

1 **Effect of algal flocculation on dissolved organic matters using cationic starch**
2 **modified soils**

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31 **Abstract:** Modified soils are being increasingly used as geo-engineering materials for
32 the sedimentation removal of cyanobacterial blooms. However, when organic
33 flocculants were used as soil modifiers, little is known about the potential impacts of
34 these materials on the treated water. This study investigated dissolved organic matters
35 in the bloom water after algal removal using cationic starch modified soils (CS-MS).
36 Results showed that the dissolved organic carbon (DOC) could be decreased by
37 CS-MS flocculation and the use of higher charge density CS yielded a greater DOC
38 reduction. When CS with the charge density of 0.052, 0.102 and 0.293 meq/g were
39 used, dissolved organic carbon (DOC) was decreased from 3.4 to 3.0, 2.3 and 1.7
40 mg/L, respectively. The excitation-emission matrix fluorescence spectroscopy and
41 UV₂₅₄ analysis indicated that CS-MS exhibits an ability to remove some soluble
42 organics, which contributed to the DOC reduction. However, the use of low charge
43 density CS posed a potential risk of DOC increase due to the high CS loading for
44 effective algal removal. When CS with the charge density of 0.044 meq/g was used,
45 DOC was increased from 3.4 to 3.9 mg/L. This study suggested that by optimizing
46 charge density of CS, the use of organic MS-CS flocculants can achieve the
47 sedimentation removal of cyanobacterial cells without increasing the content of
48 dissolved organic matters in bloom waters. For the settled organic matters, other
49 measures (e.g., capping treatments using oxygen loaded materials) should be jointly
50 applied after algal flocculation.

51 **Keywords:** Cyanobacterial bloom; Cationic starch; Modified soil; Charge density;
52 Dissolved organic carbon

53

54 **Introduction**

55 The frequent outbreak of cyanobacterial blooms in eutrophic waters is a global issue,
56 posing serious threats to aquatic ecosystem and human health (Chen et al., 2006; Liu
57 and Le, 2015; Lu et al., 2013; Wang et al., 2013c). Modified soils (MS) have
58 triggered great interest as geo-engineering materials for cyanobacterial bloom control
59 in recent years (Lürling and van Oosterhout, 2013; Mackay et al., 2014; Spears et al.,
60 2014; Tian et al., 2014). In this method, modifiers offer soil particles the abilities of

61 charge neutralization and bridging, and make them obtain flocculation potential for
62 cyanobacterial cells (Li and Pan, 2013; Pan et al., 2011a).

63 Several chemical coagulants (e.g., aluminum and ferric salts) and organic
64 macromolecular flocculants (e.g., cationic starch, chitosan and *Moringa oleifera*)
65 have been tested as soil modifiers for algal removal (Dai et al., 2015; Li and Pan,
66 2013; Pan et al., 2006; Shi et al., 2015). Compared with chemical modifiers, natural
67 polymers are often easily biodegradable, eco-friendly and are well accepted by the
68 public (Mukherjee et al. 2014; Ndabigengesere and Narasiah, 1998; Renault et al.,
69 2009). However, when organic flocculants are sprayed into natural waters, a major
70 concern is the likelihood of dissolved organic matter (DOM) increase in water column.
71 The DOM is a storage pool of nitrogen and phosphorous and an important contributor
72 of biochemical/chemical oxygen demand in eutrophic waters (Bushaw et al., 1996;
73 Qu et al., 2013). The increase of DOM may result in the lost of water quality and the
74 rapid oxygen depletion in receiving water (Evans et al., 2005; Mermillod-Blondin et
75 al., 2005). On the other hand, with the operation of charge neutralization, some
76 autochthonous soluble organic matters may be simultaneously flocculated and settled
77 during algal flocculation using the positively charged MS (Lee and Westerhoff, 2006;
78 Wang et al., 2013b), and DOM reduction may occur after algal removal. However, to
79 date, the use of organic flocculants in MS has mainly focused on algal removal effect
80 with little consideration of its impacts on DOM in the treated bloom water.

81 Algae particles are negatively charged, which can stably suspend in water column
82 (Chen et al., 2004). Charge neutralization is a main mechanism operating algal
83 flocculation using MS, which can reduce the electrostatic repulsion and promote
84 aggregation between MS and algae particles (Li and Pan, 2013; Shi et al., 2015). To
85 achieve the effective algal removal, desired amount of positive charges need be
86 provided to neutralize negative charges on algal cell surface (Li and Pan, 2015; Li et
87 al., 2015). Thus, the charge density may potentially affect the loadings of modifiers,
88 which are directly related to the residual DOM in the treated water. Identifying and
89 understanding of this effect is essential to control DOM content in MS treated bloom
90 water and develop new soil modifiers for cyanobacterial bloom mitigation.

