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Profiling of microbial community during *in situ* remediation of volatile sulfide compounds in river sediment with nitrate by high throughput sequencing

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ABSTRACT

The physiological profiling on community level and analysis of 16S rRNA V6 tag based on high throughput sequencing were used to characterize and to compare microbial community structure, diversity, and bacterial phylogeny from sediments before and after remediation with the help of nitrate. The experiment was conducted *in situ* within a heavily polluted river located in the south of China. Total 2900 m^2 area of sediment were selected on river bed for *in situ* test. The results showed that about 86.5% of nitrate on average can be consumed within 14 days since injection into contaminated sediment in river and about 86.5% on average of the odor matters of AVS (acidic volatile sulfide) can be removed. It was found that the microbial community richness and diversity decreased during remediation process after nitrate injection due to overgrowth of functional bacteria and then revived on the 14th day nearly the end of remediation process. The most abundant phyla were *Proteobacteria* (44.10%), *Firmicutes* (7.22%) and *Cloroflexi* (6.70%) while 24.90% of the sequences were from bacterial domain. *Proteobacteria* were found significantly abundant in treated sediments on the 7th day, which were resulted from significant abundance of beta-proteobacteria, epsilon-proteobacteria and gamma-proteobacteria. On the contrary, *Firmicutes*, *Cloroflexi*, *Actinobacteria* and *Deltaproteobacteria* were significantly abundant in untreated sediments and in sediments after 14 days treatment. Microbial community in sediment before remediation was closely adapted to the sediment conditions with serious sulfide pollution. Injection of nitrate into sediment played multi-roles as stimulation of nitrate reducing bacteria (NRB), inhibition of sulfate reducing bacteria (SRB) as well as increase of oxidation-reduction potential (ORP).

1. Introduction

Nitrate dosing has been proved as a cost-effective approach for sediment remediation by removing organic matters and notorious odor caused by sulfide in sediment (Murphy et al., 1999; Zhang et al., 2009; Perelo, 2010). Nitrate, a thermodynamically favorable electron acceptor, can intensify the microbial competition by stimulating indigenous nitrate reducing bacteria (NRB) (Bentzen et al., 1995). For instance, the increased redox potential due to nitrate injection inhibits the growth of anaerobic sulfate reducing bacteria (SRB), but promotes the growth of nitrate-reducing, sulfide-oxidizing bacteria (NR-SOB) which can oxidizes various sulfide compounds through nitrate reduction (Garcia-de-Lomas et al., 2006). Since function and metabolism of indigenous bacteria in sediment are seriously diverse (Dichristina, 1992; Moura et al., 1997; Shao et al., 2011), it is important to investigate the effect of nitrate injection on the bacterial community in sediment.

Variation of microbial community in hydrogen sulfide (H_2S) odor control with nitrate has been investigated in petroleum industry, wastewater treatment, and sewage system (Garcia-de-Lomas et al., 2007; Zhang et al., 2008; Kumaraswamy et al., 2011). These studies focused on examining the role played by SRB and NRB, whereas studies on the microbial community after nitrate injection were scant. Zhang et al. (2009) demonstrated that

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Table 1
Sediment properties of experimental regions.

Items		Region 1	Region 2
Density (g/ml)		1.31 ± 0.10	1.38 ± 0.11
Water content (%)		61.36 ± 1.9	55.31 ± 4.4
AVS (mg S/kg dry sediment)		3056.63 ± 222.2	3060.63 ± 303.3
Nitrate (mg N/kg dry sediment)		64.1 ± 15.8	31.3 ± 6.1
Total organic carbon (% w/w)		3.86 ± 0.66	3.90 ± 0.52
Sediment oxygen Demand (mg O/kg dry sediment)		9206 ± 1721.2	8154 ± 1127.3
Particle size distribution (%)	<0.063 mm	89.96 ± 0.70	84.62 ± 0.59
	0.063–0.12 mm	3.90 ± 0.61	4.77 ± 0.58
	0.12–0.25 mm	1.68 ± 0.06	2.19 ± 0.43
	0.25–0.55 mm	1.36 ± 0.11	2.22 ± 0.45
	0.55–1.00 mm	1.15 ± 0.06	1.51 ± 0.43
	1.00–2.43 mm	1.19 ± 0.04	2.11 ± 0.09
	2.43–4.00 mm	0.65 ± 0.03	1.39 ± 0.35
	>4 mm	0.11 ± 0.04	1.18 ± 0.02

Sulfurimonas denitrificans-like bacteria were the dominant autotrophic denitrifiers in microbial community in nitrate-induced marine sediment remediation, accounting for 69% of the total nitrate reduction. Shao et al. (2011) found that microorganisms which were phylogenetically close to *Sulfurimonas denitrificans* and *Thiohalomonas denitrificans* were the major autotrophic denitrifiers, and further revealed that denitrifiers enriched at 30 mM NO_3^- had a higher Shannon index than those at 10 and 80 mM NO_3^-.

