Flocculation of cyanobacterial cells using coal fly ash modified chitosan

Yuting Yuan^a, Honggang Zhang^a, Gang Pan^{a, b, *}

^a Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China

^b School of Animal, Rural and Environmental Sciences, Nottingham Trent University, NG25 0QF, UK

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ABSTRACT

Harmful algal blooms (HABs) have increasingly occurred worldwide and 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 algal removal efficiency plateaued over 90% in a wide dosage range from 3 to 6 mg L⁻¹. For chitosan without CFAL, removal efficiency peaked at 3 mg L⁻¹ with a maximum removal efficiency of 81%, which quickly decreased as the dosage increased (>3 mg L⁻¹) due to the fast reversal of zeta potential. This indicated that CFAL-Chitosan could maintain a better removal efficiency over a wide dosage range as a result of improved charge neutralization compared with the chitosan only treatment. The flocs of CFAL-Chitosan were larger and denser than produced in the presence of chitosan without CFAL. However, excessive CFAL beyond the optimized dose inhibited M.A. removal due to hydrolysis and declining molecular weight of chitosan that weakened the bridging–netting properties, where surface charge reversal happened within a narrow dosage range and the removal–dosage curve became parabolic. The pH and metal residuals that were assumed to pose a threat to the aquatic environment were not significantly affected by adding optimized dosage of CFAL-Chitosan. The study provides a HABs control method using a cheap material of CFA. Further studies are needed to check the potential influence of leachable metals and persistent organic pollutants in CFA under a wide range of environmental condition.

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1. Introduction

Harmful algal blooms (HABs) and lake eutrophication have been intensively studied due to their threats to aquatic organisms, human health, coastal aesthetics and aquaculture (Gan et al., 2010; Thornton et al., 2013). Many approaches have been tested to control the nutrient fluxes to the receiving waterbodies including internal and external loading management (Huser, 2012; Sondergaard et al., 2002; Spears et al., 2013a). 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 (Al) and iron (Fe) salts are widely used as geo-engineering materials

for P-sorption in eutrophic water. In addition, the Al and Fe salts can be used as a 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 and Fe salts when a ballast is included (Pan et al., 2011a). Flocculation can be a welcome technique combined with nutrient control methods for eutrophication management, which can improve the water clarity and trigger submerged macrophyte restoration in shallow waters (Pei et al., 2014; Sun et al., 2013).

In recent years, efforts have been made to utilize natural polymers as flocculants such as chitosan (Li and Pan, 2015; 2013; Pan et al., 2011a, 2012, 2011b; Zou et al., 2006) which may be biodegradable and therefore represent a lower risk to human and environmental health (Wang et al., 2016). Chitosan can be used to modify the properties of local soils with respect to HABs removal through modification of charge neutralization and bridging–netting processes (Li et al., 2015; Zou et al., 2006). However, the

* Corresponding author. Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, 100085, China.

E-mail address: gpan@rcees.ac.cn (G. Pan).

effectiveness of the modification can be confounded by various chemical components of the receiving waters, for example, 'folding' of the chitosan molecular chain in waters of high ionic strength and alkalinity (Pan et al., 2011a; Zou et al., 2005).

Commercial inorganic flocculants have been tested to assist the flocculation process of chitosan. Chitosan combined with poly aluminum chloride (PAC) can turn local soils into effective flocculants. Pan et al. (2011a) demonstrated this approach for the removal of cyanobacteria from suspensions using 10 mg L⁻¹ PAC and 10 mg L⁻¹ chitosan. 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% Al₂O₃ and 6–15% Fe₂O₃ (Ahmaruzzaman, 2010), which may potentially enhance the flocculation properties of chitosan. 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). Besides, CFA is a fine textured material and relatively widely available at the global scale which can potentially accelerate flocs sedimentation by adding frame and weight to the flocs. So far, few studies have assessed HABs removal using CFA and little is known about the effects of using chitosan and CFA on the flocculation of cyanobacteria.

In this study, HCl was used to extract Al and Fe from CFA. Chitosan was modified using CFA leachate (CFAL) to prepare a compound flocculant (CFAL-Chitosan) for M.A. flocculation. It is hypothesized that Al and Fe in CFAL can interact 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 of algal flocs, floc size and stability. Fourier Transformed Infrared Spectroscopy (FT-IR) and molecular weight analysis were conducted to elucidate the flocculation mechanisms.