91 In this study, a series of cationic starch (CS) with different charge densities were
92 used as soil modifiers (CS-MS). The flocculation of *Microcystis aeruginosa* (*M.*
93 *aeruginosa*) using each CS-MS were conducted by jar tests. Dissolved organic carbon
94 (DOC), UV absorbance at 254 nm (UV₂₅₄) and excitation-emission matrix (EEM)
95 fluorescence spectra were used to analyze the DOM after algal removal. The objective
96 of this study is to explore the effect of algal flocculation on DOM in bloom waters
97 using CS-MS.

98 **1. Materials and methods**

99 **1.1. Algal species and culture**

100 *M. aeruginosa*, a common freshwater bloom-forming cyanobacterium, was used in
101 this study. The *M. aeruginosa* cells (FACHB-905) were obtained from the Institute of
102 Hydrobiology, Chinese Academy of Sciences, and cultivated in BG11 medium. The
103 BG11 medium was composed of 1,500 mg/L NaNO₃, 75 mg/L MgSO₄·7H₂O, 40
104 mg/L K₂HPO₄, 36 mg/L CaCl₂·2H₂O, 20 mg/L Na₂CO₃, 6 mg/L Citric acid
105 monohydrate, 6 mg/L Ferric ammonium citrate, 2.86 mg/L H₃BO₃, 1.86 mg/L
106 MnCl₂·4H₂O, 1 mg/L Na₂EDTA, 0.39 mg/L Na₂MoO₄·2H₂O, 0.22 mg/L
107 ZnSO₄·7H₂O, 0.08 mg/L CuSO₄·5H₂O, 0.05 mg/L Co(NO₃)₂·6H₂O. Algal batch
108 cultures were maintained at 25 ± 1°C under continuous cool white fluorescent light of
109 2,000-3,000 lux on a 12 h light and 12 h darkness regimen in an illuminating
110 incubator (LRH-250-G, Guangdong Medical Apparatus Co. Ltd., China).

111 **1.2. Cationic starch modified soils**

112 CS was prepared by reacting corn starch (Unilever Co. Ltd., China) with cationic
113 monomer, 2,3-epoxypropyl trimethyl ammonium chloride (GTA), using the
114 microwave-assisted method (Lin et al., 2012). The details of the synthesis are as
115 follows: 0.5 g GTA was dissolved in 100 ml of 5.0 g/L NaOH solution with constant
116 stirring for 10 min. 10.0 g starch was added to the above mixture and stirring was
117 continued for another 30 min at a 70°C water-bath. Then, the reaction vessel was
118 placed on the turntable of a domestic microwave oven (Guangdong Galanz Group Co.
119 Ltd., China) and irradiated at the power of 750 W with periodic pause to avoid boiling.
120 This microwave irradiation-cooling cycle was continued until a viscous gel-like mass

121 formed. After cooled down to room temperature, the gel-like mass was washed with
 122 ethanol for three times, dried in a vacuum oven (DZF-6020, Shanghai Yiheng
 123 Instrument Co. Ltd., China) at 50°C for 6 h and then pulverized before use. The
 124 obtained product is termed CS_{0.5:10} (0.5:10 is the mass ratio of GTA to starch). Using
 125 this method, CS_{1.0:10}, CS_{1.5:10} and CS_{2.5:10} were synthesized. As the mass ratio of GTA
 126 to starch increased, the charge density and degree of substitution of CS increased, but
 127 the intrinsic viscosity and molecular weight showed no significant changes (T-test, P
 128 < 0.05) (**Table 1**).

129

130

Table 1 The properties of cationic starch

	Charge density ^a (meq/g)	Degree of substitution ^b	Intrinsic viscosity ^c (dL/g)	Molecular weight ^d (×10 ⁵)
Native starch	0	0	1.21	4.27
CS _{0.5:10}	0.044	0.073	1.31	4.67
CS _{1.0:10}	0.052	0.092	1.24	4.39
CS _{1.5:10}	0.102	0.166	1.30	4.63
CS _{2.5:10}	0.293	0.255	1.08	3.76

131 ^a Determinated using the polyelectrolyte titration method (Kam and Gregory 1999).

132 ^b Determinated using one point method (Ahmad et al. 1999).

133 ^c Determined using element analysis method (Lin et al., 2012).