Conventional culture and fingerprinting techniques, which have been widely applied for determination of microbial community in the past, had inherent limitations. For example, conventional culture technique cannot give an accurate profile of microbial community in light of the inability in cultivation and characterization of major bacteria (90–99.9%) (Amann et al., 1995); fingerprinting techniques, such as denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (t-RFLP), and phospholipid fatty acid (PLFA) analysis, are difficult to obtain a precise, accurate and comprehensive description of microbial communities (Schutte et al., 2008; Cetecioglu et al., 2009; Shao et al., 2009; Pratt et al., 2012). In this regard, an innovative next-generation sequencing method by analyzing 16S rRNA variable tags has been developed recently. This method can provide many novel, reliable information of taxonomic identification of microbes (Huber et al., 2007; Roesch et al., 2007). For example, barcoded Illumina paired-end sequencing (BIPES) based on 16S rRNA V6 tag, a high throughput and cost-effective method, is capable of generating hundreds of thousands of reads in a single flow cell run; and the tag sequences containing adequate information for a taxonomic assignment can give deeper insight into microbial diversity and dynamics (Zhou et al., 2011).

Owing to the rapid economic development of Shenzhen City over the last three decades, Shenzhen River, located in the south of China, has been seriously polluted with pungent, pernicious odor remitted from the water, causing health problems to the nearby residents and decline in aesthetic value. The present work aimed to dissect the microbial community of sediment collected in Shenzhen River using high throughput sequencing during *in situ* sediment bioremediation through nitrate injection. The finding can shed more light on the changes in microbial community during remediation and the essence of sediment remediation through nitrate injection. Furthermore, this work attempted to decipher the relationship between environment factors and microbial community during sediment remediation, which can improve our understanding of the efficiency of *in situ* sediment remediation through nitrate injection so that remediation process might be further optimized.

2. Materials and methods

2.1. Sites description and sediment collection

Shenzhen River, 37 km long with watershed area of 312.5 km^2, is located in the south of China. A pilot-scale *in situ* remediation operation with nitrate injection was conducted within two representative regions of Shenzhen River, which were severely contaminated by AVS. The two regions were located on the Luohuqiao (labeled as Region 1) and Lingang community (labeled as Region 2) of Shenzhen River. There were two neighboring sections in each region, including one treated section (Section A) and one control section (Section C). The area of each section in Region 1 (1A and 1C) was 400 m^2 (8 m × 50 m) and the area in Region 2 (2A and 2C) was 2500 m^2 (50 m × 50 m). Sediment properties of experimental regions were showed in Table 1. Calcium nitrate was evenly injected into sediments of the sections 1A and 2A with a treatment depth of around 60 cm. The nitrate dosage in the sections of 1A and 2A were 3600 and 4053 mg N/kg dry sediment, respectively, calculated by the AVS content in the sediments. No calcium nitrate was injected into the sections of 1C and 2C, which was used as the control areas to compare with the treated areas. Core sediments were collected from each section before nitrate injection, on the 7th and 14th day after nitrate injection. There was no sediment collection for 1C and 2C on the 7th day. Representative sediment layer at the depth of 20–30 cm which is less influenced by upper or lower layers of sediments were selected. Sediment samples were put into sealed plastic bag and stored in portable icebox, then transferred to lab as quickly as possible and stored at −80 °C before analysis.

2.2. Analysis of physicochemical parameters

Acid volatile sulfide (AVS) was analyzed based on the procedures with a UV spectrometer (Shimadzu UV-2450, Japan)(Allen et al., 1991; Lin et al., 1997). Total organic carbon (TOC) in sediment was measured with atitration method (Schumacher, 2002). Oxidation-reduction potential (ORP) and pH were detected with a portable multi-parameter instrument (HQ40d, HACH, USA). Nitrate was extracted from sediments by preparing a 1:4 dilution of sediment sample and deionized water and shaking for 4 h. The diluted slurry were then centrifuged, and the supernatant were filtrated with 0.45 μm membrane and were quantified for nitrate concentration with ion chromatograph (ICS1100, Dionex, USA).

2.3. DNA extraction

DNA was extracted from 1 g fresh sediment samples using the UltraClean™ Soil DNA Isolation Kit according to the manufacturer's manual (Mobio, USA). All the extracted total DNA samples were stored at −80 °C before further analysis.

2.4. PCR amplification and sequencing using HiSeq 2000

The bacterial 16S rRNA genes were amplified using the specific primers barcode-F985 (5′-CNACGCGAAGAACCTTANC-3′) and R1046 (5′-CGACAGCCATGCANCACCT-3′) targeting the V6 region based on a modified method (Zhou et al., 2011). Eight-digit error-correcting barcode sequences before the 5′ end of the F985 primer, as described by Hamady et al. (2008), are specific to the different samples. In addition, a 2 bp GT linker was added between the barcode and the 5′ end of the F985 primer to avoid the potential match of the barcode sequence and the target 16S sequences. Therefore, the forward primer was 29-base barcode-GT-F985 and the reverse primer was 19-base R1046.