

Report for 2004NJ68B: Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by Dehalorespiring Bacterial Cultures

- Other Publications:

- Poster Presentation: Liu, F.; E.-K. Son; D.E. Fennell. 2005. Dechlorination of Polychlorinated Dibenzo-p-Dioxins and Dibenzofurans by a Dehalococcoides-Containing Culture. Poster Presentation in the 105th American Society for Microbiology General Meeting, Atlanta, GA, June 5-9, 2005.

Report Follows

Problem and Research Objectives

Dioxins and dioxin-like compounds (DLCs) are a group of planar chemicals some of which are very toxic to human beings and other organisms. This group includes polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and certain co-planar polychlorinated biphenyls (PCBs).

Dioxins are of great concern to the public because of their severe toxicity, lipophilic behavior—resulting in bioaccumulation in food chains, and their extraordinary chemical stability. They are found throughout the whole environment, including air, water, soil and sediment. Food contaminated with DLCs pose a threat to the public health.

Dioxin contamination is especially a severe environmental problem in New Jersey. The Passaic River is on EPA's list of contaminated watersheds because of its dioxin contamination. The contamination sources include a former pesticide manufacturing facility (Diamond Alkali) located in Newark which was designated as a Superfund site. A Remedial Investigation and Feasibility Study (RI/FS) is being conducted to evaluate the Passaic River Study Area for its potential long term remedies. Environmental dredging has been proposed for the most contaminated portions of the river.

In the meantime, related research investigations have been carried out to investigate other remediation strategies. *In situ* bioremediation has unique strong points compared with other remedies. *In situ* operation prevents the secondary contamination that may occur during the sediment dredging and disposal process.

We are using a mixed culture which contains *Dehalococcoides ethenogenes* strain 195 and a pure culture of *Dehalococcoides ethenogenes* strain 195 to investigate microbial dechlorination of PCDD/Fs. We are also trying to determine the effects of other halogenated co-substrates on the dechlorination processes.

Methodology

Microcosm study

We selected 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) and 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF) as target compounds. Seven sets of triplicate 60 mL serum bottles were set up in each set with pre-grown mixed culture. The mixed culture was pre-grown with tetrachloroethene (PCE) and butyric acid. PCE and 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB) were added as halogenated co-substrates in some treatments.

The details were listed in Table1.

Table1. Experimental Setup

Bottle Set	PCDD/F congener (μM)	Halogenated Co-substrates (μM)	Electron Donor (μM)
(1) 1,2,3,4-TeCDD Killed control	1,2,3,4-TeCDD (31 μM)	None	None
(2) 1,2,3,4-TeCDD only	1,2,3,4-TeCDD (31 μM)	None	Butyrate (440 μM)
(3) 1,2,3,4-TeCDD with PCE addition	1,2,3,4-TeCDD (31 μM)	PCE (110 μM)	Butyrate (440 μM)
(4) 1,2,3,4,7,8-HxCDF killed control	1,2,3,4,7,8-HxCDF (5 μM)	None	None
(5) 1,2,3,4,7,8-HxCDF only	1,2,3,4,7,8-HxCDF (5 μM)	None	Butyrate (100 μM)
(6) 1,2,3,4,7,8-HxCDF with PCE addition	1,2,3,4,7,8-HxCDF (5 μM)	PCE (25 μM)	Butyrate (100 μM)
(7) 1,2,3,4,7,8-HxCDF with TeCB addition	1,2,3,4,7,8-HxCDF (5 μM)	1,2,3,4-TeCB (25 μM)	Butyrate (100 μM)

Analyses

Well-mixed samples were removed with a sterile, anoxic syringe and extracted with toluene/acetone (Fennell et al., 2004). PCDD/F congeners were analyzed by Gas Chromatography- Mass Selective Detector (Fennell et al., 2004). PCE was analyzed by Gas Chromatography- Flame Ionization Detector (Fennell et al., 2001).

Principal Findings and Significance (Progress Report)

We had previously reported the activity of *D. ethenogenes* strain 195 on a variety of chlorinated aromatic compounds. During previous studies we added tetrachloroethene as a co-substrate to ensure growth of *D. ethenogenes* strain 195 in case the aromatic chlorinated compounds tested (1,2,3,4-TeCDD, 1,2,3,4-tetrachlorodibenzofuran, 2,3,7,8-TeCDD, 2,3,4,5,6-pentachlorobiphenyl) were not supportive of growth. The mixed culture containing *D. ethenogenes* strain 195 was shown to dechlorinate 1,2,3,4-TeCDD both with and without the addition of PCE as a co-substrate. 1,2,3,4-TeCDD was dechlorinated to 1,2,4-trichlorodibenzo-*p*-dioxin (1,2,4-TrCDD) and 1,3-dichlorodibenzo-*p*-dioxin (1,3-DCDD). Rates of daughter product formation were initially slower in PCE-amended cultures relative to cultures with no added PCE. At the end of the incubation, the extent of 1,2,3,4-TeCDD dechlorination was very similar in both treatments with and without PCE addition. It seemed that PCE addition did not affect the dechlorination of 1,2,3,4-TeCDD. We further transferred the pre-grown culture at 10% v/v ratio spiked with 1,2,3,4-TeCDD alone or together with PCE addition. The results also showed that 1,2,3,4-TeCDD was dechlorinated in both treatments at similar rates. The 1,2,3,4-TeCDD dechlorination and 1,3-DCDD formation did not show significant difference in the treatments. Although the first transfer results agree with that of the pre-grown culture, we have not yet confirmed that 1,2,3,4-TeCDD is a growth substrate for strain 195.

The dechlorination of 1,2,3,4,7,8-HxCDF was observed after one month of incubation. A penta-CDF congener was detected in all three active sets: the set spiked with 1,2,3,4,7,8-HxCDF as the only halogenated substrate, the PCE-amended set and the 1,2,3,4-TeCB-amended set. The most extensive dechlorination occurred in the 1,2,3,4-TeCB-amended set where the penta-CDF was further dechlorinated to two tetra-CDF congeners. We examined the dechlorination products and found no 2,3,7,8-substituted penta- or tetra-CDF congeners formed. This confirmed that the dechlorination process detoxified 1,2,3,4,7,8-HxCDF and formed non-2,3,7,8-substituted congeners. It shows the potential for use of the mixed culture to bioaugment contaminated sites.