

Bacterial Community Analysis of an Anaerobic Digester

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In wastewater treatment, the health of anaerobic digesters is dependent on methane producing Archaea referred to as methanogens. In an anaerobic digester, methanogens share a unique relationship with the syntrophic bacteria which oxidize butyrate, propionate and other compounds to acetate, hydrogen, and carbon dioxide. These products are then utilized by the methanogens. Without the syntrophs and their metabolic products, the system breaks down and may require a long recovery period. Syntrophic bacteria therefore, are essential to the proper function of this type of wastewater treatment system. Preliminary data on the bacterial diversity based on 39 clones did not reveal the presence of syntrophs. Thus, it was hypothesized that to detect the syntrophs additional sequences of the 16S rRNA genes would have to be analyzed. The hypothesis was tested by extracting DNA and using the polymerase chain reaction (PCR) to amplify the 16S rRNA genes and further analyze the diversity of the bacteria in the digester. The diversity of the new clones was compared and added to preliminary data to obtain a more complete picture of the bacterial diversity.

This study analyzed the bacterial community inhabiting a lab scale anaerobic digester, which was routinely treated with hydrogen and carbon dioxide, located in the Marquette University Department of Civil and Environmental Engineering. DNA was extracted from biomass, and PCR was used to amplify the 16S rRNA gene from the bacteria inhabiting the biomass of the anaerobic digester. The amplified 16S rRNA gene was inserted into a plasmid and transformed into competent *Escherichia coli* cells. Direct PCR was performed on the *E. coli* cells to reamplify the 16S rRNA gene. A total of 192 clones were amplified. However, because of failure to form contiguous sequences and the formation of chimeras, only 89 sequences were available for analysis of the diversity using bioinformatics techniques.

The addition of 89 clones unveiled three new classes of bacteria not previously reported in the digester: Verrucomicrobiae, Acidobacteria, and Unknown TM7. Preliminary data did not include syntrophic bacteria, while the new data introduced at least some sequences of syntrophic bacteria. Amplification of syntroph 16S rRNA genes with more specific primers may be necessary to examine the diversity of this important group of bacteria in this digester. The percent coverage of the bacterial diversity in the digester increased from 7% to 46% and when both the preliminary and the data from this study were combined, it increased to a total 52% coverage, lending support to the hypothesis. This also indicates that additional clones must be analyzed to get a better picture of the digester's bacterial diversity.