2. Materials and methods

2.1. Algal species and culture

Microcystis aeruginosa (M.A., FACHB-469) was obtained from the Freshwater Algae Culture Collection at the Institute of Hydrobiology Chinese Academy of Sciences (FACHB, Wuhan City, China), and cultured in BG11 medium under controlled conditions. Before autoclaving, the BG11 growth medium (Wyatt et al., 2012) was adjusted to pH 8.0 using 0.5 mol L⁻¹ NaOH or 0.5 mol L⁻¹ HCl. The algae batch culture with initial density of 1.23×10^5 cells mL⁻¹ was held in a 10 L glass vessel and kept at 25 ± 1 °C under 40–60 μmol m⁻² s⁻¹ of white fluorescent light on a 12 h light and 12 h dark regime in an illuminating incubator (LRH-250-G, Guangdong Medical Apparatus Co. Ltd., China). Continuous aeration was supplied during the algae growth phase. *M. aeruginosa* cells under this condition were dispersed single cells (Li and Pan, 2013).

2.2. CFA and CFAL-Chitosan

CFA was collected from a power plant in Datong City (Shanxi province, China). The CFA was washed with deionized water three times, dried at 105 °C, then sieved through 180 mesh before use (<90 μm, pre-treated CFA). The pre-treated CFA was characterized by X-ray fluorescence (XRF-1800, Shimadzu, Japan) and X-ray Diffraction (X'Pert Pro MPD X-ray Diffractometer, Philips, Netherlands). Toxicity Characterization Leaching Procedures (TCLP, see Supplementary Materials) were carried out to determine the metal mobility of pre-treated CFA (USEPA, 1994).

Pre-treated CFA was used in two ways in this study. Firstly, 100 mg L⁻¹ of pre-treated CFA was utilized directly in the

flocculation experiments and acted as ballast to assist sedimentation processes. Secondly, the leachate of pre-treated CFA (CFAL) was obtained using HCl and used for chitosan modification. The leaching protocol was optimized through a preliminary test and set as 2% HCl (in mass fraction), solid/liquid ratio of 1 g: 5 mL, leaching time of 24 h under 25 °C at an agitation rate of 180 rpm in an oscillation incubator (HZQ-F160, HDL Electronic Technology Development Co., LTD, China). The CFAL was separated by filtration through a 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, denoted as F-0, F-12, F-20 and F-40 and the treatments of CFAL-Chitosan stock solutions were shown in Table 1. 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 an Ubbelohde viscometer (Supplementary Materials, Intrinsic viscosity). Viscosity of the CFAL-Chitosan stock solutions was quantified using a rotational viscometer (NDJ-1, Shanghai Yueping Scientific Instrument co., LTD, China).

CFAL-Chitosan solutions were dried and mixed with KBr in a ratio of 1 mg: 100 mg for FT-IR analysis (Nicolet 8700, Thermo Fisher, USA). The total Al and Fe in CFAL-Chitosan solutions (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 using ICP-OES (Optima 8300, PerkinElmer, USA). The free Fe was measured using polarograph (797 VA Computrace, Metrohm, Switzerland) and the Fe bonded with chitosan (chitosan-Fe) was calculated as the difference between free Fe and 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 $4.15\text{--}4.23 \times 10^6$ cells mL⁻¹ at the start the flocculation experiments. The algal solution was adjusted to pH 8.0 either by 0.5 mol L⁻¹ NaOH or HCl preceding the flocculation experiments and 200 mL of algal solution was transferred to a 300 mL beaker for flocculation. In the flocculation experiments, pre-treated CFA of 100 mg L⁻¹ and different dosage of CFAL-Chitosan (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 5.0 and 6.0 mg L⁻¹, in terms of chitosan concentration) were added to the algal solutions. The control was run by adding 100 mg L⁻¹ of pre-treated CFA only. The stirring process was 200 rpm for 1 min, 120 rpm for 2 min, 40 rpm for 10 min then the algal solutions were left static. After sedimentation for desired time intervals (0, 2, 5, 10, 20, 30, 40, 50 and 60 min), samples (2 mL) from 2 cm below the water surface were collected 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 using a microscope (Axioskop 2 mot plus, Carl ZEISS, Germany). The zeta potential was measured using

Table 1
Treatments of CFAL–Chitosan stock solutions.

