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Carbon isotope fractionation during dechlorination of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin by a *Dehalococcoides*-containing culture

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ABSTRACT

Carbon isotope fractionation was observed during dechlorination of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) by a mixed culture containing *Dehalococcoides ethenogenes* strain 195. Fractionation was examined when 1,2,3,4-TeCDD was added as the only chlorinated compound and when 1,2,3,4-TeCDD was added with a known growth substrate, tetrachloroethene (PCE). The 1,2,3,4-TeCDD was dechlorinated to 1,2,4-trichlorodibenzo-*p*-dioxin (1,2,4-TrCDD) which was enriched in ^{13}C relative to 1,2,3,4-TeCDD with isotope separation factors, ε_C, of $1.3 \pm 0.2‰$ and $1.7 \pm 0.4‰$ (average ± 95% confidence interval (CI)) in cultures with and without PCE, respectively. The 1,2,4-TrCDD was further dechlorinated to 1,3-dichlorodibenzo-*p*-dioxin (1,3-DCDD) which was depleted in ^{13}C relative to 1,2,4-TrCDD with ε_C of $-2.4 \pm 0.4‰$ and $-2.9 \pm 0.8‰$ (average ± 95% CI) in cultures with and without PCE, respectively. This demonstrates carbon isotope fractionation during sequential reductive dechlorination of PCDDs, where isotope fractionation during dechlorination of the intermediate was substantial and a ^{13}C depleted lightly chlorinated PCDD congener was ultimately formed during dechlorination of more highly chlorinated PCDD congeners. Despite reproducible, statistically significant differences between isotope compositions of the parent, 1,2,3,4-TeCDD and daughter, 1,2,4-TrCDD and 1,3-DCDD congeners in triplicate bottles of both treatments, fractionation factors for 1,2,3,4-TeCDD could not be determined for all replicates by regression analysis of the plot of the Rayleigh equation. It is possible that dissolution of 1,2,3,4-TeCDD imposed a kinetic limitation on dechlorination, thus masking isotope fractionation during its dechlorination.

1. Introduction

A promising tool for monitoring biotransformation of pollutants is compound specific isotope analysis (CSIA) (Meckenstock et al., 2004; Schmidt et al., 2004). CSIA detects fractionation resulting from the kinetic isotope effect (KIE) caused by different reaction rates for a compound containing the heavy isotope versus the light isotope at the location of the reaction, and from other environmental processes (Schmidt et al., 2004). CSIA was examined for fuel components (Meckenstock et al., 1999; Hunkeler et al., 2001; Gray et al., 2002; Somsamak et al., 2005); aromatic hydrocarbons (Hall et al., 1999; Richnow et al., 2003; Yanik et al., 2003); and halogenated compounds (Hunkeler et al., 1999; Bloom et al., 2000; Drenzek et al., 2001; Sherwood Lollar et al., 2001; Nijenhuis et al., 2005, 2007; Cichocka et al., 2008). CSIA could be useful for monitoring fate of the polychlorinated dibenzo-*p*-dioxins (PCDDs). PCDDs occur as mixtures of up to 75 congeners, are hydrophobic and accumulate in sediments and biota (Czuczwa et al., 1984; Hites, 1990; Bopp et al., 1991; Cai et al., 1994; Koistinen et al., 1995; Wagrowski and Hites, 2000). The 2,3,7,8-substituted PCDDs are highly toxic (Van den Berg et al., 2006) and environmental problems are global. For example, 500 ppb PCDDs were detected in the River Elbe, Germany (Götz et al., 2007); 50 ppb in the Gulf of Finland (Verta et al., 2007); 20–40 ppb in the Passaic River, NJ, USA (Bopp et al., 1991); and up to 90 ppb in Newark Bay, NJ, USA (Chaky, 2003). Freshly added (Adriaens et al., 1995; Beurskens et al., 1995; Ballerstedt et al., 1997; Vargas et al., 2001; Ahn et al., 2005) and existing PCDDs (Albrecht et al., 1999; Yoshida et al., 2005) were dechlorinated under anaerobic conditions. However, with few exceptions (Lohmann et al., 2000; Bunge et al., 2007), lightly chlorinated PCDDs are not routinely measured in environmental samples, thus there is little historical data documenting environmental PCDD dechlorination. CSIA could be used to compare isotopic signatures between PCDD parent and daughter

