



Performance of *Microcystis aeruginosa* Removal from Water Using *Moringa oleifera* Seed Presscake Extract as Natural Coagulant

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ABSTRACT

The natural *Moringa oleifera* seed presscake extract (MOSPE) purified with salt-in and dialysis process was used for coagulating removal of *Microcystis aeruginosa* (M.A.) from water in this study. The results showed that the MOSPE obtained in the dialysis tubes with three molecular weight cutoffs (2000, 3500 and 7000 Da) could remove the M.A. efficiently. M.A. removal efficiency increased with the rising temperature. There existed optimal dosage for best M.A. removal, the optimal dosages for the initial chlorophyll-a (Chl-a) concentrations of 0.60 and 1.43 mg·L⁻¹ were 5.0 and 20 mg MOSPE·L⁻¹, respectively. Adsorption and charge neutralization contributed to the removal of M.A. from solution. Compared with the polymeric aluminium chloride (PAC) at same dosage as MOSPE, the MOSPE had better algal removal performance.

INTRODUCTION

Currently, harmful algal blooms (HABs) in freshwater bodies such as lakes, reservoirs and rivers that serve as drinking water sources have become a worldwide ecological problem as a result of eutrophication, which is resulted from excessive nutrients discharged from agricultural activities, domestic wastewaters and industrial effluents (Gao et al. 2009). It was reported that more than 60% of the lakes in China suffer from eutrophication and HABs, and *Microcystis aeruginosa* (M.A.) is one of the dominant species in HABs (Pan et al. 2006). The HABs have attracted increasing attention due to the fact that the HABs can damage the aquatic ecosystem, destroy drinking water treatment and water quality, and even create health risk to water users, including human beings and animals. Specifically, harmful algae can destroy aquatic ecosystems by using up the dissolved oxygen and blocking the sunlight, both of the two factors are essential to aquatic organisms. Moreover, the cyanotoxins released by some harmful algae are hepatotoxic and neurotoxic compounds which have been proved to adversely impact the aquatic animals and human health on the cellular and organ level (Codd 2000, Carmichael et al. 2001, Landsberg 2002, Black et al. 2011). Furthermore, the exces-

sive growth of algae in source water can increase coagulant demand, clog and penetrate the filters, shorten backwashing cycle during the water treatment process (Schmidt et al. 1998, Plummer et al. 2002), and some of their metabolites can cause unpleasant taste and odour in treated water. Hence, it is of great importance to develop safe, efficient and cost effective HABs control techniques.

Several methods including chemical, electrochemical, mechanical and biological techniques have been studied for HABs removal (Pan et al. 2006, Anderson 1997). Among these techniques, flocculation-coagulation is very attractive due to its easy operation, high efficiency and immediate result. Additionally, this technique keeps the integrity of the algal cells and there is no release of intracellular toxins and the compounds, which can cause taste and odour (Hall et al. 2007, Hankins et al. 2006). However, commercial flocculants, such as polyaluminium chloride and polyferric chloride, are not eco friendly and also expensive when used in large quantities (Pan et al. 2006, Wu et al. 2011). Recently, low-cost natural coagulants such as clays have drawn increasing attention for water treatment, but the high loading and the long time for sedimentation hinder its wide use (Pan et al. 2006, Wu et al. 2011, Han et al. 2001, Sengco et al. 2004). So, it is of great importance to look for a cheap, effective, nontoxic natural coagulant for HABs control.

Moringa oleifera is a tropical plant belonging to the family of Moringaceae. *Moringa oleifera* seeds (MOS) have been used for water purification for many centuries (Pritchard et al. 2010). Since 1970s, a number of researches have been carried out to evaluate the potential of MOS as a natural coagulant (Pritchard et al. 2010, Jahn et al. 1979, Jahn et al. 1988, Muyibi et al. 1995, Muyibi et al. 2003). It has been proved that the main active components in MOS responsible for water purification are water-soluble proteins that act as natural coagulants in water treatment (Ndabigengesere et al. 1995). Compared with alum, the proteins are more efficient for turbidity removal and have less effect on pH and conductivity of the water after treatment (Ndabigengesere et al. 1995, Sarpong et al. 2010). Additionally, less sludge is produced by coagulation with *Moringa* than alum (Ndabigengesere et al. 1995). The main mechanism of coagulation with the proteins has been manifested to be adsorption and neutralization of charges when they are used for turbidity removal (Ndabigengesere et al. 1995). As MOS is a good source of edible and other useful oils due to its high oil content, the *Moringa oleifera* seed presscake (MOSP) can be used as coagulant because the proteins still remain in MOSP after oil extraction (Sutherland et al. 1994).

