

1 **Removal of *Microcystis aeruginosa* using cationic starch modified soils**

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23 **Abstract**

24 A cheap and biodegradable modifier, cationic starch (CS), was used to turn local  
25 soils into effective flocculants for *Microcystis aeruginosa* (*M. aeruginosa*) removal.  
26 The isoelectric point of soil particles was remarkably increased from pH 0.5 to 11.8  
27 after modification with CS, which made CS modified soil particles positively charged  
28 and obtain algal flocculation ability. At the soil concentration of 100 mg/L, when the  
29 CS modifier was 10 mg/L, 86% of *M. aeruginosa* cells were removed within 30 min.  
30 Lower or higher CS dosage led to limited algal removal. About 71% and 45% of *M.*  
31 *aeruginosa* cells were removed within 30 min when CS was 5 mg/L and 80 mg/L,  
32 respectively. This is because only part of algal cells combined with CS modified soil  
33 particles through charge neutralization at low dosage, while flocs formed at high CS  
34 dosage were positively charged which prevents further aggregation among the flocs.  
35 The floc stability was quantified by a floc breakage index under applied shear force.  
36 Algal flocs formed at acid and alkaline conditions were more prone to be broken than  
37 those at the neutral condition. The cost and biodegradability concerns may be largely  
38 reduced through the use of CS modified local soils. For field applications, other  
39 practical issues (e.g., re-suspension) should be further studied by jointly using other  
40 methods.

41 **Keywords**

42 Cationic starch, Modified local soil, Algal flocculation, Cyanobacterial bloom  
43 mitigation, Floc breakage

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## 45 **1. Introduction**

46 The frequent outbreak of cyanobacterial blooms in eutrophic freshwaters is a global  
47 issue, posing serious threats to aquatic life, human health, water quality, commercial  
48 fisheries, and coastal aesthetics (Falconer, 1999; Guo, 2007; Hawkins et al., 1985).  
49 Over the past several decades, great efforts have been made to develop bloom  
50 mitigation strategies around the world (Chen et al., 2012; Edzwald, 1993; Everall and  
51 Lees, 1996; Garcia-Villada et al., 2004). The use of natural clays as a means to  
52 remove algal blooms through flocculation and sedimentation has received increasing  
53 attention in recent decades (Anderson, 1997; Atkins et al., 2001; Lee et al., 2008; Pan  
54 et al., 2006). However, the low flocculation efficiency and the high clay loading  
55 (0.25–2.5 g/L) limit its wide application in fields (Lee et al., 2008; Pan et al., 2006).  
56 Coagulant/flocculent modified clays/sands/soils could largely enhance the  
57 flocculation efficiency and reduce the material loading, and are considered as  
58 potential geo-engineering materials for cyanobacterial bloom mitigation (Mackay et  
59 al., 2014; Park et al., 2013; Spears et al., 2014).

60 Several modifiers including chitosan, *Moringa oleifera* (MO), xanthan and  
61 polyaluminum chloride (PAC) have been tested to modify clay/sand/soil for algal  
62 flocculation (Chen and Pan, 2012; Li and Pan, 2013; Pan et al., 2011a). Chitosan,  
63 xanthan and MO, biodegradable natural polymers, are potentially environmental  
64 friendly (Baumgartner et al., 2008; Grabow et al., 1985; Kurniawati et al., 2014).  
65 However, economic concern may largely limit the application of the methods at large  
66 scale due to the high cost of these materials. MO is extracted from MO seeds which

67 are not easily available in many parts of the world, and it is still lack of commercial  
68 products as coagulants (Sengupta et al., 2012). For commercially available PAC, it  
69 cannot be biodegraded although it is relatively cheap, which may be a concern for the  
70 ecological sustainability. Previous studies suggest that high algal removal efficiency  
71 using local clay/sand/soil can be achieved through the two-component modifier  
72 mechanism (e.g., chitosan-PAC or chitosan-MO) (Li and Pan, 2013; Pan et al., 2011a).  
73 In this mechanism, one modifier is responsible for charge modification that makes  
74 solid particles possess net positive charge in natural waters and obtain algal  
75 flocculation ability. The other is to enhance the bridging function that aggregates  
76 small, light, and fluffy flocs into large and dense ones. It remains a challenge to find  
77 cheap and safe modifier materials that can make the two-component mechanism  
78 working. So far, there are few both cost-effective and biodegradable modifiers that  
79 can make clay/sand/soil particles obtain both charge neutralization and bridging  
80 functions for cyanobacterial bloom removal.

