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Mechanism of aerobic denitrifiers and calcium nitrate on urban river sediment remediation

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ABSTRACT

Calcium nitrate, coupled with aerobic denitrifiers, were applied to reduce nitrogen and organic pollutants of urban river sediment. 115-day remediation tests showed that total nitrogen in the sediment decreased by 16.5%, while total transferable nitrogen increased from 0.097 to 0.109 mg/g. Increased urease activity and decreased protease showed a potential for further nitrogen degradation. Although the total organic carbon (TOC) content decreased slightly, the predominant organic pollutants were removed or converted to low molecular weight compounds through microbial biodegradation. Decreased phenol oxidase activity exhibited that low concentrations of recalcitrant aromatic compounds remained in the sediment. The high-throughput sequencing results reveals that the community diversity of bacteria markedly increased. Compared with the addition of calcium nitrate or aerobic denitrifiers alone, genera mainly removed nitrogen and organic compounds, especially recalcitrant compounds, following the treatment with the combination technology. Moreover, the concentrations of nitrogen and organic matter did not increase significantly in the overlying water, which was free of undesirable secondary pollution. During the process of remediation, dosing with nitrate may provide a more favorable environment for aerobic denitrifiers and indigenous microbes in the sediment. Furthermore, the introduced aerobic denitrifiers may improve nitrogen removal and have a collaborative effect with indigenous microbes.

1. Introduction

Sediment, particularly at the bottom of large rivers, acts as a sink for a wide variety of anthropogenic contaminants, such as heavy metals, nutrients, and organic chemicals (Moreno et al., 2015). An excess of decaying organic matter in the sediment consumes much dissolved oxygen (DO), resulting in a low redox potential [oxidation reduction potential (ORP)]. Under those circumstances, the reduction of sulphate to hydrogen sulphide by microbes results in the generation of undesirable odours. High concentrations of nutrients lead to the eutrophication of water, which threatens surface and ground water quality and human health.

During remediation of urban river sediment, nitrate is often added. Nitrate can serve as an alternate higher-potential electron acceptor and can thus stimulate the biodegradation of many organic pollutants, such as silicon materials, alkanes, polycyclic aromatic hydrocarbons, and heterocyclic compounds (Murphy et al., 1999; Yan et al., 2017). Harada et al. (2014) suggested that redox potential increased with the amount of calcium nitrate added, and that the rate of denitrification increased from 21.3 to 36.4 mg/(m^2·d). Following the addition of calcium nitrate, the proportion of total organic carbon decreased by 9.7%–10.2%. Moreover, the proportion of acid volatile sulphide in the sediment decreased significantly (by 54.9%–57.1%) due to the oxidation of sulphide, and the binding capacity of P in the sediment was improved, subsequently reducing the release of P. However, the addition of calcium nitrate also resulted in high concentrations of NO_3^--N in the overlying water and in the sediment (Liu et al., 2017b).

Compared with addition of calcium nitrate, bioaugmentation has gained increasing attention in recent years, as it is relatively cost-effective and environmental-friendly (Miranda et al., 2007). It has been widely applied to enhance the degradation of target pollutants in water or sediment, such as polyaromatic hydrocarbons (PAHs), total petroleum hydrocarbons (TPH), aromatic compounds, nitrogen compounds, and heavy metals (Winquist et al., 2014; Wu et al., 2016; Liu et al., 2017a). Recently, bacteria capable of combined heterotrophic nitrification and aerobic denitrification have been investigated as potential microorganisms for biological nitrogen removal systems. Aerobic denitrification, which occurs in natural systems, is defined as the co-respiration or co-metabolism of oxygen and nitrate (Zhao et al., 2010). Aerobic denitrifiers have ability of combining heterotrophic

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nitrification and aerobic denitrification to remove nitrogen under aerobic conditions (Padhi et al., 2013). Full-scale bioaugmentation experiments have been successfully conducted using aerobic denitrifiers in the treatment of soils, municipal wastewater, and urban rivers. Heterotrophic-aerobic nitrogen removing bacteria were successfully used to treat municipal wastewater in a pilot-scale sequencing batch reactor (SBR), and the average effluent concentration of chemical oxygen demand (COD), ammonia nitrogen (NH_4^+-N), total nitrogen (TN) and total phosphorus (TP) was 20.6, 0.69, 14.1, and 0.40 mg/L, respectively (Chen et al., 2015). Zhou et al. (2016) studied the performance of indigenous aerobic denitrifiers coupled with water lifting and aeration technology in a reservoir system, and found that the TN removal efficiency in water varied between 21.74% and 52.54%, and the efficiency of TN removal from surface sediment reached 41.37 ± 1.55%. However, bioaugmentation is usually slow, especially when oxidizers or electron acceptors are insufficient (Coates et al., 1997).

To overcome limitations associated with the use of single method, combinations of different methods have been introduced to enhance removal of contaminants in sediment. For example, mobilized microorganism technology combined with aeration could greatly reduce the pollutants in water and sediment in a reactor (Wu et al., 2008). Constructed wetland microcosms inoculated with denitrifying bacteria showed good performance on the removal of organic matter, nitrogen and phosphorus during treating polluted river water and domestic wastewater (Shao et al., 2014). Bioaugmentation with the strain *Bacillus* sp. GZT, combined with adding sodium lactate or sodium propionate, could enhance 2, 4, 6-Tribromophenol (TBP) biodegradation in water/sediment microcosms (Xiong et al., 2017).

