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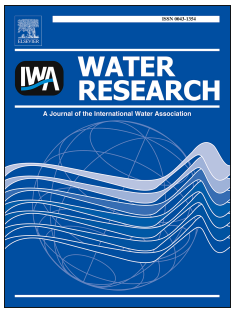

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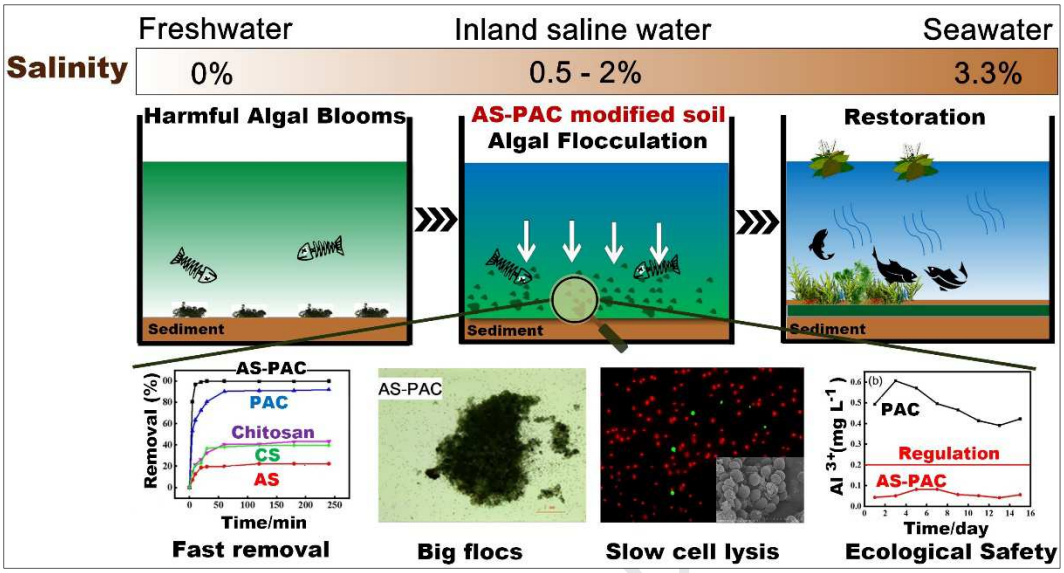
Graphic Abstract



Amphoteric starch-based bicomponent modified soil for mitigation of harmful algal blooms (HABs) with broad salinity tolerance: Flocculation, algal regrowth, and ecological safety

Xiaoguang Jin[1,2,5], Lei Bi[1], Tao Lyu*[3,4], Jun Chen[1,2], Honggang Zhang[1], Gang Pan*[1, 2, 3, 4]

[1] *Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China*

[2] *University of Chinese Academy of Sciences, Beijing 100049, China*

[3] *School of Animal, Rural, and Environmental Sciences, Nottingham Trent University, Brackenhurst Campus, Nottinghamshire NG25 0QF, United Kingdom*

[4] *Centre of Integrated Water-Energy-Food studies (iWEF), Nottingham Trent University, Nottinghamshire NG25 0QF, United Kingdom*

[5] *School of Environment, Tsinghua University, Beijing 100084, China*

** Correspondent authors: E-mail: tao.lyu@ntu.ac.uk (T. Lyu); gpan@rcees.ac.cn (G. Pan)*

Abstract

The treatment of harmful algal blooms (HABs) by in-situ flocculation is an emerging technology capable of efficiently removing HABs from natural waters. However, differences in salinity, pH and algal species in freshwaters and seawaters can influence the flocculation treatment. In this study, we developed a bicomponent modified soil using amphoteric starch (AS) and poly-aluminium chloride (PAC) in order to effectively flocculate microalgae under broad salinity conditions. Specifically, the impacts of water salinity (0-3.3%), pH (3-11), and algal species (*Microcystis aeruginosa* and marine *Chlorella sp.*) were investigated in order to evaluate efficiency, dosage and mechanisms of algae flocculation. The results showed that

AS-PAC modified soils possessed excellent resistance to salinity change due to the anti-polyelectrolyte effect of AS, which contributed to 99.9% removal efficiency of *M. aeruginosa* in fresh and saline waters, and *Chlorella sp.* in marine water, respectively. The dosage of the flocculant modifier was only 10-20% of that of another proven modifier (i.e. *Moringa oleifera*), which substantially reduced the material cost. The high salinity tolerance of algal flocculation by the AS-PAC modified soil was attributed to the synergistic processes of charge neutralization and netting-bridging. Thus, this study has developed a universal flocculant and revealed fundamental mechanisms for the mitigation of HABs under broad salinity conditions.

Keywords: Eutrophication control; HABs flocculation; lake restoration; modified local soil (MLS); sediment remediation

1. Introduction

Harmful algal blooms (HABs) have become an important global issue, and which have occurred in both freshwater rivers and seawaters (Conley et al. 2009). The main cause of HABs may be attributed to increasing anthropogenic activities (Pan et al. 2018), such as agriculture, which present a serious threat to water quality, public health, and aquatic sustainability (Carmichael and Boyer 2016, Wang et al. 2018). HABs also cause serious annual economic losses of several million pounds in the UK (Berdalet et al. 2016), $330M in Australia, and >$2 billion in USA (Dodds et al. 2009). Therefore, the development of management strategies and mitigation technologies for their removal is paramount in the protection of a significant fraction of the world's water resources, human health and economic growth.

Over the past several decades, researchers have made great efforts to develop an integrated management approach for HABs control (Khare et al. 2019). Current strategies include mechanical (e.g. flocculation (Pan et al. 2011)), biological (e.g. induce exotic species (Anderson 2009)), and chemical controls (e.g. chemical oxidation (Qian et al. 2010)). Among them, flocculation has been classified as the most cost-effective and convenient way to rapidly remove algae (Pierce et al. 2004). Since the 1990s, the ability of natural clay to flocculate HABs has been recognised, and it has started to be applied in engineering projects as a low cost and eco-friendly material (Anderson 1997). The flocculated algae are dragged down onto the sediment due to the high density of the clay, after which nutrients released from algal cell decomposition can be utilised by submerged vegetation and facilitate a switch from HABs-dominated to vegetation-dominated waters (Pan et al. 2019, Zhang et al. 2018b). However, flocculation by the sole use of natural clay needs a high dosage (0.25−2.5 g L^{-1}) in order to achieve a relatively high (>90%) removal efficiency (Pan et al. 2006a, Sengco et al. 2001). To reduce the usage of clays and improve the removal efficiency of HABs, the development of different modifiers to upgrade the natural particles, e.g. clay and soil, has attracted great attention.

