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High density oligonucleotide array monitoring of bacterial community dynamics during carbon stimulated uranium bioremediation.

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We investigated the utility of photolithographic 16S oligonucleotide arrays (Affymetrix GeneChip) in monitoring bacterial community composition during a simulated uranium bioremediation process. Initial validation of the array approach was performed using U(VI) contaminated (200 ppm) sediment from Area 2 at the Oak Ridge, TN, Field Research Center. The composition of a 16S rRNA gene amplicon pool generated from sediment extracts was assayed by clone library sequencing (764 clones) and GeneChip hybridization. Forward and reverse sequences from clones were assembled into contigs and consensus sequences compared with the same 16S database (LLNL) used to designate GeneChip OTUs and design GeneChip probes. Comparison of the clone sequence data with GeneChip "positive hits" showed that both approaches were in good agreement indicating an amplicon pool dominated by *Arthrobacter*, *Rathayibacter*, and *Actinobacterium sp*.

To demonstrate the utility of GeneChip monitoring we analyzed 16S amplicons from distinct stages (NO_3^- reduction, net U(VI) reduction, net U(IV) re-oxidation) during the U(VI) remediation process. TRFLP analysis of these stages showed bacterial community divergence. Array data indicated a stimulation of denitrifying and metal reducing bacteria (including *Hyphomicrobium, Azoarcus, Pseudomonas, Geobacteraceae*, *Shewanella*, and *Geothrix* sp.) throughout NO_3^- and U(VI) reduction stages. Metal reducing bacteria were also detected during the observed U(IV) re-oxidation stage suggesting that a loss of microbial functionality was not a factor in U(IV) re-oxidation. These results demonstrate the ability of the custom Affymetrix GeneChip to accurately follow changes in bacterial community composition and the potential of this array in monitoring remediation practices.