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# Characteristics and roles of *Microcystis* extracellular polymeric substances (EPS) in cyanobacterial blooms: a short review

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**ABSTRACT**

Cyanobacterial blooms are serious eco-environmental problem, and cyanobacteria can produce large amounts of extracellular polymeric substances (EPS). EPS, as complex polymers, possess several characteristics, such as hydrophobicity, carbon-rich, nutrient-rich, metal adsorption, and stickiness, which influence the cell surface characteristics and the cycling of carbon, nutrients, metals, and rheological property in water, particularly during large blooms. All these characteristics are related to bloom outbreak. This review provides the classification, composition, extraction, and characteristics of cyanobacterial EPS and their corresponding roles in algal blooms. Further studies should investigate transparent exopolymer particles as a special and free form of EPS and their precise roles in cyanobacterial blooms in freshwater.

## Introduction

Cyanobacterial blooms are abundant in eutrophic waters and widespread in summer throughout the world; these species are persistent in winter and appear year-round in eutrophic lakes (Ma et al. 2015). The health-affecting toxins and odorous compounds produced by cyanobacteria would restrict drinking water, irrigation, aquaculture, and fish breeding, and would seriously affect the human health (Saqrane and Oudra 2011). Thus far, the blooms have become a serious eco-environmental problem, and the mechanism of the bloom outbreak should be further investigated.

Cyanobacteria produce large amounts of extracellular polymeric substances (EPS). EPS have a defensive function and produce large quantities of EPS to reduce grazing by protists (Liu and Buskey 2000; Shnyukova and Zolotareva 2002; Sorrels et al. 2009). In addition, EPS have a metal-binding capacity (De Philippis et al. 2011) and have influence on carbon or nutrient cycling (Passow 2002; Pannard et al. 2016). They also participate in aggregate formation through adsorption and stickiness, which would influence the fate of blooms (Logan et al. 1995; Passow 2002). Thus, the in-depth study of *Microcystis* EPS attracts attention not only in terms of improving our comprehension of the roles of EPS in *Microcystis* blooms but also in improving the management of the harmful blooms by affecting the characteristics and production of EPS. Despite many efforts to elucidate the roles of algal EPS in aquatic systems, the roles of EPS in *Microcystis* blooms are still not well understood nor consistently reported. This situation may be attributed to the complex properties of EPS and the bloom outbreak mechanism. Meanwhile, studies on the algal EPS have generally been limited to

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single property. Therefore, the past research results should be summarized. This review aims to provide an overview on the classification, extraction, composition of *Microcystis* EPS and then review the characteristics and corresponding functions in cyanobacterial blooms emphatically. Finally, the main conclusions and the research needs are presented.

## Classification, extraction, and composition of cyanobacterial EPS

EPS were generally fractionated into soluble EPS (sEPS) and bound EPS (bEPS), and bEPS were further divided into loosely bEPS and tightly bEPS (Sheng et al. 2010). These two types of bEPS were separated by a mild method for loosely bEPS (e.g. high speed centrifugation, heating at low temperatures) and a harsh method for tightly bEPS (e.g. heating at high temperatures, or chemical extraction methods) based on the harvested algae samples after sEPS collection (Sheng et al. 2010; Xu et al. 2013). However, transparent exopolymer particles (TEP), which were different from bEPS and sEPS, as a special type of EPS was proposed by Thornton (2002) (Figure 1). TEP exist as non-living and discrete particles rather than as dissolved substances, capsules, or surface coatings (Alldredge et al. 1993). The role of TEP in aquatic systems differs from other forms of EPS given that these individual particles in waters can aggregate and can be collected by filtration (Passow 2002). These transparent gel-particles are sticky particles and consist predominantly of acidic polysaccharides. In fact, sEPS and bEPS of cyanobacterial *Microcystis* also contained large amounts of acidic heteropolysaccharides (Chen et al. 2016). Similar components in EPS fractions indicate that a close connection was observed between the pools of sEPS, bEPS, and TEP in pelagic ecosystems as shown in Figure 1 (Thornton 2002), and the dynamic relationship would be strengthened during the algal blooming period.

