Flocculation of Cyanobacterial Cells Using Coal Fly Ash Modified Chitosan

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Abstract

Harmful algal blooms (HABs) have increasingly occurred worldwide, which pose serious threats to water environment safety. In this study, a compound flocculant (CFAL-Chitosan) was developed for HABs mitigation where chitosan was modified by coal fly ash leachate (CFAL). When using optimized dosage of CFAL-Chitosan flocculant, the zeta potential of *Microcystis aeruginosa* (M.A.) flocs stayed close to zero and the algal removal efficiency plateaued over 95 % in a wide dosage range from 3 to 6 mg/L. For chitosan without CFAL, the removal efficiency peaked at 3 mg/L with a maximum removal efficiency of 81% , which quickly decreased as the dosage increased (> 3 mg/L) due to the fast reversal of zeta potential. This indicated that CFAL-chitosan could maintain better removal efficiency over a wide dosage range due to improved property on charge neutralization than that of chitosan alone. The flocs of CFAL-Chitosan were larger and denser than that of chitosan without

CFAL. However, excessive CFAL beyond the optimized dose inhibited M.A. removal due to the hydrolysis and declining of molecular weight of chitosan that weakened the bridging-netting property, where the surface charge reversal happened within a narrow dosage range and the removal-dosage curve became parabola. The pH and environmentally sensitive metal residuals in the algal solution were not significantly affected by the adding of optimized dosage of CAFL-chitosan. The study provides a possible way for HABs control using the cheap material of CFA. Further studies are needed to check the potential influence of leachable metals and persistent organic pollutants (pops) in CFA under a wide range of environmental condition.

Key words

Harmful algal blooms; *Microcystis Aeruginosa*; Flocculation; Chitosan; Coal fly ash.

1. Introduction

Harmful algal blooms (HABs) and lake eutrophication have been intensively studied due to their threats to aquatic organisms, human health, costal aesthetics and aquacultures (Gan et al. 2010, Thornton et al. 2013). Many approaches have been tested to control the nutrient fluxes to the receiving water bodies including internal and external loading management (Huser 2012, Sondergaard et al. 2002, Spears et al. 2013). However, in cases where nutrient management is not economically feasible or the results obtained are unsatisfactory, additional strategies are needed to reinforce the recovery such as algae harvesting (Chen et al. 2012), filtrations (Yadidia et al. 1977), fish stocking (Jeppesen et al. 2012) and algicides (Garcia-Villada et al. 2004). Aluminum and iron (Al/Fe) salts are widely used as geo-engineering materials for P-sorption in eutrophic water. In addition, the aluminum and iron salts can be used as flocculant because their hydrolysis products can overcome the electrostatic stabilization of algal cells and promote flocs formation (Gonzalez-Torres et al. 2014). Effective precipitation is generally obtained by Al/Fe salts when a ballast is included (Pan et al. 2011a). Flocculation can be a welcome techniques combined with the nutrient control methods for eutrophication restoration, which can improve the water clarity and trigger submerged macrophyte restoration in shallow waters (Pei et al. 2014, Sun et al. 2013). However, the possible accumulation of Al in aquatic food chain may pose risks to human health such as Alzheimer’s disease (Kawahara and Kato-Negishi 2011).

In recent years, efforts have been made on utilization of natural polymers as flocculants such as chitosan (Li and Pan 2013, Pan et al. 2011a, Zou et al., 2006) which may be biodegradable and less accumulated in aquatic food chain (Wang et al. 2015). Chitosan enhances HABs removal for local soil materials via charge neutralization and bridging-netting effect (Li et al. 2015, Zou et al. 2006), however, the algal removal rate may decline due to the folding of chitosan molecular chain in high ionic strength and alkalinity environment (Pan et al. 2011a).

