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The interactions of algae-bacteria symbiotic system and its effects on nutrients removal from synthetic wastewater

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ABSTRACT

The ability of *Chlorella vulgaris-Bacillus licheniformis* and *Microcystis aeruginosa-Bacillus licheniformis* consortiums to eliminate total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and soluble chemical oxygen demand (sCOD) from synthetic wastewater was studied. The highest values of dry cell weight, chlorophyll-a, and chlorophyll metabolism related genes/bacterial rRNA gene copies were obtained in the *Chlorella vulgaris-Bacillus licheniformis* system at *Chlorella vulgaris* and *Bacillus licheniformis* ratio of 1:3. On the 10th day, the *Chlorella vulgaris-Bacillus licheniformis* system at this ratio removed 86.55%, 80.28% and 88.95% of sCOD, TDP and TDN, respectively. But, the *Microcystis aeruginosa-Bacillus licheniformis* system at this ratio only removed 65.62%, 70.82%, and 21.56% of sCOD, TDP and TDN, respectively. *Chlorella vulgaris* and *Bacillus licheniformis* could coexist as an algae-bacteria consortia and quorum sensing substances (autoinducing peptides and bis (3′-5′) diguanylic acid) concentrations were measured. Finally, the interactions and communication patterns between *Chlorella vulgaris* and *Bacillus licheniformis* were depicted.

1. Introduction

The concept of an algae-bacteria consortia was initially proposed in 1981 to study uptake of nitrogen in a flocculating algae-bacterial system (Nambiar and Bokil, 1981). There were a large number of bacteria in the natural environment that formed relationships with algae. This algae-bacteria symbiotic system was the ecological basis for natural water purification. In the algae-bacteria symbiotic system, organic matter in the water body was oxidized and decomposed by aerobic bacteria to produce ammonium nitrogen (NH_3-N), phosphate and carbon dioxide. Algae used these nutrients, along with sunlight as an energy source, to photosynthetically synthesize cellular material. In the process, oxygen was released, thereby allowing bacteria to continue oxidation of organic matter (Derry and Jacobsen, 1990). The removal efficiency of nitrogen (N) and phosphorus (P) in the environment could be improved by taking advantage of the synergy between multiple species, when compared to traditional single and multi-step treatments (Brenner et al., 2008). This could be achieved by combining the ability of algae to assimilate N, P, and other nutrients in sewage with the powerful ability of bacteria to decompose organic pollutants. Exploiting the joint relationship between the carbon dioxide-oxygen cycle and the material cycle created by algal-bacterial symbiosis in combination with other treatment methods might aid in development of a new technology to solve the problem of excessive N, P and organic matter that resulted in water eutrophication of urban sewage.

The removal efficiencies of high-rate algal pond were higher than that of traditional stabilization pond and it was cost effective and easy to be operated. So, it was highly suitable for developing areas with relatively weak economies (Park et al., 2011). The activated algae method was developed in the 1970s and the removal rates of biochemical oxygen demand, chemical oxygen demand, N and P for industrial-scale sewage treatment were 97%, 87%, 92% and 74%, respectively (Jr and Mckinney, 1972; Gomez et al., 1995). A *Chlorella vulgaris-Azosprillum brasilense* system was used to treat municipal wastewater and removal efficiencies for NH_3-N, nitrate nitrogen and P were 100%, 15% and 36%, respectively (de-Bashan et al., 2004). In contrast, single algae systems without bacteria were only able to achieve removal efficiencies of 75%, 6%, and 9%, respectively. Furthermore, wastewater treatment and biomass production could be achieved simultaneously in the algae-bacteria symbiotic system. Although the coexistence of algae and bacteria has been known for some time, little research has been conducted on algae-bacteria symbiotic systems, including N and P removal, biomass production and their interaction and communication mechanisms.

In this study, synthetic wastewater was used as a nutrient medium for the algae-bacteria consortium of *Chlorella vulgaris-Bacillus licheniformis* and *Microcystis aeruginosa-Bacillus licheniformis*. The removal efficiencies for soluble chemical oxygen demand (sCOD), total dissolved phosphorus (TDP), and total dissolved nitrogen (TDN) were determined, along with

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the chlorophyll-a concentration and dry cell weight of the symbiotic systems. Moreover, the morphologies of the *Chlorella vulgaris-Bacillus licheniformis* consortium were observed by scanning electron microscopy (SEM). The relative ratio of chlorophyll metabolism-related genes to bacterial genes was analyzed by q-PCR. Furthermore, the concentrations of quorum sensing (QS) substances (autoinducing peptides (AIP) and bis (3′-5′) diguanylic acid (c-di-GMP)) in the *Chlorella vulgaris-Bacillus licheniformis* symbiotic system were measured at different algae-bacteria ratios to determine their simple interactions and analyze the communication patterns.

2. Materials and methods

2.1. Algae, bacteria and the synthetic wastewater

Microcystis aeruginosa (FACHB-315) and *Chlorella vulgaris* (FACHB-8) were obtained from the Freshwater Culture Collection at the Institute of Hydrobiology (Wuhan, China). Algae inoculums were cultivated in 1000 mL conical flasks containing 400 mL BG-11 medium under a light intensity of 120 μmol/(m^2 s) for 7 days. The cell concentrations of *Chlorella vulgaris* and *Microcystis aeruginosa* were quantified by the hemocytometer method using an optical microscope. The relationship between algal cell concentration and optical density of the algal solution at 680 nm (OD_{680}) was established, thus allowing for calculation of algal cell concentrations by measuring OD_{680} of the algae solution according to the linear relationship.

