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Bioremediation of Reclaimed Wastewater Used as Landscape Water by Using the Denitrifying Bacterium *Bacillus cereus*

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Abstract Organic matter and nitrogen removal from reclaimed wastewater used as landscape water was carried out by in situ bioremediation. A denitrifying bacterium *Bacillus cereus* DNF409 was introduced for this purpose, and the amount of *B. cereus* used was optimized. The total nitrogen (TN) content and chemical oxygen demand (COD) of the landscape water decreased from 9.86 to 3.1 mg/L (removal rate, 68.6%) and from 127 to 36 mg/L (removal rate, 71.7%). The transparency of water increased from 0.2 to 0.55 m.

Keywords *Bacillus cereus* · Bioremediation · Landscape water · Reclaimed wastewater

Due to the increasing scarcity of water resources, reclaimed wastewater is being increasingly reused for landscape water. It is also being used as supplemental water supply for urban rivers and lakes (Bixio et al. 2006). Due to the high content of pollutants in reclaimed wastewater, which could cause water body eutrophication, algal populations proliferate rapidly at high temperature during summer, resulting in the formation of water blooms. Water blooms not only deteriorate water quality, leading to the death of aquatic organisms, but also decrease the esthetic value of landscape water bodies (Yang and Abbaspour 2007).

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Phosphorus and nitrogen are mainly responsible for the eutrophication of water bodies. Studies have attempted to develop methods to eliminate these elements and improve the quality of landscape water (Wang et al. 2007). Ecological methods, such as cultivating submerged plants and rearing fish, and physicochemical methods, such as using chemical flocculants, ozone ventilation, ultraviolet radiation, and ultrasonic waves to kill algae, are currently being employed as water treatment strategies (Coveney et al. 2002). Moreover, the results of bioremediation experiments have indicated that microbial products can be used to eliminate organic pollutants and improve water quality (Burford et al. 2003; Devaraja et al. 2002; Queiroz and Boyd 1998; Vezzulli et al. 2004).

The Grandeur Garden of Beijing is the first park in Beijing City to use reclaimed wastewater as landscape water. The inflow of reclaimed wastewater has aggravated the eutrophication of water bodies in this park. *Bacillus cereus* DNF409, an effective denitrifying bacterium isolated from sludge and applied successfully in the bioremediation of various lakes in China. The use of this bacterium for the bioremediation of polluted landscape water in urban areas provided an effective, economical, and simple method for achieving biological nitrogen removal and water quality improvement. In this study, we used *B. cereus* DNF409 for the treatment of the Grandeur Garden Lake and provided a new applicable method for urban landscape water bioremediation.

Materials and Methods

The experiments were carried out in the Grandeur Garden of Beijing, China. The water bodies in this garden cover an area of approximately 10,000 m^2 (water volume, 10,000 m^3)

and are approximately 0.6–1.2 m deep (average depth, 1 m). Reclaimed wastewater rich in many nutrients was introduced in the water bodies in this garden. This inflow of nutrients led to the increased accumulation of nitrogen and phosphorus and stimulated the growth of algal populations, which resulted in the eutrophication of the water bodies. The properties of the reclaimed wastewater used in this study are shown in Table 1.

The *B. cereus* strain DNF409 was provided by the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University. The solid medium for culturing this strain consisted of rice straw powder (300 g/kg), wheat bran (700 g/kg), glucose (40 g/kg), peptone (20 g/kg), yeast extract (20 g/kg), KH_2PO_4 (10 g/kg), and CaO (5 g/kg) with an initial moisture content of 65% (Zhao et al. 2008). Cultures were incubated at 37°C for 48 h, dehydrated at 60°C for 8 h, and then powdered. The bacterial cell density of the solid powder was 1.2×10^{11} colony-forming units (CFU) m/L.

Eutrophicated water samples were obtained from the Grandeur Garden of Beijing. These samples were divided into 2 groups. In one group, the experimental water sample was inoculated with 10^9 CFU/L of *B. cereus* DNF409, while the control sample was not inoculated with the bacterium. In the other group, 100 mg/L of ethanol was added to the experimental water sample, and the sample was then inoculated with 10^9 CFU/L of *B. cereus* DNF409; the control sample contained only 100 mg/L ethanol and was not inoculated with *B. cereus* DNF409. The cultures were incubated at 28°C for 60 h, and then changes in the concentration of nitrate nitrogen (NO_3–N) and nitrite nitrogen (NO_2–N) in the samples were analyzed.

