Desalination and Water Treatment
www.deswater.com
doi:10.5004/dwt.2017.20856

88 (2017) 116–121
September

Effectiveness of denitrifying bacteria in restoring the city black-odorous river's sediment

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Received 25 August 2015; Accepted 28 March 2017

ABSTRACT

The effect of different denitrifying bacteria dosages on sediment bioremediation was examined under laboratory conditions, with the black-odorous sediment and water samples collected from Nanning Chaoyang Creek. The results showed that after six weeks of treatment, with denitrifying bacteria dosage of 0.5 g/m^3, sediment thickness reduced for 3.43 cm, and G value increased 3.11 times, for the most. The result from the group with dosage of 0.25 g/m^3, in terms of the above indexes, was second to group with dosage of 0.5 g/m^3 of denitrifying bacteria. Furthermore, for the case with dosage of 0.25 g/m^3, degradation of organic matter was 25.9%, and the chemical oxygen demand, ammonia nitrogen, total nitrogen and total phosphorus removals in overlying water were 76.5%, 94.4%, 87.8% and 79.4%, respectively, slightly higher than those from the group with dosage of 0.5 g/m^3. Therefore, the optimal dosage of denitrifying bacteria was 0.25~0.5 g/m^3.

Keywords: Black-odorous river; Denitrifying bacteria; In-situ remediation; Overlying water; Sediment

1. Introduction

Urban rivers are often regarded as the channels and places for direct discharge of urban rainwater and sewage. Nowadays, with the fast development of economy and urbanization, water pollution has become a serious problem worldwide, especially in municipal rivers in developing countries [1,2]. In the seriously polluted rivers, large quantities of pollutants are gradually deposited into the sediments and cannot be easily decomposed, which should be the cause for the black odorous problem. Because black odorous river pollution is the potential threat to aquatic ecosystems and human settlements, it affects people's daily life and urban development. There are several in-situ remediation technologies available, which can be classified into physical, chemical, and biological categories. Studies have shown that the physical and chemical techniques have certain limitations in practical application, such as the expensively engineering cost, secondary pollution problem, and so on [3–5]. However, the technologies based on biological remediation can largely avoid these shortcomings, especially the method by the addition of microbial agents.

Technologies by adding exogenous dominant bacteria or the combination of gene technology used to produce efficient bacteria have been developed [6]. U.S. Moulin Vert drains used Clear-Flo1200 agents for a three months' trial, and the result showed that the sediment continued to be mineralized, with 84% of COD removals and reduction of NH_3–N (from 0.02 mg/L to 0) [7]. Fabiano et al. [8] reported that using effective microbial compounds should be an effective way to remediate contaminated sediment, since using the effective microbial compounds could result in the increase of benthic bacterial density, which can help to increase the consumption of organic matters (with the concentration reduced from 40 to 20 mg/g or less) in a bay of Genoa. Wu et al. [9] employed the fixed "Collier" to restore sediment of Purple lake Creek in Nanjing Forestry University campus, and their results showed that sediment thickness was reduced more than 80% and COD removal in sediment reached 93%. However, the performance of restoring agents var-

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ies in different environments, thus the selection of appropriate agents with the corresponding degradation is very important.

Denitrifying bacteria belong to facultative aerobic microorganisms, with the characteristics of quickly breeding, good environmental adaptability and denitrification ability. They can use nitrite and nitrate as the nitrogen source, and organic matters in water and sediment as the carbon source, and thus directly reduce the risk of being black smelly water [10]. Kessel [11] treated two sewage ditch mud with denitrifying bacteria and found that the removals of nitrate were 97.2 and 94.5%, respectively. Wang et al. [12] isolated three species of denitrifying bacteria in the sediments of nitrate from the contaminated sediment filter, and found that the removal could be more than 95% and there was basically no accumulation of nitrate after immobilization.

The main purpose of this work was to study the in-situ remediation technology for urban black-odorous river. More specifically, denitrifying bacteria agents were adopted to remediate the black-odorous river's sediment, and to achieve the better results, optimized conditions would be figured out. The sediment and water samples from the typical black-odorous rivers in Nanning area were used in the investigation. This study will be helpful to explore a suitable in-situ technology that will be effective to remediate the black-odorous river's sediment in Nanning.