The effects and mechanism of dosing with calcium nitrate plus aerobic denitrifiers for remediation of polluted sediment were rarely reported. Therefore, this study aimed to evaluate the feasibility and synergistic mechanism of coupling calcium nitrate to aerobic denitrifiers for the removal of nitrogen and organic matter from river sediment. Different forms of nitrogen, organic structures, and enzymatic activities were studied to further investigate the mechanism of remediation. Additionally, molecular biology was used to characterize microbial community diversity and variation in the remediation process.

2. Materials and methods

2.1. Materials

The sediment used in this study was obtained from the riverbed of the old Hai River in Tianjin, China. The freshly collected sediment was stirred evenly to ensure homogeneity before putting into reactors. The main pollution index of the sediment samples was 1.09–1.14 mg/g of TN and 6.7–7.2 mg/g of TOC.

Aerobic denitrifiers were isolated from activated sludge in a SBR reactor. The strain showed good abilities of heterotrophic nitrification and aerobic denitrification in the previous study on the isolation and identification of aerobic denitrifiers. Based on 16S rDNA sequence analysis, the strain showed 99% similarity with *Citrobacter* sp. (KP068655.1), which are affiliated to the phylum *Proteobacteria*. The concentration of the bacterial solution was 10^8 CFU/mL.

2.2. Experimental design

Eight devices were prepared for the experiment, and the size of each device was 25 × 25 × 80 cm. Before starting, the sediment and clean water were placed in the devices. Compared to the collected sediment, the poured sediment was very fluffy. So the sediment stood for 2 weeks to make its physical characteristics closed to natural river sediment. The height of the sediment and overlying water was 13 and 57 cm, respectively. To simulate the flow of the river, the water was recirculated by a peristaltic pump in the laboratory-scale microcosm (Fig. S1), and the flow was controlled at 9.96 ± 0.1 L/(h·m^2).

Based on the pollution characteristics of sediment and the previous study on optimization of microbial inoculum and calcium nitrate addition, the optimal concentration of calcium nitrate in this study was 0.75 g/L (calcium nitrate mass/sediment volume), and the optimal dosage of aerobic denitrifiers was 0.03% in the overlying water and 0.09% in the sediment (bacterial solution volume/sediment volume), respectively. To reduce adverse impact of calcium nitrate addition, the concentration of calcium nitrate was decreased to 0.15 g/L when calcium nitrate and aerobic denitrifiers were added simultaneously. Four experimental microcosms were applied in duplicate: (1) unamended control (C); (2) bioaugmentation with aerobic denitrifiers (B); (3) calcium nitrate addition (0.75 g/L) (N); and (4) calcium nitrate addition (0.15 g/L) plus bioaugmentation with aerobic denitrifiers (B + N). Experiments were conducted from September to December of 2016, and the remediation effects of polluted sediment were monitored. The values determined for sediment and water before starting (day 0) were defined as the "start" values. Aerobic denitrifiers were injected into the oevrlying water and sediment directly with syringes on day 0 of the experiment. Calcium nitrate was injected into the sediment on days 0, 14 and 21, respectively. Water and sediment samples were collected from five points in each device and homogenized before testing. Water and sediment were not sampled on the same day to avoid the impact of turbulence.

2.3. Measurements

2.3.1. Water quality monitoring

Water temperature and pH were measured using a pH meter (Ohaus Instrument [Shanghai] Co. Ltd). According to the standard methods described by the National Environment Protection Agency of China (NEPAC), TN, NH_4^+-N, and NO_3^--N were monitored by colorimetric analysis with a UV-Vis spectrophotometer (Beijing Persee Instrument Co. Ltd., T6 New Century, China) (NEPAC, 2002). Chemical oxygen demand (COD_{Cr}) was measured based on the microwave digestion technology (Dharmadhikari et al., 2005).

2.3.2. Sediment quality monitoring

The ORP of the sediment was measured in situ with a Hach FJA-6 ORP Meter at a depth of 2 cm from the surface layer of sediment. TN in the sediment was measured using the Modified Kjeldahl method (SAC, 2014). TOC of the sediment was determined using the combustion oxidation nondispersive infrared absorption method (SAC, 2009).

Total transferable nitrogen consists of four forms of transferable nitrogen: ion-exchangeable form (IEF-N), weak-acid extractable nitrogen (WAEF-N), strong-alkali extractable nitrogen (SAEF-N), and strong-oxidant extractable nitrogen (SOEF-N). Different forms of transferable nitrogen in the sediment were determined using the method described by Wang (2013).

Organic matter content in sediment was determined by gas chromatography-mass spectroscopy (GC-MS) analysis (NEPAC, 2002). Organic matter was extracted from the sediment by ultrasonic-hexane extraction, and GC-MS analysis was carried out on an Agilent Technologies gas chromatograph model 7890A with a 5975C mass detector. Each sample (1 μL) was injected into a HP-5MS column (30 m × 0.25 mm × 0.25 μm) with helium as the carrier gas (1 mL/min). A split/splitless (S/SL) injector was used with 250 °C injector temperature. The GC oven temperature was programmed initially at 50 °C for 3 min, then increased to 300 °C at a rate of 10 °C/min, and finally retained for 5 min. The operating conditions of mass spectral determination were as follows: ionization voltage 70 eV; ion source temperature 230 °C; mass scan range: full scan. The percentage composition was calculated from the GC peak areas via the normalization method. Compounds were identified by comparing the mass spectral values with data available in the National Institute of Standards and Technology (NIST) library.