Commercial inorganic flocculants have been tested to improve the flocculation efficiency of chitosan. Chitosan combined with poly aluminum chloride (PAC) can turn local soils into effective flocculants. Over 90% of algal cells were removed using 10 mg/L PAC and 10 mg/L chitosan (Pan et al. 2011a). The PAC facilitates formation of small flocs which are linked by chitosan into flocs 40% larger than using PAC alone (Pan et al. 2011a). Coal fly ash (CFA) contains 25-30% Al_2O_3 and 6-15% Fe_2O_3 (Ahmaruzzaman 2010), which may potentially be a raw material for flocculation. Several studies report that CFA based flocculants prepared from acid or alkaline leachate of CFA are effective alternatives to commercial inorganic flocculants for water purification (Fan et al. 2005, Yan et al. 2012). The flocculants derived from CFA may have the potential to enhance the flocculation ability of chitosan. Besides, CFA is a fine textured material and easily accessible in many cities which can potentially accelerate flocs sedimentation by adding frame and weight to the flocs. So far, few studies are seen on HABs removal using CFA and little is known on the effects of using chitosan and Al/Fe in CFA on algae flocculation.

In this study, hydrochloric acid was used to extract Al/Fe in CFA. Chitosan was modified by the leachate of CFA (CFAL) to prepare a compound flocculant (CFAL-Chitosan) for M.A. flocculation. It is hypothesized that the Al and Fe in CFAL can interacted with chitosan and form a compound flocculant which may enhance the algal removal ability of chitosan. We evaluated the flocculation efficiency of the compound flocculant via dosage effect on removal efficiency, surface charge, floc size and stability. The FT-IR and molecular weight analysis were conducted to elucidate the flocculation mechanisms. The objective of the study is to find a new method for HABs control using chitosan and ways for CFA recycling.

2. Materials and methods

2.1 Algal species and culture

The *Microcystis aeruginosa* cell (M.A., FACHB-469) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology (FACHB) Chinese Academy of Sciences, and cultured in BG11 medium under controlled conditions. Before autoclaving, the BG11 growth medium was adjusted to pH 8.0 using 0.5 mol/L NaOH or 0.5 mol/L HCl. The algae batch culture with initial density of 1.23×10^8 cells/L was held in a 10 L glass vessel and kept at 25 ± 1℃ under 2000-3000 lx of white fluorescent light on a 12 h light and 12 h darkness regime in an illuminating incubator (LRH-250-G, Guangdong Medical Appratus Co.Ltd., China). Continuous aeration was supplied during the algae growth phase. The *M. aeruginosa* cells under this condition were dispersed single cells (Li and Pan 2013).

2.2 CFA and CFAL-Chitosan

CFA was collected in a power plant in Datong City (Shanxi province, China). The CFA was washed with deionized water three times, dried at 105℃, then sieved through 180 mesh before use (<90μm, pre-treated CFA). The pre-treated CFA was characterized by the X-ray fluorescence (XRF-1800, Shimadzu, Japan) and X-ray

Diffraction (X'Pert Pro MPD X-ray Diffractometer, Philips, Netherlands). The Toxicity Characterization Leaching Procedures (TCLP, see Supplementary materials) were carried out to determine the metal mobility of pre-treated CFA (USEPA 1994). Leachates from three different extraction fluids (pH 2.88, 4.93 and 7.50) were analyzed according to Inductively Coupled Plasma Emission Spectrometry (ICP-OES; Optima 8300, PerkinElmer, USA).

Pre-treated CFA was used in two ways in this study. The 100 mg/L of pre-treated CFA was utilized directly in the flocculation experiments and acted as ballast to assist sedimentation processes. Besides, the leachate of pre-treated CFA (CFAL) was obtained using hydrochloric acid and used for chitosan modification. The leaching protocol was optimized through a preliminary test and set as 0.55 mol/L of hydrochloric acid, solid/liquid ratio of 1 g:5 mL, leaching time of 24 h under 25℃ at agitation rate of 180 rpm in an oscillation incubator (HZQ-F160, HDL Electronic Technology Development Co., LTD, China). The CFAL was separated from the insoluble particles by 0.45 μm filter membrane. The metal concentrations in the CFAL were measured by ICP-OES (Optima 8300, PerkinElmer, USA).

