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Interpretation of the disparity in harvesting efficiency of different types of *Microcystis aeruginosa* using polyethylenimine (PEI)-coated magnetic nanoparticles

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ABSTRACT

Heteroaggregation between magnetic nanoparticles (MNPs) and cells is a crucial precondition for the harvesting and separation of algae from the aquatic environment. However, separation mechanisms have not been fully elaborated in previous studies, especially regarding cyanobacteria coated with extracellular polymeric substances (EPS). The present study was conducted to investigate the interaction between PEI-coated iron-oxide nanoparticles (IONPs) and *M. aeruginosa* as a function of cell type (FACHB-1343 and FACHB-905) and cells with/without extracellular polymeric substances (EPS). The results demonstrated that harvesting efficiency (HE) varied with the different cell types with/without EPS surrounding the cyanobacteria. The PEI-coated IONPs showed higher HE for M9 than for M1 with the same mass ratio of NPs/cyanobacteria. Furthermore, EPS significantly affected the HE because of their heterogeneous composition, which was interpreted by spectral analysis and the DLVO theory. Overall, this study provides guidance on cyanobacteria harvesting with different environmental conditions when employing magnetic separation technology.

1. Introduction

The cyanobacterial *Microcystis aeruginosa* is a widely distributed bloom-forming microorganism that deteriorates the freshwater system by e.g. the accumulation of cyanobacterial aggregation and floating on the surface, as well as the depletion of the dissolved oxygen levels by excessive respiration. This results in the death of other aquatic organisms [1,2]. Moreover, the troublesome and sticky mucilaginous *Microcystis* bloom is difficult to eliminate from eutrophic water bodies [3]. Furthermore, cyanobacterial secondary metabolite toxins have been identified as potential stress factors that pose a significant risk to ecological and human health [4–6]. However, in contrast with its negative aspects, *M. aeruginosa* is an important biofuel in the bioenergy industry [7,8]. Bioenergy is considered a type of sustainable energy that could be useful in mitigating the excessive reliance on fossil fuels; therefore, it is important to remove and harvest cyanobacteria from water bodies. Various approaches (filtration, settlement, or centrifugation) to algal harvesting have been considered as not cost-effective [9], because such methods consume large amounts of resources and energy. However, magnetophoretic separation was developed and has been proven an effective method for the harvesting of cyanobacteria from water [10–12]. This process is based on the heteroaggregation between magnetic particles and cyanobacteria cells [13]. It has the advantage that the particle-cell mixture can be separated from the water within minutes by using a magnetic field [8,10,12]. In addition to high collection efficiency, magnetophoretic separation technology offers other advantages, such as low energy usage and low cost, and renewable and reusable magnetic particles [8,14]. Significant efforts have been made to increase the collection efficiency by developing new magnetic particles or changing the surface properties of the magnetite particles [10,13,15,16].

The surface property of colloidal particles is an important factor that influences colloidal interaction. This interaction between the magnetic particles and cyanobacteria cells is a precondition for the heteroaggregation and harvesting of cyanobacteria from water [13]. Given that the harvesting efficiency is comparatively low with the usage of naked IONPs, various agents, such as polyethylenimine, cationic polyacrylamide, and poly (diallyldimethylammonium chloride) have been applied to modify the MNPs in an effort to improve the efficiency [13,17,18]. In this regard, both the surface-coated magnetic particles

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and the cyanobacteria coated with extracellular polymeric substances (EPS) should be considered to improve collection efficiency. EPS are composed mainly of polysaccharides, proteins, humic substances, and other biological macromolecules [19–21]. Cyanobacteria can produce EPS through various approaches, such as excretion, secretion, sorption, and cell lysis [22,23]. EPS act as a barrier between the cell and the ambient environment because of their heterogeneous compositions that enable EPS to interact with other substances [22,24]. Furthermore, EPS significantly affect the cell surface properties; consequently, these substances with high molecular and multiple functional groups have a strong effect on cyanobacterial float and aggregation [3]. Zeta potential and surface hydrophobicity/hydrophilicity are important surface properties that influence the aggregation of microorganisms [23].

According to the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory [25,26], the heteroaggregation process is controlled likely by different colloidal forces, which include electrostatic repulsion, van der Waals attraction, and Lewis acid-base interactions. These colloidal forces can be influenced significantly by both the electrolytic environment (such as pH, ionic strength, and ionic valence) and the colloidal particles [27,28]. For example, many studies have reported that the surface charge affect the electrostatic interactions between colloidal particles [28–30]. However, colloidal particle size also influences the heteroaggregation between the EPS-coated cyanobacteria cells and the magnetic NPs, although this has not been reported. The harvesting efficiency (HE) of cyanobacteria could vary with the types of *M. aeruginosa* in the presence of the same magnetic NPs.

The current study aims to investigate the heteroaggregation between IONPs and *M. aeruginosa* with variations in electrolyte cations and cell types. Therefore, the differences in the coating on the cyanobacteria cells were compared and the DLVO theory was applied to interpret the interaction energy of the colloidal particles.

