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The production of cyanobacterial carbon under nitrogen-limited cultivation and its potential for nitrate removal

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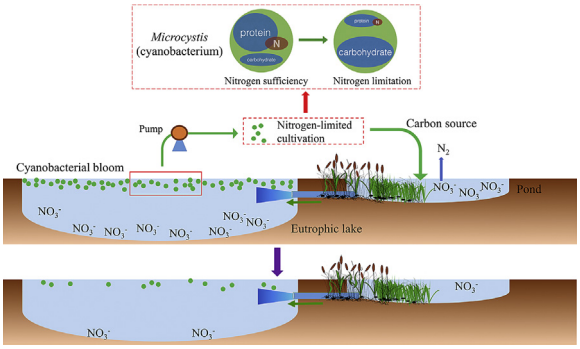
HIGHLIGHTS

- N-limitation could induce organic and carbohydrate accumulation in cyanobacteria.
- Under such stress, *Microcystis* had a higher intracellular C/N ratio.
- Its leachates were biodegradable and contained more DOC but a small amount of DTN.
- These *Microcystis* could significantly enhance the denitrification.

GRAPHICAL ABSTRACT



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ABSTRACT

Harmful cyanobacterial blooms (CyanoHABs) represent a serious threat to aquatic ecosystems. A beneficial use for these harmful microorganisms would be a promising resolution of this urgent issue. This study applied a simple method, nitrogen limitation, to cultivate cyanobacteria aimed at producing cyanobacterial carbon for denitrification. Under nitrogen-limited conditions, the common cyanobacterium, *Microcystis*, efficiently used nitrate, and had a higher intracellular C/N ratio. More importantly, organic carbons easily leached from its dry powder; these leachates were biodegradable and contained a larger amount of dissolved organic carbon (DOC) and carbohydrates, but a smaller amount of dissolved total nitrogen (DTN) and proteins. When applied to an anoxic system with a sediment-water interface, a significant increase of the specific NO_X^--N removal rate was observed that was 14.2 times greater than that of the control. This study first suggests that nitrogen-limited cultivation is an efficient way to induce organic and carbohydrate accumulation in cyanobacteria, as well as a high C/N ratio, and that these cyanobacteria can act as a promising carbon source for denitrification. The results indicate that application as a carbon source is not only a new way to utilize cyanobacteria, but it also contributes to nitrogen removal in aquatic ecosystems, further limiting the proliferation of CyanoHABs.

1. Introduction

Harmful cyanobacterial blooms (CyanoHABs) are expanding worldwide and represent a serious threat to water and habitat

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quality, drinking water supplies, and the ecological and economic sustainability of freshwater ecosystems (Corbel et al., 2014). This harmful phenomenon is usually linked to anthropogenic eutrophication and external nutrient loading (phosphorus and nitrogen) (Jančula and Maršálek, 2011; Paerl et al., 2016). High level of nitrogen can improve the proliferation of cyanobacteria blooms, and some species, such as *Microcystis*, shift from dominance of non-toxic strains to toxic strains (Davis et al., 2010; O'Neil et al., 2012).

Present studies mostly focus on influence of nitrogen on cyanobacteria; however, recent studies investigated how CyanoHABs affect the nitrogen removal. Such studies have found that there is an important link between cyanobacteria and denitrification in eutrophic lakes (Chen et al., 2012; Finlay et al., 2013). Lake investigations have suggested that with the decomposition of algae, abundant particulate and dissolved organic carbon is released, providing electronic donors for denitrifiers to convert nitrate to N_2, thus promoting the permanent removal of nitrogen from the aquatic ecosystem (Bernhardt, 2013). Based on these findings, we assumed that cyanobacteria might be an alternative carbon source for nitrate removal systems, which could be applied to water treatment systems and ponds/wetlands located at the riparian zone. If certain harmful bloom-forming cyanobacteria, such as *Microcystis,* could be utilized as a carbon source for nitrate removal, it could lead to a solution to eutrophication issues.

Cyanobacteria have relatively high carbon content, approximately 50% of their dry weight (Grobbelaar, 2004; Milledge, 2011). However, there are two key limitations of cyanobacteria as a carbon source for denitrification: (1) protein is the most abundant carbon-containing component, usually accounting for 50%—70% of their dry weight, but it is difficult for microbes to metabolize; comparatively, carbohydrates, although more degradable, only account for 10%—30% of their dry weight (Becker, 2007; Singh et al., 2011; Spolaore et al., 2006); and (2) cyanobacteria normally have a high nitrogen content, ranging from 1% up to 14% (typically approximately 5—10%) of their dry weight, and the intracellular C/N ratio is low, approximately 6:1 (Grobbelaar, 2004; Redfield, 1958). Cyanobacteria are considered to be endogenous nitrogen sources in aquatic environments. If the C/N ratio and carbohydrate content could be improved by artificial manipulation, cyanobacteria will be a promising carbon source. This proposes a new way to utilize cyanobacteria, and also contributes to nitrogen removal in aquatic ecosystems, further limiting the proliferation of CyanoHABs. Additionally, until now, no studies have developed an efficient means to obtain cyanobacterial carbon that is comparable to traditional carbon sources like glucose.

