

Using Passive Anode Cathode Technology to Assess Microbial Happiness and Boost Benzene Biodegradation Rates

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As part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), contaminated sites are amended with solutions composed of critical nutrients and alternate electron acceptors for anaerobic petroleum hydrocarbon degradation in the cold, calcareous, and clayey soils of Western Canada. Cost constraints arise when it is time to assess the microbial response to injection of biostimulant solutions. Passive Anode Cathode Technology (PACT) is a type of microbial fuel cell that can be used to estimate increases in microbial respiration. A platinum catalyst cathode and graphite anode function like a rechargeable battery. The PACT is in an open circuit where microbes can deposit electrons on the graphite anode. The circuit is then closed, and the electrons deposited by the microbes are expelled. The voltage gain from the re-deposition of electrons by the microbes is recorded and used to make inferences about increasing microbial respiration, and therefore, increases in hydrocarbon degradation.

We conducted two experiments with an anaerobic benzene degrading microbial consortia from Dr. Ania Ulrich (University of Alberta) in liquid media. The first experiment tested different electron acceptors (EA) with the PACT with treatments of no EA, ferric iron (Fe^{3+}), sulfate (SO_4^{2-}), and ferric iron and sulfate (Fe^{3+} and SO_4^{2-}). The second experiment tested the PACT effect with the addition of all EA (nitrate (NO_3^-), SO_4^{2-} , and Fe^{3+}) and treatments of PACT with no EA, PACT with all EA, no PACT with no EA, and no PACT with all EA.

Benzene degradation rates increased in the presence of the PACT and in particular, in treatments with high SO_4^{2-} . The Fe^{3+} and SO_4^{2-} treatment showed the most benzene degradation. In comparison, the addition of NO_3^- to the treatment slowed the degradation rates. The voltage readings from the PACT are a good predictor of benzene degradation with positive correlations in the SO_4^{2-} treatment ($r=0.81$, $p = 0.19$) and in the Fe^{3+} and SO_4^{2-} treatment ($r = 0.56$, $p = 0.44$). The presence of the PACT resulted in a deviation from typical benzene:EA stoichiometry. There are two surfaces in the PACT microcosm that allows for biofilm formation; the graphite anode and the iron oxyhydroxides formed from the reducing agent. Gene sequencing analysis showed genera such as *Geobacter*, *Thauera*, and *Thiobacillus*, which are all microbes capable of direct interspecies electron transfer (DIET) that can be facilitated on reactive surfaces. We predict that DIET processes are the reason for increased benzene degradation rates and unique benzene:EA ratios. The PACT improved benzene degradation rates, accurately predicted percent benzene degraded with an increase in voltage gain in liquid cultures, and changed microbial community composition. As the area of influence of the PACT is limited, transitioning this work to the field should involve an in-situ biostimulant solution that includes nano-sized graphite powder, SO_4^{2-} , Fe^{3+} , a nitrogen source, and phosphate.

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Kathlyne Hyde is a PhD student in the Department of Soil Science at the University of Saskatchewan. Her thesis is focused on understanding microbe and mineral interactions in petroleum hydrocarbon contaminated sites being amended with a biostimulant solution. Her work primarily focuses on benzene adsorption and biodegradation.