## Biodegradation of Chlorobenzenes and Nitrotoluenes at an Industrial Site in South America

Maria Lemes (CDM Smith, Denver, CO, USA) David L. Freedman (Clemson University, Clemson, SC, USA) Paola Barreto (Jacobs, Philadelphia, PA, USA) James Henderson (DuPont, Charlotte, NC, USA) Erin Mack (DuPont, Newark, DE, USA)

**Background/Objectives.** This study was conducted to evaluate remediation of a large industrial facility in South America with a complex mixture of constituents of concern (COCs) in the soil and groundwater, including chlorobenzenes and nitroaromatics. The overall objective was to evaluate interactions among the COCs during biodegradation under aerobic and anaerobic conditions, as well as the potential impact of products from sequential chemical reduction/oxidation of the source zone on biodegradation in downgradient groundwater. Interactions among these COCs and the chemical reduction/oxidation byproducts during biodegradation have not previously been evaluated.

**Approach/Activities.** Using soil and groundwater from the site, microcosms were used to assess the effect of pH and nutrients on biodegradation rates for the parent compounds. Aerobic enrichment cultures that grow on chlorobenzene (CB; 35 mg/L) and 1,2-dichlorobenzene (1,2-DCB; 7.5 mg/L), plus anaerobic enrichment cultures that use lactate as an electron donor to convert 2,6-dinitrotoluene (2,6-DNT; 11 mg/L) and 4-nitrotoluene (4-NT; 7.6 mg/L) to corresponding amines, were used to assess the inhibitory or synergistic effects of mixtures of the co-contaminants, as well as the inhibitory potential of the groundwater subjected to reduction/oxidation. Lactate was used as the electron donor for reduction of the nitrotoluenes. The enrichment cultures were then exposed to other COCs (2,4-dinitrotoluene [2,4-DNT; 0.6 mg/L], 4-isopropylaniline [4-IPA; 5.9 mg/L], 1,2-dichloroethane [1,2-DCA; 1.5 mg/L], and 1,4-dioxane [2 mg/L]) to determine their effect on the rate of biodegradation.

**Results/Lessons Learned.** pH adjustment does not appear to be necessary, while the rate of biodegradation may be modestly improved by addition of nutrients. 2,4-DNT, 4-IPA, 1,4-dioxane, and 1,2-DCA did not inhibit the rate or extent of aerobic CB biodegradation. The 1,2-DCB aerobic degradation rate was decreased only by the presence of 4-IPA, although no effect occurred at a lower level (2.8 mg/L of 4-IPA). CB and 1,2-DCB served as primary substrates for aerobic cometabolism of 2,4-DNT and 4-IPA, but not 1,4-dioxane or 1,2-DCA. This indicates that the aromatic oxygenases required for metabolism of CB and 1,2-DCB are also reactive with 2,4-DNT and 4-IPA; however, the transformation yields are not sufficient to result in a significant level of 4-IPA and 2,4-DNT cometabolism in situ. No inhibitory effects were observed on the rate or extent of anaerobic biodegradation of 2,6-DNT and 4-NT when 2,4-DNT, 4-IPA and 1,4-dioxane were added as co-contaminants at the target high concentrations. Minimal inhibitory effects were observed when 1,2-DCA was added as a co-contaminant.

Chemical reduction successfully reduced 4-NT, 2,6-DNT and 2,4-DNT in stoichiometric and higher doses. Chemical oxidation successfully oxidized 4-IPA and the daughter products of nitro group reduction at stoichiometric and higher doses. 1,4-Dioxane, 1,2-DCB and CB were partially removed and the removal increased with dose. No removal of 1,2-DCA was observed even at a dose 10 times higher than stoichiometric. Potentially positive effects of the chemical reduction/oxidation groundwater on downgradient biodegradation of CB was observed. When exposed to aerobic conditions, 2,6-diaminotoluene (DAT) was consumed at a slow rate. To be effective, sequential anaerobic/aerobic treatment will require enrichment of microbes that actively grow on 2,6-DAT. Overall, the results indicate that use of chemical reduction/oxidation products and residual contaminants is sufficiently viable to warrant additional investigation.