134 ^d Calculated based on the Mark-Houwink relationship taking '*k*' as 1.18×10^{-3} and '*a*'
 135 as 0.89 (Ahmad et al. 1999).

136

137 The soil used was collected from the bank of Meiliang Bay, Lake Taihu (China).
 138 This bay has suffered from severe cyanobacterial blooms over the past years, and MS
 139 materials have been tested to settle the blooms (Pan et al., 2006; Pan et al., 2011b).
 140 The soil sample was grounded and sieved (180 meshes) before use. For CS-MS
 141 preparation, a certain amount of CS was used to modify the soil suspension according
 142 to the dose conditions tested. The soil concentration used in all the flocculation
 143 experiments was fixed to 100 mg/L. The surface charge of soil and CS-MS particles

144 was characterized using a Zetasizer 2000 (Malvern Co. United Kingdom).

145 **1.3. Algal flocculation**

146 Flocculation experiments were conducted in a jar test apparatus (ZR3-6, Zhongrun
147 Water Industry Technology Development Co. Ltd., China) using *M. aeruginosa*
148 cultures in mid- to late-exponential growth phase. The initial cell concentration for all
149 the flocculation experiments was set to 3.2×10^9 cells/L. 200 ml of readily prepared
150 *M. aeruginosa* solution was transferred to 300-ml beaker, and then stirred at 200 r/min
151 for 1 min and 40 r/min for another 5 min after CS-MS was added. The control was run
152 in the above mentioned *M. aeruginosa* solution without adding any soil or CS. The
153 flocculation experiments were conducted at raw algal solution pH of 8.60. The pH
154 kept at 8.60 ± 0.2 after the addition of CS-MS. All the flocculation experiments were
155 conducted in triplicate.

156 Samples were collected from 2 cm below the water surface after floc sedimentation
157 for 30 min to enumerate the cell number using an Axioskop 2 mot plus microscope
158 (Carl ZEISS, Germany). The algal removal efficiency was calculated as: (initial cell
159 concentration - sample cell concentration) / initial cell concentration $\times 100\%$. After
160 algal flocculation, 10 ml water sample was also collected to investigate the cell
161 surface charge using a Zetasizer 2000 (Malvern Co. United Kingdom).

162 **1.4. DOM analysis**

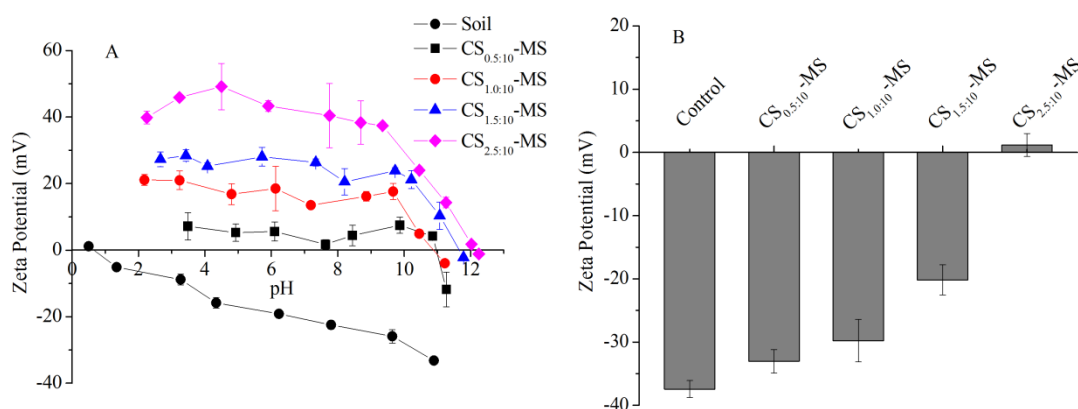
163 DOM analysis was performed after algal sedimentation by measuring DOC, UV₂₅₄
164 and EEM spectra. Water samples were filtered through 0.45- μ m membrane filters
165 before analysis. DOC was measured using a TOC analyzer (Liqui TOC II, Elementar,
166 Germany). UV₂₅₄ was determined using an UV-Vis spectrophotometer (756PC,
167 Shanghai Sunny Hengping Scientific Instrument Co. Ltd., China) at 254 nm with a
168 quartz cell path of 1 cm. The EEM spectra were measured using a fluorescence
169 spectrophotometer (F-7000, Hitachi High-Tech. Corp., Japan). The spectra were
170 collected with subsequent scanning emission spectra from 250 to 550 nm at 5 nm
171 increments by varying the excitation wavelength from 200 to 400 nm at 5 nm
172 increments. The excitation and emission slits were maintained at 5 nm and the
173 scanning speed was set at 1000 nm/min. The spectrum of pure water was used as the

174 blank.

