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Hydrophobicity and iron coagulation of extracellular polymeric substance from colonial *Microcystis*

Peiliang Zhang, Shujie Jia, Xiaohong Zhang, Jing Li, Shan Lu and Pengfu Li

ABSTRACT

The bloom-forming cyanobacterium *Microcystis* occurs mainly as colonial aggregates under the natural conditions. This paper investigated the hydrophobicity and iron coagulation of extracellular polymeric substances (EPSs) from colonial *Microcystis* in order to understand the impact of EPS on the water treatment process. The higher contents of dissolved EPS (dEPS) and bound EPS (bEPS, mucilaginous matrix around the cells), lower dEPS/bEPS ratio and greater negative zeta potential of bEPS and dEPS were found in colonial *Microcystis* compared with unicellular *Microcystis*. XAD resin fractionation analysis indicated that the hydrophobicity could be ranked in an order as follows: bEPS > dEPS > dissolved extracellular organic matter (dEOM) for all the *Microcystis* strains. Correlation analysis showed that there was a statistically significant correlation between the amounts of carbohydrate and dissolved organic carbon in the hydrophobic fraction of EOM (dEOM, dEPS and bEPS), indicating that the hydrophobicity of *Microcystis* EOM might be related to carbohydrate. The coagulation experiment showed that for each colonial *Microcystis* strain, the removal efficiency of bEPS was higher than that of dEPS within the pH range from 3 to 10. The implications of the EPS characteristics were further discussed with respect to water treatment.

Key words | coagulation, extracellular organic matter, extracellular polymeric substance, hydrophobicity, water treatment, zeta potential

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INTRODUCTION

Algae, including cyanobacteria, green algae, diatoms and so on, secrete extracellular organic matter (EOM) during growth, which can be dissolved in the water body (Henderson *et al.* 2008). EOM has an adverse impact on the water treatment process in the production of drinking water, leading to serious membrane fouling in the application of membrane separation technology and increased coagulant demand (Sharp *et al.* 2006; Babel & Takizawa 2010).

Harmful cyanobacterial bloom caused by eutrophication of water bodies has become a worldwide environmental issue (Lehman *et al.* 2005). *Microcystis* is the most commonly reported bloom-forming cyanobacterial genus in lakes and reservoirs (Paerl *et al.* 2001). Like some other cyanobacteria, *Microcystis* produces large amounts of EOM (Forni *et al.* 1997). The EOM adhering to *Microcystis* cell surface is classified as the bound EOM (bEOM), while the EOM released into culture solution is classified as the dissolved EOM (dEOM). The dEOM produced by *Microcystis aeruginosa* causes serious membrane fouling during ultrafiltration in the production of drinking water, leading to a significantly decreased membrane permeability. Hydrophobic (HPO) adhesion, cake layer formation and pore plugging are the main mechanisms for membrane fouling caused by dEOM (Qu *et al.* 2012b). The bEOM of *M. aeruginosa*, which has greater HPO adhesion ability and is less electrostatic repulsive, leads to lower flux decline but more irreversible fouling, while dEOM causes more severe flux decline and reversible fouling due to its stronger hydrophilicity (Qu *et al.* 2012a). Coagulation by the inorganic coagulants, such as ferric salts, polyferric salts, aluminum salts and polyaluminum salts, is the most common approach applied for removal of algae and algogenic organic matter in water treatment due to low cost and ease of use (Ma *et al.* 2015; Tang *et al.* 2017). The removal efficiency of dEOM of *M. aeruginosa* by coagulation with polyaluminum chloride is low, while the removal efficiency of bEOM is high (Tang *et al.* 2017).

Extracellular polymeric substances (EPSs) are high-molecular-weight components of microbial EOM. Microbial

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EPS has been previously reported to deposit in the biofouling layer and membrane pores, and was suggested to be the dominant foulant in the membrane filtration system (Zhou *et al.* 2015; Tang *et al.* 2018). Cyanobacteria produce large amounts of EPSs, which are mainly composed of polysaccharide (Pereira *et al.* 2009). EPS is classified into dissolved EPS (dEPS) and bound EPS (bEPS). bEPS is mucilaginous matrix around the cells, while dEPS is the EPS released into the surrounding aqueous solution (Pereira *et al.* 2009). It has been reported that cell-associated polysaccharide mucilage (i.e. bEPS) in *Microcystis*-dominated bloom samples collected from the epilimnion of a eutrophic freshwater lake in U.K. occupies 0.007% of lake water volume. Under intense *Microcystis* bloom conditions, levels of cell-associated mucilage in the lake may reach 0.06% (Tien *et al.* 2002). *Microcystis* EPS contains neutral sugars (galactose, glucose, xylose, mannose, rhamnose, arabinose and fucose) and uronic acids (Plude *et al.* 1991; Chen *et al.* 2016). The acetate, sulfate and pyruvic groups as well as proteins are found in EPS of *Microcystis* (Chen *et al.* 2016). It takes more than 48 days to degrade *Microcystis* EPS by the natural bacterial community (Li *et al.* 2009). EPS may exist in the water body for a long time and affect water treatment. Therefore, it is urgently necessary to further characterize *Microcystis* EPS in order to evaluate its influence on the water treatment process.

It is well known that *Microcystis* occurs mainly as colonial aggregates with thousands of algal cells constrained by mucilaginous matrix under natural conditions (Otsuka *et al.* 2000). However, during a long-term cultivation under laboratory conditions, the algal colonies disaggregate, and *Microcystis* exists mainly as single cells (Zhang *et al.* 2007). Until now, only unicellular *M. aeruginosa* has been used to characterize *Microcystis* EOM in the literature. There is a lack of information on colonial *Microcystis*. *M. wesenbergii*, *M. aeruginosa* and *M. flos-aquae* are common bloom-forming species (Kurmayer *et al.* 2003). This paper investigated the hydrophobicity and iron coagulation of EPSs from three colonial *Microcystis* strains in order to understand the impact of EPS on the water treatment process.

MATERIALS AND METHODS

Isolation and cultivation of *Microcystis*

Microcystis strains were isolated from Lake Taihu, China. Morphological classification of colonial *Microcystis* strains was conducted during isolation using optical microscope (Eclipse E100, Nikon, Japan) (Yu *et al.* 2007). Cyanobacteria were grown in BG11 medium at 25 °C (Rippka 1988). After cultured for about 18 months in the laboratory, some strains maintained their colonial form, while others lost their characteristics of colonies and existed in a unicellular form. Three colonial strains (*M. wesenbergii* TH24, *M. aeruginosa* TH129 and *M. flos-aquae* TH183) and three unicellular strains (*M. wesenbergii* TH4, *M. aeruginosa* TH177 and *M. flos-aquae* TH60) were selected and used in this study. The cultures were continuously aerated with gentle supplement of filtered air. A light intensity of 40 μmol photons m^{-2} s^{-1} was provided by cool-white fluorescent lamps on a 12:12 h light: dark cycle.

