The concern about the contamination of aquatic environments has increased especially in the case of surface waters used for human consumption. Adequate water treatment and sanitation are essential to remove turbidity and other impurities. Coagulant addition is a necessary stage in water clarification, being aluminum sulfate (alum) the most common coagulant used worldwide [1]. However, a non-biodegradable sludge is produced and remaining aluminum in water can be verified, which is related with serious drawbacks such as Alzheimer's disease [2,3]. Therefore, studies using natural coagulants such as *Moringa oleifera* are getting more attention. The use of *M. oleifera* seeds prevents the presence of dissolved aluminum in water and in the sludge, producing a biodegradable sludge with also higher nutritional value [1].

Besides presenting efficiency to remove arsenic [4], surfactants [5] and pathogens [6] from water, *M. oleifera* coagulant is also being studied to be applied to remove cyanobacteria cells. Attention to the removal of cyanobacteria in water must be taken into account once algal blooms are frequent in water reservoirs, where one of the most common cyanobacterial bloom is of *Microcystis aeruginosa* species [7–9]. The studies evaluating the removal of *Microcystis* and other cyanobacteria genera by *M. oleifera* coagulant are however still scarce [10,11] and the use of this natural coagulant could be shown an interesting alternative to the aluminum coagulants, usually applied in higher dosages when cyanobacteria blooms are present in the water.

M. oleifera Lamarck is the most distributed species worldwide when it comes to Moringaceae family. Producing 2000–20,000 seeds per year, the usage of seeds as a coagulant enables the production of 60,000 L of drinking water, when applying a 50 mg L^{-1} dosage [6]. Moreover, no toxicity is presented in the seeds and no changes at pH and conductivity are observed when using this coagulant [12]. The importation of coagulants and alkalizing

* Corresponding author.
E-mail address: maiarasdc@gmail.com (M.S. Carvalho).

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products by development countries could not be necessary if *M. oleifera* coagulant is employed once it is cultivated in most part of these countries [13].

Early studies were performed using *M. oleifera* coagulant extracted with aqueous solutions, in order to work with a simpler methodology and to reduce costs for development countries [6,14,15]. However, efficiency given by the use of aqueous *M. oleifera* extract is not comparable to saline extracts, being saline coagulants currently preferred [12,16,17]. In fact, previous studies revealed that *M. oleifera* coagulant obtained with a 1 M NaCl solution had an efficiency 7 times higher than those obtained with distilled water [18,19]. The application of saline solutions in order to obtain the coagulant provide higher efficiencies, once the coagulant active components are commonly related to soluble cationic proteins [20–23]. Studies evaluating the use of other salts than NaCl are scarce, in particular divalent salts as calcium chloride. Still, in the performed studies, there is a discordance between the authors that indicate no difference in the efficiency of the coagulant obtained with different salts and the ones that indicate divalent salts are more efficient [24,25]. It is expected that highest density of charges, due to the dissociation of salt present in the media, is described as a salting-in mechanism, which increases the solubility of seeds active components e.g. proteins [19,23]. Given that, the use of divalent salts in the extraction of the coagulant could further enhance the coagulating capability and decrease the dosage required.

This study aims to evaluate the influence of the salt used (NaCl or $CaCl_2$) in order to obtain *M. oleifera* Lam coagulant for the removal of *M. aeruginosa* cells, using DAF as clarification method. This influence was studied under two complementary points of view: a traditional study on the saline extracts to remove the major samples constituents and a discussion about the difference in salts used to extract *M. oleifera* coagulant on its coagulating capability.

2. Materials and methods

2.1. Coagulant preparation

Two coagulant solutions were prepared for this study. The first extraction was prepared with a monovalent salt, being 1 M NaCl, and the second with a 1 M $CaCl_2$, a divalent salt, solution. For both salts, it was prepared a stock solution with 10 g L^{-1} of *M. oleifera* seeds using the same extraction method.