175 2. Results

176 2.1. Surface charge of CS-MS particles

177 After CS modification, the isoelectric point of soil particles was remarkably increased
178 from pH 0.5 to above pH 10.8 (**Fig. 1A**), which made CS-MS particles positively
179 charged under most natural water conditions. However, the surface charge of CS-MS
180 particles varied with the charge density of CS. When high charge density CS was used,
181 CS-MS particles possessed high surface charge (**Fig. 1A**) and gained a high ability of
182 charge neutralization under natural water conditions. When 21 mg CS-MS (20 mg soil
183 modified with 1 mg CS) was added to 200 ml *M. aeruginosa* solution with 6.4×10^8
184 cells, an increase was caused in the surface charge of algal cells (**Fig. 1B**). The cell
185 surface charge was neutralized and increased from -37.5 mV to -33.0, -29.8, -20.2 and
186 +1.2 mV in CS_{0.5:10}-MS, CS_{1.0:10}-MS, CS_{1.5:10}-MS and CS_{2.5:10}-MS treatments,
187 respectively (**Fig. 1B**).



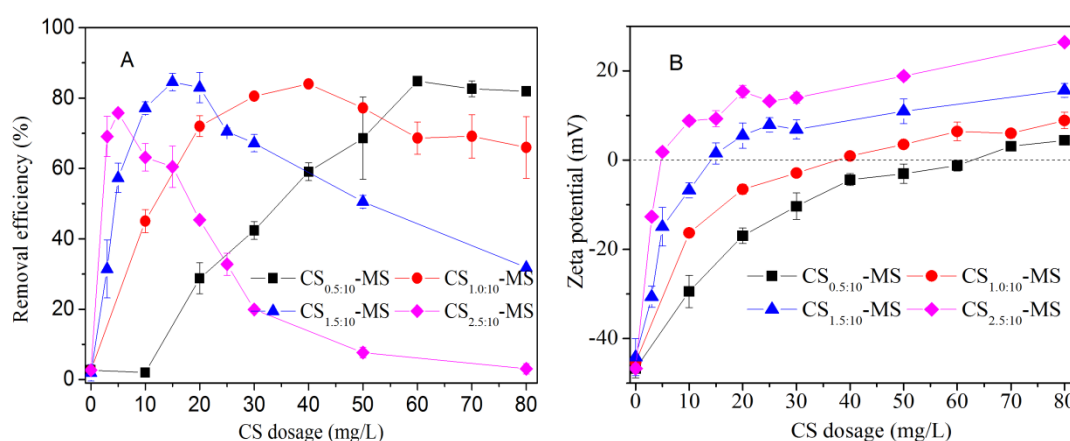
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189 **Fig. 1** – Surface charge of soil particles after CS modification (A) and the increase of
190 *M. aeruginosa* surface charge after the addition of CS-MS (B). Error bars indicate
191 standard deviations.

192 2.2. Algal flocculation using CS-MS

193 After CS-MS was added, a maximum removal efficiency of about 85% for *M.*
194 *aeruginosa* cells was achieved, but the optimal CS dosage varied greatly depending
195 on its charge density, which was 60, 40 and 15 mg/L for CS_{0.5:10}, CS_{1.0:10} and CS_{1.5:10},
196 respectively. In CS_{2.5:10}-MS treatment, the removal efficiency just maximally reached

197 76% at the CS dosage of 5 mg/L (**Fig. 2A**). Once CS was overdosed, the removal
 198 efficiency exhibited a decreasing trend (**Fig. 2A**). As the CS dosage further increased
 199 to 80 mg/L, the removal efficiency gradually decreased to 81%, 66% and 31% in
 200 CS_{0.5:10}-MS, CS_{1.0:10}-MS and CS_{1.5:10}-MS treatments, respectively; in contrast, the
 201 removal efficiency in CS_{2.5:10}-MS treatment dropped sharply and reached less than 10%
 202 at the 80 mg/L of CS (**Fig. 2A**). The surface charge of algal cells as a function of CS
 203 dosage was measured. As the CS dosage increased, the cell surface charge was
 204 increased and charge reversal occurred around the optimal dosage of CS (**Fig. 2B**). At
 205 the 80 mg/L of CS, the cell surface charge reached +4.4, +8.9, +15.6 and +26.4 mV in
 206 CS_{0.5:10}-MS, CS_{1.0:10}-MS, CS_{1.5:10}-MS and CS_{2.5:10}-MS treatments, respectively (**Fig.**
 207 **2B**).