Extraction of dEOM and EPS

After being grown for 30 days, *Microcystis* cultures of the late exponential phase were centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatants were sequentially filtered with 0.45- and 0.22-μm pore-size membranes to obtain dEOM. Furthermore, dEOM was dialyzed in dialysis tubes with a cutoff molecular weight of 3,500 Dalton against distilled water for 72 h to obtain dEPS. In order to obtain bEPS, the cell pellet harvested by centrifugation was extracted with 3% glutaraldehyde solution at 4 °C (Li *et al.* 2009). The extracted solution was centrifuged at 10,000 × *g* for 15 min at 4 °C, and subsequently the bEPS-containing supernatants were filtered and dialyzed using the above-described procedures.

EPS productivity

The extracted EPS samples were lyophilized after dialysis. The EPS content was determined by weighing the lyophilized EPS and normalized by the cell counts. Before the cell counting, the *Microcystis* colonies were disaggregated into single cells by ultrasonic treatment at 80 W and 20 Hz for 1 min (JY 92-IIN, Scientz, China). The cells were counted in a hemocytometer under an optical microscope.

Determination of dissolved organic carbon (DOC), zeta potential and specific ultraviolet absorbance (SUVA)

DOC concentration was determined with a TOC analyzer (liquiTOC II, Elementar, Germany), and potassium hydrogen phthalate ($KHC_8H_4O_4$) was used for calibration. Before DOC measurement, the sample was acidified with HCl to pH 2.0. Zeta potential was obtained by a zetasizer

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(Nano-Z, Malvern, UK) at pH 7.0. Ultraviolet absorbance at 254 nm (UV_{254}) was determined with a UV/Vis spectrophotometer (UV-1800, Mapada, China), and SUVA was calculated as UV_{254}/DOC. All the analyses were performed in triplicate.

Determination of carbohydrate and protein contents

The carbohydrate content was determined using glucose as standard by phenol-sulphuric acid method (Liu *et al.* 1973). The protein content was determined using bovine serum albumin as standard by the method of Bradford (Bradford 1976). All the determinations were conducted in triplicate.

Hydrophobicity analysis using XAD resin fractionation

Non-ionic macroporous sorbents XAD-8 (Sigma, USA) and XAD-4 (Amberlite, USA) were used to fractionate dEOM and EPS according to a previously described method (Carroll *et al.* 2000). The sample of approximately 10 mg L^{-1} DOC was acidified to pH 2.0. Clean resin volume was 60 mL in each 15-mm diameter column, and the flow rate was 300 mL h^{-1}. After passing successively through the XAD-8 and XAD-4 resins, the sample was fractionated into three fractions. The HPO and transphilic (TPI) fractions were adsorbed onto XAD-8 and XAD-4 resins, respectively. The hydrophilic (HPI) fraction passed through both resins. The HPO and TPI fractions were eluted with 0.1 M NaOH from the XAD-8 and XAD-4 resins, respectively. The DOC and carbohydrate contents of the three fractions were determined as mentioned above. Each fraction was adjusted to neutral pH prior to carbohydrate determination. Before fractionation, both resins were sequentially rinsed with 0.1 M NaOH, Milli-Q water, 0.1 M HCl, Milli-Q water, methanol and Milli-Q water. The washing procedure was repeated until the DOC of effluent from the XAD resin exhibited almost the same level as that of Milli-Q water. The recovery rate of the XAD resin fractionation was 90–99%. The experiments were carried out in triplicate.

Coagulation of EPS

EPSs, including dEPS and bEPS, of three colonial *Microcystis* strains were used in coagulation tests. The initial DOC concentration of EPSs was 5 mg L^{-1}, and 5.0×10^{-4} mol L^{-1} $NaHCO_3$ was added to provide a certain buffering capacity and maintain ionic strength. Ferric sulphate [$Fe_2(SO_4)_3$] was used as coagulant at a dosage of 10 mg L^{-1} Fe. A dosage of 10 mg L^{-1} Fe was selected because only weak coagulation of EPS occurred with 7.0 mg L^{-1} Fe in the preliminary experiment. pH ranging from 3 to 10 was adjusted by 0.1 M HCl or NaOH solution. The jar test was conducted by rapid mixing at a shear rate of 112 s^{-1} for 2 min, followed by a slow stir flocculation period at a shear rate of 18 s^{-1} for 20 min and a 30-min settling period. The supernatants taken from 2 cm below the surface were analyzed for DOC. The experiments were performed in triplicate.

Correlation analysis

The correlation analysis between the amounts of carbohydrate (percent) and DOC (percent) in the HPO fractions of *Microcystis* EOM (dEOM, dEPS and bEPS) was conducted by Pearson correlation statistic method (GraphPad Prism version 6.01).

RESULTS AND DISCUSSION

Zeta potential, SUVA and carbohydrate/protein ratio

Our study showed that dEOM, dEPS and bEPS of all *Microcystis* strains had negative zeta potential values at pH 7 (Table 1), indicating that all of them were negatively charged. For each strain of both colonial and unicellular *Microcystis*, the absolute zeta potential value of EOM could be ranked in an ascending order as follows: bEPS < dEPS < dEOM. This finding indicated that dEOM carried more negative surface charges than dEPS, while bEPS carried the least. This result also indicated that the macromolecular substances (dEPS) in dEOM carried less negative surface charges than the low molecular parts of dEOM. It has been previously revealed that cyanobacterial EPS contains uronic acids, sulphate groups, acyl groups and ketal-linked pyruvate groups, which contribute to the anionic nature of the EPS and confer a negatively charged behavior to the overall macromolecules (Pereira *et al.* 2009; Chen *et al.* 2016). Compared with the result found in this study, dEOM from *M. aeruginosa* has been previously reported to carry more negative surface charges. The zeta potential of dEOM from *M. aeruginosa* has been reported to be −26.2 mV (Qu *et al.* 2012a). The difference in zeta potential of dEOM between various strains of *M. aeruginosa* might be attributed to the difference in chemical composition of dEOM between various strains of *M. aeruginosa*.

dEPS and bEPS of all *Microcystis* strains had an SUVA value ranging from 0.28 to 0.69 L m^{-1} mg^{-1}, and