The extraction method of bEPS could affect the composition and function of EPS and could further influence the relative contents of matters in EPS matrix (D'Abzac et al. 2010; Bourven 2011; Liu, Qin, Zhang et al. 2014). We attempted to compare several common extraction methods for cyanobacterial *Microcystis* and found that some extraction methods could cause loss of some materials in bEPS (Liu, Qin, Zhang et al. 2014). Xu et al. (2013) and Qu et al. (2012) used different extraction methods to characterize the bEPS and obtained conflicting result. The extraction media of algal bEPS included hot water, NaCl, NaOH, ethylene diamine tetraacetid acid, cation exchange resin, and sonication (De Brouwer et al. 2002; Takahashi et al. 2009; Liu et al. 2014). Intracellular polymers such as 2-keto-3-deoxyoctonate (Klock et al. 2007), phycobilin proteins (Liu, Qin, Zhang et al. 2014), DNA (Brown and Lester 1980), ATP, and transmission electron microscope analysis (Takahashi et al. 2009) were used to evaluate the rupture of cells. We initially concluded that NaOH solution at pH = 10 would be the suitable extract for *Microcystis* bEPS through comparing these common methods by evaluation of excitation–emission matrix (EEM) spectroscopy and measuring the release of intracellular compounds (Liu, Qin, Zhang et al. 2014). sEPS samples were generally collected directly by low-speed centrifugation or filtration, and the supernatant or the filtrate was considered as sEPS fraction. TEP as the special type and discrete particles of EPS were filtered, and the TEP samples on the filters were then stained for further measurement (Passow 2002).

These three forms of EPS could be directly exuded by cyanobacteria. Cyanobacterial EPS mainly contained carbohydrates (or polysaccharides) and proteins (Liu et al. 2014). Monosaccharides such
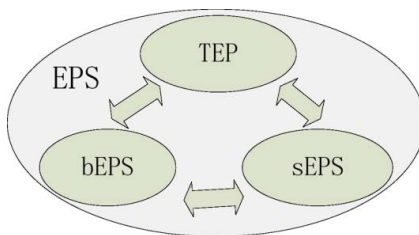


**Figure 1.** Dynamic relationship among the three pools of extracellular polymeric substances (EPS).

as glucose, fucose, mannose, arabinose, and rhamnose were included in cyanobacterial polysaccharides (Li et al. 2001). To learn more about cyanobacterial exopolysaccharides and their characteristics, readers are referred to reviews such as Li et al. (2001), De Philippis and Vincenzini (1998) and De Philippis et al. (2001).

TEP components were generally produced by both actively growing (exponential phase) and nutrient-limited (stationary/death phase) algae, and the colloidal fraction ($<0.4\ \mu$m) is likely the dominant fraction among algal-derived TEP (Villacorte et al. 2015). According to our research, the TEP concentration per cell of *Microcystis* sp. was 15 pg Xeq./cell (xanthan gum equivalents per cell) (Liu, Qin, Huang 2014), which was similar to that of diatom *Skeletonema* sp. (0.02 ng Xeq./cell) but was smaller than those of other diatoms (*Coscinodiscus granii, Eucampia zodiacus, Rhizosolenia setigera*) (Fukao et al. 2010).

The composition of EPS, characterized by fluorescence EEM spectroscopy, showed that large amounts of protein-like and humic-like substances were distributed in EPS fractions (Xu et al. 2013; Liu, Qin, Zhang et al. 2014). Among them, the tryptophan-like substances were correlated with the *Microcystis* growth, and the tyrosine-like substances showed their relationship with cell amount under nitrogen limitation (Xu et al. 2013; Liu et al. 2017). Other materials such as lipids and uronic acids were also reported in EPS fraction (Shifrin and Chisholm 1980; Verspagen et al. 2006). Besides, given that allelopathy is a manifold ecological/physiological phenomenon and it plays a crucial role in aquatic ecosystems, the active and allelopathic materials exuded by phytoplankton and the allelopathic effect were also noted and investigated widely (Zheng et al. 2013; Żak and Kosakowska 2014).

