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Analysis of a Microbial Community Denitrying Nitrate to Nitrogen Gas in a Nitrate-Contaminated Aquifer

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Abstract

Little study has been published specifically addressing the dynamics of nitrate reducing bacteria (NBR) during the bioremediation of nitrate-contaminated aquifer. In our previous study we successfully quantified fumarate-enhanced microbial nitrate reduction rate in a nitrate-contaminated aquifer by using a series of single-well push-pull tests (PPTs). In this study we analyzed the suspended population during PPTs. To monitor changes in the microbial community, PCR amplification of 16S rDNA genes and denaturing gradient gel electrophoresis (DGGE) were used to study the dynamics of the bacterial community in detail. Before the stimulation of NBR, the dominant DGGE bands obtained by PCR were affiliated with γ-Proteobacteria consisting of *Acinetobacter* spp. and *Pseudomonas fluorescens*. However, as NBR biostimulation proceeded, the dominant patterns of DGGE bands changed, and they were affiliated with *Azoarcus denitrificans* Td-3 and Flavobacterium xanthum. Azoarcus denitrificans Td-3 is known to completely reduce nitrate to nitrogen gas. The series of single-well push-pull tests in this study should prove useful for conducting rapid, low-cost feasibility assessments for in situ denitrification and provide important information about which microorganisms play a key role in bioremediation of a nitrate contaminated aquifer.

Key word : in situ determination of denitrification rate, single-well push-pull test, fumarate, a nitrate-contaminated aquifer, *Azoarcus denitrificans* Td-3

INTRODUCTION

Microbial nitrate reduction is an important means to remediate a nitrate-contaminated aquifer. Little study has been published specifically addressing the dynamics of nitrate reducing bacteria (NBR) during the bioremediation of nitrate-contaminated aquifer. Recently we successfully quantified

fumarate-enhanced microbial nitrate reduction rate in a nitrate-contaminated aquifer by using a series of single-well push-pull tests (PPTs). It would of interest to know if the introduction of fumarate during the PPTs changes the microbial community in the subsurface and what types of microorganisms play an important role in nitrate reduction. Analysis of the suspended population during PPTs may provide some information on these issues.

The objective of this study was to assess NRB population dynamics during PPTs in a nitrate-contaminated site by using molecular analysis. These finding were compared with results of macroscopic measurements of activities (e.g. rates of nitrate and fumarate degradation).

METHODS

Field Single-well PPTs. A PPT consists of the controlled injection of a prepared test solution into an aquifer followed by the extraction of the test solution/ground water mixture from the same location. The injected test solution consists of ground water containing a nonreactive tracer (Br^-) and one or more biologically reactive solutes (fumarate, nitrate, nitrite, and/or nitrous oxide) selected to investigate specific processes of interest. The test solution is injected ("pushed") into the aquifer where it flows radically outward from the well and penetrates a volume of aquifer material adjacent to the well. During the extraction phase, flow is reversed; the test solution/ground water mixture is extracted ("pulled") from the same location, and concentrations of tracer, reactants, and reaction products are measured as a function of time.

Four PPTs were performed in series as reported as Kim et al. (2004). Three groundwater samples for microbial analysis were taken during PPTs. The first one (J1) was taken before performing 1st PPT , the second one (J2) after completing 1st PPT, and the last one (J3) after completing 2nd PPT.

DNA Extraction from Groundwater. Genomic DNA extractions from groundwater (10 L) taken during PPTs were performed using a modification of the reported method (Lee et al. 1996). The modified step involved extraction of the lysate with an equal volume of phenolchloroformisoamyl alcohol (25:24:1) and chloroformisoamyl alcohol (24:1) (Lee et al., 2002). The quality of extracted DNA was checked by standard agarose electrophoresis. DNA concentrations were measured by absorbance at 260 nm.

PCR, Cloning, Sequencing and Phylogenetic analysis. The 16S rDNA variable region V3 primer set, PCR amplification, and subsequent DGGE analysis have been described previously (Muyzer et al., 1996; Simpson et al., 1999). Sequences were compared to those in databases using the Basic Local Alignment Search Tool (BLAST) algorithm (Altschul et al., 1990) to identify known sequences with a high degree of similarity.

RESULTS AND DISCUSSIONS

The presence of several bands in DGGE profiles indicated a diverse bacterial population in

groundwater (Fig. 1). The dominant DGGE bands (#2, 3, 4, 5, 7, and 8) obtained by PCR were affiliated with γ-Proteobacteria consisting of *Acinetobacter* spp. (#2, 4, 5, 7, and 8) and *Pseudomonas fluorescens* (#3). However, as PPTs proceeded, the dominant patterns of DGGE bands changed, and they were affiliated with Azoarcus denitrificans Td-3 (#1) and *Flavobacterium xanthum* (#6). Azoarcus denitrificans Td-3 is known to completely reduce nitrate to nitrogen gas. Although we cannot statistically test the significance of this observation, our results suggest that the suspended bacterial community of the dominant species changed during PPTs.

Figure 1. Comparison of DGGE patterns of 16S rDNA fragments amplified from groundwater DNA. J1 (Before 1st PPT); J2 (After 1st PPT); and J3 (After 2nd PPT).

In our previous study substantial enhancement of nitrate reduction was observed when fumarate was added into the aquifer (Kim et al., 2004). This suggests that a diverse NRB population is present. This agrees with our results from DGGE and phylogenetic analysis. More specifically, consumption of fumarate coupled to complete nitrate reduction suggests the presence of *Azoarcus denitrificans* Td-3.

CONCLUSIONS

In this study, we presented a novel combination of single-well PPTs with molecular microbiological methods. Molecular and chemical data complemented each other and provided valuable insights into microbial processes and activities in a nitrate-contaminated aquifer.

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