Two general categories of modifiers, inorganic and organic, have been developed to modify natural particles for the flocculation of HABs. Inorganic materials, such as poly-aluminium chloride (PAC) (Pierce et al. 2004) and ferric chloride (Wei et al. 2010), have been successfully used to modify soils and applied to freshwaters and oceans. The algal flocs produced by these inorganically-modified soils are mainly formed by the electrical interaction between the positively charged modifiers and negatively charged algal cells

(Sengco et al. 2001). These flocs are usually small (Beaulieu et al. 2005), and thus high dosages of flocculants (e.g. 10-15 mg L^{-1} of PAC) are needed to achieve high efficiencies of algal removal (Pan et al. 2011). By doing this, there exists the potential for the release of toxic ions, such as aluminium, to the water, with a subsequent threat to human health (Gauthier et al. 2000). Organic modifiers, such as chitosan (Pan et al. 2006b), cationic starch (Shi et al. 2016), and xanthan (Chen and Pan 2011), have also been used to modify soils for algal flocculation in freshwater. Compared with inorganic modifiers, organic modifiers incorporate netting and bridging functions, which efficiently flocculate algal cells, forming extensive and dense flocs. Furthermore, some natural organic modifiers are biodegradable and thus safe to the aquatic environment. However, the applicability of organically-modified soils are limited in seawaters, because high salinity constrains the spatial extension of these modifier chains and cause the loss of the functions of netting and bridging (Zou et al. 2005). Hence, it has become necessary to find new materials or methods which could effectively flocculate HABs across a broad range of salinity conditions.

In this study, amphoteric starch (AS) was developed to modify natural soils, together with PAC, which was employed for the flocculation of HABs in saline waters, and the performance of these materials was compared with that of two other widely-used soils, modified with chitosan and cationic starch (CS; Fig. S1). Firstly, in order to investigate the salt resistance of different flocculants, AS-PAC, AS, PAC, Chitosan and CS modified soils were prepared, and used to flocculate i) *Microcystis aeruginosa* in waters over a broad range of salinity values (0%~2%) and ii) marine *Chlorella sp.* under salinity condition of 3.3%. Secondly, the effect of pH on *Microcystis aeruginosa* flocculation, by AS-PAC modified soils, was tested.

Thirdly, the synergistic effects and flocculation mechanisms of AS-PAC bicomponent modified soils were explored by dosage experiments. Lastly, in order to prove the general feasibility of the techniques in an engineering context, the algal vitality, cell integrity, algal regrowth rates, toxic ion release from the flocculants, and materials cost, were assessed. With these results, this study has aimed to provide low-cost and eco-friendly materials in order to improve the mitigation of HABs and control eutrophication over a broad salinity range.

2. Materials and methods

2.1 Amphoteric starch preparation

Amphoteric starch was derived from corn starch (Unilever Co. Ltd., Shanghai, China) through two synthesized processes under microwave treatment. Briefly, 0.5 g NaOH and 2 g 2, 3-epoxypropyl trimethyl ammonium chloride were dissolved in 100 mL deionized water under constant magnetic stirring. The solution was heated to 75^{o}C using a water-bath and then, with continued stirring, 10 g corn starch was added. Thereafter, the 500 mL reaction vessel was placed in a microwave oven (Galanz Group Co. Ltd., Guangdong, China) and heated for 10 mins under 750 W microwave power, with repeated stops (every 2 min) to avoid boiling. This formed a viscous gel-like solution (Lin et al. 2012). Then, 40 g L^{-1} of NaOH solution (50 mL) was added under constant stirring in a 70°C water bath, followed by 2 g chloroacetic acid. The reaction vessel was placed into the microwave oven and irradiated at 750 W, again with periodic pauses to avoid boiling, and stopped after 10 mins when a viscous gel-like mass had formed. The product was left to cool to ambient temperature, and 150 mL of anhydrous acetone added. The solid phase was collected, further washed three times with 200 mL of acetone, and dried in a vacuum drying oven (DZF-6020, Shanghai Yiheng

Instrument Co. Ltd., China) at 50°C for 5h. Finally, the synthesized amphoteric starch was characterized for the degree of cationic/anionic group substitution by the Kjeldahl and alkaline titration methods (Mattisson and Legendre 1952). Fourier Transform infrared spectroscopy (FTIR; Tensor 27, Bruker, Germany) was used to determine the functional groups of the synthesized amphoteric starch and the original core starch over the wavenumber range of 400-4000 cm^{-1}.

2.2 Flocculation experiments

2.2.1 Flocculant preparation

The synthesized amphoteric starch (AS), Polyaluminum chloride (PAC), bicomponent modifier of AS and PAC (AS-PAC), chitosan and cationic starch (CS), were used to prepare the modified soil flocculants. The molecular weight of the chitosan, synthesised AS and CS were 680, 520 and 490 kDa, respectively. The soil was collected from the banks of Meiliang Bay, Lake Taihu (China), washed and screened to remove extraneous materials and suitable particle size fractions (~ 70 μm) selected. The soil was added into deionized water to prepare a suspension of flocculant with a concentration of 100 g L^{-1}. Prior the experiment, the AS was dissolved in deionized water to obtain a concentration of 1 g L^{-1}. The PAC was obtained from Dagang Reagent Plant Co. Ltd., Tianjin, China, with a basicity (B = [OH]/[Al]) of 2.4 and Al_2O_3 content of 30%, and dissolved in deionized water to obtain a concentration of 1 g L^{-1}. The chitosan was from Qingdao Yunzhou Bioengineering Co. Ltd., Shandong, China, dissolved in 0.5% acetic acid solution and further diluted with deionized water to a concentration of 1 g L^{-1}. The CS was prepared according to the method described by Shi et al. 2016, then dissolved in deionized water to obtain a concentration of 1 g L^{-1}.