So far, the published documents have mainly focused on the removal of turbidity and microorganisms such as *E. coli* by the extract (Pritchard et al. 2010, Sarpong et al. 2010, Okuda et al. 1999, Sánchez-Martín et al. 2010). However, there is no report about algal removal by the MOSP extract (MOSPE). In this project, batch experiments were carried out to investigate the feasibility of the application of MOSPE for M.A. removal.

MATERIALS AND METHODS

Preparation of MOSP coagulant: The MOSP was crushed and sieved through 0.45 mm screen. 1 g of the MOSP powder through the sieve was mixed with 50 mL of 1 mol·L⁻¹ (NH₄)₂SO₄ solution on a magnetic stirrer at 2000 rpm for 10 min. Subsequently, the mixture was separated with 0.45 μm membrane filter, and fine (NH₄)₂SO₄ powder was added slowly into the filtrate on the magnetic stirrer, mixing at the above-mentioned speed until the solution was about 90% saturated by fine (NH₄)₂SO₄ powder. The precipitate in the solution was separated by centrifuge at 4000 rpm for 10 min and then re-dispersed in 10 mL distilled water. The suspension was then put into dialysis tubes with different molecular weight cutoffs (2000, 3500 and 7000 Da). Each dialysis tube was put into a beaker with 1 L distilled water for dialysis until no white precipitate was observed in the water detected by 1 mol·L⁻¹ BaCl₂. The solution obtained in each dialysis tube was diluted with distilled water to 100 mL. The solution was referred to as MOSPE for the experiments,

and the MOSPE concentration was 10 g·L⁻¹ described by MOSP content. The polymeric aluminium chloride (PAC, offered by Zhengzhou Sino-French Water Supply Co., Ltd. where the PAC is used for drinking water treatment.) solution at the concentration of 10 g·L⁻¹ was also used to carry out the same experiments to compare its M.A. removal performance with MOSPE.

Microcystis aeruginosa culture: M.A. was purchased from the Freshwater Algal Culture Collection at the Institute of Hydrobiology, Chinese Academy of Sciences. The M.A. was incubated in sterilized 1L glass flasks containing 500 mL of BG11 nutrient solution that had been adjusted to pH 8.0 by adding 0.1 mol·L⁻¹ NaHCO₃ or 0.1 mol·L⁻¹ HCl solutions at 25±1 °C under fluorescent light (1000 lx, 12-h light/12-h dark) (Zheng et al. 2012).

Preparation of Microcystis aeruginosa solution: M.A. cells at late exponential growth stage were harvested with 0.45 μm membrane filter. The M.A. cells on the filter were washed with distilled water for 5 min and then suspended in distilled water to carry out the experiments immediately.

Coagulation experiments: Coagulation experiments were carried out using program-controlled jar test apparatus. After the prepared coagulants were added into 200 mL of M.A. solution, the solution was rapidly mixed (200 rpm) for 3 min followed by slow mixing (40 rpm) for 15 min (Szygwa et al. 2009). The mixture was then allowed to settle for 30 min. Samples were collected from 2 cm below the water surface using a syringe for analysis.

Analytical methods: The chlorophyll-*a* (Chl-*a*) concentration was used to represent the quantity of M.A. in the water solution. The Chl-*a* concentration was measured using the method that was described in the previous documents (Gao et al. 2009, Zheng et al. 2012). The M.A. removal efficiency was calculated by the following equation:

$$R = \frac{C_0 - C_e}{C_0} \times 100\% \quad \dots(1)$$

Where, *R* is the M.A. removal efficiency; *C*₀ and *C*_{*e*} are the initial and final Chl-*a* concentrations (mg·L⁻¹), respectively.

COD_{Mn}, NO₃⁻-N and NH₄⁺-N were analysed according to Standard Methods for Water and Wastewater Examination (SEPA 2002).

Infrared spectroscopy: Fourier Transform Infrared Spectroscopy (FTIR) was employed to characterize the MOSPE. The MOSPE sample was prepared by adding a drop of MOSPE solution on a KBr tablet and evaporating the water at 40°C. The spectrometer FTIR spectra were recorded on a Nicolet 5700 FTIR from 4000 to 400 cm⁻¹.