Seeds were obtained in Campina Grande, Paraiba, Brazil. The extraction followed the indicated by Beltrán-Heredia and Sánchez-Martín [5]: seeds were dehusked and reduced into powder by a domestic mill and mixed up with saline solution, and stirred for 30 min at room temperature; then the extract was filtered through commercial filter paper, followed by filtration through a 0.45 μm glass fiber membrane.

2.2. Synthetic samples

M. aeruginosa cultured cells were grown in the laboratory in ASM-1 medium, at 25 ± 3 °C, under a 12 h light/12 h dark light regimen. Cultures were harvested on the 26th day, at the exponential phase of growth.

The sample used consists on distilled water in which was added KCl and $CaCl_2$ in order to obtain a moderately to hard water (1 mM IS KCl plus 3 mM IS $CaCl_2$) [26]. Humic acid was added in the solution on the concentration of 4–8 mgC L^{-1}, which corresponds to moderate-high dissolved organic carbon (DOC) concentrations [27]. Finally, *M. aeruginosa* culture was added in order to obtain a 25 NTU initial sample.

The sample used in the present study was obtained by the addition of *M. aeruginosa* culture in a synthetic water. This synthetic sample was prepared using as reference the usual classification of natural water found in Brazilian water treatment plants inlet.

2.3. Experimental tests

2.3.1. Coagulation/flocculation/dissolved air flotation

$CaCl_2$ and NaCl coagulant extracts were used in order to evaluate their differences when it comes to coagulating potential. For both extracts, dosages of 25, 50, 100, 150 and 200 mg L^{-1} were used in the experiments.

The C/F/DAF tests were conducted in a Jar Test equipment provided with an air saturation chamber and jars equipped with a channel for the entrance of water saturated with air and dispersion of air bubbles in the media. Experiments were performed at room temperature (25 ± 2 °C), at the operational conditions: coagulation at a velocity gradient (G_C) of 1000 s^{-1} for 10 s [28]; flocculation at a velocity gradient (G_F) of 15 s^{-1} for 15 min [23]; DAF for 8 min of saturation time using a relative pressure of 4 bar, 10% of pressurized recycle and flotation velocity of 5 cm min^{-1} (72 m^3 m^{-2} day) [28].

In order to evaluate the effect of the salts on the treatment efficiency, new experiments were performed using a dosage of 50 mg L^{-1} of coagulant. For these tests, an aqueous coagulant was used, as well as the coagulant obtained using the saline solutions and only the saline solution as a coagulant. The same C/F/DAF conditions were used in this experiment considering: *M. oleifera* saline coagulants, aqueous coagulant and each saline solution as coagulant. The aqueous coagulant was prepared using the same extraction method described in the Section 2.1.

2.3.2. Analytical methods

Treatment efficiency was measured by the analysis of the following parameters: turbidity, color, pH and dissolved organic carbon (DOC) (Shimadzu TOC-V CPH analyzer) according methodology to Standard Methods [29]. In addition, cell density was obtained by direct microscope count using Neubauer counting chamber.

In order to evaluate the differences between the two coagulant extracts, the same parameters were analyzed, with the addition of zeta potential (Delsa Nano C Particle Analyzer). Protein concentration in each coagulant extract was determined using Bradford method [30]. The sludge of the experiments realized with 50 mg L^{-1} coagulant dosage, for both extracts, were dried then characterized by SEM/EDS technique (JEOL JSM 6360-LV).

2.4. Statistical analysis

Random Effects ANOVA and F-test were applied considering two factors (coagulant type and dosages) in order to observe their influence in the treatment efficiency. In addition, post hoc analysis using Tukey Test were applied to evaluate the difference among the dosages in the treatment efficiency. A confidence level of 95% was used, which indicates that *p*-values smaller than 0.05 indicates significant alterations in the treatment response (e.g. turbidity removal efficiency) when evaluating differences inside each factor evaluated. The smaller the *p*-value, the further the hypothesis of an equivalence response given by the factors is given. The tests were implemented via Action® software.

3. Results and discussion

3.1. Coagulant dosage determination

The general initial water characteristics are listed in Table 1. Note that tests were not applied at the same day, therefore even with the