2. Materials and methods

2.1. Subjects

In this study, sediment and overlying water samples were collected from Nanning Chaoyang Creek. Chaoyang Creek is an important tributary of the Yongjiang river basin, throughout the commercial center of Nanning. Water environmental degradation resulted in severe black-odorous river pollution. Pollution status of Chaoyang Creek is as shown in Fig. 1.

Fig. 1. Black-odorous Chaoyang creek.

2.2. Experimental devices

The experimental reactor was made of transparent plexiglass column, with the height of 100 cm and diameter of 20 cm. Sampling ports were set every 10 cm from the bottom-up. To simulate real river body indoors and in order to avoid the direct light to the sediment as well as water samples in devices, the experimental glass reactor was wrapped by foil. The schematic diagram of the device was shown as Fig. 2.

2.3. Experimental drug

Denitrifying bacteria agent was a kind of brown powder, made from solid fermentation and with a special flavor of fermentation. The main component was denitrifying bacteria belonging to the genus Bacillus (Bacillus).

2.4. Experimental methods

In order to investigate the restoring effect of denitrifying bacteria in urban black-odorous river's sediment, five groups were designed. The first plexiglass column was blank control, without any treatments; the second to the fifth column were treated with four different dosages of denitrifying bacteria agents, 0.25 g/m^3, 0.5 g/m^3, 1 g/m^3 and 2.5 g/m^3, respectively. This experiment was conducted over 6 weeks. The operational temperatures of the devices were within the range of 28.2~30.1°C, and dissolved oxygen (DO) ranged from 0.25 to 1.04 mg/L. Chemical oxygen demand (COD), ammonia nitrogen (NH_3–N), total nitrogen (TN) and total phosphorus (TP) and sediment thickness, organic matter and sediment biodegradability (G value) were regularly tested. According to the quality and volume ratio shown in Table 1, dosed denitrifying bacteria agents to the sediment and overlying water once a week. Black-odorous water samples should be timely supplied to the devices, and every time the same water supplement in Nanning Chaoyang Creek was used.

G value was measured by following steps: firstly, after drying the sediment, 0.20 g dry soil sample was placed in a 250 mL erlenmeyer flask, mixed with 100 mL of overlying

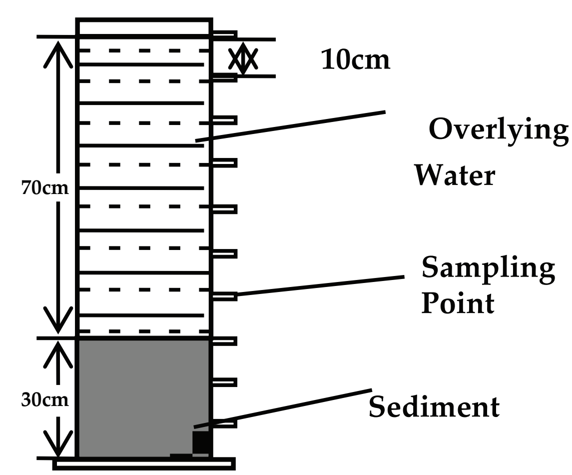

Fig. 2. Schematic diagram of experimental device.

Table 1
Dosages of denitrifying bacteria agents[1]

Dosage/(g/m³)	0.25	0.50	1.00	2.50
Dosages in overlying water/($\cdot 10^{-3}$g)	5.50	11.0	22.0	55.0
Dosages in sediment / ($\cdot 10^{-3}$g)	2.40	4.70	9.40	23.6

[1]During the experiment, different amount of denitrifying bacteria agents were added to overlying water and sediment, respectively. Thus, the second and third rows are the dosages in overlying water and sediment. Based on the volume of the reactor, the overall dosages of the system for different treatments could be calculated, as listed in the first row.

water and boiled for 10 min; and then placed it in incubation oscillator for continuous oscillation for 6 h under the temperature of 30°C; After shaking and standing for 30 min, the supernatant COD was measured before and after the experiment, and sediment G value was calculated by the following equation .

$$G = \frac{(C_1 - C_2) \times V}{10^6 \times q \times t} \quad (1)$$

where G is the sediment biodegradability, kg/(kg·h); C_1, C_2 are the COD values before and after the experiment, mg/L; V is the Overlying water sample volume, 100 mL; q is the sediment dry weight, 0.20 g; t is the oscillation time, 6 h.

