Genomic and physiological perspectives on bioremediation processes at the FRC

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Introduction

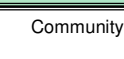
A suite of molecular and physiological studies, including metal reduction assays, metagenomics, functional gene microarrays and community sequence analyses were applied to investigate organisms involved in bioremediation processes at the ERSP Field Research Center and to understand the effects of stress on the makeup and evolution of microbial communities to inform effective remediation strategies.

Community analyses of U(VI)-reducing sediments

Introduction

Microbial reduction of U(VI) and nitrate has been successfully biostimulated in Area 3, resulting in reduction of U(VI) levels in groundwater to below U.S. EPA regulatory limits. Understanding the microbial diversity present throughout the active subsurface treatment zone is valuable to understanding the bioremediation process. Clone libraries of 16S rRNA genes from throughout the inner loop of the treatment zone were used to characterize the microbial diversity and relate the differences in microbial composition with spatial location within the treatment zone.

Methods



Sediment samples from the injection, extraction and multi-level sampling wells of below ground treatment system in the FRC Area 3 (Fig. 1) were collected by well surging. DNA was extracted and 16S rRNA gene libraries were constructed and sequenced. Sequences were then filtered for quality (80% sequence with Q=20) classified with RDP, compared with LIBSHUFF and analyzed with EstimateS and DOTUR. OTUs were defined at 95% similarity.

Figure 1. Well location in the treatment zone of Area 3 at ORNL.

The arrow indicates the direction of the subsurface transport.

Results

- In all wells examined, the microbial communities were dominated by proteobacteria with a relative contribution that ranged from 56 to 61% of the total community. Several genera of known importance were detected: *Dechloromonas* (dehalogenator, denitrifier), *Geobacter* (iron- and uranium reducer), *Desulfovibrio* (sulfate-and uranium-reducer), *Acidovorax* (nitrate reducer), and *Rhodobacter* (iron- and nitrate-reducer).
- LIBSHUFF detected significant differences in the microbial community composition among the wells. The microbial communities differed significantly in their composition with the exception of the sampling well (102-3) and the injection well (104) that were significantly similar (p=0.05).
- The denitrifying bacteria present in the sediments differed from those in the Criddle-Jardine fluidized bed denitrifying bioreactor (data not shown). In the bioreactor more *Thauera* sequences were detected while in the sediments *Dechloromonas* was the dominant denitrifier. More stringent conditions and the presence of halogenated compounds that favor *Dechloromonas* could explain this difference.

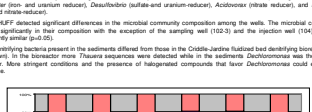
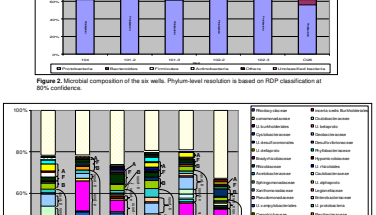


Figure 2. Microbial composition of the six wells. Phylum-level resolution is based on RDP classification at 80% confidence.


100%
80%
60%
40%
20%
0%
104
101-2
101-3
102-2
102-3
C05
Well Name
Phylum Unclassified
Unclassified Acidobacteria
Unclassified Actinobacteria
Unclassified Bacteria
Unclassified Bacteroidetes
Unclassified Chlorobi
Unclassified Cyanobacteria
Unclassified Firmicutes
Unclassified Fusobacteria
Unclassified Proteobacteria
Unclassified Spirochaetes
Unclassified Verrucomicrobia
Unclassified Candidate division TM7
Unclassified Candidate division WS3
Unclassified Candidate division OP11
Unclassified Candidate division OD1
Unclassified Candidate division OP10
Unclassified Candidate division OP3
Unclassified Candidate division TM6
Unclassified Candidate division WS6
Unclassified Candidate division OP9
Unclassified Candidate division SR1
Unclassified Candidate division TM7
Unclassified Candidate division WS3
Unclassified Candidate division OD1
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