The chitosan powder was purchased from Qingdao Yunzhou Biochemistry CO.,LTD which originates from crab shells. Four CFAL-Chitosan stock solutions were prepared as algae flocculants, denoted as F-0, F-12, F-20 and F-40. The F-0 was prepared by adding 0.5 g chitosan in 100 mL of 0.09 M acetic acid. Different volumes of CFAL (6, 10 and 20 mL) were diluted to 100 mL and 0.5 g chitosan was added to the dilutions described above to prepare F-12, F-20 and F-40, respectively. The CFAL/Chitosan ratio for F-0, F-12, F-20 and F-40 was 0 mL:1 g, 12 mL:1 g, 20 mL:1 g and 40 mL:1 g, respectively. The CFAL-Chitosan stock solutions were freshly made and diluted ten times before use.

2.3 Molecular weight and component analysis

The molecular weight (M_v) of CFAL-Chitosan was obtained from the intrinsic viscosity using Mark-Houwink-Sakurada equation reported before (Wang et al. 1991). The intrinsic viscosity was determined using 0.2 M acetic acid/0.1 M sodium acetate with Ubbelohde viscometer (Supplementary Materials, Intrinsic viscosity). The viscosity of CFAL-Chitosan stock solution was quantified by rotational viscometer (NDJ-1, Shanghai Yueping Scientific Instrument co., LTD, China).

The CFAL-Chitosan were dried and mixed with KBr in ratio of 1 mg: 100 mg for FT-IR test (Nicolet 8700, Thermo Fisher, USA). The total Al and Fe in the CFAL-Chitosan (F-12, F-20, and F-40) were measured by ICP-OES (Optima 8300, PerkinElmer, USA). The Al bonded with chitosan (chitosan-Al) was separated by Al fraction procedure (Vanbenschoten and Edzwald, 1990) and quantified by ICP-OES (Optima 8300, PerkinElmer, USA). The free Fe was measured by polarograph (797 VA Computrace, Metrohm, Switzerland) and the Fe bonded with chitosan (chitosan-Fe) was calculated as the subtraction of free Fe from the total Fe.

2.4 Algae flocculation

Flocculation experiments were set up in a jar test apparatus (ZR3-6, Zhongrun Water Industry Technology Development Co., Ltd., China). Algal cells in the mid- to late-exponential growth phase (Chen et al. 2004) were used and the cell concentration was 4.15-4.23×10^9 cells/L in the flocculation experiments. The algal solution was adjusted to pH 8.0 either by 0.5 mol/L NaOH or HCl before flocculation and 200 mL of algal solution was transferred to 300 mL beaker for flocculation. In all flocculation experiments, pre-treated CFA of 100 mg/L was added to the algal solution to assist floc sedimentation. CFAL-Chitosan of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0 and 6.0 mg/L (in terms of chitosan concentration) were added and the control was conducted without adding any flocculants. The stirring process was 200 rpm for 1 min, 120 rpm for 2 min, 40 rpm for 10 min. Samples (2 mL) from 2 cm below water surface were collected after sedimentation for 0, 2, 5, 10, 20, 30, 40, 50 and 60 min for cell counting. The removal rate was calculated as (initial cell concentration−sample cell concentration)/initial cell concentration ×100%. The cells were firstly fixed with Lugol solution (1% final conc.) and enumerated using a hemocytometer under microscope (Axioskop 2 mot plus, Carl ZEISS, Germany). The zeta potential was measured by Zetasizer 2000 (Malvern Co. UK). The floc growth during the flocculation process was monitored by a laser particle size analyzer (Mastersizer 2000, Malvern Co. UK). Samples were sent into the analyzer and back to the jar by a peristaltic pump (BT00-300M, Baoding Longer Percision Pump Co. Ltd., China) with a flow rate of 35 mL/min. The metal residuals including Al, As, Cr, Cd, Ba and Mn after flocculation were quantified with ICP-OES (Optima 8300, PerkinElmer, USA). The pH values were recorded before and after flocculation. The flocculation tests were operated in triplicate and the results were presented as mean values.

