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Analysis of the Microbial Community in a Wastewater Sequencing Batch Reactor

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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, all were negative for 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 of organisms 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. In this sample, there was a much higher number of organisms capable of reducing nitrate to nitrite and some, about 9%, were also capable of denitrifying nitrite.

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 NH3 and NO2-
Anaerobic – NO3 and NO2- reduction to N2 gas (denitrification)

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

Methods:

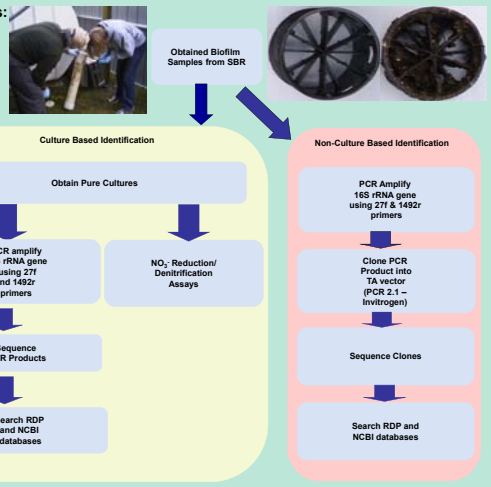

Figure 1 - Flow chart detailing the steps and procedures used in characterizing the organisms present in the biofilm at two and twelve week increments.

Results:

Table 1 – Uncultured 16S rRNA sequence identifications from biofilm after two weeks of SBR operation.

Clone:	Sequence % Match:	Most Similar Type Strain:	Phylogeny:
BM01	96%	*Mycobacterium bonickei*	Actinobacteria; Mycobacteriaceae
BM24	93%	*Chryseobacterium aecheongensee*	Bacteroidetes; Flavobacteriaceae
BM07	88%	*Terrimonas ferruginea*	Bacteroidetes; Flavobacteriaceae
BM18	86%	*Terrimonas ferruginea*	Bacteroidetes; Flavobacteriaceae
BM17	81%	*Haliscomenobacter hydrossis*	Bacteroidetes; Saprospiraceae
BM02	90%	*Haliangium ochraceum*	δ Proteobacteria; Haliangiaceae
BM35	95%	*Acinetobacter schindleri*	γ Proteobacteria; Moraxellaceae
BM21	99%	*Citrobacter brakii*	γ Proteobacteria; Enterobacteriaceae
BM22	96%	*Citrobacter brakii*	γ Proteobacteria; Enterobacteriaceae
BM06	85%	*Geobacter metallireducens*	γ Proteobacteria; Geobacteraceae
BM08	93%	*Xanthomonas cynarae*	γ Proteobacteria; Xanthomonadaceae
BM05	92%	*Pseudoalteromonas luteoviolacea**	γ Proteobacteria; Pseudoalteromonadaceae
BM14	84%	*Bacillus clausii**	Firmicutes; Bacillaceae
BM15	96%	*Clostridium baratii**	Firmicutes; Clostridiaceae
BM19	88%	*Verrucomicrobium spinosum*	Verrucomicrobia; Verrucomicrobiaceae

The * indicates that initially these bacteria were characterized in the new phylum-level division TM7. The current identifications were provided by the Ribosomal Database Project, which did not show any organisms as part of TM7. The TM7 designation actually was made using the NCBI BLAST database. This discrepancy shows the possible differences encountered when using various databases.

Table 2 – Cultured 16S rRNA sequence identifications and NO_3 reduction/ denitrification test results from biofilm after two weeks of SBR operation.

Strain:	Sequence % Match:	Most Similar Type Strain Organism:	Phylogeny:	Test for Nitrate Reduction:	Test for Denitrification:
BMI, K, N	97-99%	*Citrobacter fruendii*	γ –Proteobacteria; Enterobacteriaceae	+	-
BMG	97%	*Enterobacter asburiae*	γ –Proteobacteria; Enterobacteriaceae	+	-
BMO	98%	*Klebsiella granulomatis*	γ –Proteobacteria; Enterobacteriaceae	+	-
BM42	99%	*Klebsiella granulomatis*	γ –Proteobacteria; Enterobacteriaceae	-	-
BM32, 61	97-98%	*Klebsiella oxytoca*	γ –Proteobacteria; Enterobacteriaceae	+	-
BM47	99%	*Klebsiella oxytoca*	γ –Proteobacteria; Enterobacteriaceae	-	-
BM55	99%	*Klebsiella pneumoniae*	γ –Proteobacteria; Enterobacteriaceae	-	-
BMJ, M	98-99%	*Klebsiella pneumoniae*	γ –Proteobacteria; Enterobacteriaceae	+	-
BM10	99%	*Kluyvera ascorbata*	γ –Proteobacteria; Enterobacteriaceae	+	-
BM12, 28	99%	*Raoultella ornithinolytica*	γ –Proteobacteria; Enterobacteriaceae	+	-
BM29	98%	*Raoultella planticola*	γ –Proteobacteria; Enterobacteriaceae	+	-
BMF	97%	*Acinetobacter johnsonii*	γ –Proteobacteria; Moraxellaceae	-	-
BMW	96%	*Acinetobacter junii novum*	γ –Proteobacteria; Moraxellaceae	-	-
BMQ, S	98%	*Pseudomonas fluorescens*	γ –Proteobacteria; Pseudomonadaceae	+	-
BMR	98%	*Pseudomonas fluorescens*	γ –Proteobacteria; Pseudomonadaceae	-	-
BM09, 14, 19, 23, 70	91-99%	*Pseudomonas koreensis*	γ –Proteobacteria; Pseudomonadaceae	-	-
BMP	99%	*Pseudomonas rhodesiae*	γ –Proteobacteria; Pseudomonadaceae	+	-
BMA, D	97-98%	*Pseudomonas umsongensis*	γ –Proteobacteria; Pseudomonadaceae	-	-
BMH	99%	*Stenotrophomonas nitritireducans*	γ –Proteobacteria; Xanthomonadaceae	+	-
BMB	99%	*Stenotrophomonas rhizophila*	γ –Proteobacteria; Xanthomonadaceae	-	-
BM81, E	96%	*Exiguobacterium acetylicum*	Firmicutes; Bacillaceae	-	-