## Characteristics and ecological roles of cyanobacterial EPS in algal blooms

### Colony and bloom formation mechanism in the blooms

Recently, two conceptual frameworks as shown in Figures 2 (Xu et al. 2014) and 3 (Qin et al. 2016) were proposed to explain the colony and bloom formation, respectively.

The conceptual frameworks of two theories were both proposed to elucidate *Microcystis* bloom formation mechanism in eutrophic Lake Taihu, China. Although one used extended Derjaguin–Landau–Verwey–Overbeek theory to explain the colony formation mechanism (Xu et al. 2014), another considered the abrupt appearance of *Microcystis* blooms within a short period and placed more emphasis on the collision and aggregation theory induced by hydrodynamic forces in shallow waters (Qin et al. 2016). However, both researchers focused on the important roles of EPS in colony and bloom formation. They both identified that EPS are involved in colony and mucilage formation. The colony formation was a survival strategy of *Microcystis*, which provides a competitive advantage over other phytoplankton species (Wu et al. 2007). Xu et al. (2014) further pointed out that tightly bEPS were related to small colony formation, and loose bEPS might be closely linked with large colony or mucilage formation. Yang (2010) gave a visual effect by using the scanning microscope to observe that there was more sticky and thick material enveloping the cell surface of colonial *Microcystis* than unicellular cells (Figure 4).