3. Results and discussion

3.1. Effects on sediment thickness reduction

Sediment thickness is an important indicator of organic pollutants concentration in sediment. Under the same conditions, the lower the concentration of organic pollutants, the higher degree of mineralization in the sediment and the lower the thickness of the sediment [9]. Fig. 3 shows that after 6 weeks of reaction, the group with dosage of 0.5 g/m³ reached the highest sediment thickness decline of 3.43 cm, followed by the group with dosage of 1.0 g/m³ (2.49 cm). For the group with dosage of 0.25 g/m³, due to lack of bacterial degradation of organic matter and nutrient in the sediment, the treatment effect of the group was lower than the case with dosage of 0.5 g/m³. When dosage was greater than 1 g/m³, matrix carried by agents into the environment increased, resulting in the slow decrease trend of thickness during the reaction sediment. As compared with the above four groups, sediment thickness reduction of the control group was very small, only 0.40 cm. It indicated that dosing denitrifying bacteria agents can reduce the contaminated sediment to a certain extent.

To clarify the sediment thickness reduction trend, measurements were conducted regularly. As can be seen, when dosing 0.5 g/m³ of denitrifying bacteria agents, the sediment reduced quickly within the first 10 d, and the reduction became slower afterwards, and the total reduction almost maintained stable after 25 d of treatment. The explanation for this trend could be as follows: in the first stage (first 10 d), carbon, nitrogen and other nutrients sources were sufficient for the growth of denitrifying bacteria in the sediment,

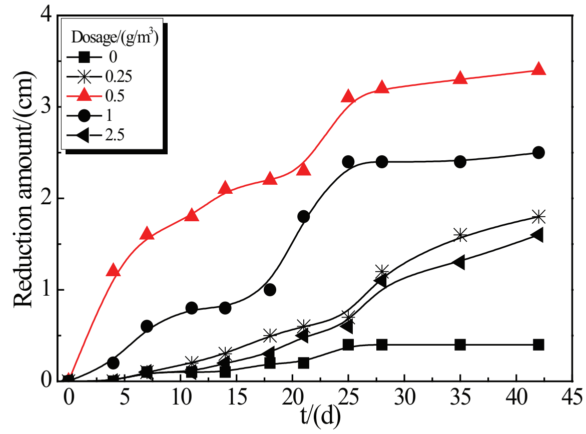

Fig. 3. Reduction effect of sediments by the agents with different dosages.

thus enhancing the microbial mineralization of sediment; since contaminants were degraded gradually, the growth of denitrifying bacteria could be inhibited to some extent, leading to reducing the sediment reduction rate. For the group of dosing 1 g/m³, during day 15 to day 25, the reduction rate was relatively high, and after that the amount of sediment reduction was negligible. This was likely due to the fact that after 15 d of acclimation, denitrifying bacteria were in the stationary phase and the number of microorganisms closed to the peak, thus accelerated the reduction rate during this period. The sediment reduction rate of other groups were not obvious, as shown in Fig. 3. In this experiment, when the dosage of denitrifying bacteria was 0.5 g/m³, sediment surface formed an oxide layer closed to 0.7 cm, and this was another reason for the fact that sediment reduction was larger than other groups. Biological oxidation of sediment reduction is according to the nitrification and denitrification process. By adding denitrifying bacteria to the system, strong denitrification environment could be created in the sediment, which is a strong contrast with nitrification environment in overlying water. By the mutual coupling process of nitrification and denitrification, the ammonia, nitrate, nitrite and organic matters could be removed from sediment and overlying water. At the same time, a brown oxide layer in the interface of sediment and overlying water was formed. Converting the role of the benthic microfauna food chain enriched in the oxide layer and sediment resulted in the rapid reduction of sediment [13].

3.2. Degradation of sediment organic matters

Sediment in natural environment is usually a mixture of clay, silt, organic matters and various minerals. That is, sediment is mainly composed of inorganic minerals and organic mobile phase [14]. As shown in Fig. 4, after 40 d of dosing agents, corresponding sediment organic matter removals for different groups with the dosages of 0.25 g/m³, 0.5 g/m³, 2.5 g/m³, 1 g/m³ were 18.26, 7.62, 7.91 and 5.85 g/kg, respectively, much higher than that from the control group. Obviously, denitrifying bacteria played an essential role of degrading organic sediments.