2.5 Floc stability

Different shear force was applied to the flocs following the slow stirring process by increasing the stirring speed to 75, 100, 150, 200 and 250 rpm for another 20 min. The corresponding velocity gradient (G) values were 28.1, 41.3, 71.3, 105.0 and 141.7 s^{-1}, respectively. The dynamic flocs size was recorded as $d_{0.5}$ during the stirring process. Referring to the empirical equation (Shi et al. 2015), the broken floc size was plotted against the average velocity gradient in a log-log scale and the slope of the curve (γ) is the main factor to quantify floc stability.

$$\log d = \log C - \gamma \log G$$

where d is the median floc diameter ($d_{0.5}$) after breakage, μm; C is the floc strength co-efficient; γ is the stable floc exponent and G is the average velocity gradient, s^{-1}.

3. Results

3.1 Characteristics of CFA and CFAL-Chitosan

The pre-treated CFA used in this study mainly consisted of SiO_2, Al_2O_3, and Fe_2O_3 (Table S1). The XRD showed the presence of quartz (SiO_2), mullite ($3Al_2O_3{\cdot}2SiO_2$), hematite (Fe_2O_3) and corundum (Al_2O_3) in pre-treated CFA (Fig.S1). The metal ions leached from the pre-treated CFA were more evident under acid conditions (pH=2.88) but less concerned when pH was 7.5 (Table 1). The total Al and Fe in CFAL-Chitosan increased with the increeasing ratio of CFAL/Chitosan and the chitosan-Al and -Fe were detected in CFAL-Chitosan (Fig.1).

Table 1 is here.

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The molecular weight (M_v) of CFAL-Chitosan was calculated from the intrinsic viscosity. The M_v of chitosan without CFAL (CFAL/Chitosan 0:1) was 682 kDa and similar to that of chitosan powder. Both M_v and viscosity of CFAL-Chitosan decreased as CFAL/Chitosan ratio increased. When CFAL/Chitosan ratio was 40:1, the M_v and viscosity decreased 21.3% and 63.5% respectively compared to chitosan without CFAL.

Fig.2 is here.

The chitosan powder and chitosan without CFAL (CFAL/Chitosan 0:1) exhibited similar FT-IR spectra (Fig.3). A broad adsorption band around 3417 cm^{-1} corresponded to the overlap of OH and NH_2 stretching vibration and peak at 2900 cm^{-1} was attributed to the stretching of CH (Ng et al. 2012). Band around 1650 cm^{-1} referred to the amide I group, and peak at 1596 and 1561 cm^{-1} was the band of amide II (Ng et al. 2012). The aliphatic OH band, acetal and glycosidic linkage were associated with peaks at 1423, 1154-1030 and 898 cm^{-1}, respectively (Ng et al. 2012, Wang et al. 2011). The spectrum of chitosan with CFAL (F-12, F-20, and F-40 in Fig.3) showed different characteristics from chitosan without CFAL. Band at 3417 cm^{-1} and amide I group shifted to lower wavenumber. The band of amide II and aliphatic OH extinguished, however, a new band emerged around 1500 cm^{-1}.

Fig.3 is here.

3.2 Dosage effect of CFAL−Chitosan

For chitosan without CFAL, the M.A. removal reached to the peak of 81.6±1.9% at 3 mg/L then decreased significantly when chitosan dosage exceeded 3 mg/L (F-0 in Fig.4). When the CFAL/Chitosan ratio was increased to 12:1, the maximum removal rate plateaued at 98.2±1.5% at 3 mg/L and remained stable until the dosage increased to 6 mg/L (F-12 in Fig.4). Removal rate of 95.0±1.5% was found at 3.5 mg/L for CFAL/Chitosan ratio of 20:1 (F-20 in Fig.4). When the CFAL/Chitosan ratio further increasing to 40:1 (F-40), the algae removal reached to the peak of 76.5±2.8% at 2 mg/L, which was quickly reduced beyond the optimal dosage of 2 mg/L (F-40 in Fig.4). The zeta potential of M.A. flocs increased as CFAL-Chitosan was added to the algal solution. For F-0 and F-40, the charge of M.A. flocs reversed at 5 mg/L and 3.5 mg/L, respectively. While the charge reversals were not observed for both F-12 and F-20 bellow the dosage of 6 mg/L. According to the dosage-efficiency curves, the CFAL-Chitosan dosage was set as 3 mg/L for the floc growth, flocculation kinetic and floc stability experiments.