81 Cationic starch (CS), a commonly used organic coagulant, has been used to  
82 flocculate negatively charged pollutants in wastewater treatment (Ellis et al., 1982;  
83 Khalil and Aly, 2004; Pal et al., 2005). The coagulant property is attributed to the  
84 positive charge and bridging function of CS polymer chain (Wang et al., 2011b),  
85 which may potentially make it qualify as a clay/sand/soil modifier for algal removal.  
86 CS is both cheap and biodegradable (Pal et al., 2005; Wei et al., 2008). If CS is used  
87 as the clay/sand/soil modifier, the cost and biodegradability concerns may be  
88 potentially reduced. Although studies on algal biomass harvesting using CS have been

89 reported (Anthony et al., 2013; Vandamme et al., 2010), the flocculation dynamics  
90 and floc stability were not well understood before, and there are no studies on the use  
91 of CS modified solid particles for sedimentation removal of cyanobacterial blooms.

92 Algal floc stability is an important property for effective algal removal. The formed  
93 flocs are often exposed to a range of stresses such as current and wind induced  
94 turbulence in fields, which may result in floc breakage and the lost of algal removal.  
95 Descriptive methods are currently used to quantify algal floc stability (e.g., floppy,  
96 fragile, dense), which have hindered further studies and applications of the technology.  
97 Flocs can be broken under an increased shear force, and the reduction of floc size and  
98 the shear force applied can be used to quantify its stability (Parker et al., 1972). So far,  
99 few studies have been seen on the characterization of algal floc stability in the area of  
100 cyanobacterial bloom mitigation.

101 In this study, CS was used to modify lakeside soil to flocculate and settle  
102 *Microcystis aeruginosa* (*M. aeruginosa*). Dosage effect on removal efficiency, surface  
103 charge and floc size was studied and the associated flocculation mechanism was  
104 investigated. Floc breakage experiments were conducted and a method was studied to  
105 quantify the stability of algal flocs. Field lake water was also collected and flocculated  
106 to test the algal removal effect of CS modified soil. The objective of this study is to  
107 develop a cheap and environmental friendly local soil modification method for the  
108 mitigation of cyanobacterial blooms.

## 109 **2. Materials and methods**

### 110 **2.1. Algal species and culture**

111 *M. aeruginosa*, a common freshwater bloom-forming cyanobacterium, was used in  
112 this study. The inoculum of *M. aeruginosa* (FACHB-905) was obtained from the  
113 Institute of Hydrobiology, Chinese Academy of Sciences, and cultivated in BG11  
114 medium in the laboratory. Algal batch cultures were performed in an illuminating  
115 incubator (LRH-250-G, Guangdong Medical Apparatus Co., Ltd., China) with  
116 continuous cool white fluorescent light of 2000-3000 lux on a 12 hr light and 12 hr  
117 darkness regimen, and the temperature was maintained at  $25 \pm 1^\circ\text{C}$ .

## 118 **2.2. Cationic starch preparation**

119 Corn starch with a moisture content of 11.4% was purchased from Unilever Co.,  
120 Ltd., China. CS was prepared using microwave-assisted method (Lin et al., 2012).  
121 Briefly, 2.0 g 2,3-epoxypropyl trimethyl ammonium chloride (GTA) was dissolved in  
122 100 mL of 5.0 g/L NaOH solution. The mixture was stirred thoroughly for 10 min.  
123 Then, 10.0 g corn starch was added into the above mixture. Stirring was continued for  
124 another 30 min at a  $70^\circ\text{C}$  water-bath. The reaction vessel was placed on the turntable  
125 of a microwave oven (WD750S, Guangdong Galanz Group Co. Ltd., China) and  
126 irradiated at the power of 750 W. Periodically, the microwave irradiation was paused  
127 at  $65^\circ\text{C}$  to avoid boiling, with the aim to prevent unwanted vapors formation. The  
128 microwave irradiation-cooling cycle was repeated for five times. Afterwards, the  
129 reaction vessel and its contents were cooled down to the room temperature. The  
130 gel-like mass left in the reaction vessel was washed with ethanol for three times, and  
131 the targeted precipitate was collected and dried in a vacuum oven (DZF-6020,  
132 Shanghai Yiheng Instrument Co., Ltd., China) at  $50^\circ\text{C}$  for 6 hr. The obtained CS was

133 pulverized before use. The degree of substitution of cationic starch is 0.18, which was  
134 determined using elemental analysis (Shi et al., 2012).

### 135 **2.3. Modified local soil**

136 The soil used was collected from the Lake Taihu north offshore (China). The soil  
137 sample was washed with deionized water, dried at 90°C for 10 h, and then grounded  
138 and sieved (74 µm) before use. The prepared CS was dissolved in deionized water to  
139 obtain a solution of 2 g/L. A certain amount of CS was used to modify the soil  
140 suspension according to the dose conditions tested. The soil concentration used in all  
141 the flocculation experiments was fixed to 100 mg/L (Fig. S1).