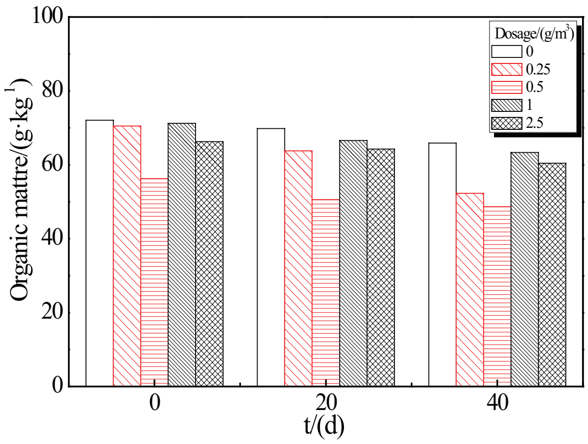

Fig. 4. Degradation of organiic matters by the agentas with different dosages.

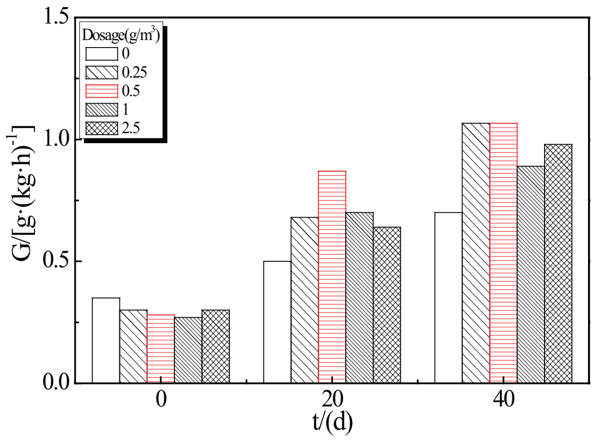

Fig. 5. Variations of G values by the agent with different dosages.

At 20 d, except for the group with dosage of 2.5 g/m^3, the organic matter of sediment was low, bacteria removal of other groups were greater than 6.0%, especially the group of 0.5 g/m^3, after 20 d it reduced 10.2% of the organic matter with largest and fastest degradation. Sediment organic matter was mainly composed of two parts, one was the original organic pollutants in sediment, the second was a variety of microorganisms contained in the sediment. Denitrifying bacteria can use organic matters as a carbon source for growth by decomposing organic matter for energy. Therefore, low dosage of denitrifying bacteria resulted in less number of microorganisms in the sediment, furthermore the degradation of organic pollutants present in the sediment was not sufficient; on the contrary, high dosage would rapidly increase the microorganisms in the sediment, resulting in the organic growth rate far greater than the speed of degradation of pollutants by denitrifying bacteria, sediment organic matter cannot be decomposed in time. Results showed that the dosage of denitrifying bacteria should not be too high, within 0.25~0.5 g/m^3 was appropriate for the degradation of organic matter in sediments.

3.3. Changes in sediment biodegradability

Sediment biodegradability is an important indicator to assess sediment microbial activity and changes of G values can be a direct reflection of sediment bioremediation [15]. As shown in Fig.5, G value of the control group increased slightly, while the increase of the other four groups were much larger. When the dosage of denitrifying bacteria was 0.5 g/m^3, the sediment G value was the highest, reached 1.07 g/(kg·h). It can be concluded that the denitrifying bacteria agents contributed to restore sediment.

Throughout the experiment, sediment G values of four groups were rising steadily, after 40 d the growth of four groups with dosages of 0.25 g/m^3, 0.5 g/m^3, 1 g/m^3, 2.5 g/m^3 were 0.77, 0.79, 0.62 and 0.68 g/(kg·h), with the rate of 257%, 282%, 230% and 227%, 1.94 to 2.25 times of the control group, and consistent with the growth trend of sediment reductions. Furthermore, with dosage of 0.5 g/m^3, G value increased 3.11 times, and the biodegradability restored was the best. And oxide layer thickness after 40 d was in an order of 0.5 g/m^3 > 1 g/m^3 > 0.25 g/m^3 > 2.5 g/m^3 > control group.

In summary, increasing dosage of denitrifying bacteria agents can enhance the decomposition of sediment organic matter, reduce the content of organic pollutants in sediment, and promote the formation of sediment surface oxide layer. On the contrary, excessive dosage would limit assimilation of denitrifying bacteria and reduce the removal efficiency of organic matters. Therefore, appropriate dosage of denitrifying bacteria agents could improve biological oxidation of contaminated sediment and enhance the ability of microorganisms to degrade organic pollutants.