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Table 1 | Zeta potential, SUVA and carbohydrate/protein ratio of EOM extracted from each strain of *Microcystis*. ± indicates the standard deviation of the mean ($n = 3$)

Strains	EOM	Zeta potential (mV)	SUVA (L m^{-1} mg^{-1})	Carbohydrate/Protein (mg mg^{-1})
Colonial strains				
M. wesenbergii TH24	bEPS	−6.4 ± 1.0	0.58 ± 0.01	15.10 ± 0.48
	dEPS	−8.7 ± 1.3	0.28 ± 0.02	14.80 ± 0.57
	dEOM	−14.6 ± 1.1	ND[a]	27.05 ± 0.53
M. aeruginosa TH129	bEPS	−11.3 ± 2.1	0.85 ± 0.04	6.29 ± 0.38
	dEPS	−12.0 ± 1.5	0.31 ± 0.01	9.89 ± 0.03
	dEOM	−15.2 ± 4.1	ND	18.38 ± 0.53
M. flos-aquae TH183	bEPS	−9.4 ± 1.3	0.83 ± 0.01	17.98 ± 0.78
	dEPS	−12.1 ± 5.5	0.63 ± 0.03	6.66 ± 0.76
	dEOM	−15.9 ± 3.0	ND	22.31 ± 1.59
Unicellular strains				
M. wesenbergii TH4	bEPS	−5.0 ± 2.6	1.05 ± 0.06	15.55 ± 0.16
	dEPS	−6.0 ± 3.0	0.60 ± 0.03	22.01 ± 0.09
	dEOM	−11.8 ± 2.6	ND	23.57 ± 1.38
M. aeruginosa TH177	bEPS	−1.3 ± 0.9	0.75 ± 0.04	7.21 ± 0.20
	dEPS	−2.3 ± 0.8	0.69 ± 0.02	8.64 ± 0.05
	dEOM	−10.9 ± 0.3	ND	19.37 ± 0.81
M. flos-aquae TH60	bEPS	−5.7 ± 2.3	0.94 ± 0.04	9.99 ± 0.37
	dEPS	−8.0 ± 1.8	0.48 ± 0.02	11.10 ± 0.54
	dEOM	−15.9 ± 5.8	ND	12.26 ± 0.30

[a]Not determined.

from 0.58 to 1.05 L m^{-1} mg^{-1}, respectively (Table 1). For all three *Microcystis* species, SUVA of bEPS from both unicellular and colonial strains was much higher than that of dEPS. The low SUVA is a result of the relatively low aromaticity (Hoyer *et al.* 1985). This finding indicated that bEPS had relatively high aromaticity compared with dEPS.

For all *Microcystis* strains, the carbohydrate/protein ratio of dEOM, dEPS and bEPS ranged from 12.26 to 27.05 mg mg^{-1}, from 6.66 to 22.01 mg mg^{-1} and from 6.29 to 17.98 mg mg^{-1}, respectively (Table 1). For all three *Microcystis* species, the carbohydrate/protein ratio of dEOM from both unicellular and colonial strains was much higher than that of dEPS and bEPS, indicating that dEOM contained more carbohydrate than dEPS and bEPS. It also showed that dEOM contained considerable amounts of low molecular weight (<3.5 kDa) carbohydrates. Consistent with the results in this study, it has been reported that bEPS of *Microcystis* is mainly composed of carbohydrate in the literature. bEPS of *M. flos-aquae* C3-40 contains no protein and predominantly consists of anthrone-reacting polysaccharide (Plude *et al.* 1991). bEPS of *M. aeruginosa* K-3A contains 66.9% carbohydrate and 12.8% protein (Nakagawa *et al.* 1987).

Hydrophobicity

The results of XAD resin fractionation showed that with respect to unicellular and colonial *Microcystis* strains, or with respect to three different *Microcystis* species, the distribution patterns of EOM (dEOM, dEPS and bEPS) in the HPO, TPI and HPI fractions were similar (Figure 1). For each *Microcystis* strain, the proportion of HPI fraction in dEOM (ranged from 45.4% to 67.3%) was higher than that of HPO fraction (ranged from 22.7% to 41.2%), and the proportions of HPO fraction in dEPS and bEPS (ranged from 50.9% to 81.5% and from 77.4% to 90.0%, respectively) were higher than those of HPI fraction (ranged from 5.5% to 43.7% and from 5.8% to 16.4%, respectively). Consistent with the result in this study, Henderson *et al.* (2008) have reported that the proportion of HPI fraction in dEOM from *M. aeruginosa* (1450/2) (57%) is higher than that of HPO fraction (30%). For each *Microcystis* strain, the proportion of HPO fraction in bEPS (ranged from 77.4% to 90.0%) was higher than that in dEPS (ranged from 50.9% to 81.5%), and the proportion of HPO fraction in dEOM (ranged from 22.7% to 41.2%) was the lowest. In contrast, for each *Microcystis* strain, the proportion of HPI fraction in bEPS (ranged from 5.8% to 16.4%) was lower than that in dEPS (ranged from 8.8% to 43.7%) (except for unicellular

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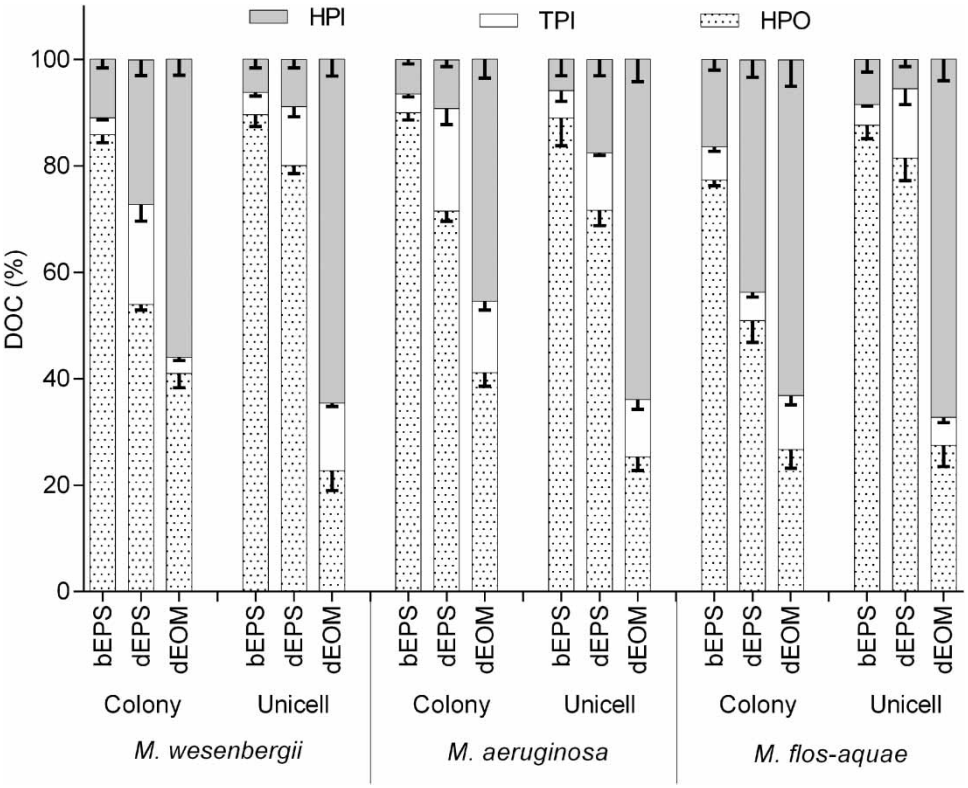

