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Comparative Molecular Analysis of the Microbial Community in a Sulfur-Based Autotrophic Denitrification Column Reactor

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ABSTRACT

As an alternative to remove nitrate, the biological autotrophic denitrification process has been receiving more attention recently because of its cost effectiveness and low sludge production. In this process, sulfur-based autotrophic denitrifying bacteria utilize elemental sulfur (S^0) as an electron donor, CO_2 as a carbon source, and nitrate as a terminal electron acceptor, resulting in the transformation of nitrate to nitrogen gas. While reducing nitrate, denitrifying bacteria oxidize elemental sulfur to sulfate (SO_4^{2-}). Since the nitrate removal mechanism relies on biochemical reactions it is helpful to understand dynamic changes of microbial population in the column reactor for maintaining a successful denitrification process. Apart from the conventional culture method for analyzing microbial community, we used a 16S rDNA-based molecular biological method which includes the cloning of 16S rDNA fragments of bacterial species present in the column followed by DGGE analysis.

The column reactor for autotrophic denitrification was packed with elemental sulfur granules and limestone with a ratio of 3:1. A sulfur-oxidizing, denitrifying bacterial consortium containing *Thiobacillus denitrificans*, which was enriched from an activated sludge, was then introduced into the packed bed column reactor. All experiments were conducted at 20°C. Synthetic groundwater was artificially contaminated with nitrate at the concentration of 30 mg NO_3^-—N/L and introduced into the column reactor from the bottom. For microbial community analysis, genomic DNA was extracted directly from sulfur granules and amplified by using 16S rRNA gene-targeted primers. Amplified DNA fragments were further purified and cloned by the T vector cloning system®, and analyzed by DGGE. To avoid redundant sequencing, PCR-amplified rDNA fragment of all clones were placed into categories by RFLP.

The data obtained from 100-day to 500-day-operated column clearly show the differentiated spatial distribution of bacterial species corresponding to their metabolic activity. When the microbial consortium enriched from activated sludge was first inoculated to the reactive media only 15% of *Thiobacillus denitrificans*, a sulfur-based autotrophic denitrifier, was present. After 100 days, *T. denitrificans* became the dominant species as time passed and occupied a large percentage (ca. 94%) at the bottom part of the column where denitrification activity was the highest. Interestingly, *Geothrix fermentans*, a heterotrophic denitrifier, was dominant at the top part of the column (ca. 90%) where nitrate was not present in a significant amount after 100 days. As a result, after 500 days *T. denitrificans* became to be dominant species (ca. 78-94%) even in top part of the column. A H_2S-oxidizing bacterium *Chlorobium limicola* appeared at the top part of the column (ca. 16%) after 500 days. The bacterium probably fed on the inorganic carbons and sulfate generated during the conversion of nitrate via sulfur oxidation by *T. denitrificans*. A vertical microbial analysis throughout the column showed that substrate-specific and column operation time-dependent microbial succession was observed.

Key words: Sulfur-based autotrophic denitrification, Cloning, DGGE