2.2.2 Microalgae species and cultivation

Microcystis aeruginosa (*M. aeruginos*a) and marine *Chlorella sp.* (marine *Chlorella*) are typical microalgae species constituting HABs in freshwater and seawater, and were therefore selected as the target species for the flocculation experiment. *M. aeruginos*a (FACHB-469) and marine *Chlorella* (GY-H6) were purchased from the Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, China and Guangyu Biological Technology Co., Ltd, Shanghai, China, respectively. The cultivation media and inoculation conditions are described in *Supplementary Materials* (S1.1).

2.2.3 Flocculation treatment

In each flocculation treatment, 200 ml of algal suspension was added into a 500 mL beaker and the experiment conducted in a test apparatus (ZR3-6, Zhongrun Water Industry Technology Development Co. Ltd., China). After adding another flocculant solution into the algal suspension, the mixture was stirred at 300 r min^{-1} for 1 min, then 120 r min^{-1} for 2 min, followed by 40 r min^{-1} for another 10 min.

Firstly, to evaluate the best composition of bicomponent (AS and PAC) modified soil for algal flocculation, two ratios of AS:PAC, i.e. 2:1 and 0.5:1, were used for flocculation of *M. aeruginos*a under simulated freshwater conditions (salinity=0% and pH=8). Under these AS-PAC ratios, the PAC concentrations in the final solutions were 0, 2, 3, 4, 8, 10 and 12 mg L^{-1}. The best flocculation efficiency was achieved using an AS:PAC ratio of 0.5:1, which was then used for subsequent flocculation tests. Secondly, different dosages of flocculants, i.e. AS, PAC, AS-PAC, chitosan, and CS modified soils, were added into the algal suspension with final

flocculant modifier concentrations of 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 mg L^{-1}. *M. aeruginosa* suspensions were adjusted to salinity levels of 0%, 0.5%, 1%, 1.5%, 2% by adding NaCl, in order to simulate different inland waters. The marine *Chlorella* culture solution was artificial seawater (Table S1) with salinity of 3.3%. Before the experiment, the algal suspension was adjusted to pH 8 in order to simulate the real operational conditions for removal of HABs. Thirdly, the best dosages of flocculants, thus obtained, were then used to evaluate the impact of the initial pH. A range of pH values (3-11) of *M. aeruginosa* growth media were prepared by adding 0.5 mol L^{-1} NaOH or 0.5 mol L^{-1} HCl before the flocculation treatment.

In all of the flocculation experiments, the concentration of soil in the final solution was kept at 1 g L^{-1}. A control group was carried out with a prepared algal solution without adding any flocculants in each experiment. Each flocculation treatment was conducted in triplicate at 25 ^{o}C.

2.3 Sampling and analysis

2.3.1 Algae removal

After each flocculation treatment, water samples (1 mL) were taken from 2 cm below the water surface at 0, 5, 10, 20, 30, 60, 90, 120, 180 and 240 min to perform algal cell counts using a hemocytometer and light microscope (Carl Zeiss Meditec AG, Jena, Germany). The difference in algal cell numbers were calculated to represent the algae removal rate (S1.2, *supplementary material*).

2.3.2 Floc formation and dimensions

An automatic continuous analysis facility was set up to monitor the algal floc growth over a period of 14 mins (Li and Pan 2013). The instrument was based on a laser particle size analyzer (Mastersizer 2000; Malvern, Worchestershire, UK) and the mean diameter, $d_{0.5}$, was used to describe the algal floc size. At 240 min, the algal flocs (1 mL) were carefully taken out and photographed using an Axioskop 2 mot plus microscope (Carl Zeiss Meditec AG, Jena, Germany).

2.3.3 Zeta potential and floc characterization

The Zeta potential of algal cells' surface charge was tested after 240 min of flocculation treatment. A 10 mL sample was collected from 2 cm below the water surface and the algal cell surface charge was measured by using a Zeta-sizer 2000 (Malvern Co., UK) with the maximum detection limit of 200 mV. The algal flocs (1 mL) were carefully taken out and analysed by field emission scanning electron microscope (FESEM; Su-8020, Hitachi, Japan). Sample preparation for FESEM analysis is described in *Supplementary Material* (S1.2).

2.3.4 Algal vitality and integrity

Algal flocs (1 mL) were carefully sampled at days 5 and 10 after the flocculation tests. The algae cell vitality was determined by the method of double staining with fluorescein diacetate (FDA) and propidium iodide (PI). In this method, FDA was dissolved in acetone to obtain a solution of 5 mg mL^{-1} and stored in a 100 mL brown bottle at 4°C. PI was diluted to 400 µg mL^{-1} in a phosphate buffer solution and stored in a 100 mL brown bottle at 4°C. The algal flocs were dispersed in the culture solution, FDA was added as a stain and the solution kept at room temperature for 5 min in the dark. PI was then added and the solution kept for

196 a further 5 min at room temperature. After dyeing, the algal cells were washed three times
197 with PBS to remove excess dye. Finally, the sample was observed according Fan et al. (2013)
198 using an inverted fluorescent Microscope (MF53, Mshot, Guangzhou, China). The algae cell
199 integrity was characterised by FESEM (Su-8020, Hitachi, Japan).

200 **2.3.5 Algal regrowth and release of metal ions**

201 After the flocculation treatment, the reaction vessels were transferred into an
202 illuminated incubator. The incubation conditions were the same as for algal cultivation.
203 Water samples (1 mL) from 2 cm below the water surface were collected every day until day
204 12. Half of the water samples were used to count the algal cell number. The other halfwere
205 used to determine aluminium concentration by Inductively Coupled Plasma Optical Emission
206 (ICP-OES; Optima 8300, Perkin Elmer Inc., USA) with a detection limit of 0.5 μg L^{-1}.