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congeners or between homolog groups as a marker for biodechlorination. Ewald et al. (2007) reported carbon stable isotope fractionation during dechlorination of trichlorodibenzo-*p*-dioxins (TrCDD) by a culture containing bacteria with high similarity to *Dehalococcoides* sp. strain CBDB1 (Bunge et al., 2003). It is important to document CSIA with different PCDD congeners and environmental conditions. Here, we examined carbon stable isotope fractionation of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) by a PCE-grown mixed culture containing *Dehalococcoides ethenogenes* strain 195 (Maymó-Gatell et al., 1997). Strain 195 grows on PCE (Maymó-Gatell et al., 1997) and the mixed culture containing strain 195 dechlorinates selected PCDD/Fs – 1,2,3,4-TeCDD, 1,2,3,4-tetrachlorodibenzofuran and 1,2,3,4,7,8-hexachlorodibenzofuran (Fennell et al., 2004; Liu and Fennell, 2008). It is not known if strain 195 obtains energy for growth on PCDD/Fs or which of its reductive dehalogenase(s) (Seshadri et al., 2005) mediates their dechlorination.

We used 1,2,3,4-TeCDD as a model PCDD, because it is dechlorinated at relatively high rates (Fennell et al., 2004), has low toxicity (Van den Berg et al., 2006) and could be added at high concentrations to enhance detection of fractionation. This congener also has similar physical–chemical properties to 2,3,7,8-TeCDD, one of the most toxic PCDD congeners. The 1,2,3,4-TeCDD is dechlorinated to 1,2,4-TrCDD and 1,3-dichlorodibenzo-*p*-dioxin (1,3-DCDD) by strain 195 (Fennell et al., 2004). We found that PCDD dechlorination was most rapid when PCDD was loaded on sediment (Fennell et al., 2004), thus 1,2,3,4-TeCDD was loaded on sediment carrier for all experiments. This approach mimicked realistic conditions since PCDDs are likely sorbed to sediment particles in the environment (Shiu et al., 1988; Hites, 1990).

2. Materials and methods

2.1. Chemicals

1,2,3,4-TeCDD, 1,2,4-TrCDD, 1,3-DCDD, and 2,2′,5-trichlorobiphenyl (2,2′,5-TrCB) were from AccuStandard (New Haven, CT). PCE (99.9+%), trichloroethene (TCE) (99.5+%), *cis*-1,2-dichloroethene (*cis*-1,2-DCE) and butyric acid (99+%) were from Aldrich Chemical Company (Milwaukee, WI, USA). Vinyl chloride (VC) (⩾99.97%) was from Linde AG (Leuna, Germany). Ethene (99%) was from Matheson Tri-Gas, Inc. (Montgomeryville, PA, USA).

2.2. Experimental setup

Carbon stable isotope fractionation of 1,2,3,4-TeCDD and PCE was investigated in a mixed culture containing *D. ethenogenes* strain 195. Culture was pregrown on PCE and butyric acid at 34 °C for PCE studies or at 25 °C for PCDD studies (Fennell et al., 1997). In the 1,2,3,4-TeCDD study, 1,2,3,4-TeCDD was present both with and without the addition of PCE as additional halogenated compound, because we do not know if 1,2,3,4-TeCDD is a growth substrate for strain 195. In the PCE study, PCE was added as the only halogenated substrate. Isotope fractionation of PCE was analyzed only in cultures where PCE was added as the only halogenated substrate.