No.	Chitosan powder (g)	CFAL (mL)	Glacial acetic acid (mL)	Deionized water (mL)	CFAL/Chitosan ratio (mL: g)
F-0	0.5	0	0.5	99.5	0
F-12	0.5	6	0	94	12:1
F-20	0.5	10	0	90	20:1
F-40	0.5	20	0	80	40:1

Zetasizer 2000 (Malvern Co. UK). The metal residuals of samples taken from the flocculated algal solutions after sedimentation for 30 min were quantified with ICP-OES (Optima 8300, PerkinElmer, USA) including Al, As, Cr, Cd, Ba and Mn. The pH values of the algal solutions were also recorded before and after flocculation using a pH meter (PHSJ-4A, Shanghai REX Instrument Factory, China). The floc growth during the stirring 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 Precision Pump Co. Ltd., China) with a flow rate of 35 mL min⁻¹. 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 sizes were recorded as *d*_{0.5} during the stirring process. Referring to the empirical equation (Shi et al., 2016), 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 SiO₂, Al₂O₃, and Fe₂O₃ (Table S1). XRD analysis showed the presence of quartz, mullite, hematite and corundum (Fig. S1). The metal ions leached from the pre-treated CFA were more evident under acid conditions (pH = 2.88) compared with pH 7.5 (Table 2). The total Al

Table 2
The concentration of metal ions leached from the pre-treated CFA in TCLP test (mg L⁻¹).

Metals ^a	Concentration of metal		
	pH = 2.88 ^b	pH = 4.93 ^c	pH = 7.50 ^d
Aluminum	17.6	0.3	0.3
Barium	4.5	2.1	0.2
Iron	0.3	—	—
Magnesium	58.6	35.1	1.0
Manganese	1.54	0.6	—

^a Elements below the detection limit (<0.01 mg L⁻¹): As, Be, Bi, Cd, Co, Cr, Cu, Ni, Sb, Sn, Ti, V, Zn, Zr, Pb.

^b Acetic acid solution.

^c Acetic acid solution.

^d Deionized water.

and Fe in CFAL–Chitosan increased with the increasing ratio of CFAL/Chitosan and the chitosan–Al and –Fe were detected in CFAL–Chitosan (Fig. 1).

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. 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.

For chitosan powder and chitosan without CFAL (CFAL/Chitosan 0:1, Fig. 3), a broad adsorption band around 3417 cm⁻¹ corresponded to the overlap of OH and NH₂ stretching vibration and the peak at 2900 cm⁻¹ was attributed to the stretching of CH (Ng et al., 2012). The band around 1650 cm⁻¹ referred to the amide I group, and the 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. The band at 3417 cm⁻¹ shifted to 3241, 3395 and 3394 cm⁻¹ and the amide I group shifted from 1642 cm⁻¹ to 1625, 1636 and 1635 cm⁻¹ for F-12, F-20 and F-40, respectively. The band of amide II and aliphatic OH extinguished, however, a new band emerged around 1500 cm⁻¹.

3.2. Dosage effect of CFAL–Chitosan

For chitosan without CFAL, the M.A. removal reached a peak of 81.6 ± 1.9% at 3 mg L⁻¹ then decreased significantly when chitosan dosage exceeded 3 mg L⁻¹ (F-0 in Fig. 4). When the CFAL/Chitosan ratio was 12:1, the maximum removal rate plateaued at 98.2 ± 1.5% at 3 mg L⁻¹ and remained stable until the dosage increased to 6 mg L⁻¹ (F-12 in Fig. 4). A rate of 95.0 ± 1.5% was found at 3.5 mg L⁻¹ for CFAL/Chitosan ratio of 20:1 (F-20 in Fig. 4). When the CFAL/Chitosan ratio further increased to 40:1 (F-40), the algae removal reached to a peak of 76.5 ± 2.8% at 2 mg L⁻¹, which was quickly reduced beyond the optimal dosage of 2 mg L⁻¹ (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⁻¹ and 3.5 mg L⁻¹, respectively. While the charge reversals were not observed for both F-12 and F-20 below the dosage of 6 mg L⁻¹. At the optimized dosage for F-0, F-12, F-20 and F-40, the concentrations of remaining cells at the end of the experiments were (77.3 ± 8.0) × 10⁴, (7.5 ± 6.2) × 10⁴, (21.0 ± 6.3) × 10⁴ and (97.8 ± 11.6) × 10⁴ cells mL⁻¹, respectively. According to the dosage–efficiency curves, the CFAL–Chitosan dosage was set as 3 mg L⁻¹ for the floc growth, flocculation kinetic and floc stability experiments.

