BIOLOGICAL AND ABIOTIC DECHLORINATION OF HIGHLY CHLORINATED PCDD/PCDF: ISSUES OF BIOAVAILABILITY AND PATHWAYS

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1. Introduction

Whereas the widespread distribution of PCDD/F in the different environmental compartments has been recognized, little is known about their fate in the ultimate environmental sinks, soils and sediments. In particular, the susceptibility of PCDD/F to biological oxidation and reduction reactions has only recently received attention. Previously, we reported on the microbially mediated reductive dechlorination of highly chlorinated PCDD/PCDF in anaerobic soils and sediments [1,2]. It was demonstrated that peri-dechlorination was the preferred pathway, resulting in the transient accumulation of 2,3,7,8-substituted congeners. However, the rate of dechlorination was found to be limited by the desorption rate of PCDD/PCDF from the sediments and the low concentrations of PCDD/PCDF spiked to the sediments; rates on the order of 10^{-4} d-1 were reported. Therefore, a set of incubations was spiked with ppm (μg/g) concentrations of octaCDD to evaluate whether faster rates could be obtained.

Reductive dechlorination of organohalogen compounds is mainly a cometabolic process whereby a fraction of the electrons generated during anaerobic (methanogenic) oxidation of the microbial growth substrate fortuitously reduce the chlorinated) compounds, resulting in a sequential dechlorination reaction process. This mechanism is dependent on PCDD/PCDF concentration, bioavailability of the organohalogen, and efficiency of electron transfer from the microorganism to the chlorinated compound, and has been shown to be stimulated by amendment with simple organic growth substrates [3]. Figure 1 represents the electron flow relevant to these systems, and identifies the following individual key-components: growth substrate (electron donor), the bacterium, the electron acceptor (carbon dioxide in this case), an external electron shuttle, and the chlorinated compound.

In this abstract, we describe some of our new findings, and a novel approach to increase the bioavailability of PCDD/PCDF, and to enhance the efficiency of electron transfer from the bacteria to the dioxins, and compare the dechlorination with an abiotic biomimetic system.

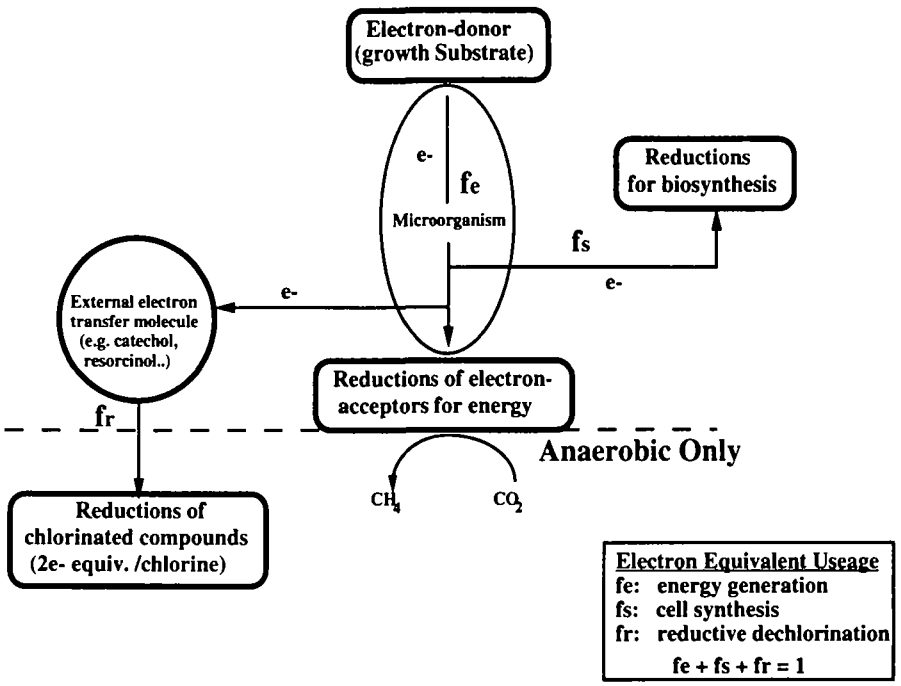

Fig. 1: Electron flow diagram during simultaneous anaerobic growth and reductive dechlorination.

