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Coagulation/Flocculation/Flotation/Nanofiltration Processes Using Moringa Oleifera as Coagulant of Eutrophized River

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This study investigated the efficiency of Moringa oleifera (MO) seeds as natural coagulant in coagulation/flocculation/dissolved air flotation (C/F/DAF), followed by nanofiltration (NF) for Microcystis protocystis and microcystin-LR removal. The methodology adopted in this work was performed in two steps: 1) coagulation/flocculation/dissolved air flotation (C/F/DAF) process using the MO extracted in saline solution of potassium chloride (KCl-1M) and sodium chloride (NaCl-1M) in optimum dosage 50 mg·L^{-1}; 2) nanofiltration process using NF90 and NF270 membrane provided Dow Chemical Company®. A working pressure of 8 bar was applied. In all samples were analyzed color, turbidity, pH, cyanobacterial cells count and microcystin concentration. The use of MO seeds as natural coagulant, obtained satisfactory results in the M. protocystis, color and turbidity removal. NF was able to completely remove cyanobacterial cells and microcystins (100 %) from M. protocystis (always under the quantification limit). Therefore, C/F/DAF+NF sequence is a safe barrier against M. protocystis and microcystins in drinking water.

1. Introduction

The conventional treatment process is concerned, some authors reported that the sequence coagulation/flocculation/sedimentation, filtration, chlorination are effective in removing cyanobacterial cells (Drikas et al., 2001), but may cause cell lysis and release of toxins into the water due to long careers of filtration and the use of some chemical coagulants and algaecides (Hoeger et al., 2004). This treatment can be useful in intracellular toxin removal, particularly if the cells maintain intact, and may have a negligible effecton the removal of extracellular toxins (RibauTeixeira and Rosa, 2010).

A recent alternative for cyanobacteria removal is the use of natural coagulants such as Moringa oleifera Lam (MO) (Lürling and Beekman, 2010). Water treated with MO seed extract, produces lower sludge volume compared to alum, produces biodegradable sludge and doesn't affect the pH of the water (Kurunziza et al., 2009). The active agents in aqueous MO are more effective coagulants than alum (Ndbigengesere et al., 1998). Besides, the cost of this natural coagulant would be less expensive compared the conventional coagulant (alum) for water purification since it is available in most rural communities in developing countries where treated water is a scarce resource (Ghebremichael, 2005).

Another problem is in the sedimentation step, where the sludge is stored for a long period of time, allowing substances such as heavy metals and cyanotoxins that have settled to remain in contact with water by this time, compromise the security of it (Drikas et al., 2001).Since algae are low-density particles and some may float, dissolved air flotation (DAF) has proven to be more effective for treating algal-rich water than the conventional clarification by settling (Ribau Teixeira and Rosa, 2007).

This system is recommended because it minimizes the disruption of flocs formed in coagulation/flocculation step and also allows the removal of a greater number of intact cells, avoiding thus the occurrence of cyanotoxins release into the water, since the float sludge is removed more frequently than the settled sludge.

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Therefore, the use of flotation can be considered not only an alternative to sedimentation units, but also an alternative method that enables increased efficiency of the filtration step. A further treatment step may therefore be required to remove extracellular toxins.
An alternative to effectively remove cyanobacteria andcyanotoxins is membrane pressure-driven filtration. Microfiltration (MF) and ultrafiltration(UF) will be adequate for removing the cyanobacterial cellsbut not cyanotoxins, due to the large pore size and high molecular weight cut-off of these membranes (Ribau Teixeira and Rosa, 2005). Reverse osmosis (RO) and nanofiltration (NF) are able to remove extracellular dissolved toxins due to their low molecular weight cut off (NF molecular weight cut-off is in the range from about 100 to 1,000 Da), the average molecular weight of microcystin is 996 Da (Dixon et al., 2011).
Considering the efficiency of DAF in removing cyanobacterial cells and the characteristics of cyanotoxins removal attributed to nanofiltration, the association of this process for the water supply treatment with cyanobacterial blooms becomes feasible. The objective of this study was to verify the processes efficiency of coagulation/flocculation with MO seeds, as a natural coagulant, followed by dissolved air flotation (DAF) and nanofiltration for the removal of M. protocystis cells and microcystin-LR.

