

PROPERTIES OF A DIOXIN-DECHLORINATING MICROBIAL
CONSORTIUM FROM AN ACIDIC SULFATE-RICH RIVER SEDIMENT

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Introduction

The Bitterfeld region (Germany) appertains to the old industrial areas in Europe. Brown coal was mined here since 1839 and served as a fuel for power plants. Since the early 20th century particularly chlorine industry (chloralkali process, organochlorine pesticide production, PVC) and aluminum industry have been settled. A characteristic property of the present landscape around Bitterfeld is the close vicinity of old industrial sites to opencast mining with the respective drainage system discharging sulfate-rich mining water. Missing investments in exhaust emission control and waste water treatment during the past decades resulted in environmental pollution on a large scale. Many stream sediments, soils and aquifers are contaminated particularly with heavy metals and organochlorine compounds, e.g. PCDD/F, with concentrations of up to 120,000 pg I-TEQ/g dry weight ¹.

Studies of microcosms by Adriaens and Grbic-Gali \square have demonstrated the capability for reductive dehalogenation of PCDD/F by anaerobic bacteria ². Further studies revealed the presence of biotic processes as well as combined biotic/abiotic reactions in different sediment samples ^{3,4,5,6}. Barkovskii and Adriaens assigned the observed *peri*-dechlorination of 2,3,7,8-substituted hepta- to penta-CDD's with the (transient) formation of 2,3,7,8-tetraCDD to the activity of non-spore-forming bacteria ⁷. On the other hand, the *peri*-lateral dechlorination pathway by pasteurized cells resulted in lesser chlorinated dioxins with non-2,3,7,8-substituted congeners as intermediates ⁷. Recently we demonstrated that anaerobic enrichment cultures from different layers of the highly PCDD/F-contaminated sediments of the creek Spittelwasser exhibited two different dechlorination pathways of freshly spiked 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD), 1,2,3-trichlorodibenzo-*p*-dioxin and 1,2,4-trichlorodibenzo-*p*-dioxin (TrCDD), respectively ¹.

The aim of the present study was to investigate the dechlorination of freshly spiked congeners by anaerobic consortia from sediments of the „Leine-Durchstich“, a less PCDD/F-contaminated tributary stream draining acidic, sulfate-rich water from coal-mining areas into the river Mulde.

Methods and Materials

A sediment sample was collected from the creek „Leine-Durchstich“ and transferred into 1-l serum bottles flushed with N₂/CO₂ (80/20) and sealed with a butyl rubber septum. The sediment was used to inoculate primary enrichment cultures in a mineral medium completed by addition of 25 μ M 1,2,3,4-TeCDD and a mixture of organic acids as described elsewhere ^{8,5}. A modified buffer system was used for the primary enrichment culture; the initial pH of the modified mineral medium was 5.5 ⁹. After a long-term incubation of 240 days at 20°C in the dark, the primary enrichment was transferred (10 % [v/v]) into fresh medium supplemented with 25 μ M 1,2,3-TrCDD and 25 μ M 1,2,4-TrCDD, respectively, and organic acids as described by Bunge *et al.* ¹. These subcultures were incubated in several aliquots of 3 ml volumes in anaerobic culture tubes. Duplicate samples were withdrawn at specific times and stored at -20°C until analysis. In addition; controls containing the same amendments were prepared by autoclaving the sediments before inoculation on three consecutive days at 121°C (25 min).

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Extraction, clean-up and analysis of selected congeners using capillary gas chromatography (GC) equipped with a DB-5 column (J&W Scientific, Folsom, CA) and an ^{63}Ni -electron capture detector (ECD) followed the methods as described elsewhere ⁵. The compounds were initially identified by matching retention times with those of authentic standards (AccuStandard, Inc. [New Haven, CT]) and quantified with a nine level calibration curve for each congener. Recovery efficiencies for PCDDs after the clean-up procedure were 65-100 % based on the internal standard used (2,4,8-trichlorodibenzofuran). The identifications of the dioxin congeners including the lesser chlorinated transformation products were confirmed by mass spectrometric detection in the selected ion monitoring mode (GC-MS-SIM) as described previously ⁵. Anion chromatography was carried out with an ICAN column (125 x 4 mm), 1.5 mM phthalic acid, 1.38 mM TRIS and 300 mM boric acid as eluent and indirect detection at 254 nm.