Figure 1 | The proportion of EOM contained within HPI, TPI and HPO fractions for unicellular and colonial *Microcystis*. Bars represent standard deviations of the mean ($n = 3$).

M. flos-aquae TH60), and the proportion of HPI fraction in dEOM was the highest (ranged from 45.4% to 67.3%). These results showed that the hydrophobicity of all the *Microcystis* strains could be ranked in order as follows: bEPS > dEPS > dEOM. dEPS was the macromolecular fraction of dEOM obtained through dialysis. Therefore, the macromolecular substances (dEPS) in dEOM were more hydrophobic than the low molecular weight parts of dEOM. Natural organic matter (NOM) with lower SUVA has been reported to exhibit lower hydrophobicity (Edzwald 1993). Consistent with this report, dEPS with lower SUVA had lower hydrophobicity compared with bEPS in this study.

The results of XAD resin fractionation indicated that for all *Microcystis* strains, the distribution patterns of carbohydrate of EOM (dEOM, dEPS and bEPS) in the HPO, TPI and HPI fractions were also similar (Figure 2). For each *Microcystis* strain, the proportion of HPI fraction in the carbohydrate of dEOM (ranged from 48.8% to 82.3%) was higher than that of the HPO fraction (ranged from 13.4% to 45.1%), and the proportion of HPO fraction in the carbohydrate of dEPS and bEPS (ranged from 58.4% to 92.0% and from 77.6% to 82.9%, respectively) was higher than that of the HPI fraction (ranged from 6.2% to 21.4% and from 8.9% to 13.5%, respectively). This finding suggested that the carbohydrates of the low molecular weight parts of dEOM were more hydrophilic than the carbohydrates of dEPS. Correlation analysis showed that there was a statistically significant correlation between the amounts of carbohydrate (percent) and DOC (percent) in the HPO fraction of EOM (dEOM, dEPS and bEPS) (Pearson correlation coefficient $r = 0.91$; $P < 0.01$) (Figure 3). This finding indicated that the hydrophobicity of *Microcystis* EOM might be related to carbohydrate. In fact, many cyanobacterial EPSs are characterized by a significant level of hydrophobicity due to the presence of acetyl groups, peptidic moieties and deoxysugars (fucose and rhamnose) (Pereira *et al.* 2009). However, treatment with proteolytic enzyme has no effect on the cell surface hydrophobicity in *Microcystis* (Yang *et al.* 2011), suggesting that the protein does not contribute to the hydrophobicity of *Microcystis* EPS. The bEPS of *M. aeruginosa* K-3A contains rhamnose and fucose (Nakagawa *et al.* 1987). The dEPS of *Microcystis* PCC 7005, *Microcystis* PCC 7941, *M. aeruginosa* NIES 87 and *M. viridis* NIES 102 contains fucose or rhamnose, and the bEPS of *M. wesenbergii* NIES 112 contains rhamnose (Forni *et al.* 1997). The acetyl group and rhamnose have

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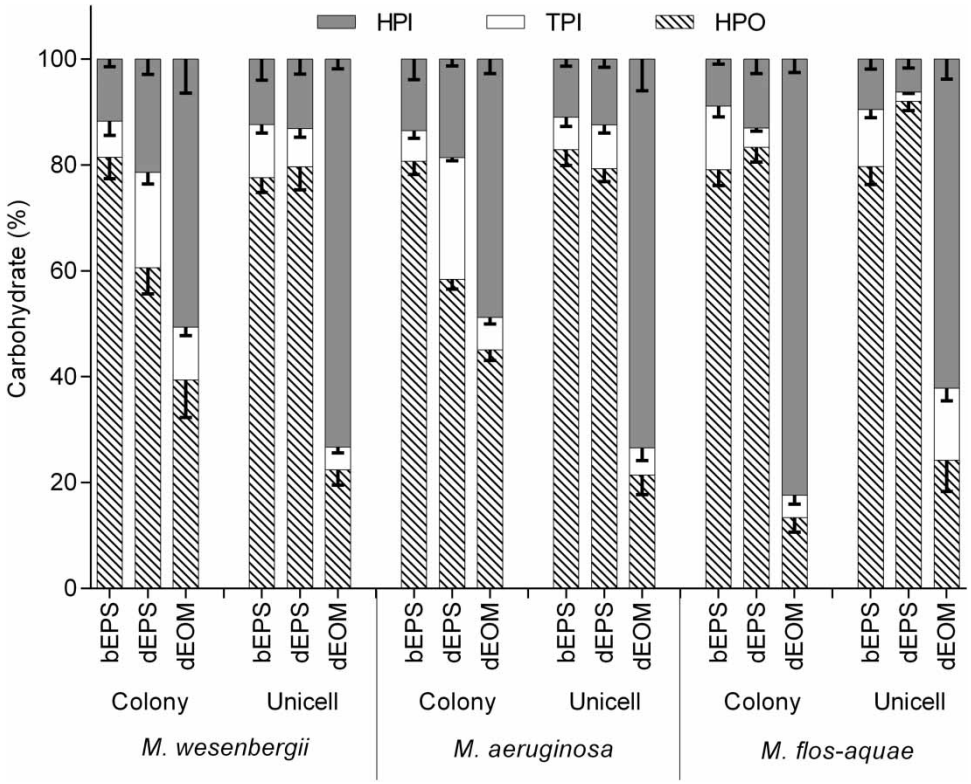

Figure 2 | Percentage of total carbohydrate of EOM present in the HPI, TPI and HPO fractions for unicellular and colonial *Microcystis*. Bars represent standard deviations of the mean ($n = 3$).