2. Materials and Methods

2.1 Samples and *Moringa oleifera* Lam saline solution-crude exctract (MO)

Synthetic water (deionized water with an inoculum of Microsystis protocystis cells) was used for tests to obtain turbidity ranging between 50 – 450 NTU.
For MO coagulant preparation, mature seeds of M. oleifera were used, from the Federal University of Sergipe (UFS, Brazil), manually removed from the pod and shelled dry. One gram of peeled seeds were weighed and crushed with 100 mL of saline solution (KCl and NaCl - 1M) in a blender. Subsequently, the solution was stirred for 30 minutes and filtered under vacuum on membranes of 0.45 µm, obtaining a solution 1.0% m/v of MO seeds (Madona et al., 2010). The dosage optima utilized during the tests was 50 $mg \cdot L^{-1}$.

2.2 Analytical methods

Whereas color and turbidity were measured in a HACH DR/2010 spectrophotometer, according to the procedure recommended by Standard Methods (APHA, 2005), pH was measured by a Digimed DM-2 pH meter according to the manufacturer's methodology. Removal degree of M. protocystis cells was monitored by the Utermöhl method (1958), according to methodology described by Lund et al., (1958), which involves the counting of sediment organisms in a special chamber using an inverted microscope.
For the collection and purification of cyanotoxin, the sample was lyophilized to obtain at least 0.5 g of dry weight. Further, an extraction with 75% methanol was performed, filtration was undertaken by a syringe filter (0.45 µm) and microcystins were counted by ELISA (Enzyme linked immune-sorbent assay) immune assay kit, which includes a kit for the quantitative analysis of microcystins in water. In this assay, the polyclonal antibodies bind to microcystins and microcystin-enzyme conjugate. Since microcystin in the sample competes with the conjugate for the same binding antibodies, a wide detection range of the compounds is achieved.

2.3 Coagulation/flocculation/DAF experiment

"Flotest" (Nova Ética- Model 218/3), the lab-scale dissolved air flotation equipment used, is composed of three acrylic vessels which work in parallel with the saturation chamber, at an intermittent flow (batch).The base of each vessel comprise two acrylic plates spaced 5 cm from one another, with channels at the bottom plates for quicker transport and distribution of previously saturated water. The pressure chamber has a 2L useful capability of water. Its saturation results from air inclusion by an air outside the laboratory. The upper part of the chamber is equipped with a pressure regulator valve with filter, needle valves for fine adjustment of pressure in the chamber, manometer and other items. The base of the chamber has three sphere valves with specific functions: to regulate the inlet of clarified water into the chamber, the air inlet and water saturated outlet for vessels.
All experiments were performed at room temperature (25 ± 2ºC), 5 bar relative pressure, 8% of recycle ratio and 8 min of retention time (Ribau Teixeira and Rosa, 2006c). Experiments were made in duplicate.

2.4 Nanofiltration test

Two flat-sheet nanofiltration membranes, NF-270 and NF-90, were used in current investigation (Dow Chemical Company®). The characteristics of these membranes, according to López-Muñoz et al., (2009), Nghiem et al., (2008) and Nghiem and Hawkes (2009).
The "synthetic water" was initially treated by the DAF process to reduce the amount of cyanobacterial cells and cyanotoxins. As observed by Ribau Teixeira and Rosa (2006a), DAF is not a very efficient process for toxin removal, except for cyanobacterial cells. The process was expected only to reduce membrane fouling.

NF experiments were performed after C/F/DAF, or rather, treated water from C/F/DAF experiments was used as NF feed water.
NF experiments were carried out in a dead-end filtration system comprising manometers, flow meter to control the trans-membrane pressure and flow rate, 5L-feed tank, valves to control the permeate output, pressure pump which supports pressure up to 5 bar and a filtration cell with a surface area of 2.58 cm^2. Membrane's filtration area was calculated according to the number of holes in the bottom wire screen and single-hole area.
In the equipment, the membrane was placed between two wire screens and then compressed. The upper screen lies inside the device and serves as support for the metal bar. It prevents the deposition of suspended materials on the membrane. Otherwise, the permeate flux and the membrane's life time are reduced.

3. Results

3.1 Results for dissolved air flotation (DAF) with the optimum concentration of KCl and NaCl (1M)

Figure 1 shows the percentages of cyanobacterial cells removal for the optimum concentration of coagulant solution, using extraction with KCl (1M) and NaCl (1M), obtained in the C/F/DAF process for different initial turbidity values.