Image capture: An optical microscope (Nikon eclipse E200,

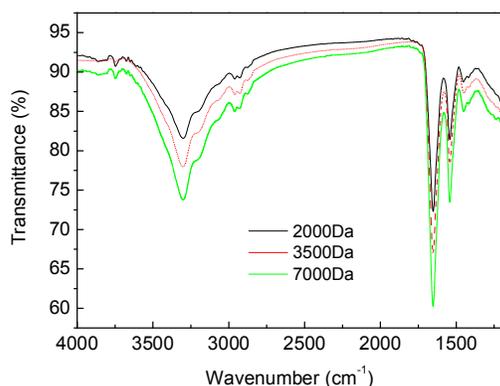


Fig. 1: FTIR spectrum of MOSPEs.

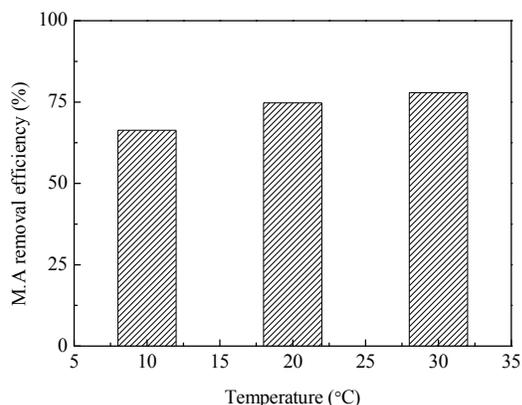


Fig. 3: Effect of solution temperature, on M.A. removal ($C_0=0.92 \text{ mg}\cdot\text{L}^{-1}$, $\text{pH}=7.2\pm 0.2$).

Japan) equipped with a video camera (Pixelink, PL-A662, Canada) was used to observe the morphology of algal flocs. A drop of sample collected at the above mentioned moment was carefully deposited on a glass slide and covered with a cover slip. The slide was observed immediately and images were taken at a magnification of 100 \times .

RESULTS AND DISCUSSION

Infrared spectroscopy: FTIR technique was employed to identify the MOSPE. Fig. 1 showed the FTIR spectra of MOSPE obtained by the dialysis tubes with different molecular weight cutoffs of 2000, 3500 and 7000 Da, respectively. The three MOSPEs had similar FTIR spectra indicating they shared same functional groups. Typically, the band at 3748 cm^{-1} was related to the -OH vibration (Mal et al. 1995), the bands at 3300 cm^{-1} were corresponded to the NH stretching (amide A) (Kong et al. 2007). The band at 2961 cm^{-1} was due to the asymmetric CH_3 stretching (Zhang et al. 2014). The band at 1652 cm^{-1} was attributed to the C=O

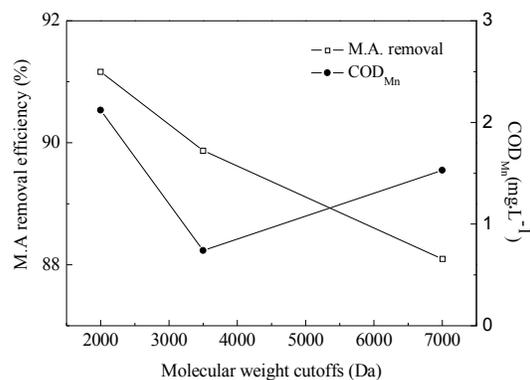


Fig. 2: Effect of MOSPEs with different molecular weights on M.A. removal ($C_0=1.56 \text{ mg}\cdot\text{L}^{-1}$, $\text{pH}=7.2\pm 0.2$, $T=30^\circ\text{C}\pm 1$).

stretching (amide I) (Kong et al. 2007). The band at 1542 cm^{-1} was related with CN stretching and NH bending (amide II) (Kong et al. 2007). The band at 1452 cm^{-1} was due to CH_2 bending vibration (Garip et al. 2007). These data indicated that the MOSPE contained various proteins, and the proteins have been proved to be active agents for water purification (Ndabigengesere et al. 1995).

