

## Abstract

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.

## Introduction

**GOAL of SBR:** Decrease the nitrogen and organic carbon levels in treated wastewater effluent by increasing the biofilm surface area for the microbial community.

**MECHANISM:** Reactor undergoes Aerobic and Anaerobic cycles  
Aerobic – chemolithotrophic oxidation of NH<sub>3</sub> and NO<sub>2</sub><sup>-</sup>  
Anaerobic – NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> reduction to N<sub>2</sub> gas (denitrification)

**Project Goal:** Evaluate the microbial community present in the SBR at two different time points.

## Methods

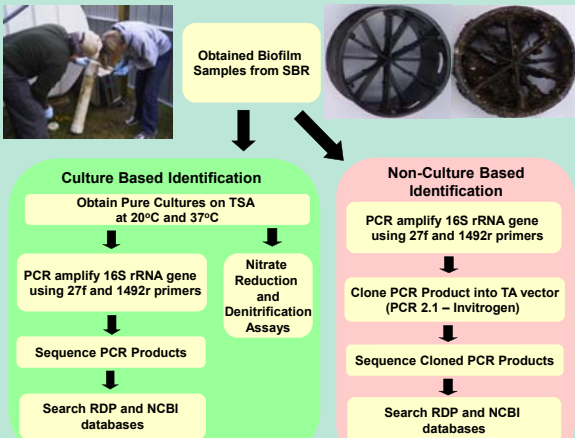


Figure 1 - Flow chart detailing the steps and procedures used in characterizing the organisms present in the biofilm at two and twelve week increments.

## Results

Table 1 – Cultured 16S rRNA sequence identifications and NO<sub>3</sub><sup>-</sup> reduction (NR)/denitrification (Denit) test results from biofilm after two weeks of SBR operation.

Strain	Seq % Match	Most Similar Type Strain	Phylogeny	NR	Denit
BM1,K.N	99.7	<i>Citrobacter freundii</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BMG	98.5	<i>Enterobacter asburiae</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BMO	98.9	<i>Klebsiella granulomatis</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM42	99.3	<i>Klebsiella granulomatis</i>	γ-Proteobacteria; Enterobacteriaceae	-	-
BM 61	97.6	<i>Klebsiella oxytoca</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM47	99.0	<i>Klebsiella oxytoca</i>	γ-Proteobacteria; Enterobacteriaceae	-	-
BM55	99.6	<i>Klebsiella pneumoniae</i>	γ-Proteobacteria; Enterobacteriaceae	-	-
BMJ,M.32	99.0-99.5	<i>Klebsiella pneumoniae</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM10	98.4	<i>Kluyvera ascorbata</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM12,28	98.6-99.6	<i>Raoultella ornithinolytica</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM29	98.9	<i>Raoultella planticola</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BMF	96.9	<i>Acinetobacter johnsonii</i>	γ-Proteobacteria; Moraxellaceae	-	-
BMW	94.8	<i>Acinetobacter junii</i>	γ-Proteobacteria; Moraxellaceae	-	-
BM14,19,23,70,R	93.2-99.3	<i>Pseudomonas korensis</i>	γ-Proteobacteria; Pseudomonadaceae	-	-
BMP	99.2	<i>Pseudomonas rhodesiae</i>	γ-Proteobacteria; Pseudomonadaceae	+	-
BMA,D,09	98.4-99.2	<i>Pseudomonas umsongensis</i>	γ-Proteobacteria; Pseudomonadaceae	-	-
BMQ,S	98.8-99.1	<i>Pseudomonas veronii</i>	γ-Proteobacteria; Pseudomonadaceae	+	-
BMSH	98.5	<i>Stenotrophomonas nitritireducans</i>	γ-Proteobacteria; Xanthomonadaceae	+	-
BMB	100.0	<i>Stenotrophomonas rhizophila</i>	γ-Proteobacteria; Xanthomonadaceae	-	-
BM81,E	96.9-98.8	<i>Exiguobacterium acetylicum</i>	Firmicutes; Bacillaceae	-	-

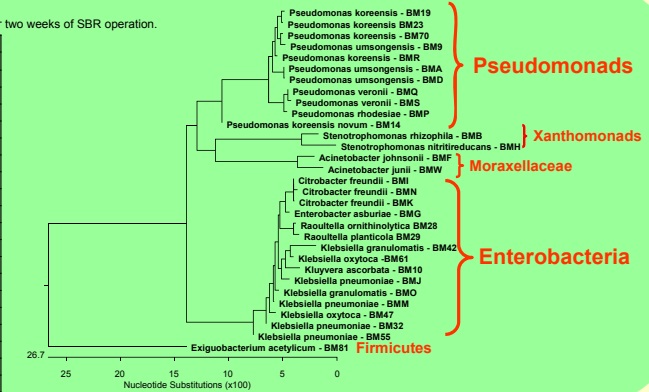


Table 2 – Cultured 16S rRNA sequence identifications and NO<sub>3</sub><sup>-</sup> reduction/denitrification test results from biofilm after twelve weeks of SBR operation.

