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Aerobic Biostimulation of Buried MC252 Oil: Metagenomic and Biogeochemical Assessment of a New Response Approach

LeeAnn Fitch (lfitch2@lsu.edu), Zachary Romaine (zromai1@lsu.edu), Vijaikrishnah Elango (velango@lsu.edu) and ***John Pardue*** (jpardue@lsu.edu) (Louisiana State University, Baton Rouge, LA, USA)

Background/Objectives. Hard structures were built across Fourchon Beach, LA to mitigate the transport of weathered MC252 oil reaching adjoining wetlands. These hard structures prevented the transport of weathered crude oil but allowed vertical transport of oil into the beach subsurface beneath the water table. This crude oi continues to persist in a highly anaerobic, hypersaline, sulfidic groundwater system. Objectives of this study are to evaluate the efficiency of aerobic biostimulation to enhance alkylated polycyclic aromatic hydrocarbon (PAHs) biodegradation in the field, develop optimized oxygen dosage for aerobic biostimulation using laboratory microcosm studies and to understand changes in bacterial community composition as a function of oxygen addition.

Approach/Activities. An aerobic biostimulation field study is being conducted at Fourchon Beach, LA covering approximately 7500 ft^2. A multi-well oxygen injection system was used to stimulate aerobic biodegradation in contaminated groundwater. Biodegradation of PAHs were quantified by weathering ratios of alkylated phenanthrenes and dibenzothiophenes to chrysenes, stable carbon isotope analysis and radiocarbon analysis. Sediments collected during sampling events were used to set up laboratory microcosms. Three aerobic treatments; 20, 35 and 50 mg O_2/month, anaerobic treatment and appropriate controls were evaluated for the laboratory study over 30 weeks. Biodegradation of PAHs in the microcosm study was evaluated by normalizing the total PAHs, C1-Phenanthrenes and C1-Chrysenes in aerobic treatments to killed control results. PAHs in sediments were analyzed using a gas chromatograph with a mass selective detector. Genomic DNA was extracted from pre aeration sediments, post aeration Geoprobe cores and microcosm sediments. 16S rRNA from genomic DNA was amplified with universal bacterial primers 515F and 806R overlapping in the V4 region, and sequenced using the Miseq Ilumina platform. Silva reference alignment were used for taxonomic alignment of sequences.

Results/Lessons Learned. Alkylated phenanthrene and dibenzothiophenes levels in pre-aeration sediments evaluated through weathering ratios to alkylated chrysenes were close to the levels in freshly-spilled MC252 oil. Post aeration measurement in sediments demonstrated increased weathering of alkylated phenanthrenes and dibenzothiophenes consistent with aerobic biostimulation. Stable isotopic and radiocarbon analysis of dissolved inorganic carbon also suggested crude oil mineralization post-aeration. Metagenomics analysis of Geoprobe-collected core sections revealed shift in the composition of the diverse, halophilic, hydrocarbon-degrading microbial population. *Marinobacter* was the dominant population in the pre aeration sediments. However, addition of oxygen increased the abundance *Halomonas*. In laboratory microcosms, significant differences ($p<0.05$) in total PAH loss was observed between anaerobic treatments and all the three aerobic treatments (20, 35 and 50 mg O_2/month) after an 18-week incubation. However, no statistical difference in total PAH losses were observed between the three aerobic treatments. Microcosm bacterial population analysis revealed *Marinobacter* as the most abundant genera in the pre-aeration sediments. After 12 weeks of incubation, *Marinobacter* abundance decreased with concomitant increase in *Rhodobacteraceae* in all the treatments. Bacterial diversity calculated by the Shannon index was not statistically different ($P> 0.05$) between pre aerated, anaerobic and aerobic microcosm sediments. Analysis of PAHs and

bacterial community in Geoprobe cores after pure oxygen addition are currently in progress and the multi-well injection system in the field is in the process of being transitioned to more aggressive oxidation. Analysis of changes PAHs and bacterial community with oxygen release compounds will be completed before the May 2017 Conference.