### 142 **2.4. Algal flocculation**

143 Flocculation experiments were performed in a jar test apparatus (ZR3-6, Zhongrun  
144 Water Industry Technology Development Co. Ltd., China) with a series of 300-ml  
145 beakers containing 200 ml of *M. aeruginosa* cultures in mid- to late-exponential  
146 growth phase. The initial *M. aeruginosa* concentration was  $3.15\text{--}3.25 \times 10^9$  cells/L.  
147 The temperature was  $22 \pm 1^\circ\text{C}$  during the flocculation experiment. After CS modified  
148 soil was added, the solution was stirred at 200 rpm for 1 min and 40 rpm for another  
149 15 min. The control was run in the above mentioned algal media without adding any  
150 soil or CS. The flocculation experiments were conducted at raw algal solution pH of  
151 8.60. The pH was relatively stable after the addition of CS modified soil and kept at  
152  $8.60 \pm 0.1$ . After sedimentation for 2, 5, 10, 20, 30, 60, 90, 120, 180 and 240 min,  
153 samples were collected from 2 cm below the surface to enumerate cell numbers with  
154 an electromotive microscope (Axioskop 2 mot plus, Carl ZEISS, Germany),

155 respectively. All the flocculation experiments were conducted in triplicate and the  
156 results were presented as the mean values and standard deviations. Cell removal  
157 efficiency was calculated as: (initial cell concentration-sample cell concentration) ×  
158 100% / initial cell concentration.

159 The zeta potential of soil, CS modified soil, algal cell and algal floc was  
160 characterized using a Zetasizer 2000 (Malvern Co. United Kingdom). Dynamic size  
161 growth of algal flocs during the flocculation reaction (15 min) was analyzed using a  
162 laser particle size analyzer Mastersizer 2000 (Malvern Co. United Kingdom). The set  
163 up of the apparatus was described previously (Li and Pan, 2013), and the mean  
164 diameter,  $d_{0.5}$ , was used to measure the floc size.

## 165 **2.5. Floc breakage**

166 This experiment was conducted to study the stability of algal flocs under different  
167 pH conditions (pH=4.0, 7.0 and 10.0). After algal flocculation was completed, the  
168 formed flocs were stirred at a shear speed of 75, 100, 150, 200, and 250 rpm,  
169 respectively, for 15 min, and the dynamic size change of algal flocs was monitored.  
170 The floc stability was evaluated by the  $\gamma$  value in the empirical relationship (Parker et  
171 al., 1972),

$$172 \quad \log d = \log C - \gamma \log G \quad (1)$$

173 where  $d$  is the median floc diameter ( $d_{0.5}$ ) after breakage ( $\mu\text{m}$ );  $C$  is the floc strength  
174 coefficient;  $\gamma$  is the stable floc exponent which is the main index to quantify the floc  
175 stability, and  $G$  is the average velocity gradient of shear speed which can be  
176 calculated according to Bridgeman et al. (2008).



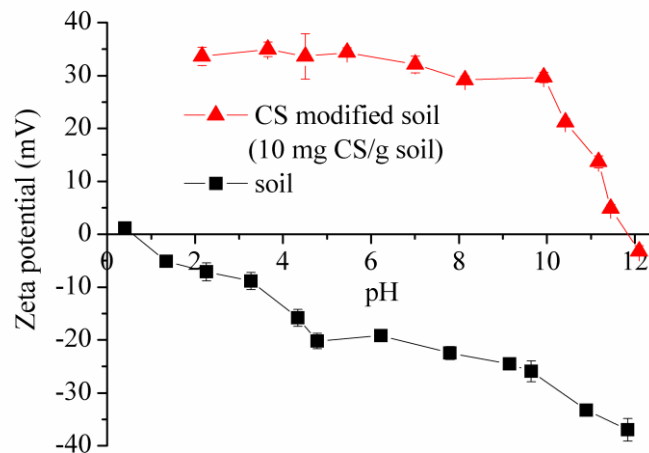
177 **2.6. Natural bloom water test**

178 Field bloom water was collected from Lake Taihu (China) in Sep. 2014 and  
179 flocculated using CS modified soil by jar tests. Algal flocs and *chlorophyll-a* (*chl-a*)  
180 content was studied after flocculation. For the floc image study, the flocs were  
181 carefully transferred on a glass slide and then photographed by an electromotive  
182 microscope (ST-CV320, Chongqing UOP Photoelectric Technology Co., Ltd., China).  
183 *Chl-a* was measured after sedimentation for 30 min using the method prescribed in  
184 Monitoring Analysis Method of Water and Waste Water (Ministry of Environmental  
185 Protection of China, 2002). The flocculation experiments were conducted in triplicate  
186 and the results were presented as the mean values.