207 **2.4 Statistical Analysis**

208 The statistical analysis was carried out using SPSS 19.0 for Windows (IBM Corp., USA).
209 Data from different flocculation treatments at the same sampling time were subjected to
210 analysis by one-way ANOVA to test for statistical differences at a significance level of $p <$
211 0.05.

212 ## 3. Results and discussion

213 **3.1 Characterization of amphoteric starch**

214 Chemical synthesis by microwave radiation has become a standard technique in
215 starch modification (Lin et al. 2013), and was therefore selected to prepare the amphoteric
216 starch in this study. The degree of substitution (DS) of cationic groups and carboxymethyl

anionic groups of the synthesized amphoteric starch reached 0.17 and 0.18, respectively. The DS values agreed with the previous study of amphoteric starch synthesis (DS value of 0.15-0.25) under microwave treatment (Lin et al. 2012). The most intense bands in the FTIR spectra (Fig. S2) from both corn starch and AS were at 3600-3000 cm^{-1}, and can be attributed to the typically broad features of hydroxyl functional groups (O-H) (Kizito et al. 2017). The spectral bands at 1148 cm^{-1} (peak D) and 1022 cm^{-1} (peak E) are typical of starch and are preserved in the spectra of both corn starch and AS (Lekniute et al. 2013). The additional band at 1415 cm^{-1} (peak C), due to the C-N stretching vibration (Peng et al. 2012), is indicative of the incorporation of the cationic moiety onto the backbone of the synthesized AS. It is noteworthy that other new bands appeared at 1572 cm^{-1} (peak B) and 1735 cm^{-1} (peak A) in the spectrum of AS, which are typically characteristic of the carboxylate symmetric stretching vibration (peak B), and the band due to the C=O group (peak A) (Lekniute et al. 2013). Changes in the spectrum for the AS, compared with the original corn starch, indicates that the AS was successfully synthesized.

3.2 Effects of AS-PAC proportion on HABs removal

To identify the optimum combination of AS and PAC for the bicomponent modified soil, two ratios (2:1 and 0.5:1) of AS-PAC modified soils were prepared for *M. aeruginosa* flocculation (Fig. 1). When the proportion of AS and PAC was 2:1, algal removal efficiency showed a positive relationship with the bicomponent dosage until values of 8 mg L^{-1} of AS and 4 mg L^{-1} of PAC were reached. By continuing the incorporation to 24 mg L^{-1} of AS and 12 mg L^{-1} of PAC, algal removal efficiency was found to decrease significantly from >99% to around 60%. However, efficiency remained at >99% for the AS-PAC proportion of 0.5:1 until

239 the highest test dosage (6 mg L^{-1} of AS and 12 mg L^{-1} of PAC). The results showed better
240 removal performance of *M. aeruginosa* cells under the treatment of AS-PAC with ratios of
241 0.5:1, compared with the ratio of 2:1. The finding was supported by the previous study, in
242 that the addition of only a small amount of the organic polymer, i.e. 10 mg L^{-1} chitosan, could
243 significantly increase algal flocs and total algal removal efficiency, than using PAC alone (Pan
244 et al. 2011).

245 FESEM images (Fig. 1), indicate that the reticular structure was the mesh bridging
246 structure formed due to the AS. Even though more reticular structures were observed for the
247 higher ratio AS-PAC (2:1) modified soil treatment, the algal removal efficiency was inferior to
248 that of low ratio (0.5:1) treatment. The results indicated that the unmatched charge
249 neutralization and mesh bridging capability had side effects on algal flocculation. During the
250 algal flocculation, the mesh bridging capability could increase along with the initial increase
251 in dosage. Then, removal efficiency decreased until the flocculant dosage exceeded the
252 optimum, the point called polymer stabilization. This might explain why CS modified soil
253 could theoretically achieve a removal efficiency of > 95% by adjusting the dosage, but only
254 reach around 85%-90% in practice (Li et al. 2015, Shi et al. 2016). Moreover, high removal
255 efficiency was still achieved when the Zeta potential of the flocs became positive. This
256 observation was not consistent with previous reports that positively-charged flocculants
257 could not effectively flocculate positively-charged flocs (Gerchman et al. 2017, Li et al. 2015).
258 This result supported the hypothesis that algal removal by AS-PAC modified soils was due,
259 not only to the effect of charge neutralization, but also to the netting-bridging functions.

3.3 Flocculation efficiency under broad salinity and pH conditions

The best dosage of different modified soils for removal of *M. aeruginosa* from simulated freshwater (salinity of 0%) is reported in Fig. 2a. The soil flocculant modified by AS-PAC (1-2 mg L^{-1}) achieved the most rapid algal removal and achieved 99.9% removal efficiency from 5 mins until the end of the experiment. Similar *M. aeruginosa* removal performance (98.5%) was also reached by PAC modified soil, however, with a larger PAC dosage (8 mg L^{-1}) and over a longer stabilization time (30 mins). The removal efficiencies of *M. aeruginosa* stabilized at approximately 80% after 30 and 120 mins for chitosan and cationic starch (CS) modified soils, respectively. The soil modified by AS alone could only remove around 29.8% *M. aeruginosa* until end of the experiment. In the simulated inland saline waters with salinity up to 2%, maximal *M. aeruginosa* removal efficiencies decreased along with the salinity increase for PAC, CS, and chitosan modified treatment (Fig. 2c). However, AS-PAC modified soil treatment achieved a 98.1% removal efficiency at a salinity of 2%. When water salinity reached 3.3% in the simulated seawater (Fig. 2b), AS-PAC modified soil also showed the fastest and highest removal efficiency (99.9%) of marine *Chlorella* followed by PAC (91.4%), Chitosan (43.1%), CS (39.6%), and AS (22.3%) modified soils.

