Bioremediation and Sustainable Environmental Technologies—2017. Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies (Miami, FL; May 2017). ©2017 Battelle Memorial Institute, Columbus, OH, www.battelle.org/biosymp. A. Barton and S. Rosansky (Chairs)

Distinct Effects and Molecular Basis of Inducing and Non-Inducing Auxiliary Substrates on 1,4-Dioxane Biostimulation

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Background/Objectives. Recent research has demonstrated that indigenous bacteria that can degrade 1,4-dioxane (dioxane) might be more widespread than previously assumed and acclimated near the source zone area due to the selective pressure. However, the intrinsic biodegradation of dioxane is typically hindered by the limited availability of carbon and energy sources and the presence of toxic co-occurring contaminants. To date, the potential to stimulate dioxane biodegradation by adding auxiliary carbon sources has received limited attention. Such auxiliary substrates might be beneficial when dioxane is present at trace concentrations that are insufficient to induce or sustain specific degraders. In this substrate interactions study, we compare the merits and limitations of biostimulation of dioxane degradation with a non-inducing growth substrate (1-butatnol [1-BuOH]) versus an inducing substrate (tetrahydrofuran [THF]).

Approach/Activities. A microcosm study was conducted to assess two biostimulation strategies (relative to natural attenuation) to bioremediate 1,4-dioxane contamination at a site in west Texas. Dioxane concentrations were relatively low (< 300 µg/L), which represents a potential challenge to sustain and induce specific degraders. Thus, biostimulation was attempted with an auxiliary substrate known to induce dioxane-degrading monooxygenases (THF) or with a non-inducing growth substrate (1-BuOH). Concentrations of dioxane, THF, and 1-BuOH in the aqueous samples were detected weekly or biweekly by GC/MS or GC/FID. Dynamics of the catabolic biomarker *thmA*/*dxmA* (coding for the active site of THF/dioxane MO) were monitored using the novel genetic probe technique developed in our group. The total microbial population size was quantified as the number of 16S rRNA genes in the recovered DNA. Experiments were also conducted with pure cultures of the archetype dioxane degrader *Pseudonocardia dioxanivorans* CB1190 to discern how these auxiliary substrates affect the induction or repression of essential genes involved in dioxane degradation, as well as the stability of pertinent catabolic plasmids under different substrate conditions.

Results/Lessons Learned. Amendment of 1-BuOH (100 mg/L) to microcosms that were not oxygen-limited temporarily enhanced dioxane biodegradation by the indigenous microorganisms. However, this stimulatory effect was not sustained by repeated amendments, which was attributed to i) the inability of 1-BuOH to induce dioxane-degrading enzymes, ii) curing of catabolic plasmids, iii) metabolic flux dilution and catabolite repression, and iv) increased competition by commensal bacteria that do not degrade dioxane. Experiments with the archetype dioxane degrader *Pseudonocardia dioxanivorans* CB1190 repeatedly amended with 1-BuOH (500 mg/L added weekly for 4 weeks) corroborated the partial curing of catabolic plasmids (9.5 ± 7.4% was the plasmid retention ratio) and proliferation of derivative segregants that lost their ability to degrade dioxane. Addition of THF (300 µg/L) also had limited benefit due to competitive inhibition; significant dioxane degradation occurred only when the THF concentration decreased below approximately 160 µg/L. Overall, these results illustrate the importance of considering the possibility of unintentional hindrance of catabolism associated with the addition of auxiliary carbon sources to bioremediate aquifers impacted with trace concentrations of dioxane.