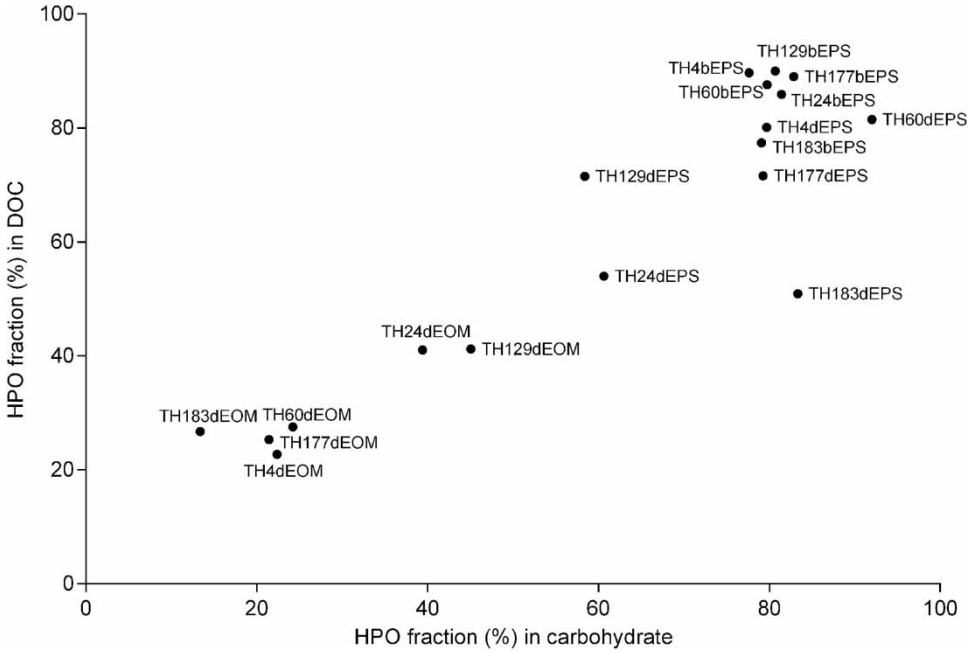

Figure 3 | Scatter plot between the amounts of carbohydrate (percent) and DOC (percent) in the HPO fractions of EOM. The points represent different types of EOM (dEOM, dEPS and bEPS) from various *Microcystis* strains (*M. wesenbergii* TH4, *M. aeruginosa* TH177, *M. flos-aquae* TH60, *M. wesenbergii* TH24, *M. aeruginosa* TH129, *M. flos-aquae* TH183).

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been found to be present in dEPS and bEPS of all determined *Microcystis* strains, including *M. aeruginosa*, *M. wesenbergii*, *M. firma* and *M. flos-aquae* (Chen *et al.* 2016). Therefore, acetyl group and deoxy sugars might play a role in the hydrophobicity of *Microcystis* EPS.

Comparison of EPS characteristics and EPS productivity between colonial and unicellular *Microcystis*

The HPO fraction in DOC, SUVA and carbohydrate/protein ratio of dEPS from unicellular strains of three *Microcystis* species were within the range of 50.9% to 81.5%, 0.48 to 0.69 L m^{-1} mg^{-1}, and 8.64 to 22.01 mg mg^{-1}, respectively, and those from colonial strains were within the range of 54.0% to 80.1%, 0.28 to 0.63 L m^{-1} mg^{-1}, and 6.66 to 14.80 mg mg^{-1}, respectively (Figure 1 and Table 1). No obvious difference in hydrophobicity, SUVA and carbohydrate/protein ratio of dEPS was observed between unicellular and colonial strains of the three *Microcystis* species. Similarly, no difference in the three characteristics of bEPS was observed between the unicellular and colonial strains. In unicellular and colonial *Microcystis*, the dEPS content was higher than the bEPS content, and the average dEPS/bEPS ratio for three *Microcystis* species was 5.60 and 3.09, respectively (Table S1, Supplementary Data).

The zeta potential of bEPS and dEPS from unicellular strains of three *Microcystis* species was within the range of −5.7 to −1.3 mV and −8.0 to −2.3 mV, respectively; that from colonial strains were in the range of −11.3 to −6.4 mV and −12.1 to −8.7 mV, respectively (Table 1). The absolute zeta potential value of bEPS and dEPS in colonial *Microcystis* was higher than that in unicellular *Microcystis*. This finding indicated that both bEPS and dEPS in colonial *Microcystis* carried more negative surface charges compared with unicellular *Microcystis*. The higher EPS content was found in colonial *Microcystis* compared with unicellular *Microcystis* (Table S1). The average bEPS content for colonial strains of *Microcystis* was 23.3-fold higher compared with unicellular strains of *Microcystis*. The average dEPS content for colonial strains of *Microcystis* was 13.0-fold higher compared with unicellular strains of *Microcystis*. Consistent with this study, it has been previously reported that the bEPS and dEPS contents in colonial *M. aeruginosa* are significantly higher than those in unicellular cells (Zhang *et al.* 2011). Additionally, the dEPS/bEPS ratio in unicellular *Microcystis* (ranged from 5.05 to 6.08) was found to be higher than that in colonial *Microcystis* (ranged from 2.78 to 3.63).

The implications of the EPS characteristics on membrane-based water treatment

Membrane filtration is increasingly applied in the water treatment process, including the production of drinking water and the reclamation of wastewater (Jacangelo *et al.* 1997). Membrane fouling is a major obstacle to maintain the efficient performance of water treatment. Membrane fouling, including flux decline and fouling reversibility, depends on the properties of the organic matter and membrane (Zhou *et al.* 2014). During membrane filtration, the zeta potential affects the electrostatic interactions between EOM and the filtration membrane. Less negative charge of EOM results in less electrostatic repulsion with the membrane (e.g. polyethersulfone membrane and regenerated cellulose membrane), which carries negative charges (Qu *et al.* 2012a). Therefore, based on the measured zeta potential of EOM in this study, bEPS adhered more easily to the membrane surface than dEPS, and in dEOM, dEPS was the component adhering more easily to the membrane surface compared with the low molecular weight parts of dEOM. The HPO fraction of organic matter has a greater tendency to adhere to the hydrophobic membrane (e.g. polyethersulfone membrane) surface during membrane filtration (Qu *et al.* 2012a). Therefore, according to the hydrophobicity of *Microcystis* EPS, bEPS would have greater HPO adhesion ability than dEPS during membrane filtration. Additionally, the macromolecular substances of EOM, including dEPS and bEPS, would adhere more easily to the membrane surface compared with the low molecular weight parts of dEOM. The average dEPS/bEPS ratio for colonial *Microcystis* was 3.09, indicating that the quantity of dEPS in the water body might be much higher than that of bEPS during a cyanobacterial bloom. Although bEPS adhered more easily to the membrane surface than dEPS according to the data on zeta potential and hydrophobicity, the dEPS/bEPS ratio was another important factor affecting the membrane fouling during membrane filtration.

