

## Anaerobic Degradation of Chlorinated Propanes and Methanes by a New Microbial Consortium

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**Background/Objectives.** Chlorinated methanes and propanes such as carbon tetrachloride (CT), methylene chloride (MC), chloroethane (CA), 1,2,3-trichloropropane (TCP), and 1,2-dichloropropane (DCP) are suspected human carcinogens and common groundwater pollutants. Some of these compounds also can have inhibitory effects on the biodegradation of chlorinated ethanes and ethenes. A mixed anaerobic culture capable of degrading all of the aforementioned compounds was enriched from anaerobic sludge, originally using TCP as a sole carbon source. The characteristics of this culture, including the range of chlorinated compounds degraded and relevant rates, degradation products, and key organisms present were evaluated.

**Approach/Activities.** The consortium, (named CPD-2) was enriched from anaerobic sludge collected from the wastewater treatment facility in Hamilton, New Jersey by sequential transfers using lactate as a sole electron donor and TCP as the electron acceptor. The initial inoculum was subsequently fermented in a 2-L reactor and then transferred to a 7-L and finally a 20-L reactor to evaluate growth conditions and kinetics. During fermentation, subsamples of the culture were periodically collected and analyzed for degradation of TCP as well as intermediate products. The culture was also tested for its ability to degrade other more-recalcitrant chlorinated organics, such as CT, MC, CA, DCP, and a range of chlorinated ethanes. The stable culture was subsequently analyzed for key dechlorinating organisms and genes, as well as the presence of methanogens and sulfate-reducing bacteria.

**Results/Lessons Learned.** The CPD-2 culture was observed to completely dechlorinate TCP to propane and propene with formation of intermediate products DCP, 1-chloropropane (1-CP) and a trivial amount of 2-chloropropane (2-CP). The degradation rate of TCP with a culture density ( $OD_{550}$ ) of  $\sim 0.9$  was  $\sim 0.5 \text{ mg L}^{-1} \text{ h}^{-1}$ , and 1,2-DCP was  $\sim 0.1 \text{ mg L}^{-1} \text{ h}^{-1}$ . The 1-CP was degraded at a slower rate than TCP or 1,2-DCP, but was observed to degrade completely in the reactor over time. The CPD-2 consortium also rapidly degraded the chlorinated methanes CT, chloroform (CF), MC, and CA to methane. Frequently, degradation of CT stalls at MC as a terminal product, but this culture completely mineralizes CT. Rates of degradation for CT, CF, MC and CA were on the order of  $0.1$  to  $0.2 \text{ mg L}^{-1} \text{ h}^{-1}$  at a cell density of  $0.7$ . Other compounds degraded by CPD-2 include 1,1-dichloroethane and chloroethane. The CPD-2 consortium did not contain *Dehalogenimonas* sp., which were previously reported to degrade TCP, but did include *Dehalobium chlorocoercia*, *Dehalobacter*, and *Dehalococcoides* sp. The unique culture is expected to have wide applicability for aquifer bioaugmentation.