3.3. Floc growth and flocculation kinetics

Using pre-treated CFA up to 100 mg L⁻¹ did not promote M.A. aggregation and the removal efficiency was zero (Figs. 5 and 6). For chitosan without CFAL, the growth of flocs plateaued at 12 min with floc size of approx. 560 μm (F-0 in Fig. 5). After sedimentation for 5 min, the removal rate reached 79.7% and did not change as the

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.

experiment proceeded for the F-0 treatment (F-0 in Fig. 6). When the CFAL/Chitosan was 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 the CFAL/Chitosan was 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 (Figs. 5 and 6).

3.4. Floc stability

The stability of algal flocs at 3 mg L^{-1} CFAL-Chitosan was tested by measuring the floc size changes after applying a shear force (Shi et al., 2016). The stable floc exponent (γ) is a quantitative measurement of floc stability. When the 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).

4. Discussion

4.1. 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^{-1}) was added and the algal cells were not removed from suspension due to the electrostatic repulsion (Figs. 4 and 6). When chitosan without CFAL (CFAL/Chitosan ratio = 0:1) was added at 3 mg L^{-1} , the removal rate reached a peak of $81.4 \pm 1.9\%$ and the zeta potential increased from -34.8 to -15.4 mV , indicating the electrostatic repulsion was reduced, which may be due to attraction between the 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 algal cells were not removed since the surface charge of algal 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^{-1}) (Li et al., 2015). Besides, M.A. removal was not stable and declined significantly at 5 mg L^{-1} due to reversal of the zeta potential ($+3.4 \text{ mV}$) and re-stabilization of algal flocs (F-0 in Fig. 4).

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^{-1} .

Fig. 5. The dynamic floc size of M.A. cells after addition of 3 mg L^{-1} CFAL-Chitosan, initial pH 8.0, pre-treated CFA concentration 100 mg L^{-1} , 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^{-1} CFAL-Chitosan, initial pH 8.0, and pre-treated CFA concentration 100 mg L^{-1} , 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.

4.2. 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^{-1} , respectively and was higher than chitosan without CFAL (Fig. 4). Moreover, the flocs of F-12 were 34% larger and more stable and sunk faster together with a ballast compared with the chitosan

only treatment at dosage of 3 mg L^{-1} (Figs. 5–7). When the flocculant dosage was beyond the optimized dosage, i.e. 3 and 3.5 mg L^{-1} for F-12 and F-20, respectively, the zeta potential of algal flocs using chitosan with CFAL (F-12, F-20) stayed near zero and algal removal efficiency plateaued over 90% and 95%, respectively. While for chitosan without CFAL (F-0), the removal efficiency was significantly lower compared to CFAL-Chitosan (F-12, F-20) due to

Fig. 7. Floc stability plots of CFAL-Chitosan at 3 mg L⁻¹ (pre-treated CFA dosage, 100 mg L⁻¹, 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.

the fast reversal of zeta potential at dosage beyond 3 mg L⁻¹ (5–6 mg L⁻¹, Fig. 4). This indicated that CFAL-Chitosan can maintain a better algal removal rate over a wide dosage range due to improved 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–NH₂ or Fe–NH₂ (Himmel et al., 2000; Wang et al., 2011). It indicated that the OH and NH₂ 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⁻¹, the electrostatic repulsion between M.A. flocs was low and M.A. removal remained over 90% (Fig. 4). However, when the CFAL/Chitosan ratio increased to 40:1, a sharp decline of algal removal occurred again at 3.5 mg L⁻¹ due to reversed charge (+2.6 mv). This indicated that excessively increasing CFAL/Chitosan ratio may result in faster reversal of algal charge and narrow the dosage range of effective algal removal. Effective M.A. flocculation was generally obtained at the dosage where the zeta potential of algal flocs was near zero. In this study, a 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. At the optimized dosage of F-12, the concentration of cells remaining in the flocculated algal solution was $(7.5 \pm 6.2) \times 10^4$ cells mL⁻¹, which was lower compared to the starting concentration ($4.15\text{--}4.23 \times 10^6$ cells mL⁻¹), but higher than the WHO recommendations (2.0×10^4 cells mL⁻¹) for safe recreational waters (2003). In addition, the pH and metal residuals in algal solution before and after flocculation were unaffected by the test conditions in this study (Table S3).