Coagulation of EPS

The coagulation experiments showed that the removal efficiency of bEPS and dEPS from colonial *Microcystis* strains was highly dependent on pH values (Figure 4). The highest removal efficiency was obtained at pH 5 for all bEPS and dEPS of colonial *Microcystis*. At pH > 5, the removal efficiency of bEPS and dEPS decreased with the increase in pH value. For each *Microcystis* strain, the removal efficiency of bEPS was higher than that of dEPS within a pH range

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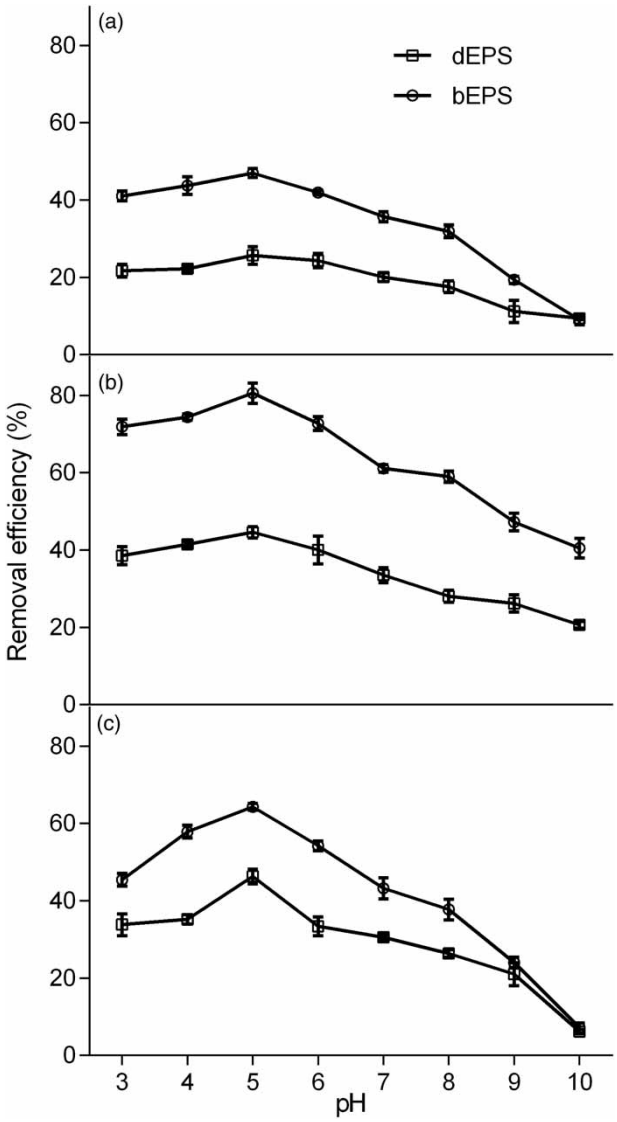

Figure 4 | Removal efficiency of *Microcystis* EPS by coagulation as a function of pH values. (a) colonial *M. wesenbergii* TH24; (b) colonial *M. aeruginosa* TH129; and (c) colonial *M. flos-aquae* TH183. Bars represent standard deviations of the mean ($n = 3$).

from 3 to 10. For *M. wesenbergii* TH24, *M. aeruginosa* TH129 and *M. flos-aquae* TH183, the highest removal efficiency of bEPS was 47.0%, 80.6% and 64.3%, respectively; and the highest removal efficiency of dEPS was 25.7%, 44.6% and 46.3%, respectively. This finding indicated that the removal efficiency of EPS from *Microcystis* was species-specific. Coagulation with metal salts is a common method to remove *Microcystis* cells from a water body (Ma *et al.* 2015). Our data in this study indicated that bEPS, which is attached to cells, could coagulate with metal salt. It is speculated that the cell removability with metal salts might be related to coagulation of bEPS with metal salts.

Consistent with the results in this study, the coagulation removal efficiency of intracellular organic matter (IOM) from *M. aeruginosa* is also highly pH dependent. The highest removal efficiency of IOM is achieved across the pH range from 4 to 6 (Pivokonsky *et al.* 2012). Therefore, the pH value of the water body is an important factor affecting the removal efficiency of organic matter from *Microcystis* during water treatment. It has been demonstrated that the underlying mechanism of the coagulation of humic acid and *Microcystis* IOM is charge neutralization and adsorption, and it is mainly charge neutralization under an acidic condition (Cheng & Chi 2002; Pivokonsky *et al.* 2012). The results of this study showed that the dEPS and bEPS of *Microcystis* had negative zeta potential values, showing negative charge characteristics. Iron coagulant forms hydrolytic products bearing a positive charge at acidic pH (Stumm & Morgan 1996). It can be speculated that the charge neutralization may occur owing to the electrostatic interactions between the EPS (dEPS and bEPS) of *Microcystis* and the coagulant under the acidic condition, leading to coagulation.

Iron-based coagulant is known to preferentially remove HPO fractions over HPI fractions of NOM (White *et al.* 1997). For each *Microcystis* strain, bEPS contained more HPO fraction than dEPS. This might be one reason why the removal efficiency of bEPS was higher than that of dEPS. In addition, zeta potential is an important parameter to measure the stability of colloidal particles. The colloid with lower zeta potential has less repulsive forces between particles, and it is unstable and prone to coagulation (Hunter 1981). The zeta potential (absolute value) of bEPS was lower than that of dEPS for each *Microcystis* strain at pH 7. This might be another reason why the removal efficiency of bEPS was higher than that of dEPS. The coagulation removal efficiency of dEOM (6.0 mg L^{-1} DOC) from *M. aeruginosa* is 18% at pH 5 at the dosage of 165 mg L^{-1} Fe (coagulant ferric chloride) (Widrig *et al.* 1996), which is lower than the removal efficiency of dEPS of the three *Microcystis* strains at pH 5 in this study (ranged from 25.7% to 46.3%). Iron-based coagulant preferentially removes high molecular weight substances (Sinsabaugh *et al.* 1986). dEOM contained the low molecular parts and macromolecular dEPS, resulting in the lower removal efficiency of dEOM compared with dEPS. In this study, the highest removal efficiency of bEPS and dEPS was 47.0%–80.6% and 25.7%–46.3%, respectively. These data indicated that *Microcystis* EPS was able to be removed by iron coagulation, and bEPS would be removed more easily than dEPS.

