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Towards understanding the role of extracellular polymeric substances in cyanobacterial *Microcystis* aggregation and mucilaginous bloom formation

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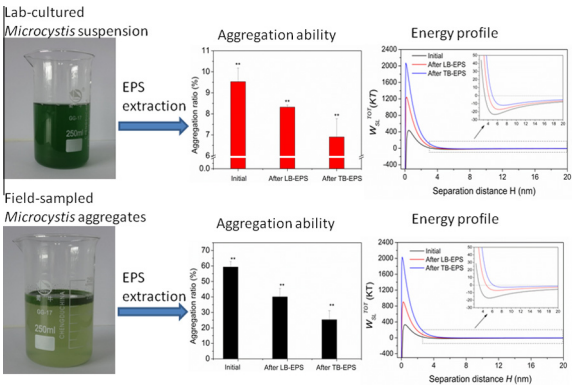
HIGHLIGHTS

- The quantitative role of EPS matrix in *Microcystis* aggregation and bloom formation was studied.
- The aggregation potential of *Microcystis* samples decreased by 27.6–57.4% after EPS extraction.
- Increased energy barrier and second energy minimum accounted for the deteriorated aggregation.
- The predominant energy contribution transformed from TB-EPS to LB-EPS during the bloom formation.
- A conceptual model about EPS function in *Microcystis* aggregation and bloom formation was proposed.

GRAPHICAL ABSTRACT



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ABSTRACT

The development of mucilaginous cyanobacterial *Microcystis* blooms is a serious environmental and ecological problem, and information on the bloom-formation mechanism has been lacking until now. The aggregation of microbial cells was attributed to the matrix of extracellular polymeric substances (EPS). In this study, the quantitative role of EPS matrix in *Microcystis* aggregation and mucilaginous bloom formation was investigated. The results showed that when EPS matrix was extracted, the aggregation abilities decreased by 27.6% and 57.4% for the lab-cultured *Microcystis* suspension and the field-sampled *Microcystis* aggregates, respectively. The extended DLVO theory revealed that EPS extraction increased the energy barrier and the values of the second energy minimum, which accounted for the deteriorated aggregation. Further analysis showed an increasing attraction energy of EPS matrix during the *Microcystis* bloom development, whereas the predominant contribution originated from tightly bound EPS (TB-EPS) and loosely bound EPS (LB-EPS) for the lab-cultured and field-sampled *Microcystis* samples. The heterogeneous energy contribution of EPS subfractions was found to be associated with the variations in organic contents. Specifically, *Microcystis* aggregates exhibited a higher organic content of TB-EPS than of LB-EPS compared with the lab-cultured *Microcystis* suspension, whereas organic content in only the LB-EPS fraction for the bloom samples was significantly higher ($p < 0.01$) than that of the *Microcystis* aggregates. Based on these results, a conceptual model of EPS function was proposed in which TB-EPS plays an

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important role in the formation of *Microcystis* aggregates, after which LB-EPS contributed to the subsequent development from *Microcystis* aggregates to mucilaginous bloom formation.

1. Introduction

In eutrophic lakes and estuaries, cyanobacterial *Microcystis* colonies usually aggregate and float upwards to form nuisance mucilaginous *Microcystis* blooms, which can then deplete the oxygen in the water, impair the recreational value of water bodies, and impact the environmental health of water resources (Carey et al., 2012; Michalak et al., 2013). Meanwhile, the carpet-like and sticky mucilaginous bloom was difficult to skim from lakes to minimize the incidental ecological disasters.

The formation of *Microcystis* blooms is induced by the biotic and abiotic factors, including eutrophication (Paerl and Paul, 2012), climate change (Zhang et al., 2012), hydrographic conditions (Tao et al., 2012) and predation (Wang et al., 2010; Yang and Kong, 2012). As a complex ecological phenomenon, the formation of mucilaginous *Microcystis* blooms is a multi-factor process, and its underlying mechanism is still under debate.

To explore the influencing factors in the formation of this bloom, several attempts were made to explore the proliferation and adhesion of *Microcystis* strains under lab-cultured conditions (Gan et al., 2012; Xu et al., 2013a). The colony size of microbial aggregates was significantly influenced by light intensity under hydrodynamic conditions (Zhang and Kojima, 1998). Elevated ionic calcium levels might also be beneficial for *Microcystis* aggregation (Wang et al., 2011). More recently, a loose *Microcystis* aggregate with a mean diameter of approximately 70 μm was observed after applying pressure such as flagellate grazing (Yang and Kong, 2012). It should be noted that these previous investigations were primarily focused on the relationship between *Microcystis* aggregation and environmental factors. The mechanism that *Microcystis* cells employ to aggregate by modifying their surface properties under environmental pressure is currently unknown.

