Regiospecific Dechlorination of Spiked Tetra- and Trichlorodibenzo-\(p\)-dioxins by Anaerobic Bacteria from PCDD/F-Contaminated Spittelwasser Sediments

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Abstract

Samples were taken from sediment of the creek Spittelwasser (district Bitterfeld, Germany), which is highly polluted with PCDD/Fs and other chloroorganic compounds. The sediment cores were separated into 10- to 20-cm thick layers, spiked with 50 µM of 1,2,3,4-tetrachlorodibenzo-p-dioxin and incubated for 8 months under anaerobic conditions in the presence of cosubstrates. Reductive dechlorination of the tetrachlorinated congener and formation of tri- and dichlorinated products was observed in all biologically active incubations. Analysis of subcultures spiked with 1,2,3- and 1,2,4-trichlorodibenzo-p-dioxin, respectively, revealed two different dechlorination pathways within the sediment cores. Pathway M was characterized by the simultaneous dechlorination of peri- and lateral chlorine atoms, whereas sequence SP was restricted to the dechlorination at positions flanked by chlorine atoms on both sides.
INTRODUCTION

Sediments are important sinks of polychlorinated dibenzo-\textit{p}-dioxins and furans (PCDD/F). Therefore, the study of transformation processes in these environments is of great concern. Reductive dechlorination of dioxin congeners might be an environmentally important reaction and has been investigated in different anaerobic microcosms derived from historically contaminated sediments. Previous studies have demonstrated the presence of biotic (Adriaens and Grbic-Galic, 1994, Beurskens \textit{et al}., 1995, Ballerstedt \textit{et al}., 1997) as well as abiotic processes (Adriaens \textit{et al}., 1996, Fu \textit{et al}., 1999) or combined reactions (Barkovskii and Adriaens, 1998). Barkovskii and Adriaens (1996) proposed different pathways leading to fewer chlorinated dioxin congeners. The peri-dechlorination of 2,3,7,8-substituted hepta- to penta-CDDs catalyzed by non-spore-forming bacteria was characterized by the (transient) formation of 2,3,7,8-tetraCDD, whereas the peri-lateral dechlorination pathway of pasteurized cells resulted in fewer chlorinated dioxins with non-2,3,7,8-substituted congeners as intermediate products (Barkovskii and Adriaens, 1996). In no case the bacteria or defined consortia involved in these dechlorination processes have been identified.

The region close to Bitterfeld belongs to the most organochlorine-polluted areas in Germany. Due to the former presence of extensive chlorine industry and the lack of effective waste water and exhaust gas purification, the surrounding environment was contaminated with chloroorganic and many other compounds. Sediments and soils of flooding areas of the rivers Elbe and Mulde and of its tributary Spittelwasser are highly contaminated with PCDD/F. In the latter case, PCDD/F concentrations of up to 3000 pg I-TEQ/g d.w. for sediments and 180,000 pg I-TEQ/g d.w. for soils were reported (Götz \textit{et al}., 1998). Our own study revealed a dioxin contamination level of 120,000 pg I-TEQ/g d.w. in non-top layers of the Spittelwasser sediment (unpublished data). Waste water of industry and households have been discharged
into the river Spittelwasser for decades. The low streaming speed favored sedimentation resulting in sediment layers of up to two meters.

The number of bacteria in sediments is known to decrease with increasing depth, while microbial activity as carbon mineralization by methanogens may have subsurface maxima (Wellbury et al., 1996). However, it is not known, if dechlorinating bacteria are present in deeper layers of freshwater sediments, where the availability of potential cosubstrates, i.e., electron donors for microbial reductive dechlorination might be low. Therefore, the objective of the present study was to investigate the capability of anaerobic bacteria obtained from different depths of the contaminated sediment of Spittelwasser to dehalogenate spiked dioxin congeners. Microcosms, inoculated with sludge of different sediment layers, were incubated with 1,2,3,4-tetrachlorodibenzo-p-dioxin (1,2,3,4-TeCDD) and subcultivated with 1,2,4- and 1,2,3-trichlorodibenzo-p-dioxin (TrCDD), respectively. Dechlorination pathways were elucidated from the analysis of the disappearance of the parent congener and the formation of specific lower chlorinated products.

MATERIALS AND METHODS

Sediment characteristics. Adsorbable organic halogen (AOX) was analyzed using the Metrohm (Switzerland) AOX analyzer 686 according to the DIN standard 38 409 (H14) after an overnight shaking of the sediment sample with acid nitrate solution and activated carbon. The chemical oxygen demand (COD) was determined using the chromate method.