### Charge characteristics and hydrophobicity of cyanobacterial EPS

To some extent, the characteristics of cyanobacterial EPS determined the surface feature of cells, which directly affect the colony and bloom formation (Liu et al. 2016). The zeta potentials as the potential at the solid surface of a colloid electrical double layer are closely related to the charges on the particle surface. The surface charged characteristics of EPS were generally characterized with the parameters of zeta potential. According to our research based on zeta potential measurement, the surface charge of cyanobacterial *Microcystis* from culture and field were both negatively charged (Liu et al. 2016), and the isoelectric value of *Microcystis* cells calculated by the authors was similar to those of EPS from *Microcystis* (Henderson et al. 2008). Uronic acids might mainly contribute to the anionic character of cyanobacterial EPS (Verspagen et al. 2006; Klock et al. 2007).
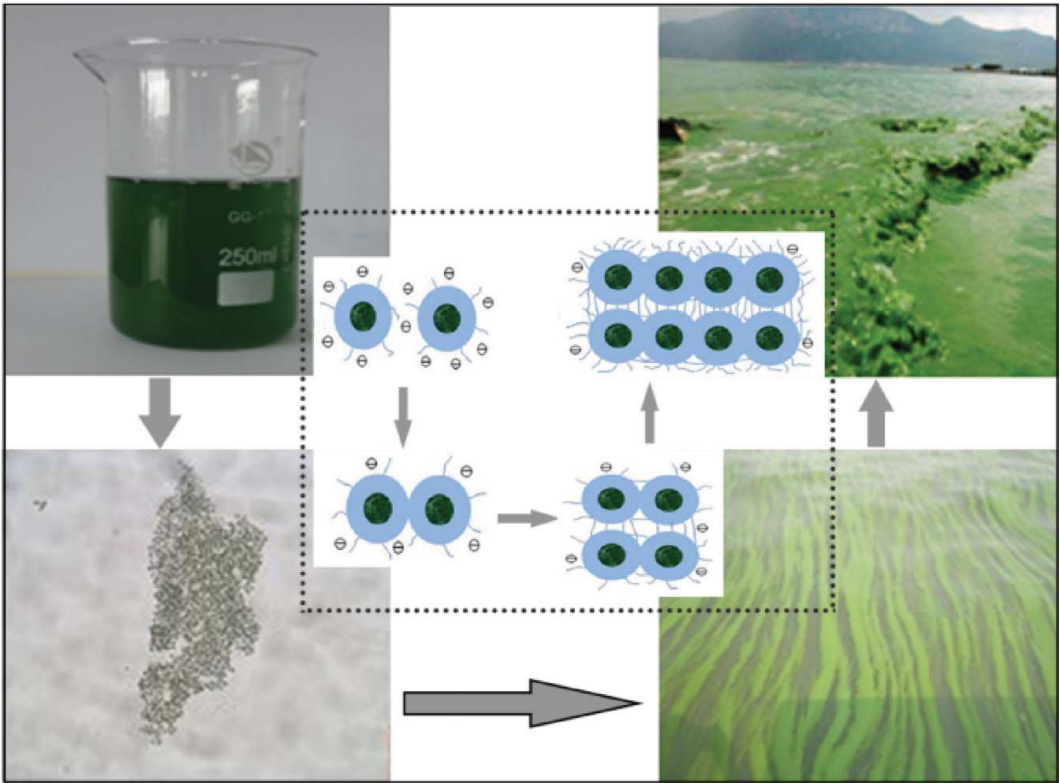
**Figure 2.** Schematic illustration of the formation of cyanobacterial *Microcystis* bloom (dark balls indicate *Microcystis* cells, blue shapes indicate extracellular polymeric substances, and circles within a line indicate the negative zeta potential).
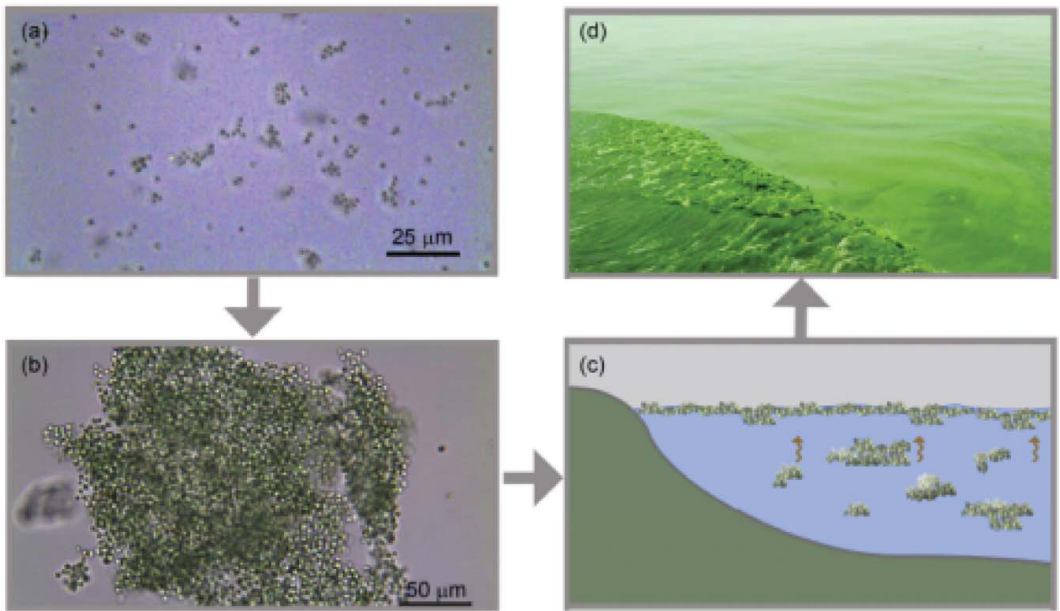


**Figure 3.** Conceptual explaining diagrams of cyanobacterial bloom 'outbreak' in Lake Taihu. (a) Cyanobacterial cell division and proliferation; (b) large colony formation through collision; (c) large colony floating upward to form visible bloom; and (d) cyanobacterial bloom transportation and migration along the onshore zone.
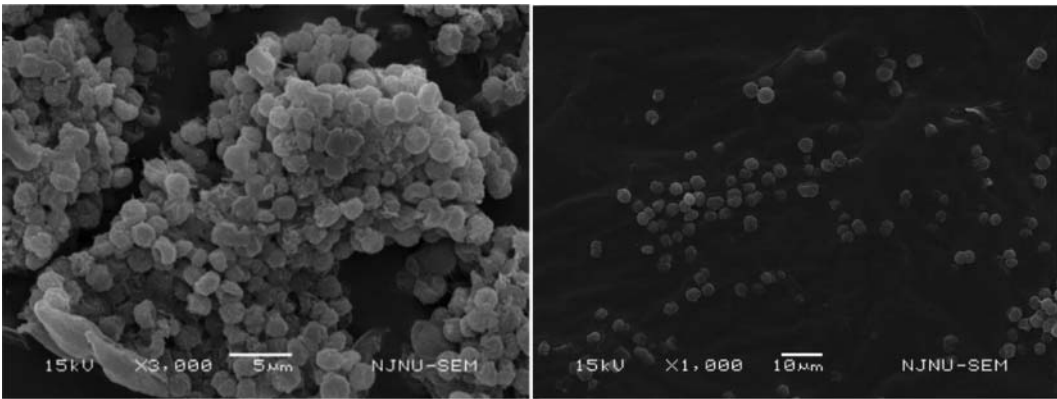
**Figure 4.** Electron micrographs of colonial (left) and single (right) *Microcystis aeruginosa* in different zoom multiples.