2. Materials and methods

2.1. Cyanobacteria growth and preparation

The cyanobacteria strains *M. aeruginosa* FACHB-1343 (M1) and FACHB-905 (M9) were obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology (FACHB), Wuhan, Hubei Province, China. Strain M1 was isolated from the Taihu Lake and M9 was isolated from the Dianchi Lake in China. The *M. aeruginosa* cells were cultured in conical flasks with a standard BG11 medium [31]. The cultures were kept at 25 ± 0.5 °C in an incubator under illumination at 2000 lx, with a 12/12 h light/dark regime. During incubation, the cultures were shaken three times per day to ensure optimum growth. The concentration of cyanobacteria in the medium was calculated by relating the optical density (OD_{660}) to the dry cell weight (DCW). Detailed information is presented in the Supporting Information section.

2.2. Preparation and characterisation of metal-oxide nanoparticles

Magnetic iron-oxide nanoparticles (IONPs), purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai), were used in this experiment. Polyethylenimine (PEI) was obtained from Aladdin Reagent (Shanghai, China). The method described by Ge et al. [13] was used to prepare the PEI-coated IONPs. To coat the naked IONPs with PEI, the IONP suspension was first disposed with a sonicator in an ice-water bath (500 W, 80 kHz, 10 min), after which the PEI was introduced to the suspension with a mass ratio of NP/0.4PEI. The PEI-coated IONP mixture was placed on a rotary shaker at 25 °C and incubated for 20 min. Subsequently, the suspension was centrifuged at 5000*g* for 10 min and the residuals were washed carefully with Milli-Q water (18 MΩ cm^{-1}, pH 7 ± 0.1). The collected PEI-coated IONPs were stored in Milli-Q water. Before the heteroaggregation experiments, the stock suspension was agitated by ultrasound (100 W, 80 kHz, 25 °C) for 15 min. Particle size and morphology were characterised by scanning electron microscopy (SEM). The hydrodynamic diameter (HDD), particle size distribution (PSD), and the zeta potential (ζ) of the PEI-coated IONPs were measured with a Zetasizer Nano ZSP instrument (Malvern Instruments Ltd., UK).

2.3. Cyanobacteria characterisation

The zeta potential of M1 and M9 were determined by the Zetasizer (Nano ZSP, Malvern Instruments Ltd., UK), and the diameter of both cyanobacteria were measured with a light microscope (Zeiss Axioskop 40, Shanghai). All the measurements were conducted at 25 °C and repeated three times by using freshly rinsed cells (10^7–10^8 cells per mL). The cell surface hydrophobicity was measured by employing the microbial adhesion to hydrocarbon (MATH) test [32,33]. The initial absorbance of the cell suspension (OD_{660}) was adjusted to 0.3 ± 0.01 to ensure standardisation across the tests with a ultraviolet-visible UV–Vis spectrophotometer (TU-1901, Persee, China) at a wavelength of 660 nm, and 1 mL of n-dodecane (laboratory grade) was added to 4 mL cell suspension in colorimetric tubes. The mixture was vortexed with a vortex generator (WH-2, Shanghai Huxi Analysis Instrument Factory, China) at full speed for 2 min and then left to stand for 20 min to allow sufficient phase separation. The hydrophobicity of each cell type was quantified as the percentage of total cells partitioned into the hydrocarbon phase [34]. All assays were performed in triplicate, and the mean percentage (%) hydrophobicity of the microbial cells was calculated with Eq. (1).

$$Hydrophobicity\% = \frac{0.3 - OD_{660}}{0.3} \times 100 \quad (1)$$

2.4. Heteroaggregation for cyanobacteria harvesting

The heteroaggregation between PEI-coated IONPs and the different cyanobacteria was assessed comparatively with different electrolyte cation conditions. Cell suspension was applied for the heteroaggregation experiments when the cells were in the exponential growth phase, after approximately 7 d of cultivation. Different amounts of electrolyte cations (0.5–100 mM) were added to the cell suspension in a 35 mL glass bottle, with various mass ratios (PEI-coated IONPs to cyanobacteria, 0.05–0.35, w/w). The initial cell concentration was ~10^7 cell/mL, with the optical densities (OD_{660}) approximately 0.3. After mixing completely for 2–3 min, a NdFeB magnet (length × width × height: 20 mm × 10 mm × 5 mm) was applied to the bottom of the glass bottle to harvest the aggregates. This process lasted 5 min and the magnetic field strength of the surface of the NdFeB magnet was 3400 G, as measured by a magnetometer (TM-701, KANETEC CO., Japan). The indicators to evaluate the collection efficiency of the cyanobacteria, including harvesting efficiency (HE) and recovery efficiency (RE) were calculated as follows [13]:

$$HE(\%) = \left(1 - \frac{C_t}{C_0}\right) \times 100 \quad (2)$$

$$RE(\%) = \left(1 - \frac{C_t/C_0}{C_t'/C_0'}\right) \times 100 \quad (3)$$

where C_0 and C_t indicate the cyanobacteria concentration (g/L) of the supernatant both before (at time 0) and after (at time t) adding the PEI-coated IONPs, and C_t' and C_t' indicate the cyanobacteria concentration (g/L) of the control group without the addition of PEI-coated IONPs. HE was applied to evaluate the algal separation performance. RE was defined as the recovery of the cyanobacteria in the presence of PEI-coated IONPs divided by the recovery of the cyanobacteria without the addition of PEI-coated IONPs, which was applied to compare different type of cyanobacteria on their stability [13,35].