3.4. Changes in water COD

Fig. 6 shows that COD concentration of each group fluctuated downward, in addition to the control group. All the trends were consistent. For the group with dosage of 0.25 g/m^3, COD degradation was the best, from 46.79 mg/L down to 11.01 mg/L, with the removal rate of 76.5%. COD removal rates were all above 59.2% for other groups with different dosages, better than the control group, suggesting that denitrifying bacteria can effectively remove COD. During the first 5 d, COD concentrations for some groups rose slightly, the more dosage of agents the greater the COD growth. This was just because when the very time denitrifying bacteria was added into the water, it took some time for them to adapt to the environment; that is, during this period, the consumption of COD by denitrifying bacteria was lower than the increase of COD caused by the addition of bacteria to the system, which improved the value of COD in the background. The 35 d due to irregularly pay of water, it added new exogenous contaminants to the overlying water, resulting in fluctuations in the concentration slightly.

3.5. Changes in water NH_3–N, TN

As shown in Figs. 7 and 8, trends of TN, NH_3–N among different groups were consistently steadily declining. The

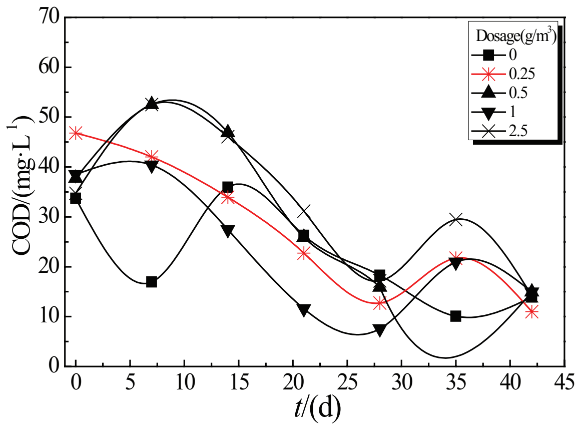

Fig. 6. Effect of different denitrifying bacteria agents dosages on cod removals.

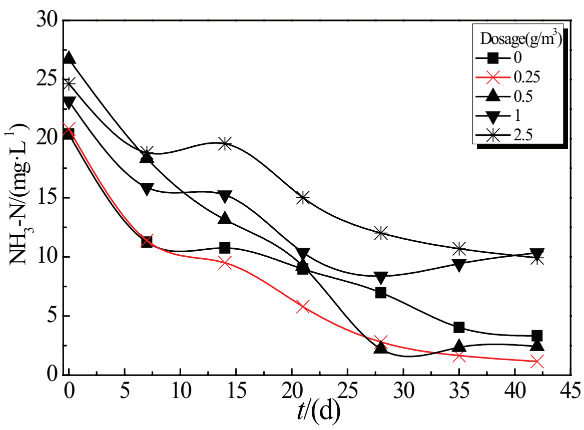

Fig. 7. Effect of different denitrifying bacteria agents dosages on NH_3–N removals.

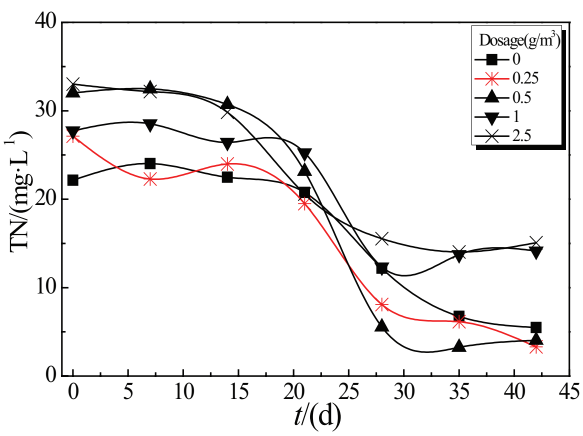

Fig. 8. Effect of different denitrifying bacteria agents dosages on TN removals.

removal rates of NH_3–N and TN of the 4 dosing groups were in the order of 0.25 g/m^3 > 0.5 g/m^3 > 1 g/m^3 > 2.5 g/m^3. For the group of 0.25 g/m^3, NH_3–N, TN removal rates were 94.4%, 87.8%, 10.8% and 12.6% higher than the control group. The NH_3–N, TN removals of group 1 g/m^3 and