187 **3. Results**

188 **3.1. Surface charge of cationic starch modified soil**

189 The isoelectric point of the native soil was pH 0.5 (Fig. 1). After it was modified by  
190 CS, the isoelectric point was remarkably increased to pH 11.8, making the soil possess  
191 net positive charge under most natural water conditions (Fig. 1). The zeta potential  
192 (ZP) of CS modified soil was relatively stable and kept about +30 mV in the wide pH  
193 range of 2.0-10.0, and then decreased to nearly zero at pH 11.8, while the ZP of the  
194 native soil gradually decreased from +1.2 to -37.0 in the pH range of 0.4-11.8.



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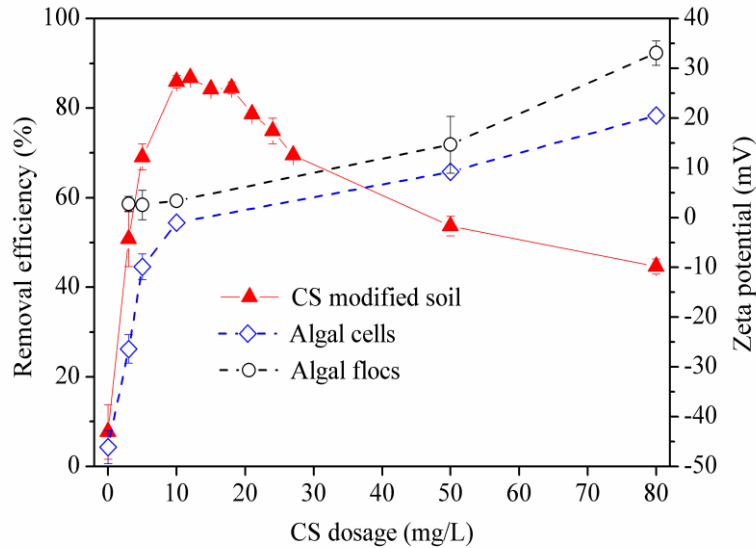
196

197 **Fig. 1** – Comparison of surface charge between the native soil and cationic starch (CS)  
 198 modified soil. Error bars indicate standard deviations.

199 **3.2. Dosage effect on algal removal**

200 When the soil concentration was fixed to 100 mg/L, the algal removal efficiency  
 201 increased from 8% to 86 % as the CS dosage increased from 0 to 10 mg/L, flattened off  
 202 at 86% in the range of 10-18 mg/L, and then decreased rapidly as the dosage further  
 203 increased (Fig. 2). When 5, 10 and 80 mg/L of CS was added, 71%, 86% and 45% of  
 204 the *M. aeruginosa* cells were removed within 30 min, respectively. According to these  
 205 results, the optimized CS dosage of 10 mg/L was used for subsequent flocculation  
 206 experiments. After 30 min sedimentation, the ZP of algal cells and algal flocs as a  
 207 function of CS dosage was measured (Fig. 2). With the increase of CS dosage, the ZP  
 208 of algal cells increased and charge reversal occurred around the optimal dosage of 10  
 209 mg/L. When 5, 10 and 80 mg/L of CS were dosed, the ZP of algal cells was increased  
 210 to -9.9, -1.0, +20.5 mV, respectively. At the same time, the formed algal flocs  
 211 became nearly electrically neutral at the low and optimal CS dosage (10 mg/L). The

212 ZP of algal flocs was +2.5 and +3.4 at 5 and 10 mg/L of CS, respectively. When CS  
213 was overdosed, the ZP of the flocs at 80 mg/L reached +33.1 mV.