Fig.4 is here.

3.3 Floc growth and flocculation kinetics

Using pre-treated CFA up to 100 mg/L did not promote M.A. aggregation and the removal efficiency was nearly zero (Fig.5 & 6). For chitosan without CFAL, the growth of flocs plateaued at 12 minutes with floc size of approx. 560 μm (F-0 in Fig.5). After sedimentation for 5 min, the removal rate of F-0 reached 79.7% and kept a stable trend as time increased (F-0 in Fig.6). When CFAL/Chitosan ratio increased to 12:1 and 20:1, the floc size increased to 750 μm (F-12 & F-20 in Fig.5), but F-12 exhibited a faster growth rate. The removal efficiency of F-12 reached 97.2% within 2 min and remained stable, while 87.5% of algal cells were removed for F-20 after sedimentation for 60 min (Fig.6). When CFAL/Chitosan ratio increased to 40:1, the floc size (380 μm) decreased compared to F-0, F-12 and F-20 and a lower removal rate of 72.8% was achieved at 60 min (Fig.5 & 6).

Fig.5 is here.

Fig.6 is here.

3.4 Floc stability

The stability of algae flocs at 3 mg/L CFAL-Chitosan was tested by measuring the floc size changes after applying a shear force (Shi et al. 2015). The stable floc exponent (γ) is a quantitative measurement of floc stability. When CFAL/Chitosan ratio was 12, the γ of flocs was 0.39, lower than chitosan without CFAL (0.49) indicating that the floc stability was improved (Fig.7). However, when excessive CFAL was added (F40), the floc stability decreased compared to CFAL-Chitosan (F-12) (Fig.7).

Fig.7 is here.

4. Discussion

4.1 The M.A. removal by chitosan without CFAL

The zeta potential of M.A. flocs was -34.8 mv when pre-treated CFA alone (100 mg/L) was added and the algal cells were not removed due to the electrostatic repulsion (Fig.4 & 6). When chitosan without CFAL (CFAL/Chitosan ratio =0:1) was added at 3 mg/L, the removal rate reached to the peak of 81.4±1.9% and the zeta potential increased from -34.8 to -15.4 mv, indicating the electrostatic repulsion was reduced, which may due to the attraction between amine groups of chitosan and algal cells (F-0 in Fig.4). Chitosan is a linear biopolymer with high molecular weight (682 kDa, Fig.2) and has a long polymer chain structure (Li et al. 2013). The flocs of large size were formed (560 μm) through electrostatic attraction and bridging-netting function by the long polymer chain of chitosan (F-0 in Fig.5). However, 18.6% of algae cells were not removed since the algae flocs were not sufficiently neutralized with zeta potential far below zero (-15.4 mv) at the optimized dosage of chitosan without CFAL (3 mg/L) (Li et al. 2015). Besides, the M.A. flocculation was not stable and declined significantly at 5 mg/L due to the reversal of the zeta potential (+3.4 mv) and re-stabalization of algal flocs (F-0 in Fig.4).