Table 1 Characteristics of landscape water

Parameter	Data
COD (mg/L)	127
TN (mg/L)	9.86
NO_3^-–N (mg/L)	8.63
NO_2^-–N (mg/L)	0.08
Transparency (m)	0.20
pH	7.6

To determine the optimal density of *B. cereus* DNF409 required for the efficient removal of total nitrogen (TN), 1,000 mL of wastewater in plastic cups was inoculated with different concentrations of *B. cereus* DNF409 (0, 10^6, 10^7, 10^8, or 10^9 CFU/L of the bacterium; 3 replicates of each concentration). The cultures were incubated at 28°C for 60 h. Water quality analyses were then performed.

Field tests were conducted from May 1 to June 1, 2007. The average water temperature ranged from 17.1 to 23.4°C. A 50 m^2 area to the north of the test site was used as the control site and was covered with waterproof plastic sheets supported by bamboo stems. The remaining area was used as the test area. The bacterial powder was soaked in water for 2 h, and the mixture was then sprayed in the test area every 5 days. A total of 50 kg of the bacterial powder was used in this study.

Water was sampled and analyzed at various intervals. Water quality was analyzed according to standard methods (AWWA 1999). Chemical oxygen demand (COD) concentration was measured by the potassium dichromate-boiling method; TN, NO_2–N, and NO_3–N, were determined by a colorimetric method; water transparency was analyzed using a Secchi disk. Water temperature and pH were measured in situ in real time using handheld meters (WTW 340i; Germany).

Results and Discussion

Decreased nitrate concentrations were noted in the water samples if the samples were supplied with an additional carbon source (Table 2). This could be explained by the fact that nitrate served as both a nitrogen source and an electron donor in microbial denitrification. These results indicate that all natural water bodies contain a certain amount of denitrifying microorganisms.

When of the water samples were not supplemented with a carbon source the concentration of nitric nitrogen decreased from 7.43 to 2.39 mg/L while that of nitrite increased from 0.08 to 0.31 mg/L. This finding suggests that the organic matter present in the natural water sample was used as a carbon source by the denitrifying bacterium. When the samples were supplement with a carbon source, the concentration of nitric nitrogen and nitrite decreased to

Table 2 Denitrification of natural landscape water by *B. cereus* DNF409

Parameter	Before	After			
		Without ethanol		With ethanol (100 mg/L)	
		Uninoculated	Inoculated	Uninoculated	Inoculated
NO_3^- (mg/L)	8.63	7.26	2.09	4.82	0.61
NO_2^- (mg/L)	0.08	0.12	0.31	0.23	0.01

0.61 and 0.01 mg/L, respectively; TN removal was 92.5%. Thus, we concluded that when the eutrophicated landscape water contained sufficient amounts of degradable organic matter, complete denitrification could be achieved by the *B. cereus* strain DNF409 and resulted in the reduction of nitrate and nitrite to N_2 (Hallin et al. 1996).

The effect of different concentrations of the inoculated bacterial powder on nitrogen removal was compared. The addition of *B. cereus* DNF409 spore powder at concentrations of 0, 10^6, 10^7, 10^8, and 10^9 CFU/L resulted in TN removals of 15.2%, 26.3%, 48.6%, 72.3%, and 72.3%, respectively. The inoculation of 10^8 and 10^9 CFU/L of the bacterium resulted in the highest removal rate. Hence, 10^8 CFU/L of the spore powder was selected as the optimal amount of inoculum for the following experiments.

The results of the field tests conducted in the Grandeur Garden of Beijing are shown in Fig. 1. The TN concentration of the control area decreased from 9.86 to 7.4 mg/L during the tests. The nitrogen removal rate was 24.9% which mainly caused by water self-purification. Compared to the control area, the TN concentration of the test area decreased rapidly from 9.86 to 4.1 mg/L in the first 15 days of the experiment, with a removal rate of 58.4%. On the 20th day, 2,000 m^3 of reclaimed wastewater (COD, 169 mg/L; TN, 9.02 mg/L) was introduced into the test area; this water served as a new nitrogen source. However, a decrease in the TN concentration continued to be observed. At the end of the experiment, the TN concentration of the test area was reduced to 3.12 mg/L.

B. cereus was also effectively used for the COD elimination. The COD concentration of the control area in which there was no inflow of external nitric compounds decreased slowly from 127 to 80 mg/L, with a removal rate of 37% (Fig. 2). On the other hand, in the test area, despite the additional inflow of reclaimed wastewater, the COD concentration decreased from 127 to 36 mg/L with a removal rate of 71.7% within 30 days of the initiation of the experiment.

