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Comparison of the performance of *Moringa oleifera* seed protein and polyaluminum chloride in removal of humic acid from water

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ABSTRACT

The water-soluble protein was extracted from *Moringa oleifera* seed press cake and tested its performance as a natural coagulant for removing humic acid (HA) from water solution when comparing to polyaluminum chloride (PAC), a commercial coagulant. Influencing factors including pH, coagulant dosage, temperature and suspended solid were investigated in this study. The results indicated that the coagulating efficiencies of HA using the *Moringa oleifera* seed protein (MOSP) were better than those by PAC under all the experimental conditions at the same dosage. MOSP could increase the zeta potential more efficiently than PAC. The Fourier transform infrared spectra revealed that no new compounds were generated during the coagulation process. Hence, adsorption and charge neutralization could be the main mechanism contributing to the HA removal using MOSP as coagulant. MOSP is an ideal natural coagulant for HA removal.

Keywords: Humic acid; *Moringa oleifera* seed protein; Polyaluminum chloride; Coagulant; Coagulation

1. Introduction

Humic substances (HSs) are natural organic macromolecules formed by the breakdown of dead animal and plant in the environment, consisting of a diverse range of components with complex chemical structures and high molecular weight [1], which are the major fraction of the natural organic matter (NOM) in fresh waterbodies [2,3]. HSs in water has received increasing attention in recent years due to the fact that they could cause unpleasant and disagreeable color, odor and taste in treated water and then lead to bacterial re-growth in water distribution systems as well [4,5]. Furthermore, the reaction of HSs with chlorine to form toxic disinfection by-products such as trihalomethanes and haloacetic acids during chlorination in the drinking water treatment process though they themselves are nontoxic [1,6]. Hence, the adverse effects of HSs on water supply speed-up the shift of research focus to NOM removal from particle removal in drinking water treatment.

In general, physical and chemical methods including adsorption [7,8], membrane filtration [9,10], coagulation [11–13], advanced oxidation [14,15], etc., rather than biotechniques are used to remove HSs from water due to their stable nature resistant to further microbial breakdown [6]. Among these techniques, coagulation is most widely used in large-scale water treatment to remove HSs because of its easy operation, high efficiency and low cost [16].

It is well known that coagulant is the key factor to determine the coagulation efficiency. Conventional inorganic coagulants including polymeric and monomeric forms of Al and Fe salts are widely adopted in water treatment. However, Al salts are thought to have adverse effects on human health, and Fe salts can cause undesirable color to treated water. Therefore, the development of new coagulant has caused great interest in water treatment.

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Natural materials extracted from some plants represent emerging coagulants and have attracted increasing interest due to their abundant source, low toxicity, multi-purposeness and bio-degradability [17–20]. Among the natural plants, *Moringa oleifera* has been recognized as a miraculous plant with multiple uses [21], whose seed has been used to purify water for centuries, and ranked as one of the most promising materials acting as coagulant for water treatment [22]. Recent researches have demonstrated that the main active agent in *Moringa oleifera* seed is a cationic protein which could purify water by coagulation. So far, the use of the active protein as natural coagulant mainly focused on the removal of turbidity from water. However, there is little knowledge about the application in HSs removal and the mechanism towards HSs removal from water by specified protein, since HSs in water usually have relatively less sizes to those of the conventional particle matters which form turbidity like clays.

In this study, humic acid (HA) [23], one of the three main components of HSs in water was selected as the target pollutant to test the ability of removal performance by the active protein extracted from *Moringa oleifera* seed press cake. Influencing factor, such as pH, protein dosage, temperature as well as suspended solid, was investigated.

2. Experiment

2.1. Preparation of coagulants

The *Moringa oleifera* seed press cake used in this project was offered by Kunming *Moringa oleifera* Bio-Tech Co., Ltd. The active protein was used as natural coagulant prepared in the way described as previously [24]. The polyaluminum chloride (PAC) solution, with the same concentration as the protein, was prepared by dissolving PAC powder (Tianjin Rui-jinte Chemical Reagent Ltd., China) into deionized water.

