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Impact of estuarine gradients on reductive dechlorination of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin in river sediment enrichment cultures

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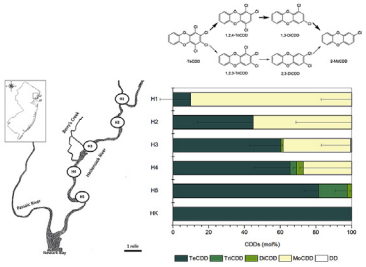
HIGHLIGHTS

- Reductive dechlorination of TecDD in Hackensack River sediment enrichment cultures.
- *Dehalococcoides* spp. abundance correlated with rate and extent of dechlorination.
- Consecutive peri- and lateral-dechlorination of 1,2,3,4-TeCDD.

GRAPHICAL ABSTRACT



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ABSTRACT

Polychlorinated dibenzo-*p*-dioxins (PCDDs) are among the most persistent organic pollutants. Although the total input of PCDDs into the environment has decreased substantially over the past four decades, their input via non-point sources is still increasing, especially in estuarine metropolitan areas. Here we report on the microbially mediated reductive dechlorination of PCDDs in anaerobic enrichment cultures established from sediments collected from five locations along the Hackensack River, NJ and investigate the impacts of sediment physicochemical characteristics on dechlorination activity. Dechlorination of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) and abundance of *Dehalococcoides* spp. negatively correlated with salinity and sulfate concentration in sediments used to establish the cultures. 1,2,3,4-TeCDD was dechlorinated to a lesser extent in cultures established from sediments from the tidally influenced estuarine mouth of the river. In cultures established from low salinity sediments, 1,2,3,4-TeCDD was reductively dechlorinated with the accumulation of 2-monochlorodibenzo-*p*-dioxin as the major product. Sulfate concentrations above 2 mM inhibited 1,2,3,4-TecDD dechlorination activity. Consecutive lateral- and peri- dechlorination took place in enrichment cultures with a minimal accumulation of 2,3-dichlorodibenzo-*p*-dioxin in active cultures. A *Dehalococcoides* spp. community was enriched and accounted for up to 64% of *Chloroflexi* detected in these sediment cultures.

1. Introduction

Urban estuarine regions support a wide variety of industrial and municipal activities which results in contamination by many pollutants including PCDDs. They are among the most notorious and persistent organic pollutants known as the “dirty dozen” compounds in the initial Stockholm Convention list. Historically, PCDDs were released as by-products from chemical manufacturing, metallurgy, paper and pulp processing, and other industries (Hites, 2011). They accumulate in sediments of estuarine water bodies,

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such as the lower Passaic River and Newark Bay (Bopp et al., 1991; Wenning et al., 1993; Huntley et al., 1998), Victoria harbor, Hong Kong (Müller et al., 2002), Tokyo Bay (Hosomi et al., 2003), and Homebush Bay, Australia (Birch et al., 2007). PCDDs of industrial origin from the upper rivers contribute to the contamination of the corresponding estuarine and marine water bodies by means of river discharge (Huntley et al., 1998; Salo et al., 2008; Friedman et al., 2012). In addition, uncontrolled anthropogenic and natural combustion events contribute to PCDD deposition level into both metropolitan and pristine estuarine watersheds (Müller et al., 2002; Hosomi et al., 2003; Sundqvist et al., 2009; Dwyer and Themelis, 2015). Due to both human and natural activities, PCCDs are ubiquitous in urban estuaries and contaminated sediments pose risks to both human health and the environment.

PCDDs are notoriously persistent but they have been known to be biodegradable via several different processes (Bunge and Lechner, 2009). Microbial reductive dechlorination under anaerobic conditions is the most important biological process that may transform PCDDs and potentially decrease their toxicity. Reductive dechlorination of PCDDs has been observed in contaminated sediments and soils. Dehalogenating bacteria belong to diverse phylogenetic groups (Maphosa et al., 2010), but so far only obligately organohalide-respiring bacteria from the genera *Dehalococcoides* and *Dehalobacter* have been found to dechlorinate PCDDs (Ballerstedt et al., 2004; Bunge et al., 2003; Bunge et al., 2008; Fennell et al., 2004; Yoshida et al., 2009; Pöritz et al., 2015). PCDD dechlorination was detected in various historically contaminated estuarine systems (Albrecht et al., 1999; Vargas et al., 2001; Ahn et al., 2005). However, limited information is available about the bacteria which drive dechlorination in these estuarine and marine environments in which the high salinity and sulfate concentrations shape the microbial communities (Zanaroli et al., 2015). Non-methanogenic, non-thermophilic, non-spore-forming microorganisms were implicated to be responsible for dechlorination of PCDDs in estuarine lower Passaic River sediment cultures, however, further characterization of these dechlorinating bacteria was not done (Barkovskii and Adriaens, 1996). Ahn et al. (2007) did not find evidence of known dechlorinating bacteria such as *Dehalococcoides* or *Desulfitobacterium* in their active dechlorinating culture established from estuarine sediments. Reductive dechlorination of PCCDs appears to be prevalent yet not fully understood in estuarine sulfate-impacted systems.