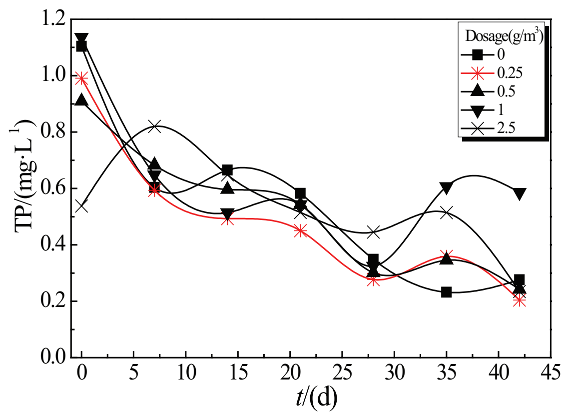

Fig. 9. Effect of different denitrifying bacteria agents dosages on TP removals.

group 2.5 g/m^3 were poor, implying that denitrifying bacteria dosage should not be too high, 0.25~0.5 g/m^3 would be better, which was consistent with other studies [16]. Nitrogen in the water is mainly from organic nitrogen degradation, and is always in the form of ammonia [17]. If the agents increased in volume, then matrix would increase the water pollution load and it cannot be timely degradated. Basically, NH_3–N concentrations had been declining within the first 25 d, and afterwards became stable. There could be two possible reasons for the decrease of NH_3–N: the first one is that anaerobic ammonium oxidation (Anammox) process occurred in the system [18]; and the second one is nitrification process occurred in the upper layer of overlying water since dissolved oxygen in this zone was relatively high. While TN concentrations of different groups decreased rapidly during day 20 to day 25, which could be attributed to the fact that after 20 d, acclimated stage of denitrifying bacteria had been completed and the number of denitrifying bacteria was high, and denitrification process became stronger, which enhanced the removal of nitrate nitrogen accumulated in the water.

3.6. Changes in water TP

Fig. 9 shows that the TP concentrations fluctuated over time, yet, exhibited a general declined tendency for each group. TP basically reduced to 0.3 mg/L or less (except for the case with dosage of 1 g/m^3), which met the III class standards of surface water. This was probably because, on the one hand, denitrifying phosphorus bacteria (DPBs) could be presented in the system. Under anoxic conditions, DPBs can use NO_3^- or O_2^- as an electron acceptor, resulting in the excess absorption of phosphorus, which leading to both TP and TN reduction in overlying water [19]; and on the other hand, pH of the overlying water ranged from 7.50–8.16 , while in the neutral environment, sediments released very small amount of phosphorus upward to overlying water, which helped reduce the phosphorus load of water bodies. Comparing the 5 groups, for the group with agents dosage of 0.25 g/m^3, TP degradation turned out to be best, from 0.99 mg/L down to 0.20 mg/L, with the removal rate of 79.4%. If the dosages were more than 1 g/m^3, with the increase amount of bacteria, more contaminated matrix would be carried into the water, leading to improving TP background values and reducing the degradation efficiency of TP.

4. Conclusions

Effectiveness of denitrifying bacteria in restoring the city black-odorous river's sediment was determined in terms of sediment thickness reduction, organics degradation, G value changes, COD removals as well as nitrogen and phosphate removals. Five dosages of denitrifying bacteria agents were evaluated. Results showed that: with the denitrifying bacteria agent dosage of, after 6 weeks, the thickness of sediment reduced 3.43 cm; G value growth rate was 282%; in addition, it formed an oxide layer of 0.7 cm closed to the sediment surface. After 25 d the basic indexes tended to be stable and 0.5 g/m^3 turned out to be a better dosage than other groups in restoring the city black-odorous river's sediment. However, organic matter degradation rate was 13.6%, less than 25.9% of the group of 0.25 g/m^3. Furthermore, when the denitrifying bacteria agent dosage was 0.25 g/m^3, the overlying water COD, NH_3–N, TN, TP removal rates were 76.5, 94.4, 87.8, and 79.4%, respectively; water quality was improved and the treatment effect was much better. Therefore, the optimal dosage of denitrifying bacteria was 0.25~0.5 g/m^3.

Acknowledgment

The authors would like to thank the Major Projects of National Water Pollution Control and Management of Science and Technology (2009ZX07317-006-02), Natural Science Fund of Guangxi (2013 GXNSFAA019278), Development Plan of Nanning City Science and Technology (20123112, 20132303), Special Fund of Nanning City Environmental Protection (Nanning City Finance and Economics [2012] 308) for their support for this study.

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