Sampling and primary incubations. Sediment samples (A and B; distance 5 m) were collected from different sediment layers of the Spittelwasser site (coordinates (x,y): 4520150; 5729500). Sediment cores (0-40 cm depth, 3.5 cm diameter) were obtained by pushing a glass tube vertically through the sediment. Individual sections of approximately 10 cm thickness
were collected from the cores and stored under N₂/CO₂ (80:20) at 4°C prior to the experiments. Anaerobic incubations of the sediment were carried out in 125-ml serum bottles capped with butyl rubber stoppers and sealed with aluminium crimps. The sediment slurries were prepared by inoculation with sediment (50 % [w/v]) into 30 ml of anaerobic mineral medium (Holliger et al., 1992) and supplemented with formate (9 mM), fumarate, pyruvate, acetate and benzoate (5 mM each), yeast extract (0.005 % [w/v]) and 1,2,3,4-TeCDD (50 µM). In addition, controls containing the same amendments were prepared by autoclaving the sediments on three consecutive days at 121°C (25 min). Dioxins were purchased from AccuStandard, Inc. (New Haven, CT) and were added from stock solutions in acetone as described by Ballerstedt et al. (1997). At time zero and after 8 months of incubation, duplicate 2-ml subsamples were removed for the analysis using sterile techniques. The slurries were mixed to secure a uniform suspension. All cultures were incubated with agitation (130 rpm) in the dark at 20°C.

**Preparation of subcultures.** The dechlorination pathway of 1,2,3,4-TeCDD was investigated using subcultures (10 % transfers of the primary culture into fresh medium, see above) which were spiked with 25 µM of the possible intermediates 1,2,3- or 1,2,4-TrCDD, respectively, and were incubated in several aliquots of 3-ml volumes in Hungate tubes containing N₂/CO₂ (80:20) in the gas phase. At regular time intervals (0, 2, 4, 6, 8 weeks), subsamples were removed in duplicate and stored at -20°C until analysis.

**Analysis.** Extraction, clean-up procedure and analysis of selected congeners by capillary gas chromatography (GC) equipped with a DB-5 column (J&W Scientific, Folsom, CA) and ⁶³Ni-electron capture detector (ECD) followed the procedures described previously (Ballerstedt et al., 1997). We used a nine point calibration curve using a quadratic fit of the data ranging from 0.78 µM to 200 µM for quantification. Dioxin congeners were identified by
matching retention times with those of authentic standards. Recovery efficiencies for PCDDs after the clean-up procedure were 65-100 % based on the internal standard used (2,4,8-trichlorodibenzo-furan). The identifications were later confirmed by mass spectrometric detection in the selected ion monitoring mode (GC-MS-SIM) as described elsewhere (Ballerstedt et al., 1997).

RESULTS

The contents of organic carbon (OC) and adsorbable organic halogen were determined in the individual layers of both sediment cores (Table 1). Sample A possessed in general higher amounts of organic carbon and a slightly higher content of AOX. Whereas sample A exhibited the highest AOX values in a depth of 20 to 40 cm, the AOX load of sample B was comparable low at this depth.

<table>
<thead>
<tr>
<th>Sediment core (layer)</th>
<th>Dry weight (%)</th>
<th>Organic carbon (%)&lt;sup&gt;b&lt;/sup&gt; (mg/kg d.w.)</th>
<th>AOX (mg/kg d.w.)</th>
<th>Relative molar distribution of congeners after 8 months of incubation (mol%)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1,3-DCDD</td>
<td>2,3-DCDD</td>
</tr>
<tr>
<td>A (30-40 cm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.8</td>
<td>14.1</td>
<td>3672</td>
<td>0.5</td>
</tr>
<tr>
<td>A (20-30 cm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.4</td>
<td>13.7</td>
<td>4019</td>
<td></td>
</tr>
<tr>
<td>A (10-20 cm)</td>
<td>14.2</td>
<td>13.1</td>
<td>2810</td>
<td>83.4</td>
</tr>
<tr>
<td>A (0-10 cm)</td>
<td>5.2</td>
<td>11.9</td>
<td>1950</td>
<td>33.1</td>
</tr>
<tr>
<td>B (30-40 cm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.9</td>
<td>0.5</td>
<td>307</td>
<td>13.5</td>
</tr>
<tr>
<td>B (20-30 cm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26.6</td>
<td>3.2</td>
<td>1696</td>
<td></td>
</tr>
<tr>
<td>B (10-20 cm)</td>
<td>18.0</td>
<td>8.9</td>
<td>1936</td>
<td>1.4</td>
</tr>
<tr>
<td>B (0-10 cm)</td>
<td>18.5</td>
<td>5.8</td>
<td>1486</td>
<td>43.8</td>
</tr>
</tbody>
</table>