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The results of this study showed that the removal efficiency of EPS from *Microcystis* was species-specific. The possible reason could be attributed to the chemical composition of *Microcystis* EPS being species-specific. Forni *et al.* (1997) have found that the uronic acid content of dEPS and bEPS from different *Microcystis* species ranges from 0.04 to 5.81 μg mg^{-1} and from 0.73 to 1.66 μg mg^{-1}, respectively. Chen *et al.* (2016) have reported that for dEPS and bEPS of nine determined *Microcystis* strains, the uronic acid content varies from 2.4% to 6.2% and from 1.2% to 2.1%, respectively; the content of the acetyl group varies from 0.5% to 2.4% and from 2.0% to 6.6%, respectively; and the content of the sulfate group varies from 3.5% to 7.2% and from 4.9% to 8.7%, respectively. The difference in the contents of these chemical groups might affect the charge of EPS, leading to affected coagulation efficiency. In the water treatment process, the coagulation removal efficiency of *Microcystis* EPS might be related to the species of *Microcystis* in the water body.

CONCLUSIONS

In the present study, higher dEPS and bEPS contents and a lower dEPS/bEPS ratio were found in colonial *Microcystis* compared with unicellular *Microcystis*. dEOM, dEPS and bEPS had negative zeta potential values at pH 7, and the absolute zeta potential value of both bEPS and dEPS in colonial *Microcystis* was higher than that in unicellular *Microcystis*. The absolute zeta potential value of EOM for each strain of both colonial and unicellular *Microcystis* could be ranked in ascending order as follows: bEPS < dEPS < dEOM. The carbohydrate/protein ratio of dEOM was much higher than that of dEPS and bEPS, indicating that dEOM contained more carbohydrate than dEPS and bEPS. It also showed that dEOM contained considerable amounts of low molecular weight (<3.5 kDa) carbohydrates. XAD resin fractionation analysis indicated that the hydrophobicity could be ranked in order as follows: bEPS > dEPS > dEOM. Correlation analysis showed that there was a statistically significant correlation between the amounts of carbohydrate and DOC in the hydrophobic fraction of EOM (dEOM, dEPS and bEPS), indicating that the hydrophobicity of *Microcystis* EOM might be related to carbohydrate. According to the data on zeta potential and hydrophobicity, bEPS adhered more easily to the membrane surface during membrane filtration compared with dEPS. Additionally, compared with the low molecular parts of dEOM, dEPS and bEPS would adhere more easily to the membrane surface because dEPS and bEPS were more hydrophobic and carried less negative surface charges. This implied that various components of EOM might have differential influences on membrane filtration during water treatment. The coagulation experiments showed that the removal efficiency of bEPS and dEPS of colonial *Microcystis* strains was highest at pH 5. The removal efficiency of EPS from *Microcystis* was species-specific. The highest removal efficiency of bEPS and dEPS was 47.0–80.6% and 25.7–46.3%, respectively. The coagulation test indicated that *Microcystis* EPS might be removed from water by the method of iron coagulation, which is commonly used during water treatment. To our knowledge, this research is the first report on hydrophobicity and iron coagulation of EPS from *Microcystis*. Our data on the characteristics of *Microcystis* EPS could provide valuable insights into understanding its influence on water treatment.

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SUPPLEMENTARY DATA

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REFERENCES

Babel, S. & Takizawa, S. 2010 Microfiltration membrane fouling and cake behavior during algal filtration. *Desalination* **261** (1–2), 46–51.

Bradford, M. M. 1976 A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72** (1–2), 248–254.

Carroll, T., King, S., Gray, S. R., Bolto, B. A. & Booker, N. A. 2000 The fouling of microfiltration membranes by NOM after coagulation treatment. *Water Research* **34** (11), 2861–2868.

Chen, M. Z., Xie, M. J., Wu, W., Yu, F. G. & Li, P. F. 2016 Chemical characteristics of capsular polysaccharide and water-soluble released exopolysaccharide from *Microcystis*. *Hupo Kexue* **28** (3), 609–615.

Cheng, W. P. & Chi, F. H. 2002 A study of coagulation mechanisms of polyferric sulfate reacting with humic acid using a fluorescence-quenching method. *Water Research* **36** (18), 4583–4591.

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Edzwald, J. K. 1993 Coagulation in drinking water treatment: particles, organics and coagulants. *Water Science and Technology* **27** (11), 21–35.

Forni, C., Telo, F. R. & Caiola, M. G. 1997 Comparative analysis of the polysaccharides produced by different species of *Microcystis* (Chroococcales, Cyanophyta). *Phycologia* **36** (3), 181–185.

Henderson, R. K., Baker, A., Parsons, S. A. & Jefferson, B. 2008 Characterisation of algogenic organic matter extracted from cyanobacteria, green algae and diatoms. *Water Research* **42** (13), 3435–3445.

Hoyer, O., Lusse, B. & Bernhardt, H. 1985 Isolation and characterization of extracellular organic matter (EOM) from algae. *Zeitschrift Fur Wasser Und Abwasser Forschung-Journal for Water and Wastewater Research* **18** (2), 76–90.

Hunter, R. J. 1981 Applications of the zeta potential. In: *Zeta Potential in Colloid Science. Principles and Applications*, 1st edn (R. H. Ottewill & R. L. Rowell eds). Academic Press, London, UK, pp. 219–257.

Jacangelo, J. G., Trussell, R. R. & Watson, M. 1997 Role of membrane technology in drinking water treatment in the United States. *Desalination* **113** (2–3), 119–127.

Kurmayer, R., Christiansen, G. & Chorus, I. 2003 The abundance of microcystin-producing genotypes correlates positively with colony size in *Microcystis* sp. and determines its microcystin net production in Lake Wannsee. *Applied and Environmental Microbiology* **69** (2), 787–795.

Lehman, P. W., Boyer, G., Hall, C., Waller, S. & Gehrts, K. 2005 Distribution and toxicity of a new colonial *Microcystis aeruginosa* bloom in the San Francisco Bay Estuary, California. *Hydrobiologia* **541**, 87–99.

Li, P. F., Cai, Y. F., Shi, L. M., Geng, L. F., Xing, P., Yu, Y., Kong, F. X. & Wang, Y. J. 2009 Microbial degradation and preliminary chemical characterization of *Microcystis* exopolysaccharides from a cyanobacterial water bloom of Lake Taihu. *International Review of Hydrobiology* **94** (6), 645–655.