Strain:	Seq % Match:	Most Similar Type Strain	Phylogeny	NR	Denit
BM2.8	97.5	<i>Microbacterium paraoxydans</i>	Actinobacteria; Microbacteriaceae	+	-
BM2.9	95.9	<i>Microbacterium novum</i>	Actinobacteria; Microbacteriaceae	-	-
BM2.24	96.5	<i>Microbacterium terregens novum</i>	Actinobacteria; Microbacteriaceae	-	-
BM2.13	98.6	<i>Rhodococcus erythropolis</i>	Actinobacteria; Nocardiaceae	-	-
BM2.1,17	99.1-99.3	<i>Bacillus pumilus</i>	Firmicutes; Bacillaceae	-	-
BM2.4	99.8	<i>Bacillus licheniformis</i>	Firmicutes; Bacillaceae	+	-
BM2.15	98.2	<i>Acidovorax defluvi</i>	β-Proteobacteria; Comamonadaceae	+	+
BM2.22	99.2	<i>Acidovorax temperans</i>	β-Proteobacteria; Comamonadaceae	+	+
BM2.23	99.5	<i>Comamonas testosteroni</i>	β-Proteobacteria; Comamonadaceae	-	-
BM2.20	98.1	<i>Enterobacter asburiae</i>	γ-Proteobacteria; Enterobacteriaceae	-	-
BM 2.3	99.6	<i>Klebsiella granulomatis</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM2.5	99.2	<i>Serratia marcescans</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM2.18	96.3	<i>Raoultella terrigena novum</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM2.19	97.5	<i>Pantoea anantii</i>	γ-Proteobacteria; Enterobacteriaceae	+	-
BM2.7,11,12,25	96.6-99.5	<i>Aeromonas media</i>	γ-Proteobacteria; Aeromonadaceae	+	-
BM2.10	98.8	<i>Pseudomonas fragi</i>	γ-Proteobacteria; Pseudomonadaceae	-	-
BM2.16	99.2	<i>Pseudomonas veronii</i>	γ-Proteobacteria; Pseudomonadaceae	-	-
BM2.21	99.9	<i>Pseudomonas alcaligenes</i>	γ-Proteobacteria; Pseudomonadaceae	+	-

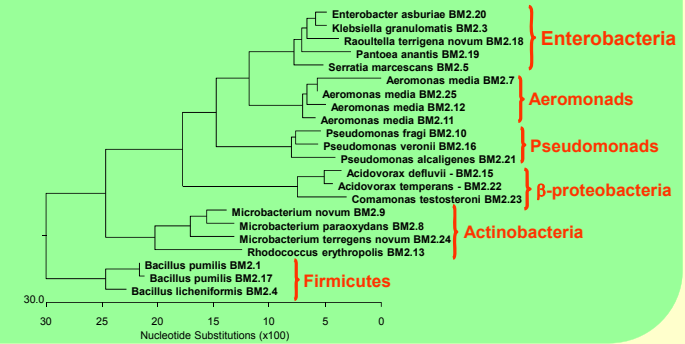


Table 3 – Uncultured 16S rRNA sequence identifications from biofilm after two weeks of SBR operation.

Clone	Seq % Match:	Most Similar Type Strain	Phylogeny
BM01	96.2	<i>Mycobacterium bonickiei</i>	Actinobacteria; Mycobacteriaceae
BM06	93.0	Unclassified ocean sediment bacterium	Bacteria; NKB19
BM18	91.6	Uncultured sludge bacterium	Bacteroidetes; Crenotrichaceae
BM24	99.3	Unclassified hydrothermal vent bacterium	Bacteroidetes; Flavobacteriaceae
BM17	92.0	<i>Halicomonobacter</i> Plant 1 Iso8	Bacteroidetes; Saprospiraceae
BM07	96.5	Uncultured penguin fecal bacterium	Bacteroidetes; Sphingobacteriales
BM21	95.6	<i>Citrobacter brakii</i>	γ-Proteobacteria; Enterobacteriaceae
BM22	96.1	<i>Citrobacter brakii</i>	γ-Proteobacteria; Enterobacteriaceae
BM35	95.2	<i>Acinetobacter haemolyticus</i>	γ-Proteobacteria; Moraxellaceae
BM08	99.6	Unclassified equine fecal bacterium	γ-Proteobacteria; Xanthomonadaceae
BM02	91.8	Uncultured Polyangiaceae bacterium	δ-Proteobacteria; Nannocystineae
BM14	93.7	TM7 uncultured landfill bacterium	TM7
BM05	94.8	TM7 unidentified lake sediment bacterium	TM7; W4-B20
BM15	93.5	TM7 uncultured lake sediment bacterium	TM7; W4-B20
BM19	88.3	<i>Verrucomicrobium spinosum</i>	Verrucomicrobia; Verrucomicrobiaceae

## Summary/Conclusions

**2 Weeks** - Cultured bacteria were primarily γ-Proteobacteria

➤ Pseudomonadaceae, Enterobacteriaceae, Xanthomonadaceae, Moraxellaceae

**12 Weeks** - Greater diversity of cultured bacteria observed

➤ Actinobacteria: Microbacteriaceae, Nocardiaceae

➤ Firmicutes: Bacillaceae

➤ γ-Proteobacteria: Enterobacteriaceae, Pseudomonadaceae, Aeromonadaceae

➤ β-Proteobacteria: Comamonadaceae

## References:

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This project was supported by the Cromglass Corporation and Lycoming College.