

## Cyanobacteria Removal by Coagulation/Flocculation with Seeds of the Natural Coagulant *Moringa oleifera* Lam

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Eutrophication of water bodies has resulted in increased population of cyanobacteria that can produce toxins capable of causing health problems in humans. In conventional water treatment, coagulant aluminum sulfate are used most commonly, although the production of non-biodegradable sludge and indications of damage to health have led to a search for other coagulants that are less harmful to the environment and to human health, such as the seeds of *Moringa oleifera* Lam. The objective of this study was to assess the efficiency of moringa seeds as a coagulant for the removal of cyanobacteria cells. To carry out the coagulation assays, deionized water was artificially contaminated with cells of the cyanobacteria *Microcystis protocystis*, in order to obtain samples with different initial turbidity. This water was submitted to coagulation/flocculation with different dilutions of a stock solution of 1% moringa seeds. In order to assess cell removal, readings were made in aliquots of 5mL of samples analyzed by triplicates counting on sedimentation chambers using an inverted optical microscope, according to the Utermöhl method. Concentrations above 200mg/L from the 1% solution of moringa seeds gave the best cell removal, about 91%. Color and turbidity removal were found to be dependent on the initial turbidity of the water sample and the concentration of coagulant. Coagulation with moringa seeds gave satisfactory results in reducing the number of cyanobacteria cells. However, since complete removal was not obtained, further studies are necessary.

### 1. Introduction

In Brazil, the large areas devoted to monoculture crops and the need to promote plant growth by the extensive use of fertilizers have caused rapid eutrophication of rivers and reservoirs. This has resulted in increased growth of cyanobacteria populations, many species of which produce toxins that can cause health problems in humans (Brazil - National Health Foundation, 2003).

In conventional water-treatment processes, the clarification stage involves the addition of chemical coagulants and flocculants to remove color, turbidity, and organic matter.

Regarding cyanobacteria removal by coagulation, good results have been reported, depending on the characteristics of organic matter present in water, the prevalent cyanobacteria species, and the type and concentration of coagulant (Heng et al, 2009; Henderson et al., 2010; Shen et al, 2011).

Aluminum sulfate is the most common of these coagulants. However, the production of nonbiodegradable sludge and indications of health hazards (Rondeau and Commenges, 2001) have led to the search for other coagulants that are less harmful to the environment and to human health. Therefore, several natural coagulants are being studied, including the seeds of the horseradish tree *Moringa oleifera* Lam.

This material is non-toxic and biodegradable. It is environmentally friendly, and unlike alum, does not significantly affect the pH and conductivity of the water after the treatment (Nkurunziza et al, 2009).

Therefore, the objective of this study was to assess the efficiency of moringa seeds as a coagulant for the removal of cyanobacteria cells.

## 2. Material and Methods

The samples consisted of deionized water artificially contaminated with inocula from a culture of cyanobacteria cells of the species *Microcystis protocystis*, in order to obtain initial turbidity of 50, 150, 250, 350, and 450 Nephelometric Turbidity Units (NTU). The amount of cells varied from  $1.0 \times 10^5$  to  $2.0 \times 10^6$  cells/mL.

A 1% stock solution of moringa seeds (concentration = 10,000 mg/L) was diluted to provide 12 different concentrations, from 25 to 300 mg/L, at pH 7.0.

Each mixture of the contaminated water with one of the diluted solutions of moringa seeds was tested in a jar test apparatus, under the following conditions: coagulation time of 180s; rapid mixing speed of 95rpm; flocculation time of 900s, and slow mixing speed of 10rpm (Cardoso et al., 2008).

To assess the treatment efficiency, the water samples were analyzed before and after the coagulation/flocculation process. Water color and turbidity were measured in a HACH DR/2010 spectrophotometer, according to the procedure recommended by the Standard Methods (APHA, 1995). To evaluate the degree of removal of *M. protocystis* cells, the cells were counted by the Utermöhl method, as discussed by Lund *et al.* (1958).

The results were examined by analysis of variance (ANOVA) using F test and response surface regression, to evaluate possible statistical interaction of initial turbidity and concentration of moringa coagulant with the measured parameters (turbidity, color, pH, and concentration of cyanobacteria cells). For this statistical analysis, values with  $p < 0.05$  were considered significant. The program 'Statistica' version 6.0/2000 was used.

