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Anaerobic biodegradation of nonylphenol in river sediment under nitrate- or sulfate-reducing conditions and associated bacterial community

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HIGHLIGHTS

- NP biodegradation can occur under both nitrate- and sulfate-reducing conditions.
- Anaerobic condition affects sediment bacterial diversity during NP biodegradation.
- NP-degrading bacterial community structure varies under different anaerobic conditions.

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ABSTRACT

Nonylphenol (NP) is a commonly detected pollutant in aquatic ecosystem and can be harmful to aquatic organisms. Anaerobic degradation is of great importance for the clean-up of NP in sediment. However, information on anaerobic NP biodegradation in the environment is still very limited. The present study investigated the shift in bacterial community structure associated with NP degradation in river sediment microcosms under nitrate- or sulfate-reducing conditions. Nearly 80% of NP (100 mg kg^{-1}) could be removed under these two anaerobic conditions after 90 or 110 days' incubation. Illumina MiSeq sequencing analysis indicated that Proteobacteria, Firmicutes, Bacteroidetes and Chloroflexi became the dominant phylum groups with NP biodegradation. The proportion of Gammaproteobacteria, Deltaproteobacteria and Choloroflexi showed a marked increase in nitrate-reducing microcosm, while Gammaproteobacteria and Firmicutes in sulfate-reducing microcosm. Moreover, sediment bacterial diversity changed with NP biodegradation, which was dependent on type of electron acceptor.

1. Introduction

The widely-used nonionic surfactant nonylphenolethoxylates (NPEOs) can be biologically degraded into nonylphenol (NP) under anaerobic condition, and NP will enter the natural environment due to the discharge of effluents from municipal wastewater treatment plants [1–3]. Because of the hydrophobicity of NP, it can be absorbed to suspended particles and sediments in rivers [4,5]. In some cases, high levels of NP can exist in river sediment ecosystems [5]. NP can act on the endocrine system by mimicking the effects of natural hormones, and thus is harmful to many aquatic organisms [6]. Therefore, an effective dissipation of NP pollution in sediment is crucial for abating its toxicological risk in aquatic environments.

Freshwater sediments harbor a huge amount of viable microorganisms and can play crucial roles in decomposition of organic pollutants [7,8]. It has been well documented that NP is ready to be biodegraded in various aerobic environments [4,9–11]. Microorganisms from a few bacterial genera have been isolated from natural environments, such as *Sphingomonas* [12–14], *Stenotrophomonas* and *Pseudomonas* [15], and *Rhizobium* and *Sphingobium* [16]. Anaerobic NP degradation in sediment is of greater importance because it is mainly anaerobic [17]. Chang and his colleagues suggested that NP could be biodegraded in anaerobic environments (e.g., sludge and mangrove sediment) [18,19]. Another previous study showed that NP could be slowly biodegraded under nitrate-reducing condition, but not under sulfate-reducing condition [17]. It is usually difficult to isolate degraders from anaerobic environments. So far only two anaerobic NP degraders have been isolated, namely *Bacillus cereus* and *Acinetobacter baumannii* [18]. In contrast, molecular biology methods have provided more detailed information on anaero-

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bic NP-degrading microbe in the environment. Based on clone library analysis, De Weert et al. [17] found that a *Pseudomonas* species was enriched with anaerobic NP biodegradation. They further suggested that it might be an anaerobic NP degrader in river sediment. Another previous study using denaturing gradient gel electrophoresis (DGGE) analysis suggested the potential role of *Clostridium* in anaerobic NP biodegradation [19]. However, these traditional molecular biology techniques have some notable disadvantages: the limited amount of data and limited accuracy of species identification [20,21].

High-throughput sequencing, especially using the recently developed Illumina MiSeq platform, can detect some rare bacterial species and yield more detailed information of microbial community with greater throughput and less cost [22,23]. However, Illumina-based sequencing has rarely been used to characterize bacterial community involved in degradation of pollutants [24,25]. Therefore, the present study was to investigate the shift in bacterial community structure associated with NP degradation in river sediment microcosms under nitrate- or sulfate-reducing conditions. The bacterial community structure was characterized using Illumina MiSeq sequencing. In addition, the potential anaerobic NP degraders in river sediment were also investigated.

2. Material and methods

2.1. Sampling and experimental setup

The sediments used for anaerobic biodegradation experiments were collected from the Wenyu River. The river annually receives a large amount of effluents from municipal wastewater treatment plants in Beijing city. NP (125–8157.9 ng/g) has been reported [26] and identified as an estrogen receptor agonist in sediments of Wenyu River [27]. The sediment samples were air-dried, homogenized, sieved through a 0.18 mm screen, and then stored at 4 °C until use. The river sediments were slightly alkaline (pH 7.6) and contained total organic carbon of 6.3 g kg^{-1}, nitrate nitrogen of 10.6 mg kg^{-1} and sulfate of 4.1 mg kg^{-1}. At the time of sediment collection, no residual linear 4-NP was detected in river sediment samples. In this study, linear 4-NP (99%; Adamas Reagent Co., Ltd.) was used in biodegradation experiments as a model NP species [17]. The mineral salt medium (pH 7.0) used in the biodegradation experiments consisted of (g L^{-1}): NH_4Cl (2.7), $MgCl_2{\cdot}6H_2O$ (0.1), $CaCl_2$ (0.08), $FeCl_2{\cdot}4H_2O$ (0.02), K_2HPO_4 (0.27), KH_2PO_4 (0.35), and resazurin (0.001) [19]. $NaNO_3$ or Na_2SO_4 (20 mmol L^{-1}) was added as an electron acceptor.