The lower Hackensack River, located in the New York/New Jersey Harbor estuary, is within a vibrant metropolitan area and one of the oldest industrial zones in the U.S. The river is contaminated by a wide range of pollutants, including PCDDs from both historical and emerging non-point sources due to industrial and municipal activities (Wenning et al., 2004). The objective of this study was to investigate the potential for reductive dechlorination of PCDDs and its relation to sampling locations along the tidal influenced sections of the Hackensack River. We also aimed to relate the relative distribution of dehalogenating bacteria belonging to the *Chloroflexi* to environmental parameters at the sampling sites.

2. Materials and methods

2.1. Sediment source

Sediments were collected by grab sampling along the Hackensack River at 5 stations (H1, H2, H3, H4 and H5) in August 2012 (See Fig. S1 for sampling locations). The uppermost station (H1) is located at 19.6 km from the river mouth and the lowermost downstream station (H5) is 2 km from the river mouth. Physicochemical parameters of sediment samples were provided by John Reinfelder and Sarah Janssen, Department of Environmental Sciences, Rutgers University (Janssen et al., 2015), and are summarized in Table S1. Temperature and pH were the same at all locations at values of approximately 28 °C and 7, respectively. Total organic matter and organic carbon were slightly lower in the sediment samples collected from stations H4 and H5 than in samples taken from the upper stations. However, salinity and sulfate concentration were substantially higher near the river mouth, indicative of the high tidal influence on water and sediments at stations H4 and H5 (Table S1).

2.2. PCCDs

Dibenzo-*p*-dioxin (DD) and its chlorinated congeners were purchased from AccuStandard Inc. (New Haven, CT, USA). Chlorinated congeners include 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD), 1,2,3- and 1,2,4-trichlorodibenzo-*p*-dioxins (1,2,3- and 1,2,4-TriCDDs), 1,2-, 1,3-, 1,4-, and 2,3-dichlorodibenzo-*p*-dioxins (1,2-, 1,3-, 1,4-, and 2,3-DiCDDs), 1- and 2-monochlorodibenzo-*p*-dioxins (1- and 2-MoCDDs).

2.3. Enrichment culture set up

Anaerobic enrichment cultures (50 mL) were established in triplicate using 10% w/v (5 g) of sediment as inoculum in 60 mL serum bottles sealed with Teflon™ lined gray chlorobutyl-isoprene septa and crimped with aluminum caps. Carbonate buffered mineral salts medium was prepared according to Monserrate and Häggblom (1997) except that sodium chloride concentration was 1.17 g/L. Silicon oxide (silica) was used to deliver electron acceptor to dehalogenating bacteria in the enrichment cultures as described below. 0.5 g of silicon oxide (200 Mesh or finer) was added to a 60 mL serum bottle and spiked with 500 μL of 2 mM 1,2,3,4-TeCDD stock solution in toluene. Toluene was evaporated leaving a layer of 1,2,3,4-TeCDD coated on silicon oxide particles. Anaerobic sediment slurry (50 mL) was added yielding a 20 μM nominal concentration of 1,2,3,4-TeCDD. A mixture of short chain organic acids (acetate, propionate, and lactate) was added at a 150 μM concentration each as electron donors and carbon sources. Enrichment cultures were set up in an anaerobic vinyl glove box (Coy Laboratory Products Inc., Great Lakes, MI, USA) and maintained under strictly anaerobic conditions under a headspace of 3%H_2/97%N_2. Electron donors were re-supplemented every 4 months, cobalamin was supplemented at 100 μg/L after 4 months of incubation to enhance dehalogenation activity and every 4 months during the course of incubation. Killed controls were established in duplicate to determine if abiotic dehalogenation occurs. They were set up the same way as the experimental cultures, but were autoclaved for 1 h on 3 consecutive days. Enrichment cultures were transferred when 1,2,3,4-TeCDD were depleted to a concentration below 2 μM. Subsets of active enrichment cultures (H1, and H2) were transferred 10% (v/v) into fresh medium amended with 1,2,3,4-TeCDD, 1,2,3-TriCDD, or 1,2,4-TriCDD. Less silica (i.e., 100 mg) was added in the second and third transfers to reduce the amount of inorganic carrier in the cultures.

To examine the role of sulfate, a set of subcultures was established in triplicate by transferring 3 mL of the original H1 enrichment culture into 27 mL fresh mineral medium. 1,2,3,4-TeCDD and the mixture of organic acids were supplemented as electron acceptor and carbon and energy sources, respectively, as described above. Sulfate was amended anaerobically to concentrations of 0, 2, 5, and 10 mM.

2.4. Sampling and analytical methods

1 mL samples of enrichment cultures were withdrawn using a syringe purged with nitrogen gas. Subsampling was performed

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