Bacillus licheniformis (No. 1.7461) was obtained from Institute of Microbiology, Chinese Academy of Sciences (Beijing, China). The bacterial cells were cultured in a 1000 mL conical flask containing 400 mL of LB medium at 28 ± 1 °C for 7 days with a 12 h/12 h light/dark cycle at a light intensity of 120 μmol/(m^2 s).

The synthetic wastewater was used in this study and the compositions were glucose 300 mg/L, NH_4Cl 22.16 mg/L, KNO_3 6.57 mg/L, $NaNO_2$ 1.08 mg/L, and KH_2PO_3 5.09 mg/L. The Physicochemical properties were shown in Table 1. All chemicals during the experimental operation were supplied by Aladdin Chemical Industries Ltd., USA.

2.2. Experimental set-up

The experimental system in a sterile environment was shown in Fig. 1 and consisted of a reactor (conical flask, 250 mL), a shaking incubator (ShanZhi, China, TS211GZ), and a sampling tap. Reactors contained synthetic wastewater (150 mL) and a defined ratio of algae and bacteria (Shown in Table 2).

2.3. Analytical methods

2.3.1. Chlorophyll-a concentration

The chlorophyll-a concentration was determined for each algae-bacteria mixture. First, samples (10 mL) were centrifuged (Dynamica, Velocity-14R, Australia) at 4000 rpm for 10 min and the supernatant was discarded. Second, the pellet was re-suspended in 90% acetone (10 mL) and then stored at 4 °C for 24 h in darkness. Finally, the suspension was centrifuged at 4000 rpm for 15 min. The supernatant was collected and used to determine chlorophyll-a concentration by ultraviolet spectrophotometry (METASH, UV-5200, China) at wavelengths of 630 nm, 645 nm, 663 nm, and 750 nm. The 90% acetone solution was used as the blank (Lorenzen, 1967). Chlorophyll-a concentration (μg/L) was calculated by the following equation:

$$Chl{-}a = [11.64 \times (OD_{663}{-}OD_{750}){-}2.16 \times (OD_{645}{-}OD_{750}) + 0.10 \times (OD_{630}{-}OD_{750})]\cdot V$$

where, V is the extraction volume (L) and OD is the optical density at λ wavelength (nm) (Römling et al., 2013).

Table 1
Physicochemical properties of the synthetic wastewater.

Characteristics	Values
pH	7.4 ± 0.12
sCOD	175.78 ± 11.26
TDN (mg/L)	31.23 ± 2.04
TDP (mg/L)	4.97 ± 0.53

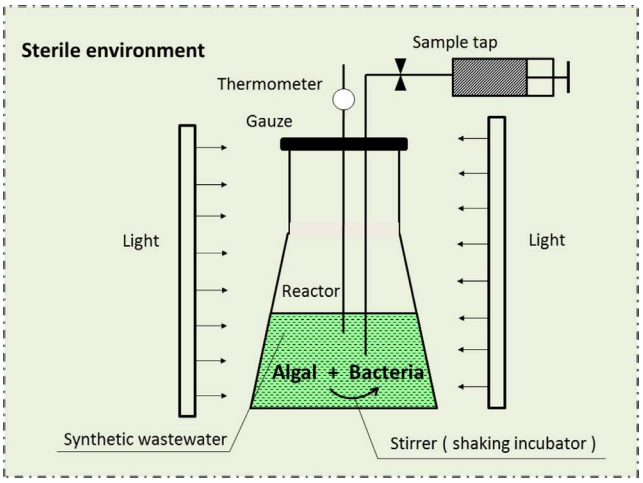

Fig. 1. Experimental set-up of the algae-bacteria symbiotic system.

Table 2
The initial additions of algae and *Bacillus licheniformis*.

Algal-bacteria ratio	*Chlorella vulgaris* Or *Microcystis aeruginosa* (×10^5 cell/mL)	*Bacillus licheniformis* (×10^5 CFU/mL)
1:0	1	0
1:1	1	1
1:2	1	2
1:3	1	3
2:1	2	1
3:1	3	1

2.3.2. Dry cell weight

To determine dry cell weight, an unused filter of 0.45 μm pore size dried for 6 h in a dryer (Lichen, 101-1S, China) at 105 ± 5 °C and then was weighed by an electronic microbalance (Sartorius, Germany). The filter was dried after filtering 5 mL sample. The net dry weight was calculated by subtracting the weight of the unused filter from the final weight.

2.3.3. SEM observations

The morphological characteristics of the algae-bacteria aggregates were observed by SEM (Nova NanoSEM NPE-207, USA). Pretreatment of the algae-bacterial consortium was completed as previously described (Wu et al., 2010). The *Chlorella vulgaris-Bacillus licheniformis* consortia were taken at the 10th day and washed 3 times using Milli-Q ultra-pure water to remove impurities and medium. Samples were immersed in glutaraldehyde (2.5%, vol%) at 4 °C for 12 h. Samples were then rinsed 3 times with Milli-Q ultra-pure water and dehydrated using an ethanol gradient of 30%, 50%, 70%, 80%, 90%, and 100%. The samples were replaced by tertiary butanol for 2 h. Finally, all samples were dried in critical point dryer (Tousimis, Samdri-PVT-3D) and coated with gold before SEM observation.

2.3.4. Measurement of water quality

The algae-bacteria solution (15 mL) was centrifuged at 4000 rpm