Increasing the Rate of Anaerobic Benzene Degradation in Enrichment Cultures

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Background/Objectives. Benzene is a widespread and toxic environmental pollutant necessitating cleanup. The fate of benzene in anoxic environments such as soils, sediments and groundwater was once thought to be controlled by the abundance of oxygen: benzene is aerobically degraded at high rates by ubiquitous microorganisms. We now know that benzene can also be biodegraded under various anaerobic electron-accepting conditions (Fe^{3+}, NO_3^-, and SO_4^{2-}) and fermentatively (i.e., methanogenic conditions). Benzene degradation rates below 1 mg/L/day have been typical of the enrichment cultures maintained in our lab for decades, while toluene degradation rates in similar anaerobic enrichments were much faster. We suspected a missing essential nutrient or accumulating inhibitor. This study investigated ways to improve benzene degradation rates in our cultures and looked for reasons behind slow degradation using biokinetics modeling.

Approach/Activities. The University of Toronto has developed anaerobic bioaugmentation cultures which specifically target and degrade benzene in methanogenic (DGG-B™) and nitrate-reducing (NRBC) cultures. We used these cultures for a benzene degradation activity study. For over two years, we maintained experimental replicates of these batch cultures taking different feeding strategies (frequency and substrate amount), some of which were further implemented in large volume batch reactors (100 L). The cultures were routinely monitored for benzene degradation activity using analytic (GC, IC) and molecular (qPCR, Next Generation Sequencing) approaches. A biokinetics model featuring modified *Monod* kinetics was developed for the methanogenic culture, and the experimental data collected herein were used for model calibration.

Results/Lessons Learned. Here, we present data showing that relatively high rates of benzene degradation can be indeed achieved in these enrichment cultures that derived from sediments from contaminated sites. Benzene biodegradation rates of methanogenic cultures increased proportionally to progressively higher initial benzene concentrations (5 mg/L to 150 mg/L), from less than 0.2 mg/L/day to over 10 mg/L/day. Benzene degradation rates in nitrate-reducing cultures also improved as the frequency of nitrate addition was increased, although benzene degradation rates never exceeded 5 mg/L/day, perhaps as a result of the difficulties in supplying nitrate consistently. In contrast, the methanogenic consortium has the advantage of not being limited by electron acceptor availability. These data indicate that slow rates of degradation are not related to limiting essential nutrients or inhibitors, but rather to benzene and electron acceptor (nitrate) availability. A companion study by Liang et al., modeling this experimental data sheds more light on rate-limiting conditions.