Results and Discussion

The sediment was characterized by a dry weight of 13 % and an organic carbon content of 20.3 mg g⁻¹ dry weight. The pore water of the sediment was acidic (pH 4.2) and contained 4712 mg l⁻¹ of sulfate and 112 mg l⁻¹ of chloride. Nitrate and phosphate were not detectable. The sediment was contaminated with 70 pg I-TEQ/g dry weight of PCDD/F. The fate of spiked 1,2,3,4-TeCDD in the primary enrichments was investigated during an 8-month experiment. The reductive dechlorination of 1,2,3,4-TeCDD started after a lag time of 85 days and resulted in the production of 1,3-DCDD and 2,3-DCDD as the main dechlorination products. The trichlorinated intermediates 1,2,3-TrCDD and 1,2,4-TrCDD were found in trace concentrations only (< 1 mol %). After 120 days of incubation, 4 mol % of 1,3-DCDD and 5 mol % of 2,3-DCDD and after 233 days, 7 and 4 mol %, respectively, were determined. The dechlorination rate was very low in comparison with other investigations ^{1,5} and was possibly influenced from the high sulfate content of the sediment. However, the initial pH of 5.5 increased to 7.5 within 20 days of incubation. Possibly, dechlorination set in after sulfate was consumed as a terminal electron acceptor by the microbial community. With regard to this presumption the following subcultures were not amended with additional sulfate and were maintained in a mineral medium at pH 7.2.

The reductive dechlorination of 1,2,3-TrCDD and 1,2,4-TrCDD, the potential intermediates of the 1,2,3,4-TeCDD dechlorination, was investigated in separate subcultures. Figure 1 shows the time course for the dechlorination of 1,2,4-TrCDD (right panel) and 1,2,3-TrCDD (left panel). Dehalogenation of the trichlorinated congeners occurred following a lag phase of about 10 days. Dichlorodibenzo-*p*-dioxins constituted the final products of dechlorination. No dehalogenation occurred in autoclaved controls, suggesting that the observed dehalogenation was microbially catalyzed. 1,2,4-TrCDD was exclusively dechlorinated to 1,3-DCDD. A fairly rapid transformation within 41 days was observed. The maximum dechlorination rate of about 1.3 $\mu\text{mol liter}^{-1} \text{ day}^{-1}$ was calculated based on the known amount of 1,2,4-TrCDD added and the increase of the molar ratio of 1,3-DCDD evolved, which is an order of magnitude higher than described for 1,2,4-TrCDD dechlorination in enrichment cultures from Saale sediment ⁵. Reductive dehalogenation of 1,2,3-TrCDD yielded two dechlorination products: 2,3- and to a lower extent 1,3-DCDD.

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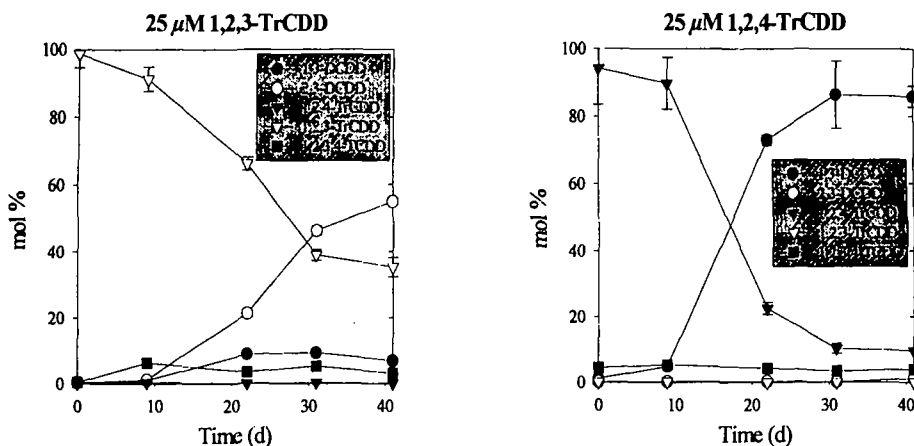