Effect of MOSPEs with different molecular weights: Although proteins have been manifested to be the active agents in the extract for water purification, the proteins varied due to the different extracting methods (Ndabigengesere et al. 1995, Ghebremichael et al. 2005). Hence, MOSPE-2000, MOSPE-3500 and MOSPE-7000 were obtained by using dialysis tubes with different molecular weight cutoffs, and their effects on M.A. removal and water quality are shown in Fig. 2. The removal of M.A. varied from 88.1% to 91.2% for the three MOSPEs, indicating MOSPEs were good for M.A. removal and removal rates of M.A. by the three MOSPEs were approximately same. It was reported that the proteins in the MOSPE had isoelectric points between 10 to 11 (Ndabigengesere et al. 1995, Ghebremichael et al. 2005), thus at the pH value set for the experiment, the proteins were positively charged while the M.A. surface was negatively charged (Gao et al. 2009, Ma et al. 2012). Therefore, electrostatic adsorption and charge neutralization took place between the proteins and the M.A. to form flocs which could be settled down by gravity, leading to the removal of M.A. from water solution. It is, therefore, assumed that coagulation by charge neutralization and electrostatically induced adsorption are contributed to the removal of M.A. from water solution. Due to the natural and complicated characteristic, MOSPE might cause side effect to water quality (Okuda et al. 2001) and it indeed resulted in increase of COD_{Mn} in treated water as shown in Fig. 2. Although the MOSPE-3500 had the second best M.A. removal efficiency, it cre-

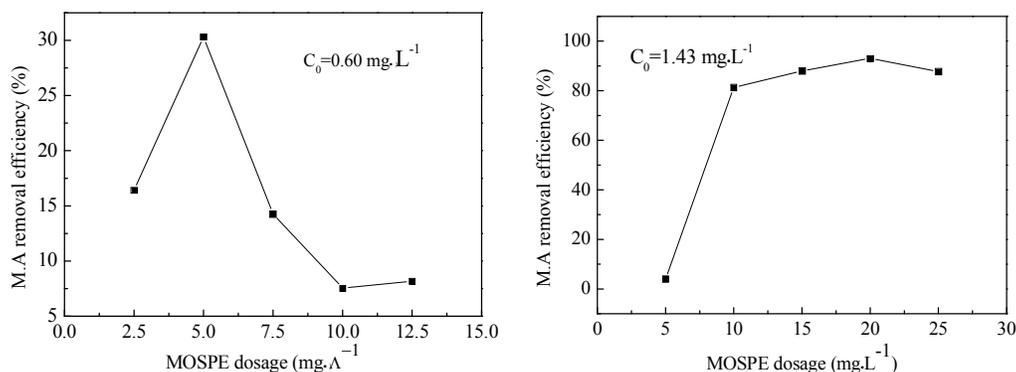


Fig. 4: Effect of initial M.A. concentration and coagulant dosage on M.A. removal (pH=7.2±0.2, T=30°C±1).

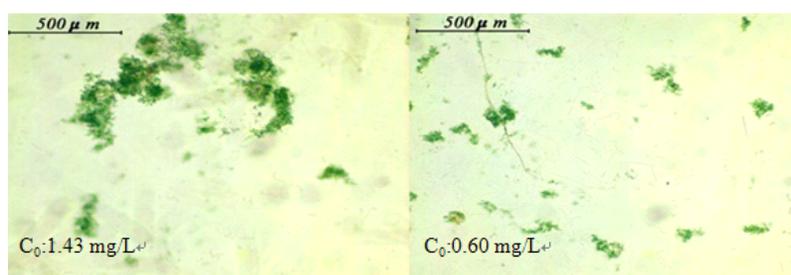


Fig. 5: Microscopic photographs of M.A. flocs.

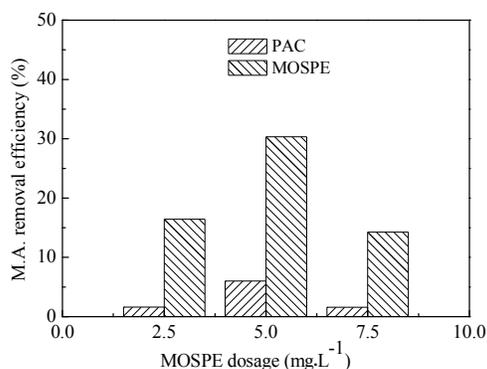


Fig. 6: Performance of M.A. removal by MOSPE and PAC (C₀=0.60 mg.L⁻¹, pH=7.2±0.2, T=30°C±1).