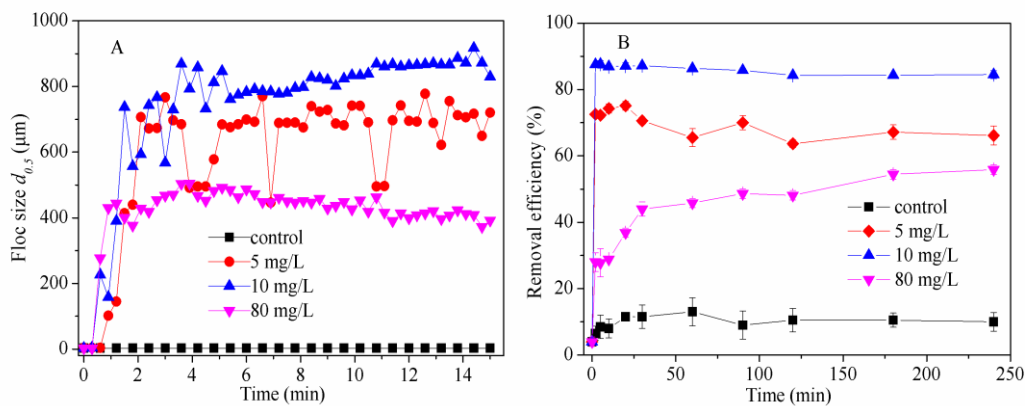


214  
215

216 **Fig. 2** – Removal efficiency (solid line) and zeta potentials of algal cells and flocs  
217 (dotted line) at different dosage of cationic starch (CS). The soil concentration was  
218 fixed to 100 mg/L. Error bars indicate standard deviations.

### 219 3.3. Floc growth and flocculation kinetics

220 After CS modified soil was added, algal flocs grew quickly within the initial 2 min.  
221 The flocs formed at the CS dosage of 10 mg/L (830  $\mu$ m) were larger than those  
222 formed at 5 mg/L (700  $\mu$ m) and 80 mg/L (440  $\mu$ m) (Fig. 3A). After flocculation, the  
223 maximum removal efficiency at the CS dosage of 5 and 10 mg/L was quickly  
224 achieved within 2 min and stayed relatively stable as time increased. At 30 min, the  
225 removal efficiency at 5 and 10 mg/L reached 71% and 86%, respectively. However,  
226 the removal efficiency at 80 mg/L increased slowly and reached only 45% at 30 min  
227 (Fig. 3B).

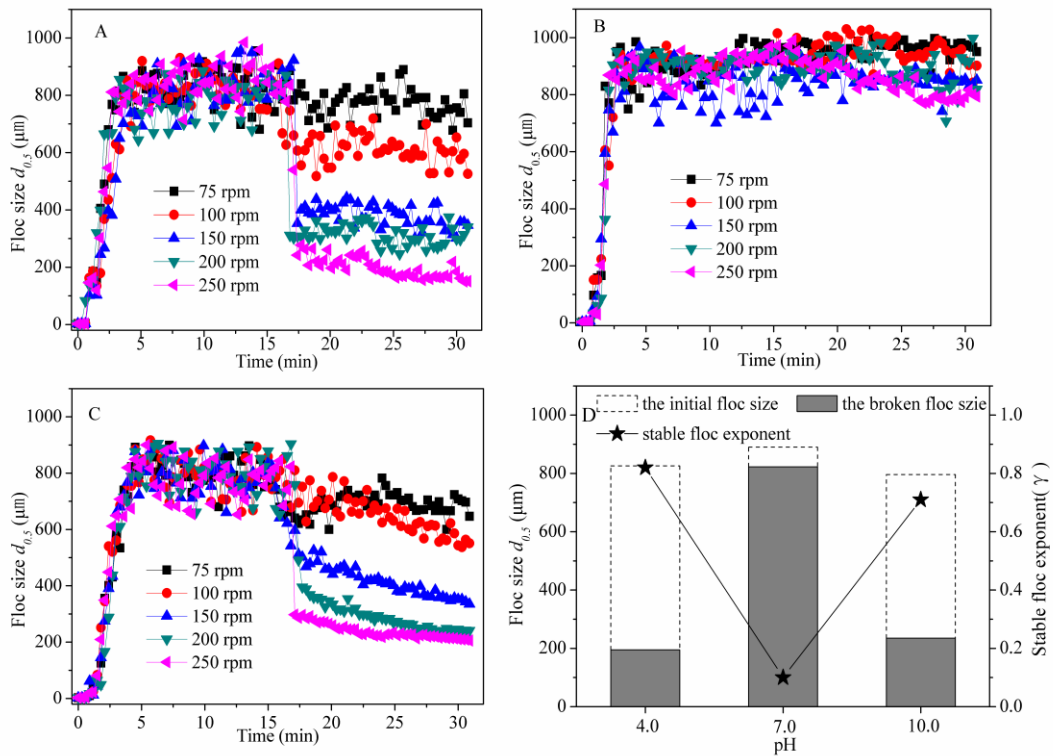


228

229 **Fig. 3** – The floc growth (A) and flocculation kinetics (B) at different dosage of  
 230 cationic starch. The soil concentration was fixed to 100 mg/L. Error bars indicate  
 231 standard deviations.