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 HCl in CFAL may trigger the hydrolysis of chitosan molecules (Vårum et al., 2001). The floc size and flocs sedimentation of CFAL-Chitosan decreased when CFAL/Chitosan ratio was 40:1, which supported a weaker bridging-netting effect (Figs. 5 and 6). HCl is a frequently used extracting agent to prepare CFA based flocculants (Choo et al., 2014; Yan et al., 2012). However, concentrated HCl can result in the hydrolysis and reduction of molecular weight of chitosan, which inhibits the bridging-netting ability (Fig. 2). 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.

4.3. Considerations of environmental risk associated with the approach

Spears et al. (2013b) recommended careful consideration of the environmental risks associated with the use of industrial waste materials for the management of eutrophication symptoms in natural freshwater ecosystems. In this study, both pre-treated CFA and CFAL were utilized. Since the heavy metal ions such as Mn and Ba (Table S4) 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 2). 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 HABs control.

4.4. Scope of the approach for environmental management

In the past decades, efforts to reduce cyanobacteria concentrations in lakes have focused on the reduction of catchment P loading such as wastewater treatment and agriculture, and internal loading such as adding P-sorption materials and sediment dredging (Jeppesen et al., 2005; Spears et al., 2016). However, many additional physical, chemical and biological methods have been developed to reinforce recovery when the 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). Chitosan as an 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 an alternative ballast to replace local soil has several advantages in some cases. Firstly, CFA is produced in large quantities and is convenient to access in industrialized and urban areas. In contrast, local soils may be difficult to source in urban areas or may be prohibited within urban planning legislation. Secondly, the CFA is a fine textured material which easily collides with algal cells (Han and Kim, 2001). We expect the pre-treatment of CFA to be minimal in comparison to other modified materials and the approach may provide added

value in terms of providing a use for a current industrial waste material. However, local planning considerations and environmental risk assessments should be conducted for all materials proposed for use in the environment in this way. Although the controlled lab experiments suggested the possibility of HABs mitigation using CFAL-Chitosan, the control of adverse effects associated with algal flocculation is complex and the use of other agents to mitigate secondary effects should be appropriately assessed.

In this study, at the optimized dosage of CFAL-Chitosan, the calculated Al dosage was 0.02 mg L^{-1} (F-12, 3 mg L^{-1}) and significantly lower compared to the effective dosage reported in other studies (Gonzalez-Torres et al., 2014; Paul et al., 2008). Introducing a small amount of CFAL can improve the flocculation efficiency of chitosan. Table S5 estimated the cost of several methods for HABs control. To achieve a cyanobacteria removal rate of over 90%, the cost of CFAL-Chitosan is $0.07 \text{ US\$m}^{-3}$, which is lower than the PAC-Chitosan ($0.23 \text{ US\$m}^{-3}$) and *Moringa oleifera*-Chitosan (MO-Chitosan, $5.19 \text{ US\$m}^{-3}$) (Li and Pan, 2013; Pan et al., 2011a). In further studies, it is possible to reduce the cost by screening cheaper biopolymers such as cationic starch and larch tannin (Shi et al., 2016; Wang et al., 2013).

5. Conclusion

In this study, we developed a compound flocculant using coal fly ash leachate (CFAL) modified chitosan for *M. aeruginosa* (M.A.) flocculation. It was found that the CFAL enhanced the flocculation ability of chitosan for M.A. removal at CFAL/Chitosan ratio of 12:1 and a good algal removal rate remained across a wide dosage range due to the improvement of charge neutralization. The algal flocs of CFAL-Chitosan were larger and denser than produced in the presence of chitosan without CFAL. However, when the CFAL/Chitosan ratio was beyond the optimal, a surplus of CFAL inhibited the M.A. removal due to hydrolysis resulting in a decline in molecular weight of chitosan which impaired the bridging–netting properties. 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 availability and ease of pre-treatment. Further studies are needed to ensure leachable metals and persistent organic pollutants (pops) in CFA pose no threat to human or environmental health across a wide range of environmental conditions.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.watres.2015.12.003>.

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