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Advanced Processes of Cyanobacteria and Cyanotoxins Removal in Supply Water Treatment

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The efficiency of *Moringa oleifera* (MO) seeds as a natural coagulant in the coagulation/flocculation process, followed by nanofiltration (NF) for the removal of cyanobacterial cells and cyanotoxins is investigated. "Synthetic water" prepared for the tests comprised de-ionized water with an inoculum of microcystis protocystis cells to obtain turbidity within the 50-450 NTU range. Methodology followed two steps: 1) coagulation/flocculation/sedimentation (C/F/S) process using MO extracted in a saline solution of potassium chloride (KCl-1M) as coagulant to determine best dosage; 2) nanofiltration process using NF-90 and NF-270 membranes, with slightly different characteristics. A 5 bar working pressure was applied. Physical, chemical (color, turbidity and pH) and microbiological (cyanobacterial cells count and microcystin concentration) parameters were analyzed in all samples. Current study shows that, as a natural coagulant, MO seeds provided satisfactory results in *M. protocystis*, color and turbidity removal and did not cause cell lysis. NF completely removed cyanobacterial cells and microcystins (100%) from *M. protocystis* (within the quantification limits). Results show that C/F/S+NF sequence is a safe barrier against *M.protocystis* and microcystins in drinking water.

1. Introduction

During the last decades, water supplies in some parts of world, including Brazil, have exhibited an increase in the number of cyanobacterial blooms affecting their water sources. Cyanobacterial blooms give an undesirable taste and odor to water and introduce operational problems in water treatment systems due to their buoyancy in water. However, the main concern with regard to the increasing occurrence of cyanobacterial blooms is the production and the release of cyanotoxins by some species. Cyanotoxins may cause serious diseases and, when their concentrations are high enough, fatal consequences to consumers. A serious public health problem is actually present.
Species of the genus *Microcystis* have caused poisoning in people and animals. The above-mentioned cyanobacteria may produce cyanotoxin microcystins with hepatotoxic activities (Lehman, 2007). To date, more than 60 structural variants of microcystins have been identified. The microcystin-LR appears to be one of the most commonly microcystins in water supplies around the world (Chorus and Bartram, 1999). Consequently, most research has focused on this particular toxin due to its most toxic congeners. As a result of the increasing concern with health implications, the World Health Organization (WHO, 2006) established a drinking water guideline rate of 1.0 µg/L for microcystin-LR (MC-LR).
A recent alternative for cyanobacteria removal is the use of natural coagulants such as *Moringa oleifera* (MO) (Lürling and Beekman, 2010). Water treated with MO seed extract produces lower sludge volume when compared to alum, produces biodegradable sludge and does not affect the pH of water (Ghebremichael, 2004; Nkurunziza et al., 2009).
Previous studies have shown that MO extracts are quite efficient in reducing turbidity and microorganisms from crude water (Nwaiwu and Lingmu, 2011; Muyibi and Evison, 1995; Ndbigengesere et al., 1995). Further, MO inhibits the growth of some species of cyanobacteria such as *Microcystis aeruginosa* (Lürling and Beekman, 2010) and the active agents in aqueous MO are more effective coagulants than alum (Ndbigengesere et al., 1995). Besides, the cost of this natural coagulant would be less when compared to

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that of the conventional coagulant (alum) in water purification since it is available in most developing countries' rural communities where treated water is a scarce resource (Ghebremichael, 2004).
An effective removal alternative of cyanobacteria and cyanotoxins is membrane pressure-driven filtration. Microfiltration (MF) and ultrafiltration (UF) are adequate for removing cyanobacterial cells, but not cyanotoxins. This is due to their large pore size and high molecular weight cut-off of the membranes (RibauTeixeira and Rosa, 2005). Reverse osmosis (RO) and nanofiltration (NF) remove extracellular dissolved toxins due to their low molecular weight cut-off (NF molecular weight cut-off ranges between 100 to 1000 Da) and average molecular weight of microcystin is 996 Da (Svrcek and Smith, 2004; Falconer, 2005; Ribau Teixeira and Rosa, 2005; Ribau Teixeira and Rosa, 2006a; Ribau Teixeira and Rosa, 2006b; Dixon et al., 2011).
Since coagulation/flocculation process and nanofiltration are respectively efficient in the removal of cyanobacterial cells and cyanotoxins, the association of this process for the water supply treatment with cyanobacterial blooms becomes feasible. Current study verifies the efficiency of the coagulation/flocculation process with MO seeds as a natural coagulant, followed nanofiltration, in the removal of cyanobacterial cells and cyanotoxins.

2. Materials and Methods

2.1 Samples

A synthetic water (deionized water with an inoculum of *Microsystis* sp cells) was used for tests to obtain turbidity ranging between 50 – 450 NTU.

2.2 Cultivation

Microcystis protocystis, supplied by Hubbard Brook Research Foundation (HBRF01), was grown in laboratory in 1L of ASM1 medium, composed of inorganic substances only. The cultivation of *M. protocystis* cells were maintained under conditions of maximum asepsis, controlled temperature around 24°C under fluorescent lamps (Philips TLT 20 W/75 s cool), with a 12 h light-12 h dark photo period. Cultures were harvested at the late exponential growth phase (Ribau Teixeira and Rosa, 2005).