4.2 The M.A. removal by chitosan with CFAL

Although CFAL alone was not effective in M.A. removal (Table S2), it enhanced M.A. flocculation of chitosan (F-12, F-20, Fig.4). The removal rate of F-12 and F-20 reached over 95% at 3 and 3.5 mg/L, respectively and was higher than chitosan without CFAL (Fig.4). Moreover, the floc of F-12 were 34% larger and more stable and sunk faster together with a ballast than chitosan without CFAL (Fig.5, 6 & 7). When the flocculant dosage was beyond the optimized dosage, the zeta potential of algal flocs using chitosan with CFAL (F-12, F-20) stayed near zero and the algal removal efficiency plateaued over 90%. While for chitosan without CFAL (F-0), the removal efficiency peaked at 3 mg/L with a lower removal efficiency than CFAL-Chitosan (F-12, F-20) and significantly decreased due to the fast reversal of zeta potential at higher dosage (5~6 mg/L, Fig.4). This indicated that CFAL-Chitosan can maintain a better algal removal rate over wide dosage range due to improved property on charge neutralization. The component analysis confirmed the formation of chitosan-Al and -Fe in the prepared flocculants (Fig.1). Compared with the FT-IR spectrum of chitosan without CFAL (CFAL/Chitosan ratio=0:1), the amide II and aliphatic OH groups disappeared when chitosan was modified by CFAL (F-12, F-20, F-40 in Fig.3). A distinct band emerged at 1500 cm^{-1} which could potentially be the characteristic of Al-NH_2 or Fe-NH_2 (Himmel et al. 2000, Wang et al. 2011). It indicated that the OH and NH_2 of chitosan might chelate with Al and Fe in CFAL.

The free Al/Fe in CFAL-Chitosan may also contribute to enhancing the charge neutralization of chitosan and it requires further studies to explore the functions of chitosan-Al and -Fe.

For F-12, when the dosage was higher than 3 mg/L, the electrostatic repulsion between M.A. flocs kept low and the M.A. removal remained over 90% (Fig.4). However, when the CFAL/Chitosan ratio increased to 40:1, sharp decline of algal removal occurred again at 3.5 mg/L due to reversed charge (+2.6 mv). This indicated that excessively increasing the CFAL/Chitosan ratio may result in faster reversal of algal charge and narrow the dosage range for good algal removal. The flocs formed at F-40 was less stable with higher γ value under the conditions tested compared to F-12. Effective M.A. flocculation was generally obtained at the dosage where the zeta potential of algal flocs was near zero. In this study, moderate amount of CFAL (CFAL/Chitosan ratio 12:1) optimized the charge neutralization of chitosan and a wide dosage range for effective M.A. removal was obtained.

The long chain structure of chitosan is largely responsible for the bridging-netting property which is positively related to the molecular weight (Li et al. 2013). When chitosan was modified by CFAL, the molecular weight (M_v) of CFAL-Chitosan decreased (Fig.2), indicating that the long chain structure of chitosan was adversely influenced and the bridging-netting ability was weakened by the over dosed CFAL. The hydrochloride acid in CFAL may trigger the hydrolysis of chitosan molecules (Vårum et al. 2001). The floc size and stability of chitosan modified by CFAL decreased and flocs sedimentation became slower under CFAL/Chitosan ratio of 40:1, which supported the weakening of bridging-netting effect (Fig.5 & 6).

4.3 Flocculation materials and methodology

Previous studies have revealed that particles with the right size can enhance the collision frequency and add frame to the flocs to accelerate sedimentation (Chen and Pan 2012, Li and Pan 2013, Pan et al. 2006, Park et al. 2013). In this study, when using CFAL-Chitosan (F-12) at 3 mg/L without pre-treated CFA, the removal efficiency of algal cells was 89.6±0.6%. This was about 8% lower than 3 mg/L CFAL-Chitosan (F-12) with 100 mg/L of pre-treated CFA. Pre-treated CFA, an alternative ballast material to local soil, facilitated algal removal when used with CFAL-Chitosan (F-12 in Fig.4). As a solid waste, the ecological safety of CFA including CFA particles and CFAL is the prerequisite for its application in natural waters. Since the heavy metal ions such as Mn and Ba (Table S3) were detected in CFAL, the dosage of CFAL used in chitosan modification should be carefully optimized which was closely related to the amount of heavy metals ions introduced to the algal solution. Although the metal mobility in pre-treated CFA under alkaline conditions was low (pH=7.50) and within the allowable limits of USEPA standard for hazardous materials (1994), it may be a concern under acid conditions (Table 1). CFA may also contain persistent organic pollutants such as PAH and dioxin. The availability of these pollutants in CFA under wide environmental conditions needs further investigation. Moreover, CFA composition varies from coal types and combustion processes. CFA screening is essential before it can be used for HAB control.

