Abstract C-15, in: A.R. Gavaskar and C.F. Silver (Symposium Chairs), *In Situ and On-Site Bioremediation—2007*. Proceedings of the Ninth International In Situ and On-Site Bioremediation Symposium (Baltimore, Maryland; May 7–10, 2007). ISBN 978-1-57477-161-9, published by Battelle Press, Columbus, OH, www.battelle.org/bookstore

Diluent Hydrocarbon Biodegradation in Laboratory Microcosms Using Anoxic Electron Acceptors

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A method was developed to examine natural hydrocarbon attenuation by anaerobic bacteria in a laboratory setting to determine the contribution of anaerobic biodegradation to in situ remediation at a former oil field near Guadalupe, CA. Mid-range hydrocarbons were used at this site as a diluent to facilitate pumping oil; diluent leaks resulted in hydrocarbon contamination of the soil and groundwater. Most previous research on hydrocarbon biodegradation has focused on aerobic microbial activity as it contributes to natural attenuation. In the current anaerobic microcosm experiments were conducted with ground water from the site to investigate the role of specific processes on biodegradation of dissolved hydrocarbons.

Groundwater was collected from a monitoring well from a where anoxic aquifer conditions exist. Microcosms were prepared in custom-made 2-L serum bottles with 100-mL gas headspaces. Four separate electron acceptors – nitrate, sulfate, manganese(IV) and iron(III)-were added separately to microcosms to test for the promotion of anaerobic biodegradation. One set of microcosms utilized a mixture of nitrate, sulfate, and Fe(III) to examine the interaction of bacterial species on biodegradation. A set of unamended microcosms was run to examine hydrocarbon biodegradation under natural attenuation conditions. For comparison of biodegradation rates, aerobic microcosms were prepared and operated side-by-side with the anaerobic microcosms. A set of killed controls was prepared with 1% sodium azide to inhibit microbial activity.

Microorganisms were supplied by site groundwater and inoculum from anaerobic soil collected at the Guadalupe site. The experiment was conducted inside of a glove-box purged with nitrogen gas, with testing performed on 80 sacrificial microcosms after 0, 26, 134, 260, and 400 days of incubation. The total petroleum hydrocarbon (TPH) concentration in groundwater was determined using gas chromatography. Ion chromatography, phenanthroline method, and formaldoxime method were used to determine the specific mechanism and electron acceptor used in the microcosm. Bacterial communities were characterized using terminal restriction fragment (TRF) analysis. Gas headspaces in the microcosms were monitored for methane and oxygen.

Results show evidence of biodegradation in all microcosms except the control. Observed anaerobic biodegradation rates were significantly less that observed aerobic rates. Unamended microcosms showed evidence of methanogenesis by detectable methane in gas headspace; unamended microcosms displayed biodegradation rates slower than those amended with electron acceptors. Experimental results suggest that the order of biodegradation kinetics as dependent on electron acceptor is as follows: aerobic > nitrate > Mn(IV) > Fe (III) > sulfate > methanogenesis.