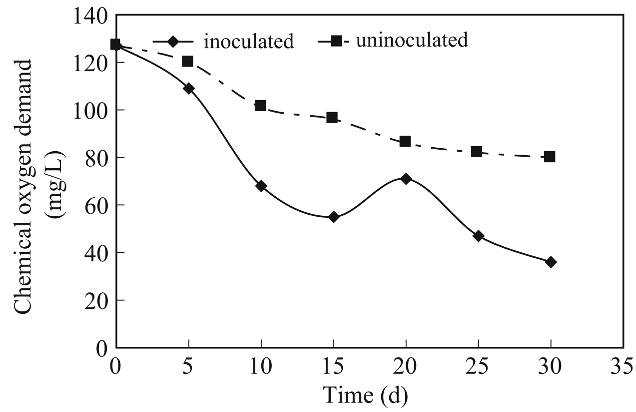

Fig. 2 Changes in chemical oxygen demand concentration over time in the 2 treatments carried out in the Grandeur Garden Lake

The water transparency was also improved dramatically by the degradation of macromolecules by microorganisms. The suspension of tiny particles such as organic detritus, bacteria, and algae in the water reduced water transparency. In the control area, the water transparency was enhanced by natural sedimentation. On the other hand, in the test area, water transparency was improved by both the decomposition and natural sedimentation of denitrifying bacteria.

Water transparency was relatively low (0.2 m) at the beginning of the experiment (Fig. 3). As the experiment progressed, water transparency increased gradually. At the end of the experiment, the water transparency of the test area was 0.55 m and that of the control area was 0.32 m; the water transparency of the test and control areas increased by 0.35 m and 0.12 m, respectively.

These results showed that the water quality of the test area greatly improved during the 30 days during which the

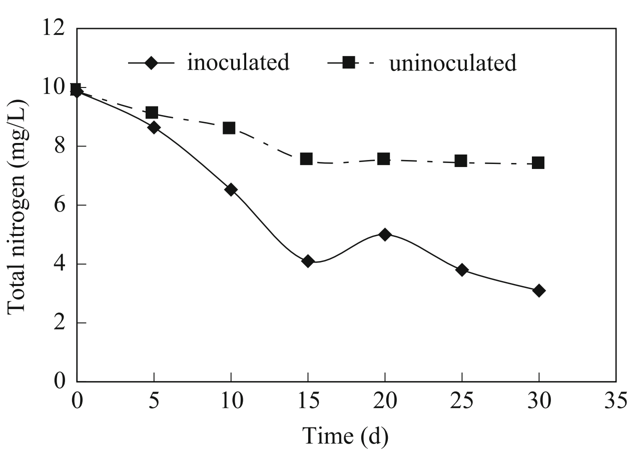

Fig. 1 Changes in the total nitrogen concentration over time in the 2 treatments carried out in the Grandeur Garden Lake

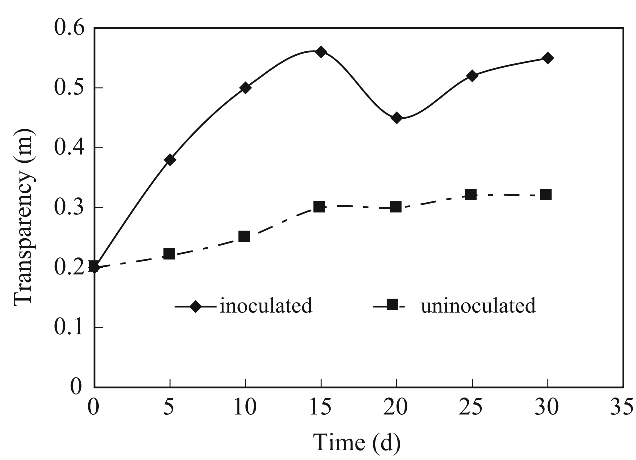

Fig. 3 Changes in water transparency over time in the 2 treatments carried out in the Grandeur Garden Lake

B. cereus powder was added to the water. The Grandeur Garden of Beijing is the first park in Beijing to use reclaimed wastewater as landscape water. Moreover, the sightseeing landscape of the Garden was greatly reduced by the severe environmental pollution caused by the introduction of the reclaimed wastewater. This study was able to solve the eutrophication problem in the Grandeur Garden of Beijing. The results obtained in this study can be applied to the treatment of other water bodies in the city of Beijing. The government should consider reusing reclaimed wastewater as landscape water instead of tap water and groundwater. This is very important from the point of view of tackling the serious problem of water shortage in Beijing.

In brief, the bioremediation of eutrophicated landscape water in urban areas can be feasibly achieved by using denitrifying bacteria. This method is easy, practical, and economical and results in the safe removal of large amounts of nitric compounds and the decomposition of organic pollutants; further, it does not cause pollution or result in the transfer of pollutants. The methods described in this study serves as a reference for improving the quality of eutrophicated landscape water in urban areas.

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