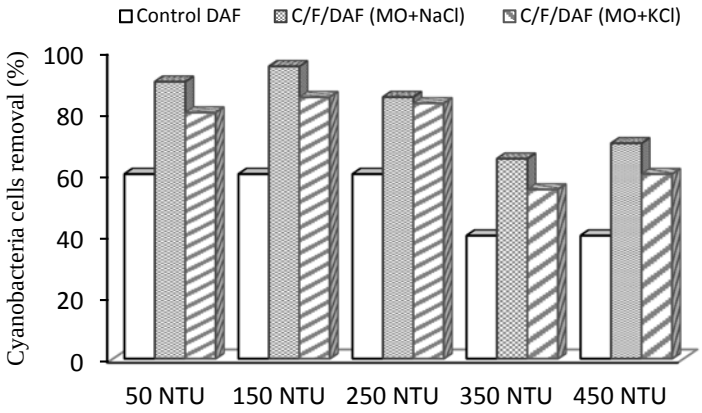

Figure 1: Percentage of cyanobacterial cells removal for the optimum concentration of coagulant solution, using extraction with KCl and NaCl (1M) for different initial turbidity rates.

Results obtained in the flotation experiments suggest that the C/F/DAF with the use of coagulation at optimal conditions may produce removal efficiencies between 80 and 95 % in M. protocystis cells. This efficiency is higher than the control experiment only in DAF.

Table 1: Physical parameters (color and turbidity) and removal efficiency of C/F/DAF process.

Turbidity (NTU)	Parameter	SW	E (%) KCl	E (%) NaCl
50	Apparent color (uH)	754	96.0	96.9
	Turbidity (NTU)	50	96.5	97.1
	pH	7.32	-	-
150	Apparent color (uH)	1,251	97.1	98.6
	Turbidity (NTU)	250	98.6	99.2
	pH	7.41	-	-
250	Apparent color (uH)	1709	99.0	98.7
	Turbidity (NTU)	350	99.2	99.5
	pH	7.50	-	-
350	Apparent color (uH)	2,351	99.2	99.5
	Turbidity (NTU)	350	99.0	99.0
	pH	7.58	-	-
450	Apparent color (uH)	2,892	99.5	99,7
	Turbidity (NTU)	450	99.2	99,5
	pH	7.64	-	-

SW: synthetic water without treatment; E (%) KCl and NaCl: removal efficiency of the parameters by C/F/DAF process

Table 1 shows that the C/F/DAF, both with KCl and NaCl saline solution, produced treated water with color and turbidity within the limits established by WHO (2006), regardless of the initial crude water turbidity. Treated water's pH was almost constant and within the limits established.
Although the DAF process has been efficient in removing cyanobacterial cells, it failed with dissolved toxins, such as microcystins. The initial concentration of the microcystin-LR in these tests was 10 $\mu g \cdot L^{-1}$ with an initial turbidity ranging between 50 - 450 NTU.
Figure 2 shows that when C/F/DAF processes were applied with coagulant salt solutions (KCl and NaCl), their efficiency was >23 % for KCl solution and >45% for NaCl solution.

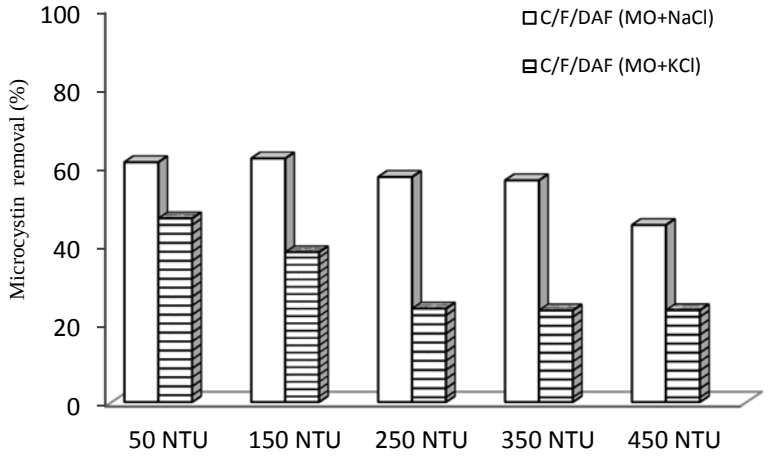

Figure 2: Percentages of microcystin removal by optimum concentration of coagulant solution, using extraction with KCl and NaCl (1M) for different initial turbidity values.

Current study showed that C/F/DAF process by natural coagulant had higher removal efficiency of cyanobacterial cells and microcystins and did not cause cell lysis when compared to that by chemical coagulant (Ribau Teixeira and Rosa, 2010).
An improvement in the performance above has also been mentioned in the literature, or rather, membrane separation process is a promising technology to obtain high efficiency in cyanotoxins removal (Teixeira and Rosa, 2006a). In this case, nanofiltration is chosen according to treatment's requirement and purpose.