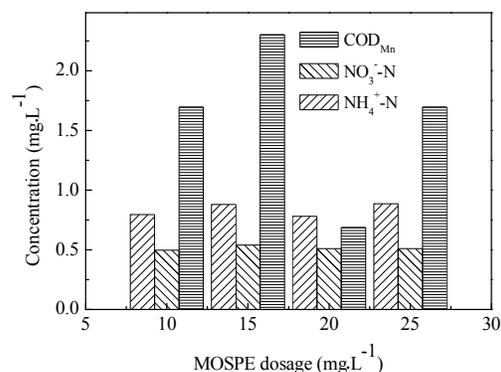


Fig. 7: Effect of MOSPE on water quality (C₀=1.43 mg.L⁻¹, pH=7.2±0.2, T=30°C±1).

ated the lowest COD_{Mn} concentration in treated water (0.74 mg.L⁻¹). Hence, the MOSPE-3500 was selected to carry out the following experiments.

Effect of solution temperature: Effect of solution temperature on M.A. removal is shown in Fig. 3. The M.A. removal increased as the solution temperature rose, indicating higher temperature was favorable for M.A. removal due to the fact that the viscosity of water rose with decreasing temperature, which in turn led to poor coagulation performance (Wu et

al. 2011). Furthermore, the solubility of gasses, such as carbon dioxide and oxygen, increased with the decreasing solution temperature. The inevitable involvement of the gases into the flocs formation led to the low density and poor settlement of the flocs, resulting in poor M.A. removal (Wu et al. 2011, Xiao et al. 2009).

Effect of initial M.A. concentration and coagulant dosage: Fig. 4 showed the effects of initial M.A. concentrations and coagulant dosage on M.A. removal. It can be seen that

there existed optimal MOSPE dosages for both of the two different initial Chl-*a* concentrations and the optimal values were 5.0 and 20 mg·L⁻¹ for the initial Chl-*a* concentrations of 0.60 and 1.43 mg·L⁻¹, respectively. The M.A. removal efficiency decreased when the MOSPE dosages exceeded the values due to the fact that the overdose of MOSPE would cause the charge reversal of the algal surface and a consequent restabilization of the algae (Ahmad et al. 2011). The excessive dosage of the extract could also lead a poor turbidity removal because of the same reason (Gidde et al. 2008).

Fig. 5 shows the flocs formed by MOSPE and M.A. It can be seen that larger flocs formed by M.A. with higher concentration, which might due to fact that M.A. with higher concentration offered more opportunity for collision among the M.A. cell and reaction between the M.A. cell and the MOSPE. The larger flocs are easier to be settled down due to gravity, resulting in higher M.A. removal.

Performance of M.A. removal by MOSPE and polyaluminum chloride (PAC): Fig. 6 shows a comparative performance of MOSPE to an equivalent dose of alum for M.A. removal. The MOSPE had higher M.A. removal efficiency than PAC at all the three same coagulant dosages, meaning that MOSPE was better than PAC for M.A. removal.

Effect of MOSPE on water quality: Previous studies revealed that both the water and salt extract could introduce undesirable matters into the treated water (Sánchez-Martín et al. 2010, Ndabigengesere et al. 1998, Bawa et al. 2001). Hence, three parameters including COD_{Mn}, NO₃⁻-N and NH₄⁺-N were tested to investigate the effect of MOSPE on treated water. Fig. 7 shows that all the three pollutants appeared in the treated water and their concentrations varied with the MOSPE dosages. The lowest concentrations of COD_{Mn}, NO₃⁻-N and NH₄⁺-N were 0.69, 0.51 and 0.78 mg·L⁻¹, respectively. The lowest concentrations of all the three pollutants appeared when the optimum MOSPE dosage of 20 mg·L⁻¹ was applied.

The result indicated that the extracting and purifying process including salt-in and salt-out by (NH₄)₂SO₄ in conjunction with dialysis could not remove inactive components from the MOSPE completely. However, MOSPE had very significant algal removal performance and was more effective than the PAC at the same dosage. Hence, it offered an alternative solution to algal removal from water. In the further study, the coagulant will be extracted from the MOSPE more adequately and further purified to remove the useless agents causing water deterioration. Then, the MOSPE will be surely more attractive for algal removal.

CONCLUSION

This study investigated the performance of MOSPE as natu-

ral coagulant for M.A. removal. The results showed that the MOSPE was very efficient for M.A. removal and more efficient than PAC at the same dosage. Higher temperature was favorable for better M.A. removal. There existed optimal dosage for best M.A. removal and the M.A. removal efficiency decreased when the proteins dosages exceeded the values. Adsorption and charge neutralization was the main mechanism for the coagulating removal of M.A. by the MOSPE. The slight side effect of the MOSPE on treated water quality could be eliminated by improving extracting method. Hence, the MOSPE is a potential natural coagulant for HABs control.

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