Anaerobic sediment microcosms consisted of mineral salt medium (10 mL), sediment (2 g) and supplementary electron acceptor ($NaNO_3$ or Na_2SO_4) in serum bottles (150 mL). Six different treatments in triplicate were performed: (A) sediment + 20 mmol L^{-1} $NaNO_3$; (B) sterilized sediment + 100 mg kg^{-1} NP + 20 mmol L^{-1} $NaNO_3$; (C) sediment + 100 mg kg^{-1} NP + 20 mmol L^{-1} $NaNO_3$; (D) sediment + 20 mmol L^{-1} Na_2SO_4; (E) sterilized sediment + 100 mg kg^{-1} NP + 20 mmol L^{-1} Na_2SO_4; and (F) sediment + 100 mg kg^{-1} NP + 20 mmol L^{-1} Na_2SO_4.The headspace in bottles was vacuumed for 10 s and then refilled with purified N_2 gas three times as previously described [28], and the butyl rubber stoppers were used to cap the bottles. The sterile controls were obtained by autoclaving at 120 °C repeatedly (20 min, three successive days). Microcosms were incubated at 28 °C on a horizontal shaker (140 rpm) for 90–110 days.

2.2. Chemical and molecular analyses

Sediment samples were collected after 6 hours' incubation (day 0) in order to enable the NP partition equilibrium between liquid and solid phases. Moreover, microcosms were usually sacrificed every 15 days for the analysis of residual NP. The residual NP in sediment was extracted twice using methanol (10 mL) with an ultrasonic processor. The mixture was filtered with a 0.22 μm syringe filter and the filtrate was used for high-performance liquid chromatography (HPLC) analysis. The mobile phase contained methanol and water (90:10) and flowed at a rate of 1 mL min^{-1}. NP was detected using a Venusil XBP C18 column (Agela Technologies) at 276 nm and the retention time was 6.9 min [9]. The levels of nitrate and sulfate in liquid phase were measured with ion chromatography (Dionex ICS-500, USA), using an Iopac ASI4 analytical column as previous described [28].

Total genomic DNA of each sediment sample was extracted using Powersoil DNA extraction kit (Mobio Laboratories) according to the manufacturer's instructions. PCR amplicon libraries were constructed with the primers 515F (5′-GTGCCAGCMGCCGCGG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) that targeted the V4 hypervariable regions of bacterial 16S rRNA genes [22]. FLASH (V1.2.7, http://ccb.jhu.edu/software/FLASH/) was used to merge the reads from the original DNA fragments and the quality filtering of reads was performed according to the literature [29]. UPARSE pipeline was used to pick operational taxonomic units (OTUs) and sequences were grouped into OTUs at 97% similarity. A representative sequence for each OTU was selected and its identity was classified using the RDP classifier [30]. OTU-based community diversity indices (Chao1 estimator and Shannon index) and rarefaction curve of each sample were generated based on three metrics calculated by the UPARSE pipeline [31]. Based on OTU level, weighted unifrac with QIIME (http://qiime.org/index.html) was used for weighted pair group method with arithmetic mean (WPGMA) clustering. The gene sequences obtained from high-throughput analysis in the current study were deposited in the NCBI short-read archive under accession numbers SRR1519305 and SRR1519306.

2.3. Statistical analysis

Analysis of variance (one-way analysis of variance) using the software SPSS 20 was applied to check for the quantitative differences in the residual NP. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. NP biodegradation

In this study, the levels of sulfate and nitrate were measured by ion chromatography. Sulfate loss was observed in the sulfate-amended sediment microcosm (with treatment F) (approximately 2 mmol L^{-1} on day 110), which verified the occurrence of sulfate-reduction. A marked nitrate loss (approximately 1.8 mmol L^{-1} on day 90) was also observed in the nitrate-amended sediment microcosm (with treatment C). The anaerobic biodegradation of NP was performed under either nitrate- or sulfate-reducing conditions.

Because of its hydrophobicity, the total amount of NP in liquid phase was negligible, compared with that in sediment during the incubation period (data not shown). Under nitrate-reducing condition, the NP residual rate significantly decreased (about 25%) after 30 days' incubation in the microcosm with treatment C ($P < 0.05$), compared with a lower decline (about 20%) in the microcosm with treatment B (Fig. 1a). Moreover, the residual NP in the microcosm with treatment C dropped to 45.9% on day 60, and further to 16.3% on day 90. In contrast, the residual NP of sterilized sediment (with treatment B) still remained about 70% after 90 days' incubation.

The residual NP in the microcosms with treatments E and F slightly changed during the first 45 days of incubation. However,