### 232 3.4. Effect of floc breakage

233 The algal removal using CS modified soil was not significantly influenced by the  
 234 pH condition in the wide pH range of 4.0-10.0 (Fig. S2). However, the algal floc  
 235 stability was greatly affected by the pH condition. When the shear speed was 75, 100,  
 236 150, 200 and 250 rpm, the floc size at pH 4.0 dropped sharply from 826  $\mu\text{m}$  to 777,  
 237 615, 372, 313 and 195  $\mu\text{m}$ , respectively (Fig. 4A); and the floc size at pH 10.0  
 238 dropped from 796  $\mu\text{m}$  to 687, 659, 428, 288 and 235  $\mu\text{m}$ , respectively (Fig. 4B). In  
 239 contrast, the sharp drop of floc size was not observed for the flocs formed at neutral  
 240 pH. The floc size only reduced slightly from 890 to 823  $\mu\text{m}$ , even when the highest  
 241 shear speed of 250 rpm was applied (Fig. 4C). The broken floc size was plotted  
 242 against the G value on a log-log scale according to Eq. (1). The value of floc stability  
 243 exponent ( $\gamma$ ) was obtained from the linearization of the equation, which was 0.82,  
 244 0.10 and 0.71 at pH 4.0, 7.0 and 10.0, respectively (Fig. 4D).



245

246

247 **Fig. 4** – Floc breakage profiles at different pH conditions (A, pH = 4.0; B, pH = 7.0;

248 C, pH = 10.0) and the relationship between stable floc exponent and floc breakage

249 (shear speed = 250 rpm) as a function of pH (D). The dosage of the modified soil was

250 10 mg/L cationic starch - 100 mg/L soil.

### 251 3.5 Algal flocculation using lake bloom water

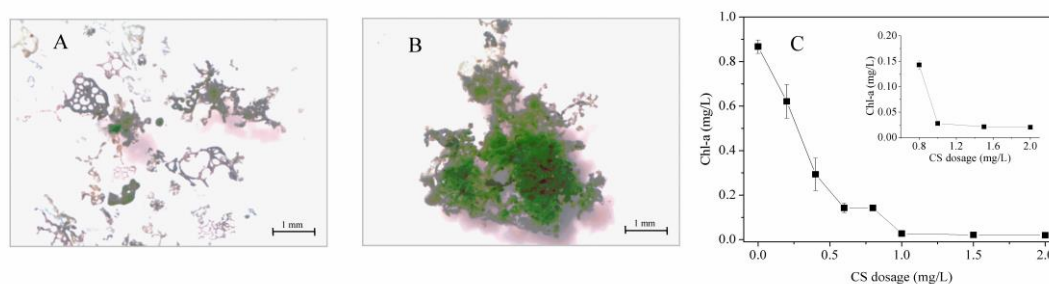
252 *M. aeruginosa* in the field is often in colonial form with several hundred

253 micrometers in diameter (Fig 5A). After CS modified soil was added, large flocs of

254 about 4 mm were formed (Fig 5B). With algal cells settled, the *chl-a* concentration in

255 water column was reduced. The optimized dosage of CS was 1.0 mg/L, where the

256 *chl-a* was decreased from 0.8 to 0.03 mg/L (Fig 5C).



257

258 **Fig. 5** – Flocculation of lake bloom water using cationic starch modified soil. (A)

259 field colonial *Microcystis aeruginosa* (Lake Taihu, China); (B) the formed flocs; (C)

260 *Chlorophyll-a* (*Chl-a*) concentration at different dosage of cationic starch (CS). The

261 soil concentration was 120 mg/L. Error bars indicate standard deviations.

## 262 4. Discussion

### 263 4.1. Charge neutralization and algal flocculation

264 When the soil was modified by CS, the surface charge of CS modified soil was

265 switched from negative to positive under wide pH range less than 11.8 (Fig. 1). This is

266 essential for the modified soil particles to obtain flocculation potential for negatively

267 charged algal cells, since charge neutralization can reduce the electrostatic repulsion

268 between algal cells, and allow aggregation to occur. The long chain of CS is critical

269 for the formation of large flocs through bridging function. Thus, the two-component

270 modifier mechanism of charge and bridging can be realized by CS alone, which is

271 more convenient for practical application.

272 At the low CS dosage of 5 mg/L, limited algal removal efficiency of 71% yet

273 reasonably large flocs of 700  $\mu\text{m}$  were achieved (Fig. 3A, B). This is because when

274 CS was lowly dosed, only parts of algal cells were combined with the modified soil

275 particle surfaces through charge neutralization. The adsorption of algal cells