## 3. Results and Discussions

The results of color, turbidity, and cyanobacteria cells removal, as well as pH values of the treated water, are presented in Table 1.

*Table 1. Removal efficiency of cyanobacteria cells (CyR), color, (CR) and turbidity (TR) and pH values for different concentrations of solution of moringa seeds.*

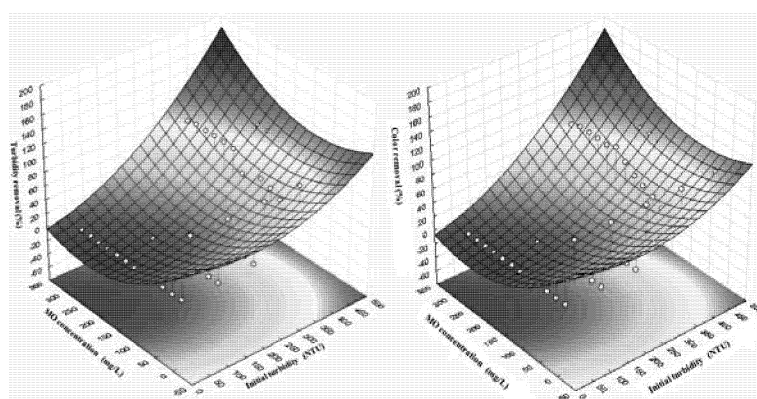
Concentration (mg/L)	Parameter	Initial turbidity (NTU)				
		50	150	250	350	450
25	TR (%)	0.0	0.68	3.1	49.4	53.0
	CR (%)	0.0	0	0.0	49.0	39.0
	CyR (%)	0	60.9	63.3	8.3	0.0
	pH	7.90	8.25	7.65	7.78	8.21
50	TR (%)	0.0	0.0	3.9	62.8	61.0
	CR (%)	0.0	0	0.0	54.8	47.8
	CyR (%)	0	74.2	61.2	24.9	0.0
	pH	8.22	8.43	7.65	7.87	7.77
75	TR (%)	0.0	0.0	2.7	70.8	75.0
	CR (%)	0.0	0	0.0	62.3	61.6
	CyR (%)	0	72.7	49.9	41.4	0.0
	pH	8.11	8.52	7.46	8.15	7.65
100	TR (%)	0.0	0.0	3.1	75.9	79.9
	CR (%)	0.0	0.77	0.0	69.8	68.5
	CyR (%)	36.5	26.8	51.7	57.8	0.0
	pH	8.16	8.60	7.44	7.77	7.61
125	TR (%)	0.0	0.0	9.3	55	92.0
	CR (%)	0.0	0	0.51	75.5	88.3
	CyR (%)	0	20.7	87.4	26.3	0.0
	pH	8.21	8.51	7.3	7.81	7.66
150	TR (%)	0.0	0.0	7.0	66.5	94.0
	CR (%)	0.0	0	0.0	86.3	91.5
	CyR (%)	0	35.4	82.5	58.1	1.4
	pH	8.19	8.62	7.37	7.74	7.63
175	TR (%)	0.0	0.0	7.0	93.7	97.2
	CR (%)	0.0	0	0.0	99.2	96.1
	CyR (%)	48.4	69.2	84.9	38.5	0.0
	pH	8.24	8.56	7.28	7.82	7.59
200	TR (%)	0.0	0.0	6.2	95	97.4
	CR (%)	0.0	0	0.0	94.0	96.1
	CyR (%)	13	79	83.2	50.5	1.4
	pH	7.98	8.33	7.31	7.75	7.62
225	TR (%)	0.0	0.0	8.9	96	97.2
	CR (%)	0.0	0	0.38	95.4	96.4
	CyR (%)	74.7	33.3	83.5	35.7	0.0
	pH	7.89	8.31	7.33	7.73	7.66
250	TR (%)	0.0	3.1	3.1	95.8	97
	CR (%)	0.0	0.0	0.0	95.3	96.0
	CyR (%)	88.3	91.0	91.0	45.6	0.0
	pH	8.11	7.35	7.35	7.76	7.61

Table 1. Removal efficiency of cyanobacteria cells (CyR), color, (CR) and turbidity (TR) and pH values for different concentrations of solution of moringa seeds.