Soil particles modified only by PAC have already been proven to provide high efficiency flocculation (>95%) of freshwater microalgae (Wu et al. 2011), which supports the similar performance observed in the present study (Fig. 2a). However, the cell sizes of marine *Chlorella* (~2 μm) are much smaller than those of *M. aeruginosa*, which is always a challenge for flocculation treatments under solely neutral functionality (Ryther 1954). It becomes the main reason of the lower algal removal efficiency by PAC modified soils in seawaters (Fig. 2b)

compared with from freshwater (Fig. 2a). Organically-modified soils (chitosan and AS modifiers), could only remove up to 43% of HABs, which agreed well with the previous studies (20%-60%), which were only based on the netting and bridging function of the polymer chain (Pan et al. 2011). The decreased viscosity of chitosan and CS solution along with the improved salinity (Fig. S3) demonstrated their constrained polymer chain and cause the loss of netting and bridging functions under high salinity conditions, so chitosan and CS modified soil had low efficiency in removing algae at high salinity. However, the stable viscosity of AS indicated the anti-polyelectrolyte property of AS (Dai et al. 2017) and lead high algae flocculation performance. Thus, the synergistic functions of charge neutralization and netting-bridging by the bicomponent AS-PAC modified soil could extend the algal removal efficiency to > 99% under a wide salinity condition.

Although the pH of natural water is around 7, the pH usually have a daily fluctuation with a range up to 10-11 in eutrophic waters. pH is also one of the vital factors which could have a significant effect on algal removal rates (Divakaran and Pillai 2002). Hence, the ability of a method to remove algal blooms under broad pH conditions is essential to its practical viability. As shown in Fig. 2d, chitosan modified soil underperformed under basic conditions, which is coincident with other research (Divakaran and Pillai 2002). The current used PAC with basicity of 2.4 has been proved relative stable of the species distribution under alkaline condition (Zhang et al. 2014), which supported the high algae removal performance (90%). Moreover, due to the synergistic effect of AS and PAC, the algal removal efficiency by AS-PAC modified soil remained at 99% over the range of pH 6-11.The results indicated that AS-PAC modified soil may also be suitable for the removal of HABs from eutrophic natural waters

304 over a wide range of pH.

305 **3.4 Algal floc formation and growth**

306 The algal flocs formed by the AS-PAC modified soil were the most rapid and largest,
307 compared with other modified soils treatment in all simulated freshwater (Fig. 3a), saline
308 water (Fig. 3b), and seawater (Fig. 3c) scenarios. It can be explained that the small flocs were
309 rapidly formed through charge neutralization attributable to the PAC (Li and Pan 2013), and
310 would then grow into larger flocs by the netting and bridging functions attributable to the AS
311 (Wu et al. 2016). The addition of soil particles increased the instantaneous concentration of
312 particles and improve the collision frequency between particles, which can contribute to the
313 formation of algal flocs. It may lead rapidly algal flocs formation by PAC-only modified soil,
314 however, the flocs are smaller than those by AS-PAC treatment due to the absence of
315 netting-bridging functions. Without the assistance of PAC, AS-only modified soil cannot form
316 visible algal flocs with the only netting-bridging function. After 240 mins of the flocculation
317 experiment, the largest flocs size formed by AS-PAC modified soil reached 1250, 880, and 590
318 μm in freshwater, saline water and seawater, respectively.

319 During the initial stages, the small flocs formed by charge neutralization might be
320 positive, negative or neutral, which depended on the usage of the flocculant (Shi et al. 2016).
321 When the Zeta potential of flocs became positive (Fig. 1), the attraction between algal flocs
322 and the traditional cationic flocculants, like CS and chitosan, would be weakened by electrical
323 repulsion, and algal flocs would be smaller and looser (Yuan et al. 2016). In contrast to
324 traditional cationic flocculants, AS contained both positive and negative groups in the

325 molecular chain (Peng et al. 2016), which attracted with both positive and negative flocs.
326 Hence, AS-PAC modified soil could remove algal cells and form larger flocs over a wider Zeta
327 potential range.

328 **3.5 Algal vitality and cell integrity after treatment**

329 After algal flocculation, rapid lysis of algal cells would release algal toxins and dissolved
330 organic matter (Mucci et al. 2017), with adverse effects on the safety of drinking water and
331 might even cause new HABs (algae regrowth). However, if cell degradation processes
332 occurred only gradually, the nutrients released could be utilised by submerged vegetation
333 and thus achieve ecological restoration (Zhang et al. 2018a). In this study, the ratio of living
334 *M. aeruginosa* cells in the flocs formed by the AS-PAC and chitosan modified soils were 20.6%
335 (Fig. 4a) and 1% (Fig. 4c) after 5 days, respectively. Algal cells were generally intact after both
336 treatments, despite decreased vitality. After 10 days, the algal cells flocculated by AS-PAC
337 modified soil were still intact with 4.6% of cells living (Fig. 4b). However, a lot of debris was
338 observed from the *M. aeruginosa* cells flocculated by chitosan modified soil and all cells
339 were observed not to be viable (Fig. 4d). After treatment of marine *Chlorella*, the same
340 tendencies of cell vitality and integrity were found. The ratio of living cells in the flocs
341 flocculated by AS-PAC and chitosan modified soil was 35% and 21.73% at 5^{th} day, 10.3% and
342 12.6% at 10^{th} day, respectively. The FESEM images illustrates the good integrity of algal cells,
343 although, a little 'wrinkled' in appearance over 10 days. Compared with chitosan modified
344 soil, AS-PAC modified soil had only a small influence on the degradation of *M. aeruginosa*
345 and marine *Chlorella* cells. Hence, mitigation of HABs by AS-PAC modified soil would provide
346 a period for subsequent processing and ecological recovery of the waterbody treated.

3.6 Ecological sustainability and safety

It is envisaged that the modified soil would carry algal flocs to the benthic sediments, due to the effects of gravity, which may improve the water clarity and create a period for growth of submerging vegetation. However, a period of slow algal cell lysis may potentiate a second HAB, with resumption of growth of the live algal cells from the flocs. In order to estimate this effect, the cell concentration of *M. aeruginosa* in the remaining supernatant was measured two weeks after the flocculation treatment. Compared with chitosan, CS, and PAC modified soils, cell concentrations were always lowest after AS-PAC modified soil treatment (Fig. 5a). Synthetic aluminium flocculants also have a potential negative effect on the environment if the release of toxic aluminium attains critical levels (Gauthier et al. 2000). The concentration of residual aluminium in the waters after the AS-PAC modified soil treatment remained at <0.08 mg L^{-1} for 15 days, which was much lower than the current Chinese drinking water standard (0.2 mg L^{-1}) (Fig. 5b). Nevertheless, further study should also focus on the evaluation of long-term release aluminium associated with the flocs after AS-PAC modified soil treatment.