2.2. Preparation of HA solution

The stock solution used in this project was synthesized by adding 1.0 g of HA (Shanghai Aladdin Bio-Chem Technology Ltd., China) into 1,000 mL NaOH solution (0.01 mol/L) under continuous stirring for 2 h and then stored in refrigerator for the following study use. The stock solution was diluted with deionized water to obtain the HA solutions with different concentrations for the experiments. The pH of the experimental solutions was adjusted by adding 0.1 M KOH and 0.1 M HCl solutions and then measured by a pH meter (PHS-3C, Leici Ltd., China). The solution temperature was determined by a mercury thermometer. All the reagents used in this study were of analytical grade.

2.3. Jar tests

Coagulation experiments were carried out on a programmable jar test apparatus (ZR4-6, Zhongrun Water Industry Technology Development Co. Ltd., Shenzhen, China) containing 0.2 L HA solution. The solution was mixed in the same way as described before [24]. At the end of mixing, sample was collected from 2 cm below the water surface using a syringe. The sample was then rapidly centrifuged at 4,000 rpm for 10 min. The residual HA concentration in the supernatant was determined by a spectrophotometer (UV-5100, Yuanxi Instrument Co. Ltd., Shanghai, China) at the wavelength of 420 nm [25]. The HA removal rate was calculated from the following equation:

$$R = (C_0 - C_e)/C_0 \times 100\% \tag{1}$$

where C_0 and C_e are the initial and final HA concentrations (mg/L), respectively.

2.4. Particle size distribution study

The particle size distribution of HA in the solution was analyzed by using Malvern laser particle analyzer (Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK) at room temperature.

2.5. Zeta potential study

The zeta potentials of HA solution and the solution containing the flocs formed by HA and *Moringa oleifera* seed protein (MOSP) were measured by using a zeta potential analyzer (zeta plus, Brookhaven Instrument Corporation, USA).

2.6. FTIR analysis

Fourier transform infrared (FTIR) spectra of HA, MOSP and flocs formed by HA and MOSP were obtained by using a FTIR spectrum (Nicolet 5700, Thermo Nicolet Ltd., USA) from the band of 4,000 to 400 cm^{-1}. The MOSP and the flocs used for FTIR analysis were evaporated at 40°C under infrared light.

3. Results and discussion

3.1. Effect of pH

3.1.1. Particle size distribution

Fig. 1 shows the particle size distribution curves of the cumulative volume percentage corresponding to particle size

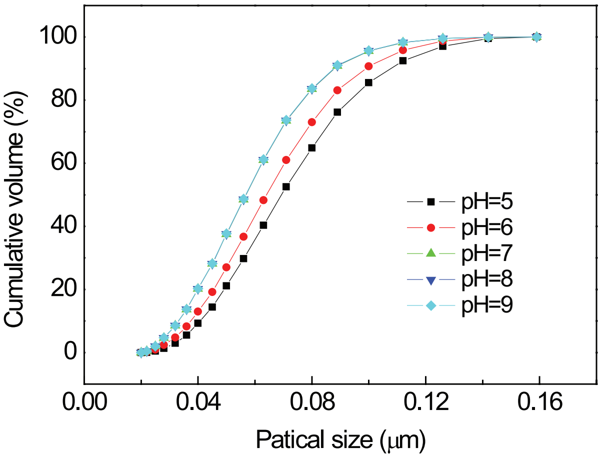

Fig. 1. Particle size distribution of HA as a function of pH (C_0 = 25 mg/L).

of HA as a function of pH, and Table 1 summarizes the statistical information of each sample. Both the graphs of size distribution in Fig. 1 and data in Table 1 show that the HA particle size increases with the rise of pH from 5 to 7, and then the particle sizes remain constant as the pH increases from 7 to 9, indicating that pH could affect the particle size of HA, and HA particle tends to agglomerate in acidic environment, which make the particle to be settled down more easily by gravity.

3.1.2. Removal of HA

Fig. 2 shows the HA removal as a function of pH. The HA removal by MOSP and PAC varies from 64.6% to 71.3% and 23.2% to 42.7% in the pH range of 5–10, respectively, and MOSP is more efficient than PAC for HA removal at every pH value. Additionally, compared with the HA removal by PAC, the HA removal by MOSP changes slightly with pH variation, revealing MOSP could work efficiently in a wider pH range.