<sup>a</sup>The layers from a depth of 20-30 and 30-40 cm were mixed before use as inoculum, <sup>b</sup>As calculated from COD-measurement, <sup>c</sup>Mean values of duplicate samples representing the molar fraction of the total concentration of all congeners measured, <sup>d</sup>Not detectable.
To study the capability of anaerobic bacteria to dechlorinate dioxins, cultures were inoculated with material of the different layers and were spiked with 1,2,3,4-TeCDD. The deepest layers (30-40 and 20-30 cm) of core A and B, respectively, were combined before inoculation. After 8 months of incubation reductive dechlorination of 1,2,3,4-TeCDD was observed in all sediment layers investigated (Table 1). Recovery efficiency obviously decreased during the incubation time and differed between samples, resulting in 5-30 % of the total PCDD measured at time zero. Therefore, the concentration of each congener was reported as mole percent of the sum of all congeners measured. 1,2,3,4-TeCDD was predominantly dechlorinated to 1,2,4-TrCDD, 1,3-dichlorodibenzo-p-dioxin (1,3-DCDD) and 2,3-dichlorodibenzo-p-dioxin (2,3-DCDD) by 243 days. 1,2,3-TrCDD was not detected as an intermediate transformation product. Dechlorination was negligible in sterile controls (below 1 mol% of 1,3-, 2,3-DCDD and 1,2,4-TrCDD) during the incubation period with the exception of autoclaved sample B (10-20 cm), where 4 mol% of 2,3-DCDD was formed. However, dechlorination activity in the live sediment slurries differed in the extent and position of chlorine removal as indicated by the formation of different less chlorinated products.

The most extensive dechlorination of 1,2,3,4-TeCDD to mainly 1,3-DCDD (> 80 mol%) occurred in enrichment cultures from core A at a depth of 10-20 cm. The highest dechlorination activity of core B was found in the surface layer (0-10 cm), but besides 1,3-DCDD noticeable amounts of 2,3-DCDD were formed, and 1,2,4-TrCDD accumulated to a large extent in these enrichment. Formation of appreciable amounts of 2,3-DCDD (ratio of 2,3-DCDD:1,3-DCDD greater than 1:4) was only found in enrichment cultures from the top layers (0-10 cm) of both sediment cores. Accumulation of 1,2,4-TrCDD as the final dechlorination product from 1,2,3,4-TeCDD (indicating that only one lateral chlorine was removed from 1,2,3,4-TeCDD) was detected in primary incubations from layers of both cores.
Subcultures were inoculated from the primary sediment incubations, spiked with the possible intermediary trichlorinated congeners 1,2,3-TrCDD and 1,2,4-TrCDD, respectively, and analyzed at intervals of two weeks for the occurrence of reductive dechlorination. Subcultures from the two upper layers (0-10 cm; 10-20 cm) of core A exhibited no dechlorination within a period of 8 weeks and were not further studied. Subsamples from sediment layers of core B (0-10 cm and 20-40 cm) reductively dechlorinated both congeners (Fig. 1 a, b). 1,2,4-TrCDD was exclusively dechlorinated to 1,3-DCDD. A fairly rapid transformation within 56 days (dechlorination rate of up to 0.8 \( \mu \text{M d}^{-1} \) on the basis of the appearance of the lesser chlorinated compound, Fig. 1 b) was observed. Dehalogenation of 1,2,3-TrCDD resulted in the formation of 1,3- and to a very low extent of 2,3-DCDD.

In contrast, two enrichment cultures from core A (20-40 cm) and core B (10-20 cm) transformed 1,2,3-TrCDD to 1,3-DCDD as the only product. 1,2,4-TrCDD was not degraded by these cultures (Fig. 1 c, d). These results were in good agreement with the identification of 1,2,4-TrCDD as the only lesser chlorinated congener during the long-term primary incubation with 1,2,3,4-TeCDD (Table 1).

**Figure 1** Reductive dechlorination of 1,2,3- and 1,2,4-TrCDD in subcultures inoculated with the primary incubations of the individual layers of sediment cores A (c) and B (a, b, d). The 1,2,3,4-TeCDD determined originated from the inoculum. Mean values of duplicate samples are shown. The error bars indicate the standard deviation.
DISCUSSION