The hydrophobic interaction could play a key role in the EPS assembly from phytoplankton (Ding et al. 2009), and the hydrophobicity of colony was stronger than that of single cell (Yang et al. 2011; Liu et al. 2016). Yang et al. (2011) further specified that the extracellular polysaccharides may play a role in adhering cells together to form colonies. Zhang et al. (2007) observed that the colonial *Microcystis aeruginosa* cells have thicker polysaccharides envelope than the unicellular cells. Monosaccharides such as fucose and rhamnose were considered to confer the hydrophobic property (Klock et al. 2007). However, the composition of monosaccharides of extracellular polysaccharides between the colonial and unicellular *M. aeruginosa* cells was not significantly different (Yang 2010).

### Roles of carbon, nutrients, and other materials in EPS

EPS have the function of the accumulation of nutrients; therefore, EPS as the complex materials could be characterized by carbon, nitrogen, and phosphorus (Comte et al. 2006). A significant portion of photosynthetic production in phytoplankton is released as EPS into waters (Fogg 1983), which contributed to the nutrients and carbon pool. Marine phytoplankton generally excretes 5% to 20% of the carbon they fix (Mague et al. 1980), but more may be released under stress (Fogg 1983). EPS released by phytoplankton are carbohydrate-rich and can thus increase the carbon-to-nutrient ratios of the organic materials. The organic carbon of EPS of cyanobacterium *Synechococcus* sp. was 0.19–0.27 pg/cell (Ikegaya et al. 2015), and the ratio of dissolved acidic polysaccharides (dAPS) to dissolved organic carbon (DOC) of cyanobacterium *Microcystis* sp. could reach up to 46% (Liu et al. 2015). Polysaccharides were considered as the largest fraction of organic carbon in seawater (Passow 2002; Hung et al. 2003). Ye et al. (2012) reported the total dissolved polysaccharides in fresh lakes accounted for 26% of DOC during summer cyanobacterial *Microcystis* blooms in Lake Chaohu, China. The contribution of dAPS to DOC could reach up to 40% during the blooms in Lake Taihu, China (Liu, Qin, Zhu et al. 2014). These results suggested that sEPS are the large fraction of dissolved carbon in waters. Besides, the existence of non-living organic carbon (such as TEP) is two orders of magnitude larger than the biomass-carbon (Hara and Koike 2000). The C:N ratios of TEP were above the Redfield ratio in marine system (Engel 2001). TEP in Lake Taihu account for 25% of particulate organic carbon in waters and even reach up to 40.5% during the summer blooms (Liu et al. 2014). TEP were reported to be photo-degraded by UV radiation, and the products of TEP photo-degradation may be low molecular weight compounds and dissolved inorganic carbon (Ortega-Retuerta et al. 2009; Xavier Mari et al. 2017). Thus, EPS would continue to affect the biological carbon pump in aquatic waters and the regulation of atmospheric $CO_2$, in turn, would be connected with global warming. The cyanobacterial bloom outbreak prefers hot climate (Paerl and Huisman 2008), and the bloom expansion and risk increase with global warming (Paerl et al. 2011).

Therefore, EPS would play an important role in the biogeochemical cycling of carbon, even further regulate the climate change and influence the bloom outbreak.