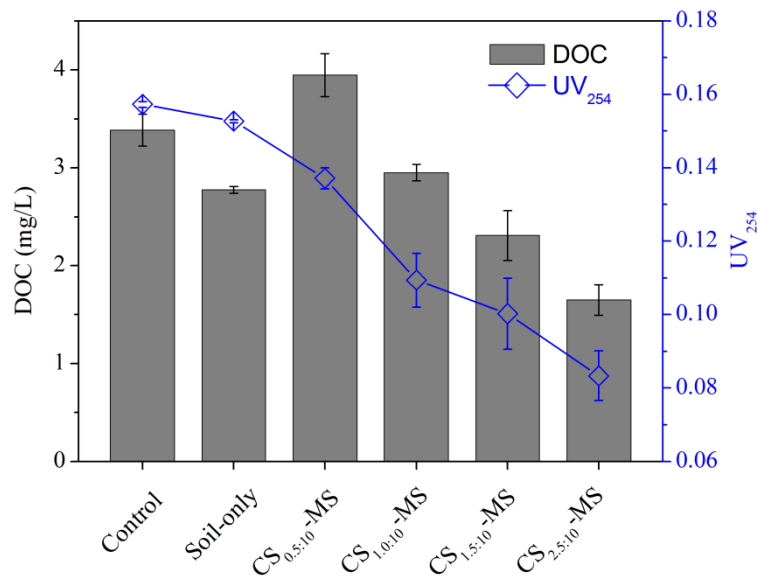


208
 209 **Fig. 2** – Algal removal efficiency and surface charge of algal cells at different dosage
 210 of CS. The soil concentration was fixed to 100 mg/L. Error bars indicate standard
 211 deviations.

212 **2.3. DOC and UV₂₅₄**

213 After algal removal, the DOC in algal solution showed a decrease and the use of
 214 higher charge density CS yielded a greater DOC reduction. The DOC was decreased
 215 from 3.4 to 3.0, 2.2 and 1.7 mg/L in CS_{1.0:10}-MS, CS_{1.5:10}-MS and CS_{2.5:10}-MS
 216 treatments, respectively. However, the use of low charge density CS resulted in the
 217 DOC increase after algal removal. The DOC in CS_{0.5:10}-MS treatment was increased
 218 from 3.4 to 3.9 mg/L (**Fig. 3**). The UV₂₅₄ reduction was observed in all the treatments,
 219 which was enhanced by higher charge density CS. After algal removal, the UV₂₅₄ was

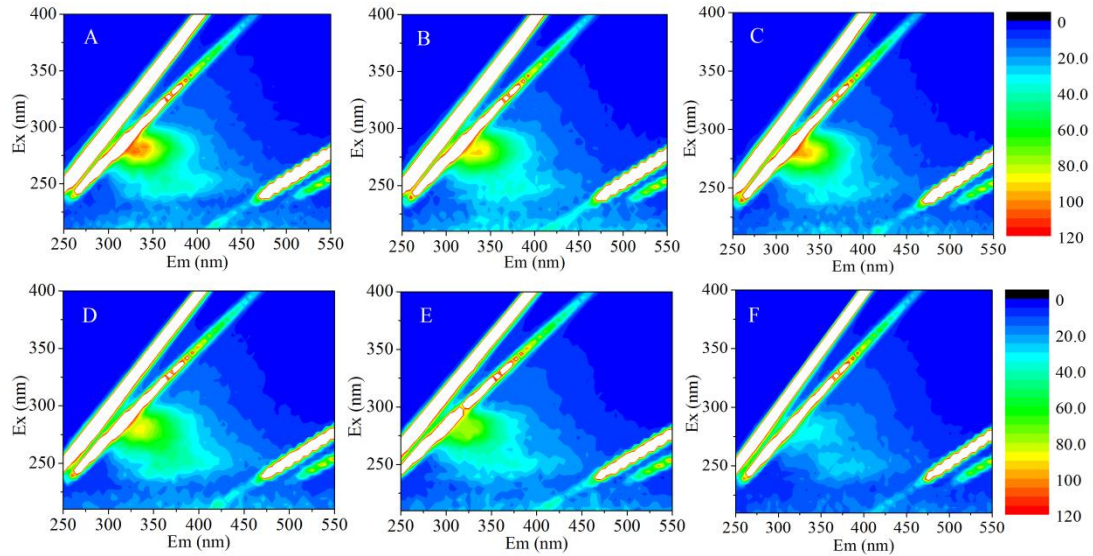
220 decreased from 0.16 to 0.14, 0.11, 0.10 and 0.08 in CS_{0.5:10}-MS, CS_{1.0:10}-MS,
 221 CS_{1.5:10}-MS and CS_{2.5:10}-MS treatments, respectively. The soil-only treatment could
 222 also lead to limited DOC (from 3.4 to 2.8 mg/L) and UV₂₅₄ (from 0.16 to 0.15)
 223 reductions in algal solution (**Fig. 3**).



