

Do Different Conditions in Bioreactors Alter *mcrA* (Methanogen) Diversity?

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Crucial in wastewater treatment is a group of obligate anaerobic Archaea that produce methane: the methanogens. Methanogen diversity under different conditions was studied using the *mcrA* gene, a functional gene for the enzyme that catalyzes the final step in methane production. Two bioreactors in the Department of Civil and Environmental Engineering had been seeded with the same biomass from an anaerobic digester at the South Shore Sewage Treatment Plant and provided with the same nutrients, except that one was also exposed to air. DNA was extracted from each bioreactor, and the polymerase chain reaction (PCR) was performed with specific primers to amplify the *mcrA* genes in the DNA from each bioreactor. The PCR product was cloned into plasmids and these were transformed into chemically competent *E. coli* bacteria. A second round of PCR was performed on DNA from the transformed *E. coli* to reamplify the *mcrA* gene. *mcrA* genes from about 100 different clones from each reactor were amplified in this fashion and Restriction Fragment Length Polymorphism (RFLP) was used to analyze the gene diversity in each reactor. Each cloned segment was digested to completion separately with *Sau96I*, *RsaI*, and *Taq α I* restriction enzymes. It was found that gene richness and overall diversity as measured by the Shannon-Weaver index varied very little between the two reactors (2.409 vs. 2.566). However, the RFLP showed that there were only a few restriction patterns common to both reactors. RFLP of *mcrA* from the two bioreactors showed that exposure to air did not greatly affect the overall gene diversity, but caused changes in the *mcrA* composition of methanogen communities.