2. Methodology

Biphasic microcosms : To increase the bioavailability of the strongly sorbing PCDD/PCDF, biphasic systems have been established in 30 ml vials containing 20 mL of prereduced medium [1,2], inoculated with (9±2) g (DW) of sediments, or (20 to 50) mg of protein in the case of sediment-derived cell suspensions. Both sediments and sediment-derived mixed cultures are taken from previously dioxin- or Aroclor-contaminated environments, shown to exhibit dechlorinating activity under methanogenic conditions. The prereduced anaerobic nutrient medium used in all incubation studies has been described earlier [1]. A mixture of aliphatic and aromatic carboxylic acids was added as the primary substrates.

Organic solvents, previously shown to have no negative impact on methanogenic activity, in particular decane and methanol, are added to the sediments at 5, 10, and 20 % to enhance the bioavailability of the PCDD/PCDF. Different external electron shuttles with molecular structures similar to those found in soil organic matter, are added to help enhance electron transfer efficiency: catechol, p-hydroxybenzoate, and resorcinol. The microcosms are spiked with octaCDD, octaCDF, 1,2,3,7,8-pentaCDD/CDF, and 2,3,4,7,8-pentaCDF to a final concentration of 500 ng/g from

tetrahydrofuran (THF) stock solutions. THF was chosen based on its water solubility and solubilizing capacity of PCDD/PCDF.

Three replicate microbial cultures for each combination of primary substrates, electron shuttles PCDD/F, and inocula are examined; additionally, duplicate live biological controls without PCDD/F, duplicate killed (Sodium azide, 500 mg/l) biological controls, duplicate chemical controls without inocula, and duplicate controls without electron shuttle, are used. Microcosms are monitored over time for active methanogenic growth by measuring the increase in protein concentration, and methane evolution, and are sacrificed at selected time points (i) to assess the extent of microbial dechlorination, (ii) to identify the accumulating lesser chlorinated products, and (iii) to differentiate between sorption/partitioning and microbial degradative activity. Analytical methods are similar to those described earlier [1]. During the incubation time, the inocula are supplied with the primary substrate mixture on a biweekly basis to maintain microbial activity and a steady supply of electrons generated from anaerobic oxidation.

Biomimetic Dechlorination Experiments : Parallel to the previous experiments, a select number of PCDD/PCDF is incubated in a biomimetic system containing an electron donor and electron transfer shuttle. As implied by the name, a biomimetic system mimicks biological activity, using an electron transfer macromolecule commonly found in methanogenic bacteria, vitamin B_{12}. Reductive dechlorination of octaCDD/F and 2,3,7,8-substituted pentaCDD/CDF will be assayed in aluminum foil wrapped serum vials containing a buffered anoxic water/1,4-dioxane medium. Titanium citrate (5 mM) will be used as the reducing agent, and vitamin B_{12} (1.2 mM) as the electron transfer mediator [4]. PCDD/PCDF is spiked to final concentrations ranging from 0.1 to 10 ppm, and dechlorination is monitored over time periods of up to one year. Three replicate samples have been incubated, along with controls containing only titanium citrate, and samples without vitamin B_{12}. All samples are sacrificed at each time point and analyzed for congener groups, as well as for the appearance of 2,3,7,8-chlorinated products.

3. Results and Discussion

Previously established microcosms containing PCDD/F-contaminated Passaic River sediments, and spiked with octaCDD to a final concentration of 4,500±300 µg/kg sediment were incubated for 6 months, and monitored on a monthly basis. Background analysis of congener groups showed concentrations of PCDD as follows (in µg/kg): octaCDD, 147.8; heptaCDD, 10.6; hexaCDD, 116.1; pentaCDD, 10.6; tetraCDD, 281.6. Whereas the octaCDD is the most abundant congener, the tetraCDD is the most abundant congener group, which is consistent with previous reports [5]. Analytical results of two time samples are given in Table 1. The most striking observation is that, whereas the spiked octaCDD does not significantly decrease or dechlorinate, the previous contamination of PCDD decreases over two months, most notably what penta- and