3.7 Cost evaluation

Economic cost is one of the most important factors which will influence the field implementation of any newly developed material/technique. To best of our knowledge, only extraction by *Moringa oleifera* (MO), combined with chitosan-modified natural particles, has been successfully tested for the mitigation of HABs in both freshwater and seawater (Li and Pan 2013). Compared with the higher usage of MO, gleaned from literature sources, AS-PAC modified soil requires a much lower rate of application (10-20% of MO) in order to achieve

similar removal efficiency of HABs (>99%). The low dosage also gives the proposed AS-PAC modified soil a significant cost advantage, especially in the mitigation of marine HABs. Table 1 shows a summarised cost of materials, mainly based on Chinese market. The cost of using AS-PAC to flocculate HABs is 0.00315 US$ m^{-3} in freshwater and 0.0063 US$ m^{-3} in saline marine waters, which are significantly lower than other materials necessary to achieve similar removal efficiencies. Thus, these results indicate that AS-PAC modified soil is a cost-effective flocculant for HABs mitigation in both freshwater and seawater.

4. Conclusions

AS-PAC modified soil has been demonstrated to be able to attain a high removal capacity of HABs by flocculation, under a broad range of salinity and pH conditions, due to the synergistic processes of charge neutralization and netting-bridging. Limited algal regrowth and low re-release of toxic aluminium after treatment demonstrated the ecological safety of the technique. A low dosage requirement and readily accessible, natural, raw materials also give the proposed material a significant advantage on the basis of cost-effectiveness. Moreover, observation of algal cell vitality and morphology indicates that the flocculated algae will undergoes gradual lysis, which will benefit the restoration of submerged vegetation.

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Table 1

The costs of soil/sand modifiers

Modifiers	**Production Location**	**Costs (US$/ton)**
Amphoteric starch (AS)	This study	1,850
Cationic starch (CS)	This study	1,650
Chitosan	China	22,800
Poly aluminium chloride (PAC)	China	650
Moringa oleifera (MO)	China	96,074

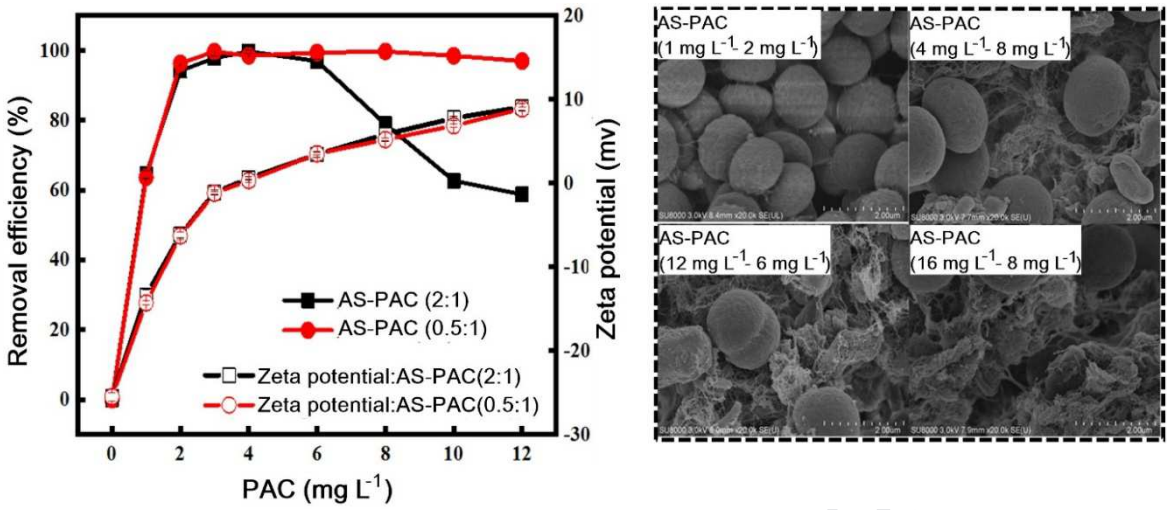

Fig. 1. The removal of *M. aeruginosa* and floc Zeta potential for different proportions of modifiers and dosage of AS-PAC modified soil (Left); and FESEM images of algal flocs after 240 mins of the treatment (Right). Experiment condition: pH=8, temperature=25 ^{o}C, salinity=0%.

