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Microbial Ecology of a Perchlorate-Reducing, Hollow-Fiber Membrane Biofilm Reactor

Many oxidized water contaminants, such as selenate, chromate, uranium (VI), and perchlorate, can be reduced by specialized bacteria to form innocuous or less mobile species. When the contaminant concentration is below its growth threshold (S_{min}), it must be removed as secondary electron acceptor, along with a primary acceptor that supports biomass. Key questions are whether the primary acceptor will select for bacteria capable of utilizing the secondary acceptor, and, if not, whether the secondary acceptor can provide a selective pressure for those bacteria. These questions were addressed for a hollow-fiber membrane biofilm reactor (MBfR) in which perchlorate was a secondary and nitrate a primary acceptor. Perchlorate-reducing bacteria (PCRB) are ubiquitous and many are denitrifiers. Perchlorate is reduced to chloride with a free energy similar to denitrification, and can be reduced concurrently with nitrate. The MBfR consists of a bundle of hydrogen-filled hollow fiber membranes within a tube. Water flows along the fibers and recirculation provides completely mixed conditions. Hydrogen diffuses through fiber walls to a biofilm naturally forming on the fiber surface. To address the above questions, four identical MBfRs were inoculated with a mixed culture and continuously supplied with 300-μM nitrate. Steady state was reached after 5 days with 3 μM effluent nitrate. At 3 weeks, 0 μM, 1 μM, 10 μM, or 100 μM perchlorate was added to the influents. Some perchlorate reduction occurred immediately, but the reduction increased significantly over several weeks, suggesting a small initial PCRB population and a subsequent enrichment. To further investigate, samples prior to and after 50 days of perchlorate addition were collected, and 550-bp partial sequences of 16S rDNA were amplified and subjected to Denaturing Gradient Gel Electrophoresis **(**DGGE). Banding patterns were similar before and after perchlorate addition and also among the different reactors, but one "main" band increased in intensity for reactors with higher influent perchlorate. Based on the 550 bp partial sequence, the main band belonged to the *Dechloromonas* genus, which includes many PCRB. Fluorescent *In-Situ* Hybridization (FISH) was applied to the sample collected after perchlorate addition, using an oligonucleotide probe designed to hybridize with rRNA from the main band species but not with other known *Dechloromonas*. 14%, 22%, 31%, and 49% of total bacteria hybridized with the probe in the reactors with 0 μM, 1 μM, 10 μM, and 100 μM perchlorate, respectively. Results of activity tests also were consistent with enrichment in reactors with higher influent perchlorate. These results suggest that PCRB were present without perchlorate, but even small amounts of perchlorate caused a significant enrichment for PCRB in a denitrifying biofilm, accounting for the greatly improved perchlorate reduction efficiencies.