3.2 Nanofiltration results with the optimum concentration of KCl and NaCl (1M)

At this stage, the membranes NF-270 and NF-90 were evaluated according to their efficiency in removing cyanotoxins. For these tests, two types of water, one with high (450 NTU) and the other with low (50 NTU) turbidity, were chosen due to their high efficiency (>95 %) obtained in C/F/DAF process within the turbidity range 50 – 450 NTU.
The membranes had the same basic configuration and were basically differentiated by nominal cut-off and permeate flow. López-Muñoz et al. (2009) estimated an average pore radius equal to 0.44 nm for membrane NF-270 and 0.38 nm for membrane NF-90, according to their respective ability to retain neutral organic compounds. Results showed that the membrane NF-90 was more "closed" than membrane NF-270.
A 5 bar pressure only was applied in the optimal concentrations of KCl and NaCl (1M) so that the flow behavior for the same pressure could be compared.
Higher pressures were not evaluated in terms of the equipment capacity. In all tests, the membrane compaction with ultra-pure water was initially undertaken and then the "synthetic water" filtration with cyanotoxins (microcystins) was carried out to verify the possible occurrence of membrane fouling.
Microcystins are very large (996 Da) when compared to the membrane pore size and are highly rejected by the membrane, with an efficiency of MC-LR (always 100%), below the quantification limit by NF, after C/F/DAF pre-treatment (Table 2).

Table 2: Concentrations and removal percentages of MC-LR toxins by NF-270 and NF-90 membranes at different times in the process.

Membrane	Time (min)	MC-LR (µg.l^{-1})	Removal with pre-treatment (%)	
			MO+KCl/DAF[b]	MO+NaCl/DAF[c]
NF- 270	Initial[a]	10.0	-	-
	t = 0	0.00	100.0	100.0
	t = 30	0.00	100.0	100.0
	t = 60	0.00	100.0	100.0
NF – 90	Initial	10.0	-	-
	t = 0	0.00	100.0	100.0
	t = 30	0.00	100.0	100.0
	t = 60	0.00	100.0	100.0

Initial[a]: concentration after C/F/DAF.
MO+KCl/DAF[b]: MO saline solution (KCl-1M)/ DAF
MO+NaCl/DAF[c]: MO saline solution (NaCl-1M)/ DAF

Thus, the nanofiltration process for different membranes NF-90 and NF-270 was efficient in microcystin removal, with a decrease in fouling during the operation (Table 3).

Table 3: Fouling percentage of membrane NF-90 and NF-270.

Initial turbidity (NTU)	NF-270 fouling (%)		NF-90 fouling (%)	
	KCl test	NaCl test	KCl test	NaCl test
50	48.1	48.1	53.6	53.6
150	35.2	32.2	48.0	50.2
250	42.2	42.2	58.9	58.9
350	48.9	48.9	64.2	63.8
450	40.7	40.7	50.6	52.4

In C/F/DAF+NF sequence, Ribau Teixeira and Rosa (2006b) obtained a 100% removal efficiency for chlorophyll-a and microcystin. Dixon et al. (2011) showed that membrane filtration of cyanobacterial metabolites (microcystins) in treated water was effective by NF90, NF270 and DK.

4. Conclusions

Current assay investigated the ability of C/F/DAF+NF sequence with the natural coagulant MO to remove cyanobacteria and associated microcystin-LR from water samples with blooms containing the toxic *M. protocystis*.
The C/F/DAF process, featuring optimal conditions with saline extraction KCl and NaCl (1M) may efficiently (between 80 and 95%) remove *M. protocystis.* The process, however, was not effective for dissolved toxins, such as microcystins-LR.
Good results in terms of NF fluxes, with membranes NF-270 and NF-90 at 5 bar pressure, overall removal efficiencies and final water quality were achieved with C/F/DAF+NF sequence. Total removal of all analytical parameters evaluated, including the toxins, was achieved after the nanofiltration step.
The pH of the treated water did not vary widely after the combined process C/F/DAF+NF with coagulant MO.
As far as the final water quality is concerned, the C/F/DAF+NF sequence guaranteed a full removal of cyanobacterial biomass (100%) and microcystin-LR with membranes NF-270 and NF-90. The determination of the best C/F/DAF process parameters, the choice of the best operating conditions, and the nanofiltration membrane used may provide satisfactory results in terms of water quality for public supply. Microcystin-LR concentrations in the treated water were always below the WHO guideline rate of 1 µg/L of MC-LR in drinking water.

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