276 neutralized the positive charge of the modified soil (+2.5 mV) and reduced  
277 electrostatic repulsion between the formed flocs, making the flocs easily bridged into  
278 reasonably large ones (700  $\mu\text{m}$ ) by the long chains of CS (Fig. 3A). The  
279 sedimentation of these large flocs was therefore fast (Fig. 3B). For algal cells left in  
280 the overlying water, the ZP was increased from -46.2 to -9.9 mV (Fig. 2), indicating  
281 that flocculation is only happened among parts of algal cells. At the CS dosage of 10  
282 mg/L, enough positive charges were provided by CS modified soil to catch up more  
283 algal cells, which led to a high removal efficiency of 86%. The charge neutralization  
284 reduced electrostatic repulsion between the formed flocs (+3.4 mV) and promoted the  
285 flocs into large ones (830  $\mu\text{m}$ ) with the bridging of CS chain (Fig. 3A). The  
286 flocculation kinetics was therefore fast (Fig. 3B). At the high CS dosage, dispersion  
287 re-stabilization was observed. Excess positive charges provided by CS caused the  
288 formed flocs positively charged (+33.5 mV, Fig. 2) and re-established electrostatic  
289 repulsion between flocs. The flocs were thereby hardly bridged into large ones (440  
290  $\mu\text{m}$ , Fig. 3A), which led to the low removal efficiency at high CS dosage of 80 mg/L  
291 (45% in 30 min, Fig. 3B). Thus, the combination of charge neutralization and  
292 bridging mechanisms operates the algal flocculation using CS modified soil.

293 A jar test using the field samples is always necessary to assure the algal removal  
294 effect and optimize the material dosage before practical application. Algal  
295 flocculation using natural bloom water from Lake Taihu (China) indicated that large  
296 flocs could be formed and colonial *M. aeruginosa* could be effectively removed using  
297 CS modified soil (Fig. 5). The *chl-a* concentration was decreased from 0.8 to 0.03

298 mg/L at the optimal CS dosage of 1.0 mg/L (Fig. 5C). Compared with dispersed  
299 single *M. aeruginosa* in the lab, colonial ones in the field often have large size and  
300 low hydrophilicity, which make them easily flocculated (or need low CS loading).

301 Soil particles may have great influence on algal flocculation kinetics. In addition to  
302 providing the mass or ballast to speed up the floc sedimentation, soil particles not  
303 only play as carriers to maintain the modifier concentration on solid surfaces (rather  
304 than dissolve large amount of flocculants in the water column), but also enhance the  
305 collision frequency between particles, which is crucial to flocculation dynamics (both  
306 particle size and concentration). If the modifiers are used alone without soil particles,  
307 the formed flocs may still float in the water column with the aid of buoyancy (Fig. S3).  
308 Harvesting measures such as air flotation and mechanical collection will be needed to  
309 achieve algal removal, which inevitably adds substantial extra work and costs.  
310 Although soil particles may consume parts of CS (9% in this study, Fig. S4), it is  
311 worthwhile to slightly increase the loadings of cationic starch to achieve the  
312 sedimentation removal of algal cells. With algal blooms settled by the modified soil,  
313 water transparency can be increased and excess nutrients are transferred from water to  
314 sediment under the capping layer with the aid of capping treatment (Pan et al., 2011b;  
315 Pan et al., 2012). The enhanced water transparency creates a favorable environment  
316 for the growth of submerged vegetation. It is possible for the sealed algal biomass to  
317 be turned into fertilizers for the growth of submerged vegetation (Pan et al., 2011b;  
318 Zhang et al., 2010).

#### 319 **4.2. Floc stability**



320 The  $\gamma$  value quantitatively describes how the floc size changes when flocs are  
321 exposed to a series of shear rates. Generally, a larger  $\gamma$  means the floc stability is  
322 lower and the floc is more prone to be broken (Jarvis et al., 2005). The  $\gamma$  value was  
323 0.82 and 0.71 at pH 4.0 and pH 10.0, respectively, which were much higher than the  $\gamma$   
324 (0.10) at pH 7.0 (Fig. 4D). This indicated that the flocs formed at acidic and alkaline  
325 conditions are less stable and more prone to be broken into smaller fragments than  
326 those formed at the neutral condition. At the shear rate of 250 rpm ( $G=141.7 \text{ s}^{-1}$ ), the  
327 floc size at pH 4.0 and pH 10.0 dropped sharply from 826 to 195  $\mu\text{m}$  and from 796 to  
328 235  $\mu\text{m}$ , respectively, while the floc size at pH 7.0 only slightly reduced from 890 to  
329 823  $\mu\text{m}$  (Fig. 4). The surface charge of algal cells was affected by pH conditions. The  
330 cell surface is less negatively charged at acid conditions and more negatively charged  
331 at alkaline conditions (Fig, S5). This may introduce some repulsion in algal flocs and  
332 weaken the adsorptive binding in algal flocs, which leads to the low floc stability  
333 (Slavik et al., 2012).