Anaerobic enrichment cultures inoculated with material of two highly PCDD/F-contaminated sediment cores were all able to reductively dechlorinate spiked 1,2,3,4-TeCDD. The products formed differed in the number and position of chlorines removed. Only one of the two possible intermediary trichlorodibenzo-\(p\)-dioxins (1,2,4-TrCDD) was detected. Studying the fate of 1,2,3- and 1,2,4-TrCDD in subcultures revealed the existence of two different dechlorination pathways. One dechlorination sequence was designated as Process M (referring to enrichments from river Mulde sediment, where this process was also found, Bunge et al., 1999). It was characterized by the formation of 1,3-DCDD and 2,3-DCDD as the final dechlorination products of 1,2,3,4-TeCDD. Almost the same 2,3-/1,3-DCDD ratios (1:4 and 1:10, respectively) were formed from spiked 1,2,3-TrCDD by the respective subcultures (B [0-10 cm] and B [20-40 cm]), suggesting that the main dechlorination route proceeded via 1,2,3-TrCDD rather than 1,2,4-TrCDD (which was exclusively dechlorinated to 1,3-DCDD). Fig. 2 shows the proposed dechlorination pathway. This process resembles the previously published dechlorination of 1,2,3,4-TeCDD by a methanogenic enrichment culture from Lake Ketelmeer (Beurskens et al., 1995), indicating a combination of simultaneous lateral and peri-dechlorination activities (Fig. 2). These observations were in contrast to the dechlorination of 1,2,3,4-TeCDD in primary enrichment cultures obtained from Saale river (Ballerstedt et al., 1997), where 1,3-DCDD was exclusively formed via 1,2,4-TrCDD. This sequence required a successive dechlorination activity in lateral and peri-positions and was designated as Process S (Bunge et al., 1999). A change in the selectivity of chlorine removal from peri- to lateral positions was also described for the dechlorination of OCDD via 2,3,7,8-TCDD to 2-monochlorodibenzo-\(p\)-dioxin (Albrecht et al., 1999, Barkovskii and Adriaens, 1996).
Another dechlorination activity designated as Process SP (detected for the first time in Spittelwasser sediment) was restricted to positions flanked by chlorines on both sides. Therefore, 1,2,4-TrCDD accumulated from 1,2,3,4-TeCDD and resisted further dehalogenation. 1,2,3-TrCDD added to subcultures was transformed to 1,3-DCDD, and no 2,3-DCDD was detectable. The regiospecificity of these dehalogenation reactions is in accordance with the thermodynamically most favorable reductions ($\Delta G^\circ$ values were calculated to be -174.8 and -162.7 kJ/reaction [redox pairs 1,2,3,4-TeCDD/1,2,4-TrCDD and

**Figure 2** Comparison of two 1,2,3,4-TeCDD-dechlorination processes (M, SP) observed in slurries of Spittelwasser sediment. Removal of chlorine atoms in peri- (P) and lateral (L) positions.
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1,2,3,4-TeCDD/1,2,3-TrCDD and -165.2 and -152.1 kJ/reaction [redox pairs 1,2,3-TrCDD/1,3-DCDD and 1,2,3-TrCDD/2,3-DCDD, respectively]; Huang et al., 1996). It should be noted, however, that we observed dechlorination pathway SP exclusively in those layers of the Spittelwasser sediment, which exhibited the highest AOX contents within cores A and B (4019 mg/kg d.w. and 1936 mg/kg d.w., respectively). One could speculate, that the high level of sediment contamination with chloroorganic compounds (e.g., up to 6 mg/kg d.w. of hexachlorocyclohexane, 3 mg/kg d.w. of chlorobenzenes and others) affected the dechlorination process. It seems, that halogenated contaminants may select for a specific dehalogenation activity or for the bacteria involved in this process. Priming of distinct dechlorination processes by other halogenated compounds has been recently described for PCBs (Van Dort et al., 1997, DeWeerd and Bedard, 1999). Wu et al. (1999) showed, that priming with 2,6-dibromobiphenyl promoted the growth of PCB dechlorinating microbes and thus accelerated the dechlorination process. The Process SP exhibits a potential for detoxification of dioxin contaminations, assuming that chlorines in lateral (flanked) positions can be also removed from higher chlorinated 2,3,7,8-substituted congeners.

Our data clearly show the occurrence of different dechlorination pathways within a single sediment core, suggesting that different dechlorinating populations are involved in these processes. Position and congener specificity of microbial PCDD reductive dechlorination might depend on specific bacteria. The different regiospecificities of microbial PCDD and PCB dehalogenation present in one sediment sample could be separated by various treatments, e.g. pasteurization (Barkovskii and Adriaens, 1996, Ye et al., 1992) or incubation at different temperatures (Wu et al., 1997). More research is needed to better understand the role of microorganisms that mediate this process in order to predict the behavior and fate of chloroorganic contaminants in the environment. Further work will be carried out to investigate the
significance of the microbial PCDD/F degradation in situ to evaluate the suitability of this attractive alternative to conventional treatment strategies for bioremediation of large-scale contaminated sites.

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REFERENCES


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