The aggregation of microorganisms is highly influenced by surface properties including the zeta potential and hydrophobicity/hydrophilicity (Sheng et al., 2010), which can be described by the extended DLVO theory (Liu et al., 2008; van Oss, 1995). Based on this theory, some results and phenomena have been explained, such as microbial adhesion (Bayoudh et al., 2009), microbial flocculability (Li et al., 2012; Liu et al., 2008), sludge stability (Liu et al., 2010; Yang et al., 2013), and membrane fouling potential (Chen et al., 2012; Li et al., 2013; Su et al., 2013).

Microbial extracellular polymeric substances (EPS), which are produced via a number of approaches including excretion, secretion, sorption and cell lysis, among others, represent a heterogeneous polymer and primarily consist of proteins, carbohydrates, humic substances and other biological macromolecules (Sheng et al., 2010; Xu et al., 2013a). Due to its high molecular weight and multiple functional groups, EPS can affect the surface properties of microbial cells through electrostatic binding (Liu and Fang, 2002), polymer bridging (Vogelaar et al., 2005) and sweep strategy (Yu et al., 2009), thus exhibiting a strong impact on microbial aggregation. As a kind of microbial aggregation, *Microcystis* blooms are made up of *Microcystis* colonies embedded in a cloud of EPS matrix (Xu et al., 2013b). And the colony-forming cyanobacteria usually float upwards to form the mucilaginous blooms due to the gas vacuole and large EPS sheaths (Hajdu et al., 2007). It should be noted that the aggregation contribution could be weakened and/or disappear when EPS matrices are extracted from the microbial surfaces. Therefore, the quantitative role of EPS in *Microcystis* aggregation and bloom formation can be determined by employing the extended DLVO theory and surface property comparison before and after EPS extraction. However, this information has been lacking till now to the best of our knowledge.

Two *Microcystis* samples, a lab-cultured *Microcystis aeruginosa* suspension and a field-sampled *Microcystis* aggregate, were studied. Their aggregation ratios and surface thermodynamic characteristics including contact angles and zeta potential in response to EPS extraction were systematically evaluated. The extended DLVO theory was then applied to interpret the results, and the roles of EPS and its subfractions in *Microcystis* aggregation were explored. Finally, surface *Microcystis* blooms with different growth stages were sampled to verify the role of EPS in mucilaginous *Microcystis* aggregation. Results obtained would help to explain the mechanism of *Microcystis* aggregation and bloom formation in aquatic environments.

2. Materials and methods

2.1. Sample preparation

Lab-cultured *Microcystis aeruginosa* (*M. aeruginosa* 7820), which was isolated from cyanobacterial *Microcystis* blooms in eutrophic lakes, was cultivated in conical flasks in batch mode. The culture grew in BG_{11} medium under a light–dark regime of 12:12 h with a light irradiance of 32 μmol photons m^{-2} s^{-1} at 25 °C (Xu et al., 2013a). After 20 d of cultivation, a stable *M. aeruginosa* suspension with a biomass of 1.68 ± 0.11 g L^{-1} was obtained.

Field-sampled *Microcystis* aggregates were collected from Meiliang Bay, a severely eutrophic region in northwestern Lake Taihu (30°55′40″–31°32′58″N and 119°52′32″–120°36′10″E), the third largest freshwater lake in China (Fig. S1 in the Supporting Information, SI). Samples were collected in the spring-summer period (May, 2013), during which obvious algal aggregation was observed on the water surface. The pH, diameter, and algal biomass of the samples were 6.97 ± 0.03, 250 ± 30 μm, and 1.35 ± 0.08 g L^{-1}, respectively. To verify the role of EPS in bloom formation, early-stage and mature *Microcystis* blooms were sampled in June and August of 2013, from Lake Taihu. More detailed information on the sample collection protocol can be found elsewhere (Xu et al., 2013b).

2.2. EPS extraction

The EPS matrix was characterized by the shear-sensitive properties, thus can be fractionated into loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) fractions in terms of binding with microbial cells (Li and Yang, 2007). The detailed fractionation procedure has been described elsewhere (Xu et al., 2013a,b), and only a brief description will be given here. The *Microcystis* samples were first centrifuged at 2500*g* for 15 min to remove the soluble components. The residue was then dissolved in a 0.05% NaCl solution to the original volume and centrifuged at 5000*g* for 15 min, with the liquid collected carefully for measurement of LB-EPS. The harvested algal samples were then re-suspended in the NaCl solution, heated at 60 °C for 30 min and centrifuged at 15 000*g* for 20 min, with the collected liquid as the TB-EPS fraction.