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The Use of Omic-based Tools to Aid in the Assessment of Monitored Natural Attenuation of MTBE Contaminated Sites with Biobarrier Oxygen Injection Systems

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Background/Objectives. A long-term performance of a two-tiered biobarrier system previously used at the 22 Area of Marine Corps Base (MCB) Camp Pendleton to treat methyl tertiary-butyl ether (MTBE) in groundwater was evaluated. The system used pure oxygen injection/sparging to create a two-tiered biobarrier approach. The project's objectives include evaluating if microbial activity as a result of the biobarrier continues to support monitored natural attenuation (MNA) of residual MTBE at the site. Findings from proteomic and metagenomic analyses coupled with existing site information were used to evaluate MNA of MTBE contaminated site during post sparge activities.

Approach/Activities. Groundwater samples were collected from two locations: Area 22 (main site with low levels of MTBE), and Area 13 (a positive control site with high levels of MTBE) for geochemical and omic analyses in the contaminant plumes upgradient and downgradient of the two biobarriers as well as within the MNA zone between the two biobarriers and in areas outside of the plume. Metagenomic analyses of collected samples will allow an understanding of the microbial composition at each area of the sites, whereas proteomic analysis will provide a direct measure of microbial biodegradation activity. Both omic analyses will aid in the effort of determining if the biobarrier system had a long-term (post shutdown) impact on the microbial composition and activity within the area of treatment. This effort will provide further evidence of the natural attenuation capacity of the system for MTBE degradation. The overarching goal was to confirm that the biobarriers changed the microbial communities as well as functional proteins, including those responsible for MTBE- degradation.

Results/Lessons Learned. The initial set of analyses revealed that composition and activity of microbial communities can be successfully measured using metagenomics and proteomics, and that organisms capable of degrading MTBE were present in both tested areas. However, the vast majority of identified MTBE degraders from Area 22 were classified as MTBE co-metabolic degraders. These finding were confirmed by proteomic analysis, which identified low levels of proteins expressed from the most abundant organisms within the community. In contrast, our positive control Area 13 revealed microbial populations that could completely mineralize MTBE (in addition to co-metabolic degraders). Further, proteomic analysis revealed the presence of several key enzymes involved in the biodegradation pathway of MTBE, confirming the direct metabolism of MTBE. Upon completion of the second round of sampling, additional omic and geochemical analyses will be performed. The targeted proteomics developed during this work aids accurate detection of specific peptides in environmental samples as well as has the potential to provide a wealth of new information on protein function and activities within the subsurface. The data presented demonstrate validity of a step wise approach in which metagenomic sequencing aid targeted proteomic in detection of key peptides involved in the biodegradation of MTBE. In the end, we are developing a comprehensive listing of microbial species involved in the degradation of MTBEs as well as other organisms that might be influential in syntrophic degradation, as well as identifying proteins involved in the biodegradation of MTBE. This information will help to evaluate the role of continued microbial activity post sparging to support ongoing attenuation of residual MTBE. This omic-based

information may also help improve understanding of the effect of these microbial biofilms on the aquifer.