2.3 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.

2.4 Coagulation/flocculation/sedimentation experiments (C/F/S)

The coagulant saline solution was prepared with 1 g of MO peeled seeds, crushed in a blender with 100 mL saline solution KCl (1M). After grinding, the solution was stirred for 30 min and the vacuum filtered (Madrona et al., 2010). Twelve concentration levels, or rather, 25 mg/L, 50 mg/L, 75 mg/L, 100 mg/L, 125 mg/L, 150 mg/L, 175 mg/L, 200 mg/L, 225 mg/L, 250 mg/L, 275 mg/L and 300 mg/L were used from the solution. Coagulation/flocculation/sedimentation tests were conducted in jar-test equipment (model 218-6 LDB; Nova Ética), in six buckets, with mixing rods rotation regulator. The experimental conditions for the coagulation/flocculation process were rapid mixing gradient (100 rpm), rapid mixing time (3 min), slow mixing gradient (10 rpm), slow mixing time (15 min) and settling time (60 min) (Madrona et al., 2010).

2.5 Nanofiltration tests (NF)

Two flat-sheet nanofiltration membranes, NF-270 and NF-90, were used in current investigation (Dow Chemical Company®). The synthetic water was initially treated by the coagulation/flocculation process to reduce the amount of cyanobacterial cells and cyanotoxins. As observed by Ribau Teixeira and Rosa (2006a), C/F/S 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/S, or rather, treated water from C/F/S 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.

3. Results and Discussion

3.1 Results for application of MO extract with KCl (1M)

Figures 1a to 1c show the percentage of cyanobacterial cells, turbidity and color removal, respectively, for different concentrations of coagulant solution used in C/F/S process for different initial turbidity. Figure 1d shows that mean pH was stable in all concentrations.

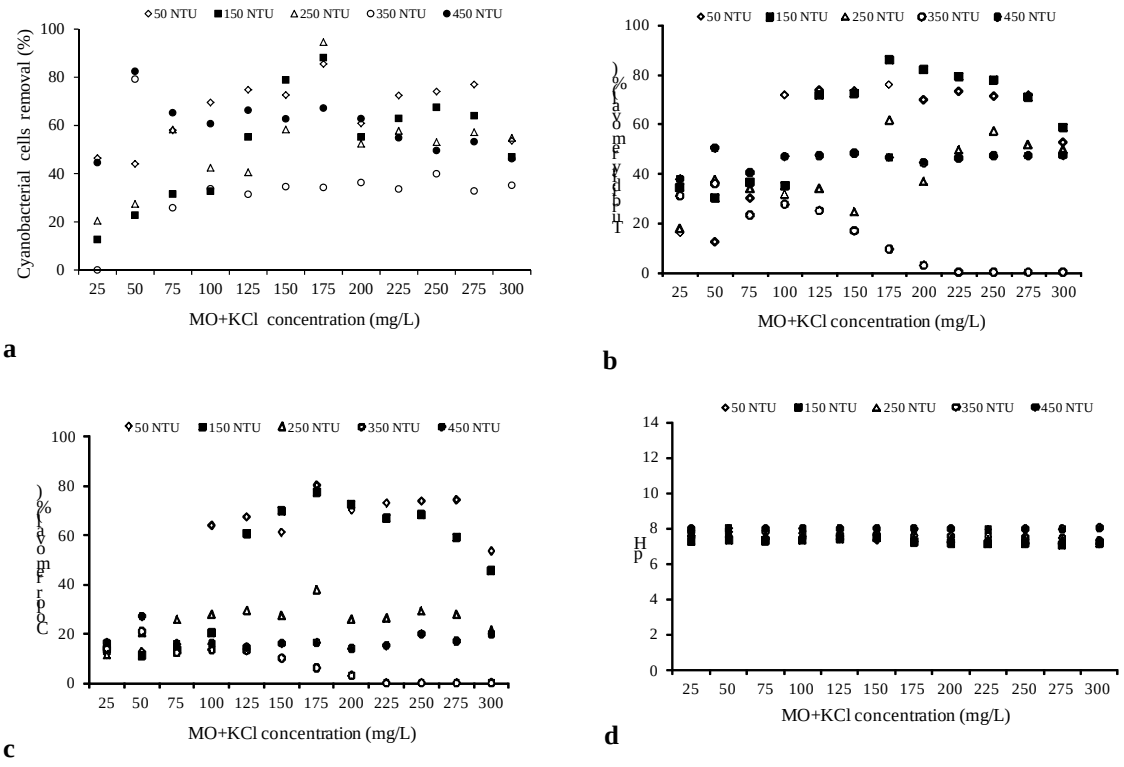


Figure 1 - Percentage of (a) cyanobacterial cells removal, (b) turbidity removal, (c) color removal, and (d) pH range for different concentrations of coagulant solution, using extraction with KCl 1M for different initial turbidity values evaluated.

