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Biostimulatory Solutions for Petroleum Hydrocarbon Impacted Sites in Cold Regions: Effects of C: N-P Ratios on Degrader Prevalence and Potential Activity in Clayey Soils

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Background/Objectives. Optimal nitrogen (N) and phosphorus (P) concentrations for in situ biostimulation of petroleum hydrocarbon contaminated sites have been extensively investigated. However, it has not been assessed how C: N: P ratios effect microbial communities and are linked to degrader prevalence and activity. In addition, most laboratory studies on biostimulation have proven results in the lab and lower success rates in the field. We believe this disconnect is due to the alteration of soil that occurs in microcosm experiments. In most laboratory studies, soils are dried, sieved, and then spiked. This process alters the soil surface area coming into contact with biostimulatory solution, soil structure, fractured flow, microbial population and habitat, and hydrocarbon adsorption and desorption. To avoid these alterations, we mimicked field conditions by collecting a duplicate borehole during Phase II Assessment. The cores were sub-sampled using a 2 x 1.5 (OD) inch slotted PVC pipe and placed into a 125 ml amber jar with a biostimulatory solution. Our overall objective, as part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), was to determine how C: N-P ratios are linked to hydrocarbon degrader prevalence and potential activity in clay soils.

Approach/Activities. This research was conducted in support of a field scale in situ biostimulation remediation project at three PHC impacted petroleum facilities in western Canada. Soil samples were collected from bulk transfer and gasoline stations in southern Saskatchewan. To assess how C: N-P ratios effected the microbial community we left the P ratio constant at a rate where formation of new P minerals, such a brushite was not predicted to occur. The N ratios were based off of hydrocarbon content and soil moisture. Nitrogen levels were kept below 2000 mg N kg^{-1} H_2O to eliminate any negative effects from osmotic stress. Soils were incubated anaerobically in the dark for four weeks at 10°C. To determine how the solution effected the microbial community the C: N-P solutions were replaced and analyzed at the beginning of the incubation and weekly for nutrients (NO_3^-, NO_2-, PO_4^-, Fe^{2+}, and SO_4^-), BTEX and F1. Soils were sampled before and after incubation for nutrients (NO_3^-, NH_4^+, PO_4^-), catabolic gene prevalence (*bamA, bzdN, bcrC, assA, and rpoB*), and microbial community composition. The BTEX and F1 in the soil was determined by a linear relationship of BTEX and F1 in the soil vs solution. DNA was extracted and quantified by 16sRNA gene sequencing. For catabolic genes, the quantity was determined using a quantitative polymerase chain reaction. Biological community composition was assessed using Illumina MiSeq sequencing and 1392R/926F primers.

Results/Lessons Learned. Results will be presented at the conference and will be used to improve amendment solutions for site-specific data across Canada.