Utilization of Molecular Biological Tools to Assess Performance of Biosparge Pilot System for Sulfolane Degradation

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Background/Objectives. Sulfolane is an industrial solvent used in the chemical industry for the sweetening of sour gas. Sulfolane has properties conducive to groundwater transport. Sulfolane has been reported to biodegrade under aerobic conditions including some organisms within the family Comamonadaceae. A former gas plant in Canada, was piloted for enhanced attenuation via biosparging to increase the aerobic biodegradation capacity of sulfolane in groundwater. Biosparging was performed to augment the groundwater with oxygen to enhance the growth of sulfolane-degrading microorganisms and sulfolane degradation rates. Molecular biological tools (MBTs) and culture-based techniques were employed to assess enhancement of sulfolane-degraders due to biosparging, evaluate shifts in the overall microbial community and to improve decision-making for future remedial strategy.

Approach/Activities. DNA and culture-based techniques were employed to characterize the microbial community and its potential for sulfolane degradation under aerobic conditions. Groundwater samples collected with spatial (upgradient, in sparge zone, and downgradient) and temporal variation were used to assess changes in sulfolane concentration, geochemistry, and overall microbial community composition and sulfolane-degrader concentration. Collected groundwater was also used to inoculate selective solid media to quantify, isolate and identify putative sulfolane-degraders. Genomic DNA was extracted from cultured isolates and was amplified via PCR (16S rRNA gene) for identification. The overall microbial community of groundwater was assessed via next generation sequencing (NGS) of the 16S rRNA gene using an Illumina MiSeq platform. NGS and plate count data was used to understand changes in the relative abundance of isolated putative sulfolane-degraders due to biosparging.

Results/Lessons Learned. Following the initiation of biosparging and resulting increased dissolved oxygen levels in the subsurface, total plate counts of sulfolane-degrading bacteria increased by more than two orders of magnitude while their percentage of the total bacterial population also increased. Putative sulfolane-degrading isolates were identified within the Comamonadaceae family and the Moraxellaceae family, which both contain organisms reportedly implicated in sulfolane degradation. Analysis of plate counts and NGS data showed increases in relative abundance of colonies and OTUs closely related to putative Comamonadaceae sulfolane-degraders after biosparging began, within and downgradient of the sparging zone. Increases in dissolved oxygen in groundwater, concurrent increases in relative abundance of putative sulfolane-degraders and decreases in sulfolane concentrations provide multiple lines of evidence to evaluate performance of the biosparging pilot system. Next steps required to confirm sulfolane degradation ability of current isolates and the potential for development of quantitative PCR methods to more efficiently track sulfolane-degrading populations will be discussed, including a focus towards using these data for development of remedial strategies.