Results show a high removal of cyanobacterial cells (Figure 1a) with different initial turbidity. Whereas for initial turbidity 50, 150 and 250 NTU, the optimum dosage was 175mg/L, with an average removal of 85.5%, 88.1% and 94.5%, respectively, in the case of initial turbidity 350 and 450 NTU, optimum dosage was 50 mg/L, with an average removal of 79.2% and 82.4%, respectively.

The coagulation efficiency in the cyanobacterial cells removal may have been affected by several factors, such as size and shape of the cyanobacteria, the composition of the cell wall and the presence or absence of mucilage sheath, among others. According to Benhardt and Clasen (1991), the cyanobacterial cells removal by coagulation/flocculation is governed by the same principles applied to the removal of colloidal in suspended particles. Cyanobacteria with more or less spherical structures and smooth surface may be destabilized by the coagulation mechanism adsorption and charge neutralisation (Henderson et al., 2010), as is the case of cyanobacteria in current study.

Figure 1b shows that turbidity removal rate was close to that of cyanobacterial cells (Figure 1a). Turbidity removal percentages for the best coagulant concentrations were 76% (50 NTU), 85.9% (150NTU) and 61% (250 NTU); in the case of waters with turbidity 350 and 450 NTU, they ranged between 35 and 50%. Percentages for color removal (Figure 1c) were similar to those of turbidity removal, or rather, 80% (50 NTU), 77% (150 NTU) and 37% (250 NTU) and > 27% for 350 and 450 NTU. As initial turbidity increased, there was a decrease in removal efficiency.

Further, pH is one environmental factor that influences the development of cyanobacteria. Whereas their maximum growth occurs in habitats with pH ranging from 7.5 to 10 (Giraldez-Ruiz et al., 1999), they are inhibited by pH rates below 5 (Brock, 1973). Figure 1d shows that the addition of coagulant does not influence the pH of the medium, which remained stable at all concentrations, with neutral values, as confirmed in previous studies (Ndabigengesere et al., 1995).

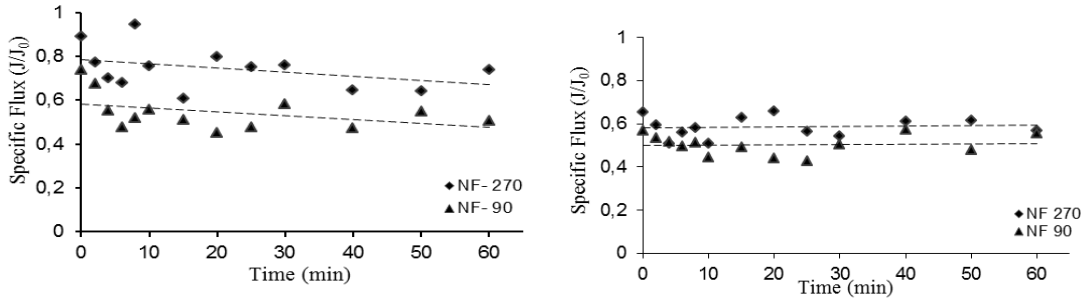
According to Madrona et al. (2010) who employed a KCl saline solution, there is a relative improvement in the time of shelf-saline extract when compared with that of the aqueous extraction. The concentration of salt solution 1M was used and the authors evaluated the protein content of MO seeds. When compared to the aqueous extraction and to salt solution KCl at different molar rates (0.01, 0.1 and 1 M), they reported a more efficient removal of color, turbidity and compounds with absorption in UV-254nm for higher molarity. This means that KCl (1 M) solution for high water turbidity (550 NTU) will remove approximately 99.7%.

3.2 Nanofiltration results with the optimum concentration of KCl (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/S 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 0.44nm for membrane NF-270 and 0.38nm 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.

The concentration and removal percentages of MC-LR toxins by NF-270 and NF-90 membranes at different times in the process (0, 30 and 60 min) showed an efficiency of 100%.

Figures 2 show permeate flux graphs for the tests with nanofiltration membranes (NF-270 and NF-90) by salt solution extraction KCl, respectively, at 5 bar pressure.



a **b**

Figure 2– NF flux for 5 bar pressure after C/F/S, by salt solution extraction (KCl) with low (a) and high (b) initial turbidity for different membranes (NF-270 and NF-90).

When compared to NF-90 membrane, the greater decrease in permeate flux in NF-270 membrane may be directly related to the phenomenon of size exclusion. As described by Eagles and Wakeman (2002), particles smaller than the membrane pores tend to deposit on the pore walls. This fact may effectively reduce pore diameter and flux. The flux decline observed in NF-270 membrane may be attributed to the accumulation of organic matter on the membrane surface and to the clogging of the membrane pores by particles that are similar to or smaller than the pore's average diameter. In the case of NF-90 membrane with its smaller average pore diameter, surface deposition was highly possible, with a correspondingly lower influence on the permeate flux.

4 Conclusions

The C/F/S 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/S+NF with coagulant MO.

As far as the final water quality is concerned, the C/F/S+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/S 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.

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