Liu, D., Wong, P. T. S. & Dutka, B. J. 1973 Determination of carbohydrate in lake sediment by a modified phenol-sulfuric acid method. *Water Research* **7** (5), 741–746.

Ma, X. X., Wang, Y. A., Feng, S. Q. & Wang, S. B. 2015 Comparison of four flocculants for removing algae in Dianchi Lake. *Environmental Earth Sciences* **74** (5), 3795–3804.

Nakagawa, M., Takamura, Y. & Yagi, O. 1987 Isolation and characterization of the slime from a cyanobacterium, *Microcystis aeruginosa* K-3A. *Agricultural and Biological Chemistry* **51** (2), 329–337.

Otsuka, S., Suda, S., Li, R. H., Matsumoto, S. & Watanabe, M. M. 2000 Morphological variability of colonies of *Microcystis* morphospecies in culture. *Journal of General and Applied Microbiology* **46** (1), 39–50.

Paerl, H. W., Fulton, R. S., Moisander, P. H. & Dyble, J. 2001 Harmful freshwater algal blooms, with an emphasis on cyanobacteria. *The Scientific World Journal* **1**, 76–113.

Pereira, S., Zille, A., Micheletti, E., Moradas-Ferreira, P., De Philippis, R. & Tamagnini, P. 2009 Complexity of cyanobacterial exopolysaccharides: composition, structures, inducing factors and putative genes involved in their biosynthesis and assembly. *FEMS Microbiology Reviews* **33** (5), 917–941.

Pivokonsky, M., Safarikova, J., Bubakova, P. & Pivokonska, L. 2012 Coagulation of peptides and proteins produced by *Microcystis aeruginosa*: interaction mechanisms and the effect of Fe-peptide/protein complexes formation. *Water Research* **46** (17), 5583–5590.

Plude, J. L., Parker, D. L., Schommer, O. J., Timmerman, R. J., Hagstrom, S. A., Joers, J. M. & Hnasko, R. 1991 Chemical characterization of polysaccharide from the slime layer of the cyanobacterium *Microcystis flos-aquae* C3-40. *Applied and Environmental Microbiology* **57** (6), 1696–1700.

Qu, F. S., Liang, H., He, J. G., Ma, J., Wang, Z. Z., Yu, H. R. & Li, G. B. 2012a Characterization of dissolved extracellular organic matter (dEOM) and bound extracellular organic matter (bEOM) of *Microcystis aeruginosa* and their impacts on UF membrane fouling. *Water Research* **46** (9), 2881–2890.

Qu, F. S., Liang, H., Wang, Z. Z., Wang, H., Yu, H. R. & Li, G. B. 2012b Ultrafiltration membrane fouling by extracellular organic matters (EOM) of *Microcystis aeruginosa* in stationary phase: influences of interfacial characteristics of foulants and fouling mechanisms. *Water Research* **46** (5), 1490–1500.

Rippka, R. 1988 Isolation and purification of cyanobacteria. *Methods in Enzymology* **167**, 3–27.

Sharp, E. L., Parson, S. A. & Jefferson, B. 2006 Coagulation of NOM: linking character to treatment. *Water Science and Technology* **53** (7), 67–76.

Sinsabaugh, R. L., Hoehn, R. C., Knocke, W. R. & Linkins, A. E. 1986 Removal of dissolved organic carbon by coagulation with iron sulfate. *Journal of the American Water Works Association* **78** (5), 74–82.

Stumm, W. & Morgan, J. J. 1996 *Aquatic Chemistry*. John Wiley Sons, New York.

Tang, X. M., Zheng, H. L., Gao, B. Y., Zhao, C. L., Liu, B. Z., Chen, W. & Guo, J. S. 2017 Interactions of specific extracellular organic matter and polyaluminum chloride and their roles in the algae-polluted water treatment. *Journal of Hazardous Materials* **332**, 1–9.

Tang, X. B., Pronk, W., Ding, A., Cheng, X. X., Wang, J. L., Xie, B. H., Li, G. B. & Liang, H. 2018 Coupling GAC to ultra-low-pressure filtration to modify the biofouling layer and bio-community: flux enhancement and water quality improvement. *Chemical Engineering Journal* **333**, 289–299.

Tien, C. J., Krivtsov, V., Levado, E., Sigee, D. C. & White, K. N. 2002 Occurrence of cell-associated mucilage and soluble extracellular polysaccharides in Rostherne Mere and their possible significance. *Hydrobiologia* **485** (1–3), 245–252.

White, M. C., Thompson, J. D., Harrington, G. W. & Singer, P. C. 1997 Evaluating criteria for enhanced coagulation compliance. *Journal of the American Water Works Association* **89** (5), 64–77.

Widrig, D. L., Gray, K. A. & McAuliffe, K. S. 1996 Removal of algal-derived organic material by preozonation and

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coagulation: monitoring changes in organic quality by pyrolysis-GC-MS. *Water Research* **30** (11), 2621–2632.

Yang, H. L., Cai, Y. F., Xia, M., Wang, X. Y., Shi, L. M., Li, P. F. & Kong, F. X. 2011 Role of cell hydrophobicity on colony formation in *Microcystis* (cyanobacteria). *International Review of Hydrobiology* **96** (2), 141–148.

Yu, G. L., Song, L. R. & Li, R. H. 2007 Taxonomic notes on water bloom forming *Microcystis* species (Cyanophyta) from China – an example from samples of the Dianchi Lake. *Acta Phytotaxonomica Sinica* **45** (5), 727–741.

Zhang, M., Kong, F. X., Tan, X., Yang, Z., Cao, H. S. & Xing, P. 2007 Biochemical, morphological, and genetic variations in *Microcystis aeruginosa* due to colony disaggregation. *World Journal of Microbiology & Biotechnology* **23** (5), 663–670.

Zhang, M., Shi, X. L., Yu, Y. & Kong, F. X. 2011 The acclimative changes in photochemistry after colony formation of the cyanobacteria *Microcystis aeruginosa*. *Journal of Phycology* **47** (3), 524–532.

Zhou, S. Q., Shao, Y. S., Gao, N. Y., Li, L., Deng, J., Tan, C. Q. & Zhu, M. Q. 2014 Influence of hydrophobic/hydrophilic fractions of extracellular organic matters of *Microcystis aeruginosa* on ultrafiltration membrane fouling. *Science of the Total Environment* **470**, 201–207.

Zhou, Z. B., Meng, F. G., He, X., Chae, S. R., An, Y. J. & Jia, X. S. 2015 Metaproteomic analysis of biocake proteins to understand membrane fouling in a submerged membrane bioreactor. *Environmental Science & Technology* **49** (2), 1068–1077.

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