Fig. 1. Reductive dechlorination of 1,2,3- and 1,2,4-TrCDD in subcultures derived from primary enrichments with sediments of the creek „Leine-Durchstich“. Error bars indicate standard deviations of duplicate samples. The residual 1,2,3,4-TeCDD originated from the inoculum.

Based on the appearance of the dechlorinated products, a pathway for the transformation of 1,2,3,4-TeCDD has been proposed (Figure 2). It can be assigned to the Process M which is characterized by the formation of 1,3- and 2,3-DCDD as the final transformation products of the 1,2,3,4-TeCDD-dechlorination^{1,10}. A striking difference to earlier observations of Process M in enrichment cultures from Spittelwasser sediments is the ratio of the formation of 1,3- and 2,3-DCDD from 1,2,3-TrCDD. Here, the formation of 2,3-DCDD exceeds that of 1,3-DCDD, indicating a preferred *peri*-dechlorination probably caused by a different composition of the dechlorinating community. In contrast, the dechlorination of 1,2,3,4-TeCDD resulted in the opposite ratio of transformation products, suggesting, that the dechlorination proceeded simultaneously via 1,2,4-TrCDD and 1,2,3-TrCDD, the respective products of lateral and *peri*-dechlorinating activities. The different regioselectivity of chlorine removal might be due to different subpopulations within the dechlorinating mixed culture.

The dehalogenating culture was sequentially transferred seven times in the mineral medium without addition of sediment. The results demonstrate, that dechlorinating microbes were present in acidic, sulfate-containing sediments. As it has been shown earlier, stable dioxin-dechlorinating mixed cultures can be enriched from sediments contaminated with very low PCDD/F levels⁵.

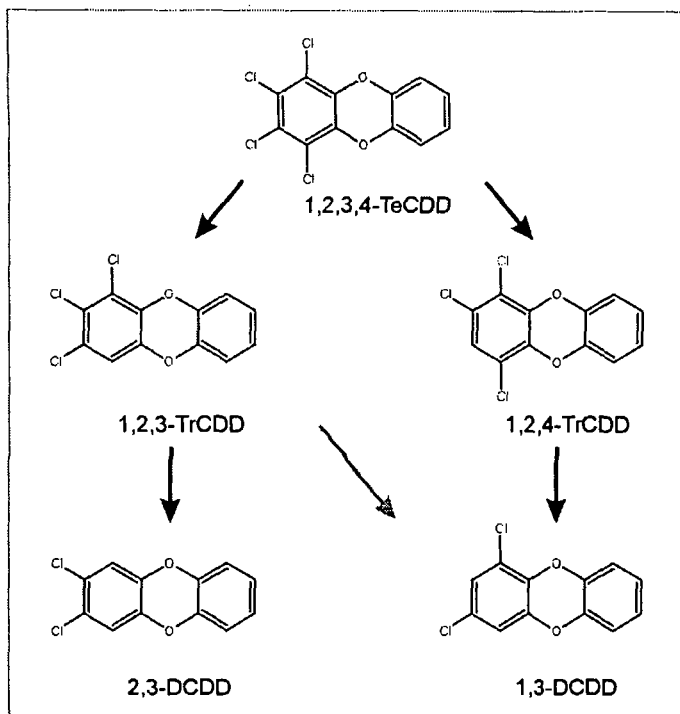


Fig. 2. Pathway of microbial dechlorination of spiked dioxin congeners in enrichment cultures from creek „Leine-Durchstich“. The sequence marked with a grey arrow contributed only to a minor extent to the formation of 1,3-DCDD.

Acknowledgments

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