Hassler et al. (2014) indicated that EPS for other nutrients would enhance the phytoplankton growth due to EPS, which contain micronutrients to some extent. However, most studies were focused on the effect of nutrients (mainly N or P) on EPS production (Shnyukova and Zolotareva 2002; Wang et al. 2011); information about the various nutrient contents in EPS was limited. Proteins as nitrogen source with assistance from other organisms were generally considered as one important part of EPS (Sheng et al. 2010; Liu, Qin, Zhang et al. 2014). Nitrogen-rich compounds (e. g. Tryptophan-like and tyrosine-like substances) were abundant in *Microcystis* EPS (Liu et al. 2017). High nitrogen contents in cyanobacterial EPS were also reported (Klock et al. 2007). Besides, although TEP were mainly composed of polysaccharides, they could adsorb the organic nitrogen (Engel 2001); thus, the nitrogen fraction was also found in TEP. Pannard et al. (2016) indicated that the presence of phytoplankton that produces large amount of EPS could control the elemental ratios (C:N, C:P) of the organic materials in pelagic ecosystems, with potential repercussions on the trophic network. EPS exuded in waters as the form of sEPS or TEP could participate in aggregation formation, which have been widely investigated in oceans (Passow and Alldredge 1995). Berman et al. (2007) revealed that modulation of TEP formation could regulate the fate of marine cyanobacterial blooms (Berman-Frank et al. 2007). TEP in freshwaters might be used as efficient nutrient storage during the beginning of *Microcystis* blooms by investigating TEP abundance during spring in Lake Taihu, China (Huang et al. 2016). Abundant *Microcystis* detritus associated with TEP were also found in Lake Taihu, which would further affect the behavior and fate of cyanobacterial blooms or suspended particles (Huang et al. 2016).

Moreover, EPS as the storage of nutrients can be absorbed directly by cyanobacterial cells, and the labile materials in EPS would also be used by heterotrophic bacteria as carbon and energy sources (Sheng et al. 2010). However, the refractory materials in EPS could be photo-degraded through UV absorption (Ortega-Retuerta et al. 2009). Large amounts of fluorescent substances (humic-like, tryptophan-like, and tyrosine-like), which could absorb UV-radiation, were found in *Microcystis* bEPS and sEPS (Liu, Qin, Zhang et al. 2014). These materials not only can degrade into low-molecular weight materials, which could be further used by bacteria or even decompose into carbon, N, P, and other nutrients (Kieber et al. 1989), but also could form 'sunscreen' – scytonemin during the photo-degradation process (Sorrels et al. 2009), which would explain that the buoyant-blooming *Microcystis* could still prevail on the water surface during summer under the strong ultraviolet radiation. Thus, carbon and nutrients in EPS can be involved in element cycling, which would further influence the bloom outbreak.

*Microcystis* has allelopathic ability. Zheng et al. (2013) indicated that extracellular secretion from *Microcystis* would inhibit submerged macrophytes and stronger allelopathic effects during active growth of cyanobacterial blooms than during breakdown. The exudate solution from *Microcystis* was prepared by filtration through a glass fiber filter, and these exudates were essentially sEPS as mentioned above. That is, algal allelopathic substances could be considered as one component of EPS. Żak and Kosakowska (2014) found that dissolved extracellular secretion of *Microcystis* sp. could influence the growth of green algae. Wang et al. (2017) demonstrated that exudates of *M. aeruginosa* from the exponential growth phase and the stationary phase significantly inhibited the growth of green algae and diatom. This kind of characteristic would be used as the power 'weapon' and would benefit the proliferation of blooming cyanobacteria.

### *Metal adsorption*

Owing to the presence of a large number of negative charges on EPS, EPS that produce cyanobacteria have been considered as chelating agents for the removal of positively charged heavy metal ions from waters (Cavoukian and Stoianov 2011; De Philippis et al. 2011). sEPS are the most efficient fraction in the metal adsorption (Cavoukian and Stoianov 2011). A high diversity of ligands on EPS can displace the metals that are weakly bound to EPS and increase the metal buffering capacity of

the aquatic environment, which supports higher metal inputs before toxic effects are detected in the biota (Tonietto et al. 2014). TEP as the discrete type of EPS might aggregate, move downward, or upward due to the low density of TEP (Passow 2002; Azetsu-Scott and Passow 2004; Xavier Mari et al. 2017). The metals would be involved in various forms of element cycling via association with TEP rather than be distributed by water movement alone.