Table 1: Preliminary analysis of Passaic River samples spiked with octaCDD[1]

Time (mo.)	octaCDD	heptaCDD	hexaCDD Recovered	pentaCDD	tetraCDD
	µg/kg				
0	1,237.0±386.0	70.4±0.0	66.9±0.0	168.0±0.0	168.0±9.0
2	1,118.0±296.0	3.5±0.0	53.5±0.0	24.4±0.0	131.5±45.5

[1] Spiked at 4,500±300 µg/kg.

tetraCDD is concerned. The decreased recovery was not due to sample loss as recovery efficiencies of the 1,2,3,4-tetraCDD were consistently in the 40 to 50 percentile, after extensive sample cleanup. Though the increase in lesser (tri- and di-)chlorinated dioxins was not measured at the time of this abstract, several arguments support our claim that the decrease in penta- and tetraCDD is likely due to dechlorination. Firstly, we observed earlier that 'aged contaminants, such as the 'endogenous' PCDD, are strongly sorbed and thus not very susceptible to significant biological activity [1,2]. However, when high concentrations (ppm) of similar highly oxidized compounds are spiked to the sediment, the electron transfer process is initiated, and triggers dechlorination of the previously present contamination, as has been observed with Aroclor 1260-contaminated sediments [6]. Results from further time points as well as quantitation of the tri- and dichlorinated PCDD will be presented.

Two types of biphasic microcosms were established: (i) microcosms containing eluted cells (40 µg protein) from anaerobic Passaic River sediments, and (ii) microcosms containing sediments. Whereas it is too early to draw conlusions from (ii), the solvents used (decane and methanol) were found to not inhibit microbial activity, based on increases in protein and accumulation of methane during the one month incubation prior to spiking with octaCDD. Moreover, the presence of both the solvent and the electron shuttles catechol and resorcinol appeared to increase dechlorination, as pentaCDD were observed after only one month of incubation (Table 2). This stands in sharp contrast to the regular microcosms, where after one month only heptaCDD were observed, similar to the biphasic system without electron shuttle. Hydroxybenzoate did not have a marked effect on the dechlorination of octaCDD, presumably due to the stabilizing effect of the carboxyl-group on the aromatic compound, which does not accomodate keto/enol-tautomerism and electron transfer to the octaCDD.

The biomimetic system proved to be reasonably effective in mediating dechlorination of octaCDD. However, whereas the lowest chlorinated congener observed was a pentaCDD, the overall extent of dechlorination was very low; only 8-10% of the octaCDD added was

Table 2: Level of octaCDD dechlorination during different microcosm incubation techniques

Microcosm Type	Lowest Chlorine Level	Dechlorination Pattern
Regular	tetra	peri mainly
Biphasic		
+ catechol	penta	ND[1]
+ resorcinol	penta	ND
+ hydroxybenzoate	hepta	ND
w/o electron shuttle	hepta	peri
Biomimetic		
Titanium citrate + vitamin B_{12}	penta	ND
w/o vitamin B_{12}	penta	ND

1 ND, Not Determined

dechlorinated within a three month period. An interesting observation was that vitamin B12 was not necessary as an electron transfer molecule to mediate reductive dechlorination of octaCDD, suggesting that direct electron transfer from the titanium citrate to the dioxin can occur. This stands in sharp contrast to the vitamin B_{12}-mediated dechlorination of alkyl halides such as carbon tetrachloride, where complex formation between the cobalt ion and two carbon tetrachloride molecules has been postulated [7].

Overall, whereas the concept of biologically controlled dechlorination systems for PCDD is still new and largely unexplored, these systems merit attention as they could be stimulated in situ in PCDD/F-contaminated environments, once the mechanism of electron transfer is fully understood. Eventually, both biological and abiotic reductive dechlorination of PCDD/F may present economically feasable and cheaper remediation alternatives to the currently employed physical chemical methods.

4. Literature Cited

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