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Microbial Ecology of Emerging Contaminant Bioremediation

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Introduction

Perchloate is a strongly oxidized agent, and has been used as a propellant of rockets and explosives in aerospace industries. Because of its high solubility and chemical stability, it has been spread quickly and found primary in the surface water and groundwater. Perchlorate possibly inhibits the production of thyroid hormones, which are needed for prenatal and postnatal growth and development, as well as for normal metabolism in adults. Because of these reasons, prechlorate has been regarded as one of the high priority emerging contaminants. Recently, the California Department of Public Health set a maximum contaminant level (MCL) of 6 ppb in drinking water as of October 18, 2007. This will have impact on other countries' perchlorate regulation policy sooner or later.

In groundwater, nitrate is another widely-spread pollutant. An ion exchange process is typically used to remove perchlorate and nitrate together from groundwater. However, the ion exchange process generates brine waste in which nitrate and perchlorate and other salts are accumulated at 10-100 times enhanced concentrations. Microorganisms are known to efficiently degrade the nitrate and perchlorate co-contamination via microbial reduction processes in high salinity conditions [1]. However, understanding about microbial ecology in such high salt environment is still limited. In this presentation, we summarize the results of microbial community and functional analysis from denitrifying perchlorate degrading (i) up-flow packed bed bioreactors (by heterotrophs) and (ii) membrane biofilm bioreactors (MBfRs) with hydrogen utilizing microbes under high salinity conditions.

PART I: Perchlorate and nitrate reduction in packed bed bioreactors by heterotrophs

Under acetate growing conditions, four denitrifying up-flow packed bed bioreactors were acclimated with a typical municipal wastewater activated sludge (MLSS = 13,125 mg/L), and then perchlorate (6.25 mg/L) and salinity were continuously supplied into each reactor. To explore the effect of salinity on the

nitrate and perchlorate reduction, the concentration level of salinity was varied at 0.0, 0.5, 1.0, and 3.0% NaCl, respectively. After the perturbed supply of perchlorate and salinity, nitrate (1,400 mg/L NO_3^-) was completely reduced in the biofilter reactors within 1~2 days of operational periods, and complete perchlorate degradation was observed between 20 and 50 days. The portions of microbial communities near the influent port (within 1.4 cm) were found to be actively degrading perchlorate and nitrate. The functionally active microbial communities were analyzed for further microbial ecology analysis. According to terminal restriction fragment length polymorphism (T-RFLP) analysis, the degree of diversity was found to be decreased by the addition of perchlorate, exhibiting chemical-mediated toxicity. The increase of salinity did not affect the degree of microbial diversity but significantly influenced the structure of communities. As salinity increased, Proteobacteria populations significantly grew. When perchlorate degrading and denitrifying populations were quantified using quantitative PCR with chlorite dismutase (*cld*) and nitrate reduction (nirS) gene specific primers, respectively, both functional populations were found to be abundant in the acclimated activated sludge, and the increase of salinity up to 3% did not have inhibitory effect on the growth of both perchlorate and nitrate reducing populations.

PART II: Perchlorate and nitrate reduction in MBfR by hydrogen-utilizing microbes

MBfRs were inoculated with microbial communities from three different marine sediments (Freeport [FP], Texa; Great Salt Lake (GSL), Utah; Salton See (SS), California), and hydrogen was fed as the sole electron donor in the MBfRs. In a semi-batch mode, a synthetic brine waste with nitrate (244 mg/L $N\text{-}NO_3^-$) and perchlorate (18.4 mg/L ClO_4^-) was treated with the hydrogen-based MBfRs. Nitrate and perchlorate removal fluxes reached as high as 5.4 $gNm^{-2}d^{-1}$ and 5.0 g ClO4 $m^{-2}d^{-1}$, which are similar to values obtained from freshwater MBfRs. Perchlorate reduction was increased when decreasing nitrate loading, indicating a competition between the two reducing populations. In addition, nitrate and perchlorate reductions were decreased with increasing salinity. After 90 days of MBfR operation, the portions of microbial communities were analyzed with T-RFLP, and cloning & sequencing analysis. Although the tree sediment microbial communities (the sources of inoculums) showed distinctively different structures of communities, the hydrogen-based MBfR treatments resulted in a similar community structures after the 90 days of MBfR operations. In the MBfRs, alpha and gamma *Proteobacteria* populations were stimulated by hydrogen. According to the following phylogenetic analysis with amplified 16S rDNA sequences, no previously-known perchlorate reducing populations were found from the MBfRs. Since the previously-known perchlorate bacteria are denitrifying heterotrophs, the hydrogen-utilizing perchlorate reducing populations were found to be novel denitrifying perchlorate degraders.

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Reference

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