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Sequencing Batch Reactors (SBRs) increase the biofilm surface area in sewage treatment units that decrease the nitrogen content of wastewater. Our hypothesis is that the species composition of the SBR biofilm will change during the course of operation. Early, denitrification-positive biofilm samples were collected after two weeks of SBR operation and 16S rRNA genes from uncultured organisms were amplified, cloned, and sequenced. One third of the clones corresponded to previously cultured Gamma Proteobacteria, Bacteroidetes, and Actinobacteria. The remaining sequenced clones were less than 95% identical to GenBank database sequences and therefore represented new genera. These were most similar to 16S rRNA sequences from uncultured Proteobacteria, Bacteroidetes, Actinobacteria, Verrucomicrobia, and the recently identified phylum-level division TM7.

Biofilm samples were also suspended in water, diluted and cultured on tryptic soy agar at different temperatures. Amplified 16S rRNA gene sequences from pure cultures showed that Gamma Proteobacteria dominated the collection, which included 49% in the class Enterobacteriales, 39% in the class Pseudomonadales, and 6% in the class Xanthomonadales. The remaining 6% of identified isolates were Firmicutes. Of the identified isolates, 52% were capable of nitrate reduction to nitrite; however, none were capable of denitrification.

The twelve week sample was also suspended in water, diluted, and cultured on TSA plates at different temperatures. Organisms cultured from the biofilm samples after twelve weeks of operation showed a greater diversity than after the two week operation time. Gamma Proteobacteria decreased to about 55% of the isolates identified. Actinobacteria made up about 18% of the sample while both Firmicutes and Beta Proteobacteria made up about 14% each. A significantly higher percentage of organisms were capable of reducing nitrate to nitrite and some, about 9%, were also capable of denitrifying nitrite.