In 1,2,3,4-TeCDD experiments 1 g sterile sediment (Arthur Kill, NJ, USA; 5.4% total carbon) added to 160-mL serum bottles (Vargas et al., 2001; Fennell et al., 2004) was wetted by addition of 0.5 mL of 6200 μM 1,2,3,4-TeCDD–toluene solution; and toluene was volatilized under sterile N_2, leaving a coating of 1,2,3,4-TeCDD on the sediment. Next, 100 mL culture was added under anoxic, sterile conditions to achieve a final concentration of 31 μM 1,2,3,4-TeCDD on a bulk volume basis. We routinely recovered only about 6 μM 1,2,3,4-TeCDD, because 2 mL of the mixed slurry was removed at each sampling from a total 100 mL slurry, resulting in incomplete sampling of the PCDDs sorbed to the bottle surfaces. [Note that complete extraction of 10 mL sediment-culture slurries from 28 mL glass tubes recovered 70% of PCDDs using the extraction method described here (Liu, 2007).] The sediment to culture ratio was 10 g L^{-1}. Assuming a maximum aqueous-phase solubility of 1.95 nM 1,2,3,4-TeCDD (Shiu et al., 1988), the aqueous-phase 1,2,3,4-TeCDD would account for 0.0063% of added 1,2,3,4-TeCDD, with greater than 99.99% sorbed to sediment, colloid matter, and the vessel.

One set of triplicate cultures received 1,2,3,4-TeCDD as the only chlorinated substrate. A second set of triplicate cultures was additionally amended with 110 μM PCE on days 0, 32, 51, 81, 111, and 132, because we do not know if 1,2,3,4-TeCDD supports growth of strain 195 and we were unsure of the ability of the culture to dechlorinate 1,2,3,4-TeCDD in the absence of a known growth substrate. Cultures received 440 μM butyrate as a hydrogen source and 40 μL of 50 g L^{-1} yeast extract, at the same time PCE was added. A third set of cultures with 1,2,3,4-TeCDD only was autoclaved at 121 °C as killed controls. Incubation was in the dark at 28 °C at 120 rpm. PCDDs were analyzed on days 0, 7, 27, 39, 55, 77, 98, 120 and 153.

The PCE study was performed in 250 mL serum bottles, containing 100 mL medium inoculated with 10% volume:volume (vol:vol) culture after growth on 500 μM PCE. Cultures were amended with 500 μM PCE and 2.75 mM butyrate and incubated at 34 °C. On days 0, 1, 4, 8, 11, 14 and 17, 0.5 mL headspace samples were removed from the bottles and added to helium-flushed autosampler vials sealed with Teflon® coated butyl rubber septa for determination of chloroethenes and ethene concentrations. Simultaneously, three 0.5 mL headspace samples were removed sequentially from each bottle for CSIA.

2.3. Analytical methods

Two millilitre of culture-sediment mixture was removed from each bottle for PCDD analysis and placed into 7 mL glass vials. Samples were centrifuged, the aqueous portion was removed to a separate vial, 2,2′,5-TrCB was added as a surrogate standard to the sediment residue, and the sediment phase was rinsed with 1 mL of acetone to remove water. The sediment phase was extracted overnight with 3 mL of 2:1 vol:vol toluene:acetone, then for 4 h with 1 mL of 2:1 vol:vol toluene:acetone, and then rinsed with 1 mL toluene. The solvent phases were combined with the aqueous-phase and back extracted by NaCl. The solvent phase was passed through a 2 mL pipette filled with Florisil (Sigma–Aldrich, St. Louis, MO, USA) and eluted with three volumes toluene per volume extract. The extract was concentrated to 2 mL. PCDDs were analyzed using an Agilent 6890 GC equipped with a 5973 N mass selective detector (GC–MS) (Agilent Technologies, Inc., Santa Clara, CA) and a HP-5MS (Agilent Technologies, Inc., Santa Clara, CA, USA) column (30 m × 0.25 mm I.D.). PCDDs were detected based on retention times of standards and identification of molecular ions (*m*/*z*: 1,2,3,4-TeCDD, 322; 1,2,4,-TrCDD, 286; 1,3-DCDD, 252; and 2,2′,5-TrCB, 256). A qualifying ion was monitored to assure correct identification of congeners (1,2,3,4-TeCDD, 320; 1,2,4-TrCDD, 288; 1,3-DCDD, 254; and 2,2′,5-TrCB, 186). The response factors for PCDD congeners compared to the surrogate, 2,2′,5-TrCB, were calculated over four concentrations from 0.5 to 10 μM as a linear calibration curve.

Ethene and chloroethenes were quantified using a Varian Chrompack CP-3800 gas chromatograph (GC) (Middelburg, The Netherlands) with flame ionization detection (FID) using a 30 m × 0.53 mm GS-Q column (J&W Scientific, Waldbronn, Germany) (Cichocka et al., 2008).