3.1.3. Zeta potential study

Zeta potential is a very important parameter to describe the destabilization of colloids in water treatment. The zeta potential variation as a function of pH is shown in Fig. 3. The zeta potential for HA in the pH range of 5–9 varied from –27.8 to ~–20.5 mV. Both of PAC and MOSP can increase the zeta potential to some extent, and MOSP can increase the zeta potential (from –10.0 to ~–1.12 mV) more dramatically than the PAC (from –23.2 to ~–18.9 mV) at the same dosage, which might be a reason why MOSP is better than PAC for HA removal. Previous study has demonstrated that the isoelectric point of MOSP was between pH value of 10 and 11 [26]. Hence, the MOSP is positively charged in the pH range of 5–9 set for the experiments, which are favorable for the electrostatic interaction between the MOSP and HA molecules. It could be deduced that the electrostatic adsorption and neutralization of charges is the dominant mechanism that regulates HA removal in water by MOSP.

3.2. Effect of coagulant dosage

Fig. 4 shows the effect of coagulant dosage on HA removal at two different initial HA concentrations. The corresponding removal rate varies in the range of 5.1%–41.1% and 9.7%–69.6% for PAC and MOSP when the dosage increases from 2.1 to 19.0 mg/L (C_0 = 25 mg/L), and the corresponding removal rate varies in the range of 2.6%–70.7% and 9.5%–77.2% for PAC and MOSP when the dosage increases from 4.5 to 45.4 mg/L (C_0 = 50 mg/L). Though the removal of HA do not keep increasing with rising dosages of MOSP and PAC, MOSP is better than PAC for HA removal at each dosage for both of the two initial HA concentrations.

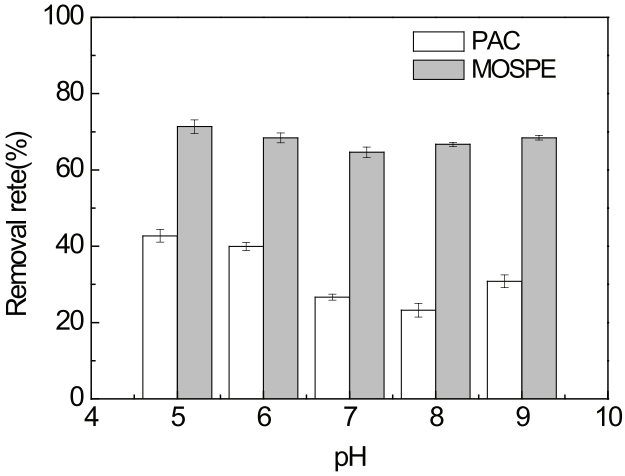

Fig. 2. HA removal by two coagulants as a function of pH (T = 35°C, C_0 = 25 mg/L).

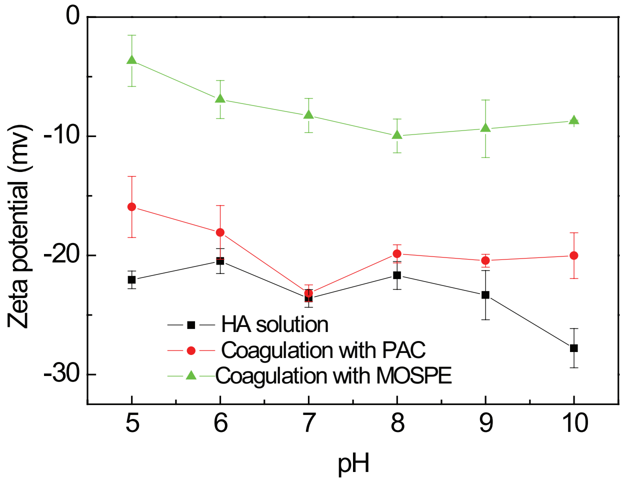

Fig. 3. Zeta potential variation as a function of pH (C_0 = 25 mg/L).