The pH and metal residuals in algal solution before and after flocculation were not significantly influenced (Table S4) at the conditions tested here. Hydrochloric acid is a frequently used extracting agent to prepare CFA based flocculants (Choo et al. 2014, Yan et al. 2012). In this study, hydrochloric acid can extract Al/Fe in CFA which improve the charge neutralization for chitosan. However, concentrated hydrochloric acid can result in the hydrolysis and decrease of molecular weight of chitosan, which inhibits the bridging-netting ability (Fig.2). For CFAL/Chitosan ratio of 40:1, the M.A. removal was 73.6±3.6% at 3 mg/L although the M.A. cells were neutralized with zeta potential near zero (-2.8 mv, Fig.4). During the preparation of CFAL-Chitosan, CFAL was diluted suggesting that the acid concentration used for CFA leaching can be reduced in practical application to alleviate the negative impacts on chitosan structure. There was a balance between the charge neutralization enhancement and structural influence of chitosan when modified by CFAL. It is likely that the M.A. removal can be potentially improved by screening mild extracting agents which not only extract Al/Fe but also maintain the chitosan structure.

4.4 Environmental implications

In the past decades, efforts have been made to reduce the external loading via improving environmental standards such as wastewater treatment and agriculture, and internal loading such as adding P-sorption materials and sediment dredging

(Drabkova and Marsalek 2007). However, many additional physical, chemical and biological methods have been developed to reinforce recovery when obtained results are unsatisfactory. Flocculation can quickly remove the suspended algal cells down to the sediments and improve water transparency which provides favorable conditions for photosynthesis and/or submerged macrophytes restoration in shallow waters (Bakker et al. 2013). The usage of CFAL-Chitosan as algal flocculant may have positive side-effects such as killing the settled algal cells since the breakdown products of chitosan are suspected to have antibacterial activities(Wisniewska-Wrona et al 2007) but the latter requires further studies. In addition, using pre-treated CFA as alternative ballast to replace local soil has several advantages in some cases. Firstly, CFA is produced in large quantity and convenient to access for places with thermal power plant. While local soils may be not easily available especially in developed urban areas (prohibited by urban planning/regulations). Secondly, the CFA is a fine textured material which easily collides with algal cells (Han & Kim 2001). The pre-treatment of CFA described in this study such as washing, drying and sieving may not be needed in practical application and CFA may be used directly without processing after careful check of heavy metal and persistent organic pollutants. While the handling cost of local soils could be substantial when using labor for digging, grinding, sieving, and washing. Thirdly, CFA is a solid waste of low value and the cost of CFA disposal may be a burden for the producing factories. While local soils are important resources for urban planning, landscape conservation and agriculture. In cases where CFA is available and local soil is prohibited to be collected at large scale, CFAL-chitosan method may provide a possibility to utilize CFA for HAB control.

Controlled lab stirring condition is essential for repeating and revealing the mechanisms of algal flocculation. However, in the field, the flocculation behavior could be influenced by many factors such as the type of algae (single or colonial cells), pH, salinity, vertical and horizontal mixing of water etc. Preliminary jar tests are required before field application. Moreover, the flocs were prone to break under turbulent conditions (Fig.S2) and this can be a problem in shallow lakes where wind-oriented turbulence is inevitable. The degradation of algae may damage the cell membrane integrity which might stimulate the release of microcystins and consume dissolved oxygen. In addition, accumulation of algal flocs on lake sediments could influence the redox condition of the sediment and thereby influence pollutant fluxes from sediment to overlying water such as nutrients fluxes. It was reported that capping materials may be helpful in solving these problems (Pan et al. 2012). The microorganism modified capping materials could be effective for decomposing microcystins released from the broken *M. aeruginosa* (Li and Pan 2015). Capping materials loaded with oxygen nanobubbles may improve the hypoxia condition near the sediment and alleviate pollutants released from sediments (Pan and Yang 2012). In some cases, it is possible to utilize the settled flocs as fertilizer for the restoration of submerged macrophytes (Pan et al. 2012, Pan et al. 2011b). The control of adverse effects after algal flocculation is a very complex issue and the possibility to manipulate them using geo-engineering methods needs further studies.