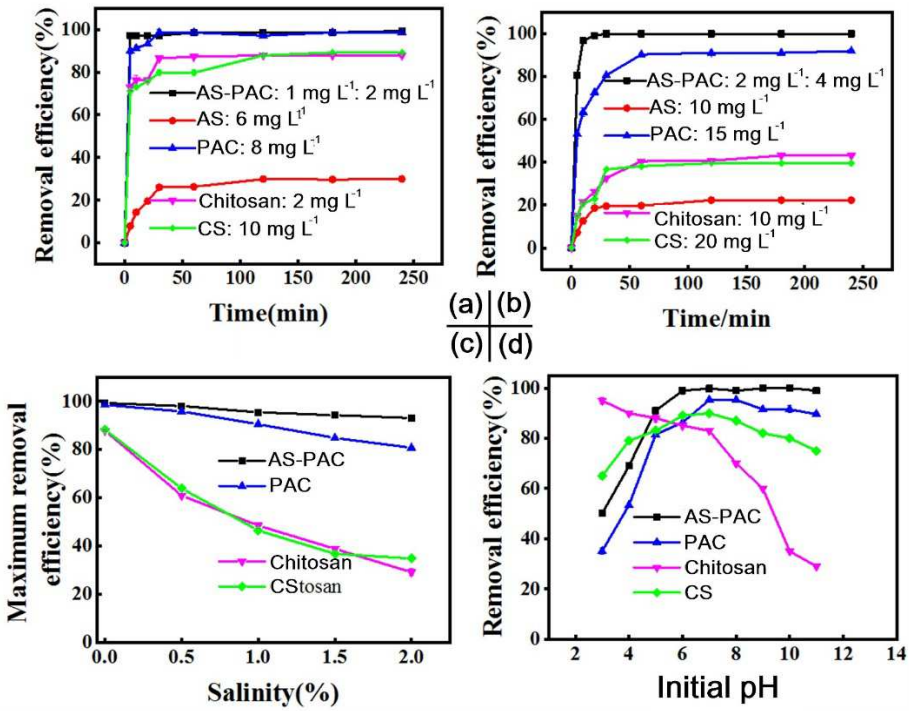

Fig. 2. The removal performance of (a) *M. aeruginosa* in freshwater with salinity of 0%, and (b) marine *Chlorella* in seawater with salinity of 3.3% during algal flocculation experiments. The maximal removal efficiency of *M. aeruginosa* under (c) salinities of 0-2% and (d) pH values of 3-11. Experiment condition: temperature=25 ^{o}C.

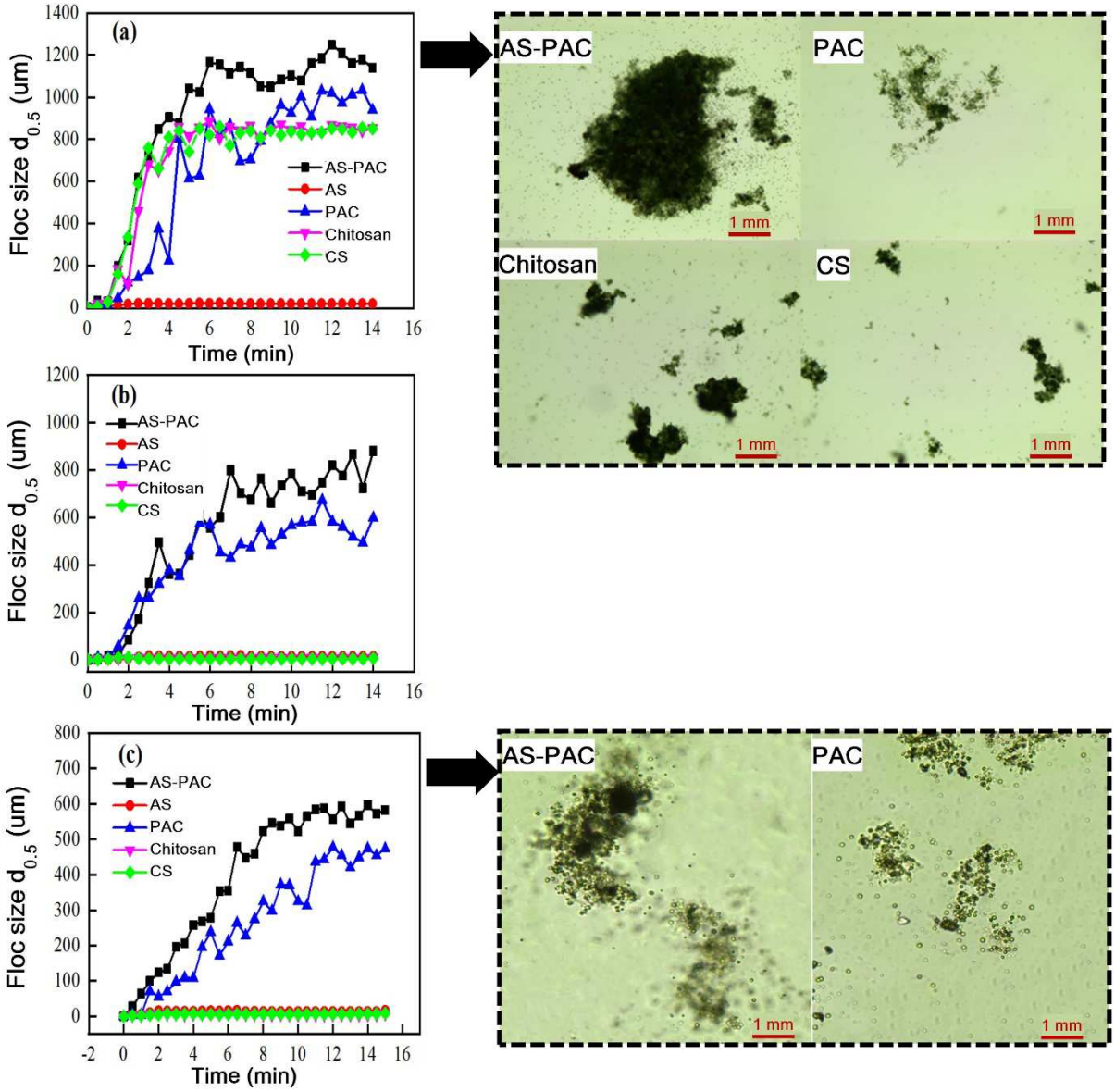

Fig. 3. The floc growth of (a) *M. aeruginosa* under salinity of 0%, (b) *M. aeruginosa* under salinity of 2%, and (c) marine *Chlorella* under salinity of 3.3% (Left); and the FESEM pictures of the algae flocs after 240 mins of the treatment (Right). Experiment condition: pH=8, temperature=25 ^{o}C.