334 Cyanobacterial blooms often elevate water pH and sometimes increase the pH as  
335 high as 9.5 (Wang et al., 2013). Since quaternary amine on the polymer does not  
336 easily dissociate as pH changes (Wang et al., 2011a), the surface charge of CS  
337 modified soil was stable in the pH range from 2.0 to 10.0 (Fig. 1), and algal removal  
338 is less affected by the pH condition within this range (Fig. S2). However, the floc  
339 breakage might occur when flocs are exposed to high turbulence. The broken flocs are  
340 often subject to re-suspension and lead to the lost of algal removal. For practical  
341 application, additional measures such as capping might be helpful in solving the

342 re-suspension problem (Pan et al., 2012).

### 343 **4.3. Cost evaluation**

344 Economic cost is often a limiting factor affecting large scale application of the  
345 method in fields, and the cost reduction can be critically dependent on technical  
346 breakthrough. Although many modifiers can be used to turn soils into effective algae  
347 flocculants (Li and Pan, 2013; Pan et al., 2011a), there may be a great difference in  
348 cost. For example, the use of MO would be economically impractical at the places  
349 where MO is non-indigenous, since MO can be very expensive when they are  
350 exported to some places (Table S1). In this study, the cost of CS is estimated to be  
351 1650 US\$/ton, which is more expensive than PAC (650 US\$/ton) but much cheaper  
352 than chitosan (22,800 US\$/ton) and MO (seeds, 96,074 US\$/ton) in China (Table S1).  
353 The modifier cost of using CS modified soil to achieve algal removal efficiency of  
354 ~86% is about 0.02 US\$/m<sup>3</sup> at the optimal CS dosage of 10 mg/L. The similar algal  
355 removal could be achieved by 2 mg/L chitosan-10 mg/L PAC modified soil or 2 mg/L  
356 chitosan-3 mg/L MO modified soil, where the modifier cost is 0.05 and 5.72 US\$/m<sup>3</sup>,  
357 respectively.

### 358 **4.4. Environmental implications**

359 In recent years, geo-engineering has triggered much interest as a tool for  
360 eutrophication control, which can offer the promise of rapid effects (Lürling and  
361 Faassen, 2012; Lürling and van Oosterhout, 2013; Meis et al., 2013). Economic cost  
362 and ecological safety are among the major concerns in its application (Spears et al.,  
363 2014; Spears et al., 2013). As the raw material, starch is globally distributed, allowing

364 the mass production of cheap CS. The biodegradability and the flocculation effect of  
365 CS make it possible to be used at low dosage together with soil particles for natural  
366 bloom water treatment. When combined with soil particles, the biotic toxicity of  
367 cationic starch can be significantly reduced, which was specifically studied in another  
368 study (Wang et al., in this issue). However, the long-term effect on aquatic ecological  
369 system even at low dosage is unclear. Further study is needed to evaluate its impacts.  
370 Previous studies indicated that, despite the distinct properties, the soil of different  
371 origin can often obtain algal flocculation ability after suitable modification (Zou et al.,  
372 2006). The local soil collected from lakeside may reduce the transportation cost  
373 However, contaminated soil (by heavy metals and fertilizers etc.) is not recommended  
374 to be used. In fields, washing and particle fractionation approach can be used to select  
375 large amount of fine soil particles, and suitable engineering facilities (such as screw  
376 turbine) may be used for mixing.

377 Although CS is biodegradable, it might be a source of oxygen demand and some  
378 settled algal cells may be liberated as it decays in field applications. For these  
379 practical problems, it cannot be solved based on flocculation treatment alone. Other  
380 measures, such as capping treatments (especially oxygen nanobubble modified one),  
381 should be jointly applied after flocculation (Pan et al., 2012; Pan and Yang, 2012).  
382 The improved water (by flocculation) and sediment (by capping) environment may  
383 create a window period for the restoration of submerged vegetation. The sediment  
384 manipulation and submerged vegetation restoration may further affect C, N and P  
385 fluxes across the sediment-water and air-water interfaces (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub> and N<sub>2</sub>O etc.),

386 which may trigger multi-disciplinary studies in the future.

## 387 **5. Conclusions**

388 Dispersal of CS modified local soils achieved effective removal of cyanobacterial  
389 cells with the operation of charge neutralization and bridging mechanisms. Water pH  
390 condition did not significantly influence algal removal effect except the floc stability.  
391 The flocs formed at acid and alkaline conditions were more prone to be broken than  
392 those at the neutral condition. This method greatly reduces the cost and  
393 biodegradability concerns by using cheaply available and environmental friendly  
394 materials such as local soils and cationic starch. With some additional studies, this  
395 approach may be practically useful as a geo-engineering tool for cyanobacterial  
396 bloom control.

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