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Microcosm Study of Aerobic Biodegradation of Bis(2-chloroethyl)ether by *Xanthobacter* sp. Strain ENV481 Relevant to Remediation of a Former Disposal Area

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Background/Objectives. Groundwater at a former disposal area is impacted with bis(2-chloroethyl)ether (BCEE, up to 140,000 µg/L) in addition to 1,2-dichloroethane, benzene, chlorinated aromatics, and 1,4-dioxane. The plume is located within an anaerobic saprolite layer at a depth of approximately 50 feet below ground surface (ft bgs). Traditional in situ remediation technologies can address most of the constituents; however, degradation methods for BCEE are poorly understood, as is the feasibility of available remediation technologies for addressing this contaminant. Previously published work[1] on BCEE biodegradation resulted in the isolation of two aerobic bacterial strains that were capable of degrading BCEE under ideal laboratory conditions at an initial rate of 343 mg BCEE h^{-1} g (dry weight)$^{-1}$. A bioaugmentation microcosm study was undertaken to determine the feasibility of BCEE biodegradation by *Xanthobacter* sp. strain ENV481 in the presence of impacted groundwater and saprolite from the former disposal area.

Approach/Activities. Soil and groundwater samples were collected from the site and used for the microcosm study to best simulate the conditions for preliminary feasibility studies. Additionally, BCEE-spiked lab water microcosms were prepared to identify any inhibition of biodegradation due to the presence of other constituents found at the site. A nuclear magnetic resonance (NMR) study was performed to better understand the role of pH and temperature on abiotic BCEE hydrolysis.

Results/Lessons Learned. Rapid biodegradation of BCEE by strain ENV481 was observed with concentrations of 20,000 µg/L in the aerobic saprolite-groundwater microcosms. An initial increase in the concentration of 2-(2-chloroethoxy)ethanol (2CEE), followed by full degradation of this species, was indicative of the hydrolytic metabolic pathway proposed previously for strain ENV481.[1] Also, 1,4-dioxane, which was initially present in the groundwater sample, was fully degraded in the same timeframe as BCEE and 2CEE. Neither inhibition of BCEE biodegradation by other constituents, nor biodegradation of the other constituents was observed within the timescale of the microcosm study. Under anaerobic conditions, strain ENV481 does not degrade BCEE, suggesting that successful bioremediation in the field will require air sparging to establish and maintain an active culture. Through NMR spectroscopic studies, abiotic hydrolysis was eliminated as a source of BCEE degradation under the pH and temperature conditions found at the site.

1. McClay, K.; Schaefer, C. E.; Vainberg, S.; Steffan, R. J., Biodegradation of bis(2-chloroethyl) ether by Xanthobacter sp. strain ENV481. *Appl. Environ. Microbiol.* **2007,** *73* (21), 6870-5.