Concentration (mg/L)	Parameter	Initial turbidity (NTU)				
		50	150	250	350	450
275	TR (%)	0.0	0.0	3.3	96.4	96.7
	CR (%)	0.0	0	0.0	95.6	95.6
	CyR (%)	49.1	72.7	88.9	38.6	21.9
	pH	8.07	8.30	7.34	7.73	7.56
300	TR (%)	0.0	0.0	4.5	92.5	94.7
	CR (%)	0.0	1.03	0.0	91.3	92.8
	CyR (%)	17.2	47.7	89.6	68.3	15.5
	pH	7.95	8.18	7.33	8.13	7.64

Turbidity removal ranged from 0 to 97.4%, color removal varied from 0 to 99.2%, and the removal of cyanobacteria cells ranged from 0 to 91%. Best turbidity and color removal was obtained for samples of higher initial turbidity, 350 and 450 NTU. The absence of removal or increase in water color and turbidity may have occurred due to the addition of the moringa solution, increasing the organic load. This can be explained considering that *M. oleifera* is an oleaginous plant rich in organic substances such as oil, protein, fat, vitamins, etc. Similar increases in color and turbidity in water treated with *M. oleifera* have been observed in other studies, particularly when the initial color and turbidity are relatively low (Ndabigengesere and Narasiah, 1998).

Applying the analysis of variance, it was observed that the initial turbidity and the concentration of the moringa solution had a statistically significant interaction with final turbidity and color ( $p < 0.05$ ). Analyzing the response surface (Figure 1) one can see that to maximize the removal of these parameters, the concentration of the moringa solution should be raised proportionately to the initial water turbidity.



■ > 180 ■ < 180 ■ < 140 ■ < 100 ■ < 60

Figure 1: Response surface for turbidity and color removal as a function of moringa solution concentration and initial turbidity (MO concentration: concentration of *Moringa oleifera* solution).

No statistically significant interaction was observed regarding pH ( $p=0.99$ ). This feature of no change in pH of treated water is considered one of the advantages of *M. oleifera*.

There was also no statistically significant interaction with the concentration of cyanobacteria cells ( $p=0.24$ ). The efficiency of *Microcystis* cells removal varied from 0 to 91%. The best removal efficiency was observed at the highest concentrations of moringa solution, above 200mg/L, for the water samples with lower initial turbidity (50, 150, and 250 NTU).

Only a few works are found in literature using *M. oleifera* for cyanobacteria removal. Other types of coagulants have been studied for this application. Sens et al. (2004) used about 30 mg/L aluminum sulfate to remove cyanobacteria from water from Lake Peri in Florianópolis, Santa Catarina, Brazil. Sales et al. (2004) found that a concentration of 70 mg/L provided the best removal of cyanobacteria from water from a reservoir in Fortaleza, Ceará, Brazil.

Chow et al. (1998), testing another chemical coagulant, ferric chloride, in water contaminated with a *Microcystis aeruginosa* cell culture, observed an increase in the number of cells after the treatment, and concluded that the coagulant apparently stimulated the growth of these cells in the studied locality.

A study performed in water supply and purification plants in Hangzhou city, China, reported the use of alkaline aluminium chloride as coagulant, coupled with chlorination and addition of potassium permanganate. When the algae density in raw water was less than  $1.0 \times 10^6$  cells/L, more than 98% of algae were removed with 13 mg/L of coagulant. When the algae density was higher, the coagulant concentration had to be increased (20 mg/L) (Shen et al., 2011).

These different performances of the coagulation and flocculation processes for cyanobacteria removal can be explained by the different kinds of coagulating agents, the presence or absence of organic material, the species and density of cells, as well as their morphology (Bernhardt and Clasen, 1991).

#### 4. Conclusions

- The process of coagulation/flocculation with *M. oleifera* seeds gave satisfactory results in reducing the number of cyanobacteria cells (best removal 91%).
- There are no statistically significant interactions between the removal of *Microcystis* cells and the initial water turbidity or the coagulant concentration.
- Turbidity and color removal efficiencies of up to 97.4% and 99.2%, respectively, were obtained, especially for water with high initial turbidity and with high concentrations of moringa seeds.
- The pH of the treated water did not present major variation after the coagulation/flocculation process with *M. oleifera*.
- The use of *M. oleifera* seeds is advantageous and it can be considered a promising step to improve the coagulation/flocculation of water containing cyanobacteria cells.

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