Microbial Recovery after Eutrophic Conditions during Biostimulation at Hydrocarbon-Impacted Sites

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Background/Objectives. Excess N and P during biostimulation of PHC-impacted sites can cause eutrophic conditions. High nutrient concentration can negatively impact the microbial community, and their ability to degrade hydrocarbons. It can increase the overall microbial population causing more competition between microorganisms for essential nutrients which may suppress hydrocarbon degrading organisms. By understanding microbial responses to eutrophic conditions, we can make recommendations on how to continue site remediation on sites where there has been excess nutrients added. Our overall objective, as part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), was to determine if microbial communities and their ability to degrade hydrocarbons, can recover after a site has been exposed to eutrophic conditions.

Approach/Activities. This research was conducted in support of a field scale in situ biostimulation remediation project at three PHC impacted bulk transfer and gasoline stations in Saskatchewan, Canada. We created a microcosm design that mimicked field conditions at each field site. Duplicate soil cores were collected during Phase II Assessment activities, and were sub-sampled using a slotted PVC pipe and placed into a microcosm containing a biostimulatory solution. Microcosms were prepared in groups of two and then randomly assigned for destructive sampling at four or eight weeks. Five treatments were then applied to each group of two. The treatments were: 1) eutrophic conditions for four weeks, followed by four weeks in optimal concentration, 2) eutrophic conditions for four weeks, followed by four weeks in unammended water, 3) uncontaminated soil in eutrophic conditions for four weeks, followed by four weeks in optimal conditions 4) unammended water for eight weeks, 5) optimal nutrient conditions for eight weeks. The unammended water was city tap water that was UV sterilized and decholoronated. Soils were incubated under nitrate and sulfate reducing conditions for four weeks at 10°C and amendment solutions were replaced weekly. Each week the amendment solution was analyzed for BTEX. Soil samples were taken initially and after four and eight weeks for catabolic gene prevalence, most probable number of diesel degraders (MPN), and microbial community. Soil DNA was extracted and quantified by 16sRNA gene sequencing. For catabolic genes, the quantity was determined using QPCR. Biological community composition was assessed using Illumina MiSeq sequencing and 926F/1392R primers.

Results/Lessons Learned. In the first four weeks, there was lower BTEX degradation in eutrophic conditions compared to the optimal treatment. After week four, when solutions were changed to optimal nutrient concentrations, samples that were previously exposed to eutrophic conditions continued to have a lower degradation rates in comparison to samples in the optimal nutrient amendment for the full eight weeks. However, samples that had excess nutrients then were put in unammended water had the highest degradation rates. Catabolic gene prevalence, MPN's, and microbial community composition results will be presented at the conference.