224
 225 **Fig. 3** – The changes of DOC and UV₂₅₄ in algal solution after algal removal at the
 226 optimal dosage of CS. The soil concentration was fixed to 100 mg/L. Error bars
 227 indicate standard deviations.

228 **2.4. EEM fluorescence spectra**

229 One main peak at 270|285/320-345 nm (Ex/Em) was observed from the EEM
 230 fluorescence spectra of algal solution (**Fig. 4**), which were identified as soluble
 231 microbial by-product-like organics, including tyrosine-, tryptophan and protein-like
 232 components (Osburn et al., 2012; Guo et al. 2010). After algal removal, the peak
 233 intensity showed a decrease, suggesting that the soluble organics were removed by
 234 CS-MS during flocculation-sedimentation process. The removal of these organics was
 235 enhanced by CS with the higher charge density (**Fig. 4**). When CS_{0.5:10}, CS_{1.0:10},
 236 CS_{1.5:10} and CS_{2.5:10} were used in MS, the peak intensity was decreased from 110.4 to
 237 98.5, 78.5, 73.1 and 33.9, respectively. In soil-only treatment, the peak intensity was
 238 also decreased to 80.2 (**Fig. 4**).



239

240 **Fig. 4** – EEM fluorescence spectra of algal solution after algal removal using CS-MS.
 241 (A) Control, (B) Soil-only, (C) CS_{0.5:10}-MS, (D) CS_{1.0:10}-MS, (E) CS_{1.5:10}-MS, (F)
 242 CS_{2.5:10}-MS.

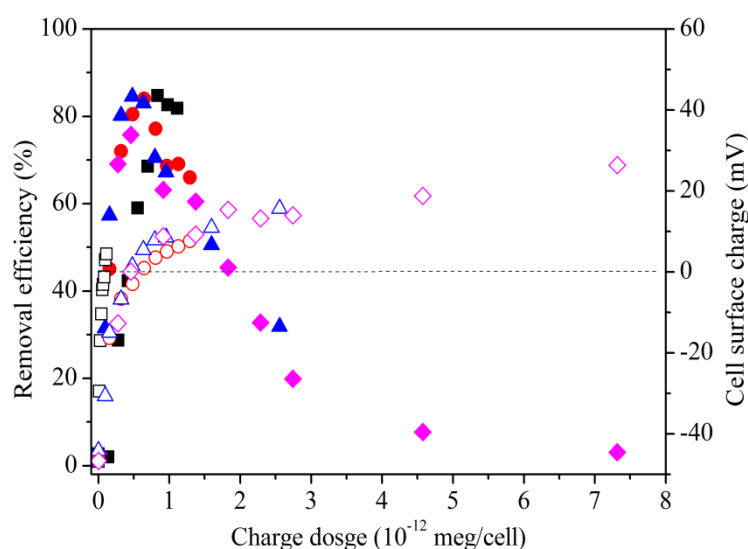
243 3. Discussion

244 3.1. Charge density and the optimal dosage of CS

245 Charge neutralization is essential in creating algal flocculation potential by
 246 eliminating energy barrier for the aggregation between flocculants and algae particles
 247 (Liu et al., 2010; Phoochinda et al., 2004). When CS with the higher charge density
 248 was used, CS-MS particles possessed the higher surface charge (**Fig. 1A**) and gained
 249 a higher ability of charge neutralization (**Fig. 1B**). The optimal algal flocculation
 250 occurred around the point of total charge neutralization (zeta potential close to zero,
 251 **Fig. 2B**). Thus, CS-MS with the higher charge neutralization ability achieved
 252 effective algal removal at a lower CS loading, simply because, for a given dosage,
 253 they can deliver more positive charges to the cell surface. At the soil concentration of
 254 100 mg/L, the optimal CS dosage for the removal of 3.2×10^9 *M. aeruginosa* cells/L
 255 followed the order of CS_{0.5:10} > CS_{1.0:10} > CS_{1.5:10} > CS_{2.5:10}, which was 60, 40, 15 and
 256 5 mg/L, respectively (**Fig. 2A**).