In another aspect, the characteristic of metal adsorption can induce algal cells to compete in waters (Hassler et al. 2014). The metal adsorption of EPS could be affected by surface charges of EPS. More negative charges, larger capacity of binding cations, and hence the competition for trace elements such as iron ions were increased. *M. aeruginosa* could carry more negative charges than *Microcystis flos-aquae* (Liu et al. 2016) and this phenomenon might elucidate to some extent why *M. aeruginosa* exists in lakes with the largest scale and the longest time and was dominant in number and frequency of occurrence (Zhai et al. 2013).

### Stickiness and rheological properties of EPS

TEP mainly composed of acidic polysaccharides have been reported to be highly sticky (Passow 2002), would adhere to different materials including the *Microcystis* cells or colonies. Therefore, large amounts of acidic polysaccharides in bEPS fraction would also make the surface of cells or colonies sticky. Sticky phytoplankton would enhance the efficiency in which two cell particles that collide stick together afterward (Jackson 1990) and would be more easier to change from small particle into larger sized colonies. The stickiness of *Microcystis* cells was reported in the range of 0–0.47 (Verspagen et al. 2006), and the stickiness values of single cells in culture were nearly zero in our study (Liu et al. 2016); this result was confirmed with the bath-cultured *Microcystis* cells that were consistently in the form of single cells (Wu et al. 2007). The stickiness of colonial *Microcystis* sp. must be further investigated.

EPS are generally flexible and elastic; thus, disentangling these polymers may require high energy to totally detach the EPS (Villacorte et al. 2015) and they can induce viscosity in waters and cause elasticity (Jenkinson et al. 2015). Viscosity controls most hydrodynamic processes at the micro-scale, within which most currently investigated planktonic processes occur (Jenkinson et al. 2015). In seawater, the biological modifications of viscosity are compatible with a habit modification that creates more favorable, turbulence-free, critical conditions for the development of plankton (Jenkinson and Sun 2010). Jenkinson et al. (2015) indicated that rheological effects might modulate the sinking and rising of organic and other materials, associated with marine or lake snow, dynamics of encounter in swimming plankton, adhesion processes, allelopathy, and the dynamics of micro zones of nutrients, toxins, and dissolved allelopathic substances. Large amounts of EPS were produced during the blooming waters. These effects would be magnified, and the specific influence should be investigated.

## Conclusions and prospects

From the results reported in this review, EPS played multiple roles in cyanobacterial blooms. Given that EPS contain carbon, nitrogen, metals, and other nutrients, their production and utilization can influence the flux and cycling of many elements, such as carbon, nutrients, metals in aquatic environments, all these were closely related to the growth and proliferation of cyanobacterial *Microcystis* sp. The compositions (e.g. acidic polysaccharides, uronic acids) and characteristics (e.g. zeta potentials, hydrophobicity, and stickiness) of EPS were the crucial factors in affecting the colony formation of *Microcystis*, which was a key session to form the blooms.

EPS as complex materials, many unknown components, and corresponding characteristics must be further identified. Considering that the importance of EPS and cyanobacterial blooms appear year-round in eutrophic waters, EPS may play crucial roles in maintaining bloom-forming cyanobacterial persistence throughout winter; the specific mechanism also deserves further study. TEP as a special form of EPS are an important agent in the transport of energy

and nutrients through pelagic ecosystems and play a significant role in the biogeochemical cycling of elements. However, their roles in cyanobacterial blooms are also poorly identified. In addition, the role of EPS in modulating water viscosity is poorly established. Water viscosity increased by EPS would affect the motor behavior of organisms (e.g. phytoplankton, zooplankton, et al.) and change the collision chances and the formation of aggregates, the sinking and rising of organic or other materials, and the dynamic of micro zones of nutrients. All these factors could further affect the bloom outbreak.

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