Table 3 – Cultured 16S rRNA sequence identifications and NO3 reduction/ denitrification test results from biofilm after twelve weeks of SBR operation.

Strain:	Sequence % Match:	Most Similar Type Strain Organism:	Phylogeny:	Test for Nitrate Reduction :	Test for Denitrification:
BM2.8	97	*Microbacterium paraoxydans*	Actinobacteria; Microbacteriaceae	+	N/A
BM2.9	95	*Microbacterium novum*	Actinobacteria; Microbacteriaceae	-	-
BM2.24	97	*Microbacterium terregens*	Actinobacteria; Microbacteriaceae	-	-
BM2.13	97	*Rhodococcus erythropolis*	Actinobacteria; Nocardiaceae	-	-
BM2.1, 17	99	*Bacillus pumilis*	Firmicutes; Bacillaceae	-	-
BM2.4	99	*Bacillus licheniformis*	Firmicutes; Bacillaceae	+	N/A
BM2.15	98	*Acidovorax defluvii*	β –Proteobacteria; Comamonadaceae	+	+
BM2.22	99	*Acidovorax temperans*	β –Proteobacteria; Comamonadaceae	+	+
BM2.23	99	*Comamonas testosteroni*	β –Proteobacteria; Comamonadaceae	-	-
BM 2.3	98	*Klebsiella pneumoniae*	γ –Proteobacteria; Enterobacteriaceae	+	N/A
BM2.5	98	*Serratia marcescans*	γ –Proteobacteria; Enterobacteriaceae	+	N/A
BM2.18	97	*Raoultella terrigena*	γ –Proteobacteria; Enterobacteriaceae	+	N/A
BM2.19	98	*Pantoea anantis*	γ –Proteobacteria; Enterobacteriaceae	+	N/A
BM2.20	98	*Pantoea agglomerans*	γ –Proteobacteria; Enterobacteriaceae	-	-
BM2.7, 11, 25	97-99	*Aeromonas media*	γ –Proteobacteria; Aeromonadaceae	+	N/A
BM2.12	97	*Aeromonas hydrophila*	γ –Proteobacteria; Aeromonadaceae	+	N/A
BM2.10	98	*Pseudomonas fragi*	γ –Proteobacteria; Pseudomonadaceae	-	-
BM2.16	98	*Pseudomonas veronii*	γ –Proteobacteria; Pseudomonadaceae	-	-
BM2.21	99	*Pseudomonas alcaligenes*	γ –Proteobacteria; Pseudomonadaceae	+	N/A

Summary:

Two Weeks: The majority of cultured organisms were γ –Proteobacteria and were representative of the 4 different families Pseudomonadaceae, Enterobacteriaceae, Xanthomonadaceae and Moraxellaceae. The only other cultured organism was identified as a Firmicute in the family Bacillaceae. γ –Proteobacteria made up about 94% of the total sample while the single Firmicute isolate made up the other 6% of the sample.

Twelve Weeks: In this sample, there was much more diversity seen in not only the family level but also at the phylum level since there are 4 phylums represented instead of two. These phylums are Actinobacteria, Firmicutes, γ –Proteobacteria and β –Proteobacteria. There are also 7 different families in the four phylums identified. These families are Microbacteriaceae, Nocardiaceae, Bacillaceae, Comamonadaceae, Enterobacteriaceae, Pseudomonadaceae and Aeromonadaceae.

Conclusions:

- There were a total of 4 families found after the initial two week incubation whereas there were 7 families found after the twelve week incubation period.
- The diversity of the SBR microbial community increased with time. This can be seen not only in the diversification of families, but also in the increased diversity in the phylum level identifications.

References:

Ribosomal Database Project - http://rdp.cme.msu.edu/index.jsp

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