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# Aerobic Cometabolic Degradation of 1,4-Dioxane by Isobutane-Metabolizing Bacteria

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**Background/Objectives.** 1,4-Dioxane (14D) is a pervasive contaminant frequently detected in ground water at sites contaminated with chlorinated solvents such as 1,1,1-trichloroethane (111TCA) and trichloroethylene (TCE). Although there are several bacterial strains that can grow on 14D, the concentrations of 14D encountered at solvent-impacted site are often at ppb (µg $L^{-1}$) levels and are too low to support substantial growth and activity of 14D-metabolizing bacteria. In this study we have examined the ability of diverse isobutane (2-methylpropane)-metabolizing bacteria to cometabolically degrade both 14D and several of the chlorinated co-contaminants frequently encountered with 14D. Our long-term aim is to exploit the activities of isobutane-metabolizing bacteria for the in situ biodegradation of 14D.

**Approach/Activities.** Pure cultures of isobutane-metabolizing bacteria were obtained from commercial culture collections or were newly isolated from isobutane-fed enrichment cultures. The 14D-degrading activity of each strain was determined in resting cell assays containing isobutane-grown cells and 100 µg $L^{-1}$ 14D. Degradation of 14D was monitored using GC/MS with a heated purge and trap pre-concentration step. Cells were also incubated with $^{13}C_4$-14D and the intermediates generated during 14D degradation were analyzed by $^{13}$C-NMR and headspace GC/MS analyses. Degradation of chlorinated co-contaminants was also determined by headspace GC analyses.

**Results/Lessons Learned.** A total of nine isobutane-utilizing strains were examined including four commercially sourced strains and five environmental isolates. The majority of these were either *Rhodococcus* or *Mycobacterium* strains. With the exception of one strain that grew poorly on isobutane, all of the tested strains grew rapidly on isobutane and rapidly biodegraded 100 µg $L^{-1}$ 14D in standardized small-scale reactions. In most cases ≤90% of the 14D was degraded within 3 hours and in several cases 14D was removed to below the limit of detection (~0.15 µg $L^{-1}$). In all cases degradation of 14D was inhibited by acetylene, suggesting that a monooxygenase enzyme catalyzes 14D degradation. The activities of one strain, *Rhodococcus rhodochrous* ATCC 21198, were examined in more detail. Isobutane-grown cells were incubated with $^{13}C_4$-14D and the time course of 14D degradation was monitored by $^{13}$C-NMR. A single metabolite with a spectrum identical to that of 2-hydroxyethoxyacetic acid accumulated during 14D degradation but was also further consumed and no other multicarbon metabolites permanently accumulated during the reaction time course. Analysis of the reaction headspace using GC/MS revealed that $^{13}CO_2$ was also produced during 14D degradation indicating that this strain can slowly mineralize 14D. Although strain 21198 does not grow on 14D, it does grow on the 14D analog, tetrahydrofuran (THF). Activity-based protein profiling studies demonstrated that 14D induces expression of the same monooxygenase expressed by this bacterium during growth on either THF or gaseous alkanes. Isobutane-grown cells also degraded, at varying rates, several chlorinated compounds including vinyl chloride, 1,1-dichloroethene, *cis*-1,2-dichloroethene, 1,2-dichloroethane, 1,1-dichloroethane and 111TCA. In contrast, TCE was only very slowly oxidized by this bacterium. Overall, our results suggest that isobutane-metabolizing bacteria are remarkably consistent in their ability to cometabolically degrade low concentrations of 14D and that isobutane may therefore be an effective stimulant for promoting in situ biodegradation of 14D and several of its associated chlorinated co-contaminants.