Table 1
Statistical summary of the results of the particle size distribution analysis of HA as a function of pH

Variable	pH = 5	pH = 6	pH = 7	pH = 8	pH = 9
Specific surface area (m²/g)	102	111	126	126	126
Surface mean diameter D[3,2] (μm)	0.059	0.054	0.048	0.048	0.048
Volume mean diameter D[4,3] (μm)	0.070	0.065	0.057	0.057	0.057
D (0.1) (μm)	0.041	0.037	0.033	0.033	0.033
D (0.5) (μm)	0.069	0.064	0.057	0.057	0.057
D (0.9) (μm)	0.107	0.099	0.088	0.088	0.088

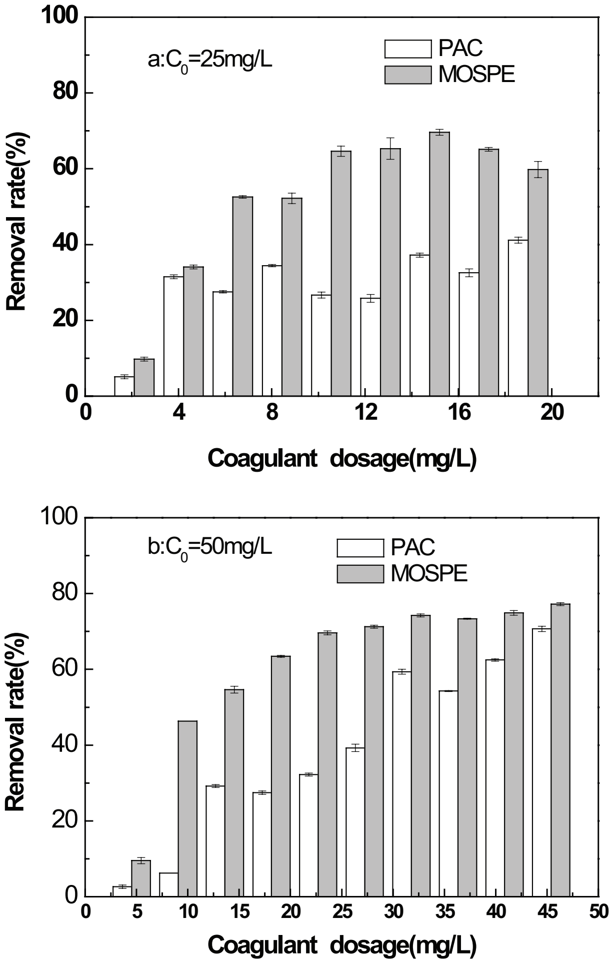

Fig. 4. HA removal as a function of coagulant dosage (pH = 7, T = 35°C).

3.3. *Effect of reaction temperature*

The effect of temperature on HA removal is shown in Fig. 5. The HA removal by PAC increases from 11.5% to 26.7% with the rising temperature from 15°C to 35°C. Whereas, the HA removal by MOSP reaches its maximum of 66.5% at 25°C and slightly falls to 64.6% when temperature increases to 35°C. MOSP is better than PAC for HA removal at each temperature.

3.4. *Effect of suspended solid*

Suspended solid and NOM always coexist in natural waterbodies. Therefore, the effect of suspended solid on HA removal was investigated (Fig. 6). The HA removal by MOSP increases from 64.6% to 72.8% as the kaolin dosage increases from 0 to 100 mg/L, indicating that the existence of suspended solid can increase the HA removal due to the fact that the suspended solid in the water act as the nuclei when HA reacts with MOSP to form flocs. However, the HA removal by PAC do not increase with rising suspended solid dosage, and the removal varies in the range of 23.9%–40.1%. MOSP is better than PAC for HA removal at each kaolin dosage.

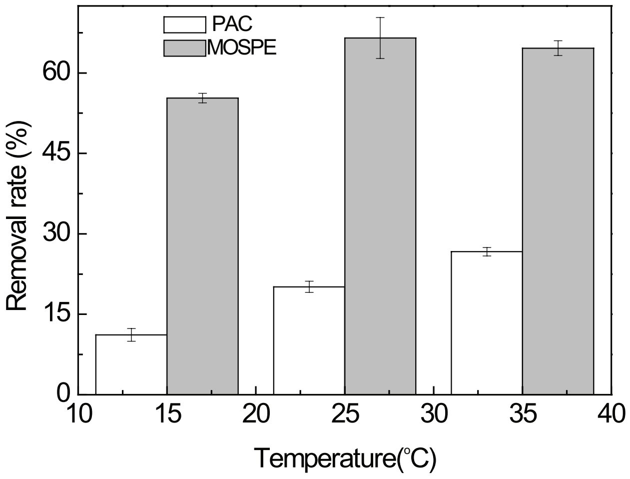

Fig. 5. HA removal by two coagulants as a function of temperature (pH = 7, C_0 = 25 mg/L).