The use of non-biodegradable chemicals such as $FeCl_3$ and PAC, or alum may pose risks to human health such as Alzheimer's disease through bio-accumulation (Kawahara and Kato-Negishi 2011). In this study, at the optimized dosage of CFAL-Chitosan, the calculated Al dosage was 0.02 mg/L (F-12, 3 mg/L) and significantly lower compared to the effective dosage reported in other studies (Gonzalez-Torres et al. 2014, Paul et al. 2008). Introducing small amount of CFAL can improve the flocculation efficiency of chitosan and CFAL-Chitosan to some extent decreased the use of bulk chemicals. Table S5 estimated the cost of several methods for HABs control. To achieve removal rate over 90%, the cost of CFAL-Chitosan is 0.07 US$/m^3, which is lower than the PAC-Chitosan (0.23 US$/m^3) and *Moringa oleifera*-Chitosan (MO-Chitosan, 5.19 US$/m^3) (Li and Pan 2013, Pan et al. 2011a). In further studies, it is possible to reduce the cost by screening cheap biopolymers as chitosan alternatives such as cationic starch and larch tannin (Shi et al. 2015, Wang et al. 2013).

5. Conclusion

In this study, we developed a compound flocculant using coal fly ash leachate (CFAL) modified chitosan for *Microcystis aeruginosa* (M.A.) flocculation. It was found that the CFAL enhanced flocculation ability of chitosan for M.A. removal at CFAL/Chitosan ratio of 12:1 and good algal removal rate remained in a wide dosage range due to the improvement of charge neutralization property. The algal flocs of

CFAL-Chitosan were larger and denser than chitosan without CFAL. However, when CFAL/Chitosan ratio was increased beyond the optimal, surplus of CFAL inhibited the M.A. removal due to the hydrolysis and declining of molecular weight of chitosan which impaired the bridging-netting property. New mild extracting methods should be studied in the future which not only extract Al/Fe in CFA but also maintain the chitosan structure at the same time. CFA combined with CFAL-Chitosan can be a possible economical way for HABs mitigation owing to its easy availability and pretreatment processes. Further studies are needed to check the potential influence of leachable metals and persistent organic pollutants (pops) in CFA under a wide range of environmental condition.

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FIGURE CAPTIONS

Fig.1-The Al and Fe in CFAL-Chitosan, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

Fig.2-The molecular weight (kDa) and viscosity (cps) of chitosan powder and CFAL-Chitosan, F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

Fig.3-The FT-IR spectra of chitosan powder and CFAL-Chitosan, a: F-12 CFAL/Chitosan 12:1, b: F-20 CFAL/Chitosan 20:1, c: F-40 CFAL/Chitosan 40:1, d: F-0 CFAL/Chitosan 0:1, e: chitosan powder.

Fig.4-Algal removal efficiency and zeta potential of M.A. flocs as function of CFAL-Chitosan dosage. F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1, initial pH 8.0, pre-treated CFA concentration 100 mg/L.

Fig.5-The dynamic floc size of M.A. cells after addition of 3 mg/L CFAL-Chitosan, initial pH 8.0, pre-treated CFA concentration 100 mg/L, F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

Fig.6-The flocculation kinetics of M.A. cells after addition of 3 mg/L CFAL-Chitosan, initial pH 8.0, and pre-treated CFA concentration 100 mg/L, F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.

Fig.7-Floc stability plots of CFAL-Chitosan at 3 mg/L (pre-treated CFA dosage, 100 mg/L, initial pH=8.0, Shear time, 16 min). F-0: CFAL/Chitosan ratio 0:1, F-12: CFAL/Chitosan ratio 12:1, F-20: CFAL/Chitosan ratio 20:1, F-40: CFAL/Chitosan ratio 40:1.