257 Based on the dosage and charge density of CS, algal removal efficiency and cell
 258 surface charge are re-plotted against positive charges in **Fig. 5**. It further indicated
 259 that positive charges play a key role in algal flocculation and the data sets take on a

260 similar shape in each CS-MS treatment. As the positive charge dosage increased, algal
 261 removal efficiency increased and reached the maximum at the charge dosage of 0.9
 262 $\times 10^{-12}$ meq/cell. The cell surface charge was simultaneously increased from -46.2 to 0
 263 mV. Once positive charges were overdosed, the removal efficiency decreased, since
 264 the reversal of cell surface charge (**Fig. 2B**) potentially reintroduced the electrostatic
 265 repulsion and prevented aggregation between CS-MS and algae particles (Jangkorn et
 266 al. 2011; Mosse et al., 2012; Shi et al., 2015). Hence, to achieve effective algal
 267 removal using MS, the modifier loading mainly depended on the charge density. The
 268 loadings of low charge density organic modifiers are often high to provide enough
 269 positive charges, which potentially introduce substantial amount of organic matters
 270 into the receiving water. It is important to notice that flocculants with excessively
 271 high charge density are either not recommended to be used as soil modifiers. The low
 272 modifier loadings can not offer adequate macromolecular chains for bridging effect in
 273 algal flocculation and may lead to limited algal removal (e.g., CS_{2.5:10} in **Fig. 2A**) (Li
 274 and Pan, 2013; Zou et al., 2005). Additionally, a small change in the loading of high
 275 charge density modifier can lead to significant differences in the amount of positive
 276 charges provided, which results in a narrow dosage range for effective algal removal
 277 (e.g., CS_{2.5:10} in **Fig. 2A**). This hinders the application of MS in fields where precise
 278 dosage control is often difficult.



279
 280 **Fig. 5** – Algal removal efficiency and cell surface charge at different dosage of

281 positive charge. The closed and open symbols represent algal removal efficiency and
282 cell surface charge, respectively. CS_{0.5:10}-MS (squares), CS_{1.0:10}-MS (circles),
283 CS_{1.5:10}-MS (triangles), CS_{2.5:10}-MS (diamonds).

284 **3.2. DOM after algal removal**

285 One main aim of algal removal is to prevent the occurrences of water quality
286 deterioration and oxygen depletion in bloom waters. The application of organic algal
287 flocculants poses a potential risk of DOM increase in water column, making algal
288 removal become meaningless. However, DOC analysis indicated that the DOC
289 content in algal solution was decreased after algal removal using CS-MS. The DOC
290 was decreased from 3.4 to 3.0, 2.3 and 1.7 mg/L in CS_{1.0:10}-MS, CS_{1.5:10}-MS and
291 CS_{2.5:10}-MS treatments, respectively (**Fig. 3**). The EEM fluorescence spectroscopy
292 (**Fig. 4**) and UV₂₅₄ analysis (**Fig. 3**) indicated that some soluble organic matters could
293 be removed by CS-MS during flocculation-sedimentation process, which contributed
294 to the DOC reduction. Although CS_{10:0.5}-MS also exhibited the ability to remove some
295 autochthonous DOM (**Fig. 4** and **Fig. 1**), the high CS loading for the effective algal
296 removal potentially introduced substantial amount of allochthonous organic matters
297 into water column and increased DOC from 3.4 to 3.9 mg/L after algal removal (**Fig.**
298 **3**). Thus, in practice, organic flocculants with low charge density are not
299 recommended to be used as soil modifiers in the MS method.

300 By optimizing the charge density of modifiers, algal cells can be flocculated and
301 settled by MS without increasing DOM in water column. This is necessary although
302 not sufficient for the ecological restoration in eutrophic waters due to the
303 decomposition of the settled organics at surface sediment. Other measures, such as
304 capping treatments using oxygen loaded materials, should be jointly applied after
305 flocculation. Previous studies indicated that nutrient fluxes across sediment-water
306 interface can be reduced or even reversed and surface sediment conditions can be
307 improved by proper capping treatments within a certain period of time (e.g., weeks)
308 (Pan et al., 2012; Pan and Yang, 2012). This may create a window period for the
309 restoration of submerged vegetation. Then, it is possible for the sealed organic matters
310 in the capping layer to be turned into fertilizers for the growth of submerged

311 vegetation (Pan et al., 2011b; Zhang et al., 2010).

312 **3.3. Co-removal potential of algal cells and DOM**

313 During algal blooms, substantial amount of DOM are often accumulated due to the
314 release and lysis of algal cells, which is an important nutrient pool in eutrophic waters
315 (Nguyen et al., 2005; Ye et al., 2011). The co-removal of algal cells (particulate
316 nutrients) and DOM (dissolved nutrients) may maximize the nutrient reduction and
317 benefit the ecological restoration in eutrophic waters.