Nitrogen-limited cultivation is a very simple method, and it is one of the most effective and widely explored strategies regarding the facilitation of lipid accumulation in algal cells (Li et al., 2008; Mandal and Mallick, 2009). This study was conducted to investigate that if it could be an efficient method to convert *Microcystis* into a promising carbon source. The common bloom-forming cyanobacterium, *Microcystis aeruginosa,* was cultivated under nitrogen-limited conditions to test its growth, intracellular C/N ratio, and potential for use as a carbon source, as well as the leachates of its dry powder. Furthermore, simulation experiments were conducted using the dry powder of *Microcystis* to evaluate its potential for enhancing denitrification.

2. Methods and material

2.1. Cyanobacteria strain

Nontoxic *Microcystis aeruginosa* (FACHB 526) was provided by the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. *Microcystis* were incubated with an autoclaved standard BG-11 medium, which contains an initial concentration of nitrate (NO_3^--N) at approximately 247 mg L^{-1} (1.5 g $NaNO_3$ L^{-1}). The *Microcystis* culture was maintained in an artificial climate chamber at 25 ± 1 °C, with a light-dark cycle of 14:10 h and illumination of 50 μmol photons m^{-2} s^{-1}.

2.2. Microcystis cultivation with different concentrations of nitrate

2.2.1. Experimental apparatus

An aeration cultivation experimental apparatus was designed for *Microcystis* cultivation, as illustrated in Fig. 1. The air pump supplied ambient air, and the air was successively passed through a gas diverter and a 0.45-μm filter into the cultivation system. The bottles contained approximately 1000 ml of cyanobacteria culture. Aeration was continuous only during the light cycle at an airflow rate of approximately 2 L min^{-1}.

2.2.2. The growth and features of Microcystis under nitrogen-limited cultivation

Nitrate modified BG-11 medium was inoculated with *Microcystis* (pre-cultivated in standard BG-11 medium) in an exponential growth phase. $NaNO_3$ was used for nitrate (NO_3^--N). The treatments included an addition of NO_3^--N at initial concentrations of 100 mg NO_3^--N L^{-1}, 50 mg NO_3^--N L^{-1}, 25 mg NO_3^--N L^{-1} and 10 mg NO_3^--N L^{-1}, respectively, using three replicates for each concentration. The initial Chl a concentration was approximately 0.5 mg L^{-1}.

The experiment lasted 12 d. During the cultivation, culture samples were taken to measure the Chl a and NO_3^--N concentrations in the culture medium. At the end of the cultivation, 20 ml of *Microcystis* culture was taken, of which 2 ml of thr culture was used to measure the specific density of *Microcystis* and 10 ml was filtered through a 0.45-μm membrane filter. Then the filtrate and the remaining culture were used to measure COD_{Cr}, respectively. The potential carbon source (mg COD L^{-1}) was expressed as the intracellular COD, which was calculated by subtracting the COD value of the filtrate from that of the culture.

Microcystis cells were harvested by low-speed centrifugation (Sorvall ST 16R, Thermo, Unite States). The harvest rate was calculated as follows:

$$\text{Harvest rate } (\%) = (1 - C_{after}/C_{before}) \times 100\% \quad (1)$$

where C_{before} is the Chl a concentration before centrifugation and C_{after} is the Chl a concentration of the supernatant after centrifugation.

Harvested cells were washed with purified water three times to remove nitrate from the culture medium and then freeze-dried by Vacuum Freeze Dryer (ZX-27, Zhixin Instrument, China). Dry powder was collected for analysis of the dry weight and content of nitrogen and carbon and was then used in the following experiments.

To better describe the features of *Microcystis,* raw data were further processed, and some specific terms were used as follows: (1) intracellular C/N ratio (by atoms), calculated by dividing the mole number of carbon by the mole number of nitrogen in the dry powder; mole numbers were calculated by dividing carbon and nitrogen contents by the respective atomic weights; (2) potential carbon source in biomass (dry weight), mg COD mg^{-1} dry weight, calculated by dividing the intracellular COD (mg COD L^{-1}) by the dry weight (mg L^{-1}); and (3) yield of the potential carbon source per consumed NO_3^--N, mg COD mg^{-1} NO_3^--N, calculated by dividing the intracellular COD (mg COD L^{-1}) by the consumed NO_3^--N (mg L^{-1}), which was the difference between the initial and final concentrations of NO_3^--N in the culture medium.