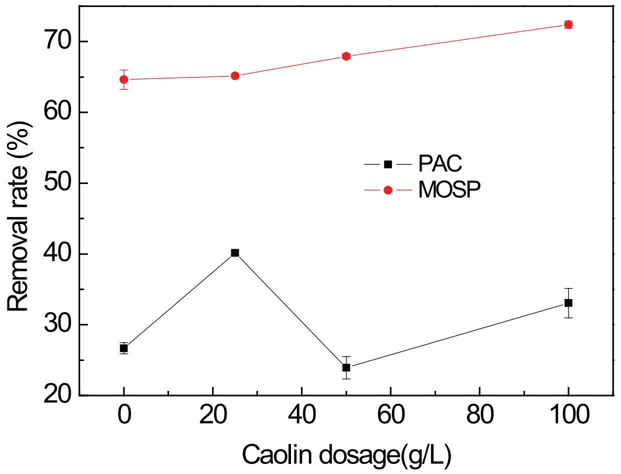

Fig. 6. HA removal by two coagulants as a function of suspended solid (pH = 7, C_0 = 25 mg/L, T = 35°C).

3.5. *FTIR study*

FTIR was performed to further investigate the removal of HA by MOSP. As shown in Fig. 7(a), the typical characteristic peaks of free phenolic hydroxyl, asymmetric stretching vibration of aromatic ring and COO-symmetric stretching of HA at 3,435, 1,593 and 1,381 cm^{-1}, respectively, could be observed [27,28]. Fig. 7(b) shows the spectrum of the floc formed by HA and MOSP, the characteristic peak of hydroxyl shifted to 3,386 cm^{-1}, which is caused by the association of free hydroxyl of HA with hydrogen-bonding acceptor (such as unprotonated carboxylic acids) of MOSP to form the hydrogen bond interactions. Moreover, the characteristic bands at 1,659 and 1,543 cm^{-1} appeared in Fig. 7(b) are assigned to amide I (C=O stretching) and amide II (CN stretching and NH bonding), respectively [29]. It was noteworthy that all these characteristic peaks could be definitely confirmed in FTIR spectrum of MOSP (Fig. 7(c)). Thus, the FTIR spectra offer the evidence for the coagulation of HA with MOSP.

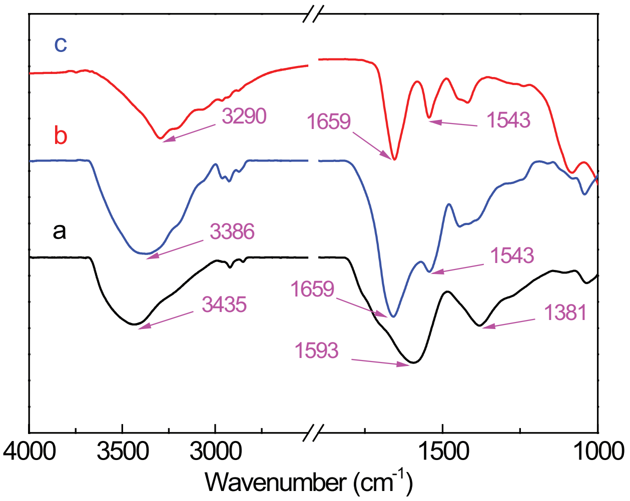

Fig. 7. FTIR spectra of HA (a), HA–MOSP floc (b) and MOSP (c).

4. Conclusion

Collectively, the role of this study was carried out to investigate the HA removal characteristics of MOSP from water solution when comparing to PAC at the same dosage. The results indicated that the MOSP was better than PAC for HA removal under all the experimental conditions. The main mechanism responsible for the HA removal using MOSP as coagulant could be adsorption and charge neutralization, indicating that MOSP could be a good bio-coagulant for HA removal from water.

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