318 During algal flocculation using CS-MS, the decrease in both UV₂₅₄ value (**Fig. 3**)
319 and EEM fluorescence peak intensity (**Fig. 4**) was observed, indicating that CS-MS
320 flocculation has the potential to achieve the co-removal of algal cells and DOM.
321 When CS with the higher charge density was used, more DOM could be removed at
322 the lower optimal CS dosage for algal removal. The UV₂₅₄ was decreased from 0.16
323 to 0.14, 0.11, 0.10 and 0.08 (**Fig. 3**); and the fluorescence peak intensity at the Ex/Em
324 of 275-280/320-345 nm was decreased from 110.4 to 98.5, 78.5, 73.1 and 33.9 (**Fig. 4**)
325 in CS_{0.5:10}-MS, CS_{1.0:10}-MS, CS_{1.5:10}-MS and CS_{2.5:10}-MS treatments, respectively.
326 This suggested that the use of higher charge density CS makes MS not only possess
327 the higher ability of algal removal but also gain a higher ability of DOM removal.
328 Previous studies reported that DOM flocculation is mainly operated by charge
329 neutralization mechanism, which is similar to algal flocculation (Hussain et al., 2013;
330 Lee and Westerhoff, 2006). There may exist competitive binding of algal cells and
331 DOM to MS, and the optimal flocculation of algal cells and DOM may occur at
332 different dosage of CS. Further studies are required to explore the co-removal effect
333 of algal cells and DOM using CS-MS flocculation in bloom waters. Soil particles play
334 an important role in the DOC reduction during CS-MS flocculation. In the soil-only
335 treatment, the DOC and UV₂₅₄ decreased from 3.4 to 2.8 mg/L and from 0.16 to 0.15,
336 respectively (**Fig. 3**); and the fluorescence peak intensity at the Ex/Em of
337 275-280/320-345 nm decreased from 110.4 to 80.2 (**Fig 4B**). However, contaminated
338 soil (by heavy metals and fertilizers etc.) is not recommended to be used. In fields,
339 washing and particle fractionation method can be applied to select large amount of
340 fine soil particles (Li and Pan, 2013).

3.4. Implications for the development of soil modifiers

In the MS method, modifiers make soil particles obtain charge neutralization ability to create algal flocculation potential. Our results demonstrated that the charge density of modifier affects its loading for effective algal removal and thereby DOM content in the treated bloom water. It is necessary to screen flocculants with right charge density to be used as soil modifiers. The charge density can be optimized by regulating the cationic monomer dosage in the production. Confirmation of the optimum one can be determined by jar tests before field applications. Some high charge density materials can be jointly used to facilitate charge neutralization to reduce the loadings of organic modifiers according to the bicomponent modification method (Li and Pan, 2013, 2015b). Besides charge density, the molecular size and structure of modifier chains may affect the bridging function of MS and thereby algal flocculation. Further study is needed to evaluate this effect.

There are various polymers available for the production of cationic flocculants as soil modifiers, such as chitosan, tannin, polysaccharides and polyacrylamides (Yang et al., 2012; Wang et al., 2013a; Wang et al., 2013b). To produce eco-friendly geo-engineering materials, biodegradable natural polymers (e.g., polysaccharides) are preferred, while synthetic polymers (e.g., polyacrylamide) are not recommended to be used, since most synthetic polymer structures are resistant to biodegradation (Bolto and Gregory, 2007). Economic cost is often a main factor limiting large scale application of the method in fields. Cheaply available polymers (e.g., corn starch) should be used to reduce the material costs. Water pH often fluctuates during algal blooms and sometimes even increases as high as 9.5 (Wu et al., 2014). Cationic monomers with quaternary ammonium groups are preferred, since they can not be easily dissociated after grafted onto the polymer backbone as the pH condition changes (Bolto and Gregory, 2007).

4. Conclusions

CS could turn soil particles into effective flocculants for the sedimentation removal of cyanobacterial blooms. The charge density of CS has great influence on DOM content in the treated bloom water. Some soluble organic matters could be removed during

371 CS-MS flocculation and lead to DOM reduction. However, the use of low charge
372 density CS posed the potential risk of DOM increase in the receiving water due to the
373 high CS loading for effective algal removal. By optimizing the charge density of CS,
374 it is possible to achieve algal removal without increasing DOM content in eutrophic
375 waters.

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