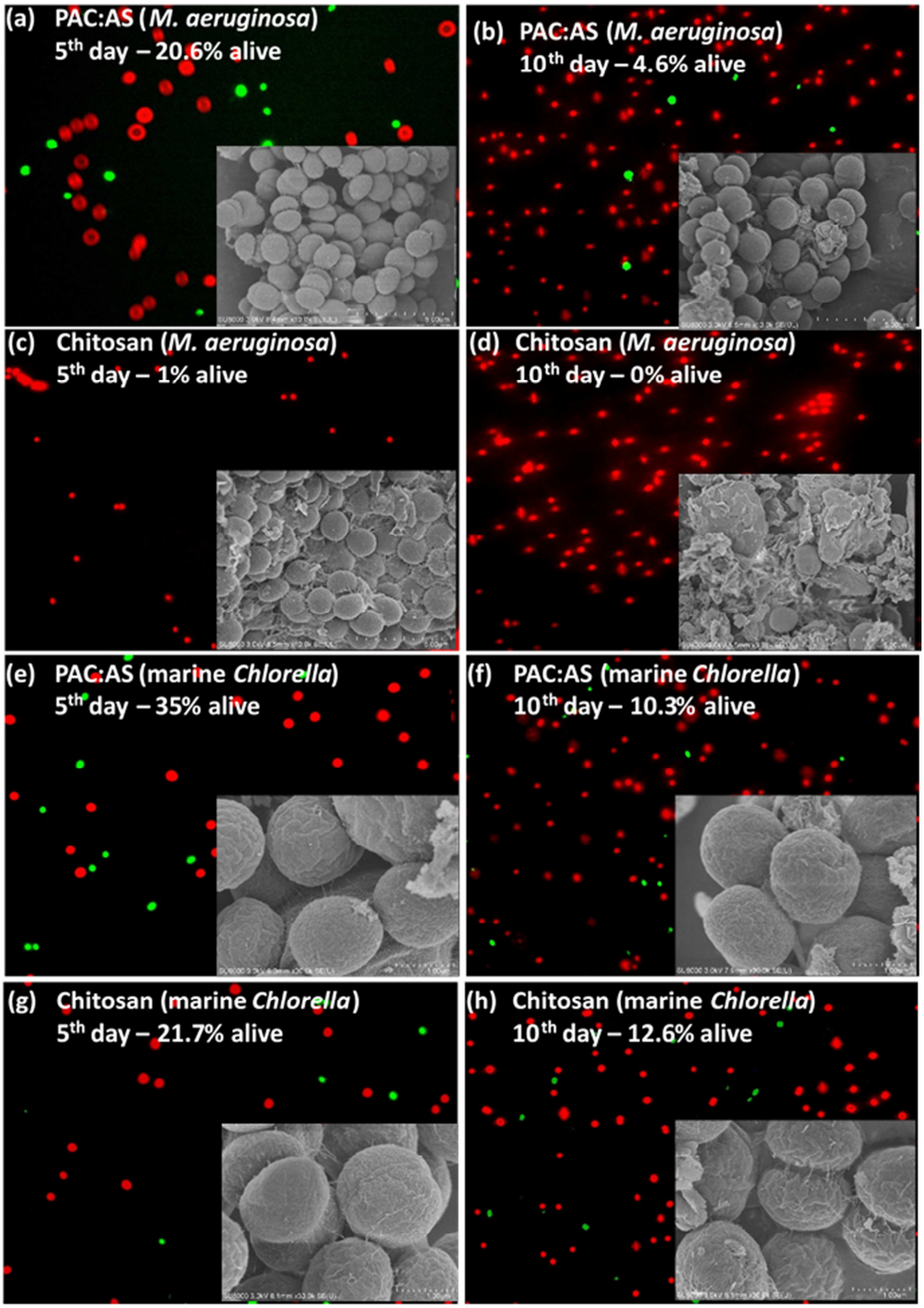

Fig. 4. Fluorograms of *M. aeruginosa* cells (a～d) and marine *Chlorella* cells (e～h) after flocculating by AS-PAC and chitosan modified soil, and the FESEM pictures of *M. aeruginosa cells* (a`～d`) and *Chlorella* cells (e`～h`) after flocculating by AS-PAC and chitosan modified soil.

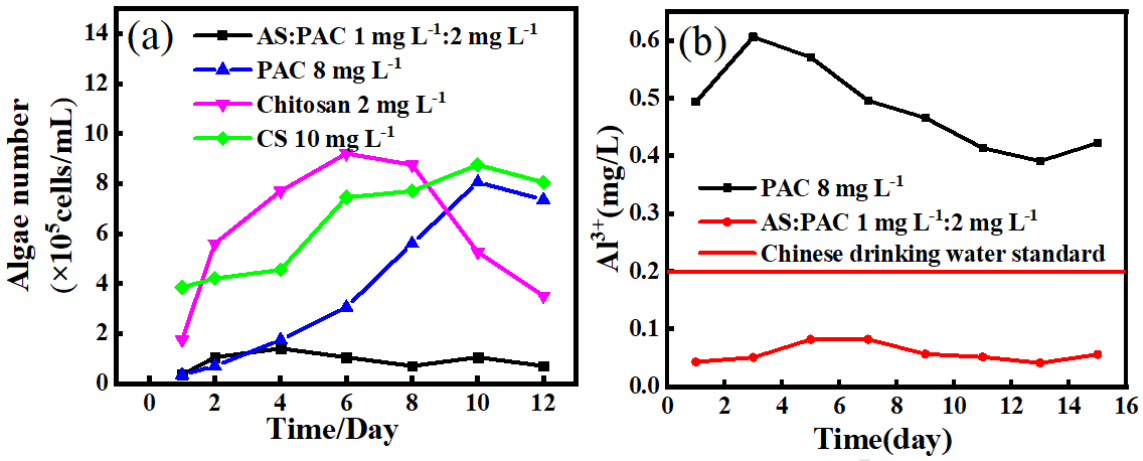

Fig. 5. *M. aeruginosa* concentration in supernatant after flocculation treatment (a); and (b) the concentration of residual aluminium in the supernatant after flocculation by AS-PAC and PAC modified soil. Experiment condition: pH=8, temperature=25 ^{o}C, salinity=0%.

Highlights

- The bicomponent amphoteric starch-PAC modified soil was tested for HABs mitigation
- Synergistic charge neutralization and netting-bridging enhanced algae flocculation
- Relatively long-term algal cell integrity reduced cell lysis and algal regrowth
- Low dosage of the innovated flocculant enhanced eco-safety by limiting Al^{3+} release

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: