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Comparisons of Microbial Communities in a Sequencing Batch Reactor (Cromaglass® Corporation) at Various Time Increments:

Sequencing Batch Reactors are used in sewage treatment plants to increase the surface area on which a biofilm's microbial community can reduce nitrate and denitrify. The goal of this particular study is to analyze the microbial community, with particular emphasis on nitrate reducing and denitrifying bacteria. Because only a small percentage of the microbes present can be cultured, both cultured and uncultured samples were examined to identify microbes present. Biofilm samples were collected from the SBR two weeks after the apparatus was installed and functioning and the 16S rRNA genes from the uncultured organisms were amplified using PCR and cloned. Plasmid DNA from the various clones was then purified and sequenced. Most of the clones showed the greatest similarity to other uncultured organisms. The sequences were then compared to the type strains in the 16S rRNA database to determine the closest phylogenetic matches.

SBR samples were also spread onto TSA plates and incubated at different temperatures. Colonies were then streaked onto various media, such as Lysine Iron, `Litmus Lactose, Bacto Endo Agar and EMB Agar, to observe phenotypic differences and choose which cultures would be identified by 16S rRNA analysis. Nitrate reduction and denitrification tests were also conducted on the organisms that were sequenced and ammonia oxidation plates were used to grow up the original cultures to verify which cultures use nitrogen as their electron transport acceptor. Among the cultured organisms, Pseudomonades and Enterobacteria, both gamma – Proteobacteria, were the most common.

While some nitrate reducers were found in the first sample, after the two week incubation, there was no evidence of denitrifying bacteria. Upon analysis of the twelve week Biofilm sample, the communities will be compared.