DIFFERENT REGIOSELECTIVITIES OF REDUCTIVE DIOXIN-DECHLORINATION BY ANAEROBIC BACTERIA FROM RIVER SEDIMENTS OF THE BITTERFELD DISTRICT (GERMANY)

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Introduction

The region close to Bitterfeld (Saxony-Anhalt, Germany) is an old industrial site. Due to the former presence of extensive chlorine chemistry and lack of effective waste water purification the surrounding environment was heavily contaminated with chloroorganic and many other compounds. Industrial waste waters were discharged into the river Spittelwasser, that into the river Mulde, a tributary of the river Elbe. Sediments and soils of flooding areas of the Spittelwasser are highly contaminated with PCDD/F (up to 3000 and 180 000 ng I-TEQ/kg d.w., respectively[1]).
Under anaerobic conditions, dioxins are reductively dechlorinated by abiotic and biotic processes[2-4]. Little is known about the microorganisms involved in dioxin dechlorination[5], whereas some bacteria are known, which dechlorinate other chlorinated compounds, e.g. members of the genus *Desulfitobacterium* which *ortho*-dehalogenate chlorophenols[6]. *Dehalospirillum multivorans*, an organism of the *Sulfurospirillum* group (epsilon-Proteobacteria) grows by chlororespiration with tetrachloroethene[7].
The objective of the present study was to compare sediment samples obtained from different, (highly) contaminated sites in the Bitterfeld district concerning the microbial capability to dehalogenate 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (TCDD), 1,2,4- and 1,2,3-trichlorodibenzo-*p*-dioxin (TrCDD) as model congeners. Differences in regioselectivity of chlorine elimination will be presented for samples of different origin. The occurrence of species of *Desulfitobacterium* and *Sulfurospirillum* was indicated by fluorescence-*in situ* hybridization.

Material and Methods

Samples were taken from historically contaminated sediments of the rivers Mulde (sample sites 1 and 3), Leine and Spittelwasser (sample sites A and B; different sediment layers) and inoculated (50% [w/v]) into anaerobic mineral medium[8]. The medium was supplemented with formate (9 mM), fumarate, pyruvate, acetate and benzoate (5 mM each) and yeast extract (0,005% [w/v]). The experiments were carried out in 20 to 50 ml-volumes in 125 ml-serum bottles or in several replicates of 3 to 10 ml-volumes in Hungate tubes containing N_2/CO_2 (80:20) in the gas phase and incubated at 20°C in the dark. The primary enrichment cultures were spiked with 50 µM 1,2,3,4-TCDD, the subcultures (10 % transfers of the primary culture into fresh medium) with 25 µM 1,2,4- or 1,2,3-TrCDD**.** Samples (2 ml) were taken periodically and extracted with hexane. The parent congener and the lower chlorinated products were analyzed by GC-ECD and GC-MSD as described elsewhere[4].
Bacteria of the genus *Desulfitobacterium* and of the *Sulfurospirillum* group were identified in subcultures by *in situ*-hybridization with probes directed against the 16S rRNA[9]. The fluorescent-dye labelled probes DSO1a (Cy3-5'-CCATCCATTAACGATAGCAT-3') and DSO2a (TAMRA-5'-ATCCATCTACTAACGATA-3') were specific for different members of the genus

Desulfitobacterium. The probe SUG2b (6Fam-5'-AAAGCCCCATCCTTTAGC-3') identified organisms of the *Sulfurospirillum* group. The relative abundance of the target organisms was determined using epifluorescence microscopy by comparison with the total number of bacteria visualized after DAPI-staining.

Results and Discussion

Anaerobic consortia from river sediments of different sample sites near Bitterfeld (Table 1) were compared concerning their potential to reductively eliminate chlorine atoms from the dioxin molecule. Reductive dechlorination of 1,2,3,4-TCDD was observed in all sediments investigated, whereas dechlorination did not occur in autoclaved controls. However, dechlorination activity differed in the extent and position of chlorine removal as indicated by the formation of different lesser chlorinated products.

The dechlorination pathway of 1,2,3,4-TCDD leading to less chlorinated congeners was investigated using subcultures, which were spiked with the possible intermediates 1,2,3- and 1,2,4-TrCDD. Three different pathways were observed (Fig. 1, Table 1).

Process „S" (Mulde 1) was characterized by the formation of 1,3-dichlorodibenzo-*p*-dioxin (1,3-DCDD) as the main dechlorination product of 1,2,3,4-TCDD and 1,2,4-TrCDD, indicating a successive lateral-*peri* removal of chlorine atoms. This pathway followed the thermodynamically most favourable reactions[10] and was first described for an enrichment culture obtained from sediment of the river Saale[4].

Process „M" resulted in 1,3-DCDD and 2,3-DCDD, indicating a combination of simultaneous lateral and *peri*-dechlorination activities as was also observed in enrichment cultures derived from Lake Ketelmeer sediment[3].

Another dechlorination activity designated process „SP" was exclusively found in sediment samples of Spittelwasser. 1,2,3,4-TCDD was dechlorinated to 1,2,4-TrCDD, which was not further transformed. In accordance with these observations, separately spiked 1,2,4-TrCDD was not degraded. In contrast, 1,2,3-TrCDD was dechlorinated to 1,3-DCDD as the only product. These results indicated, that removal of chlorines was restricted to positions which are surrounded by chlorines on both sides. We observed dechlorination pattern „SP" only in those layers of the Spittelwasser sediment, which exhibited the highest AOX contents (4019 mg/kg d.w. and 1936 mg/kg d.w.), possibly due to high levels of contamination with hexachlorocyclohexane and chlorophenols.

The regiospecificities of chlorine removal were maintained after transfer of the subcultures into fresh medium. Different dechlorination pathways might be caused by different populations of dechlorinating bacteria, as was demonstrated for the dehalogenation of highly chlorinated dioxins with pasteurized and non-pasteurized cells[5].

The enrichment cultures obtained from different sediments were studied for the presence of bacteria known to dehalogenate chlorinated compounds (Table 1). Members of the genus *Desulfitobacterium* were detected in most cultures with a relative frequency of 1 to 13 %. This points on their ubiquitous distribution in anaerobic river sediments. These bacteria obviously resist high levels of chloroorganic contamination as was demonstrated by their occurrence in Spittelwasser sediment. The nutritional versatile *Sulfurospirillum* group was only associated with those enrichment cultures, which exhibited the dechlorination pattern „M". Further studies should provide insight into the actual role of these bacteria in the dechlorination process.

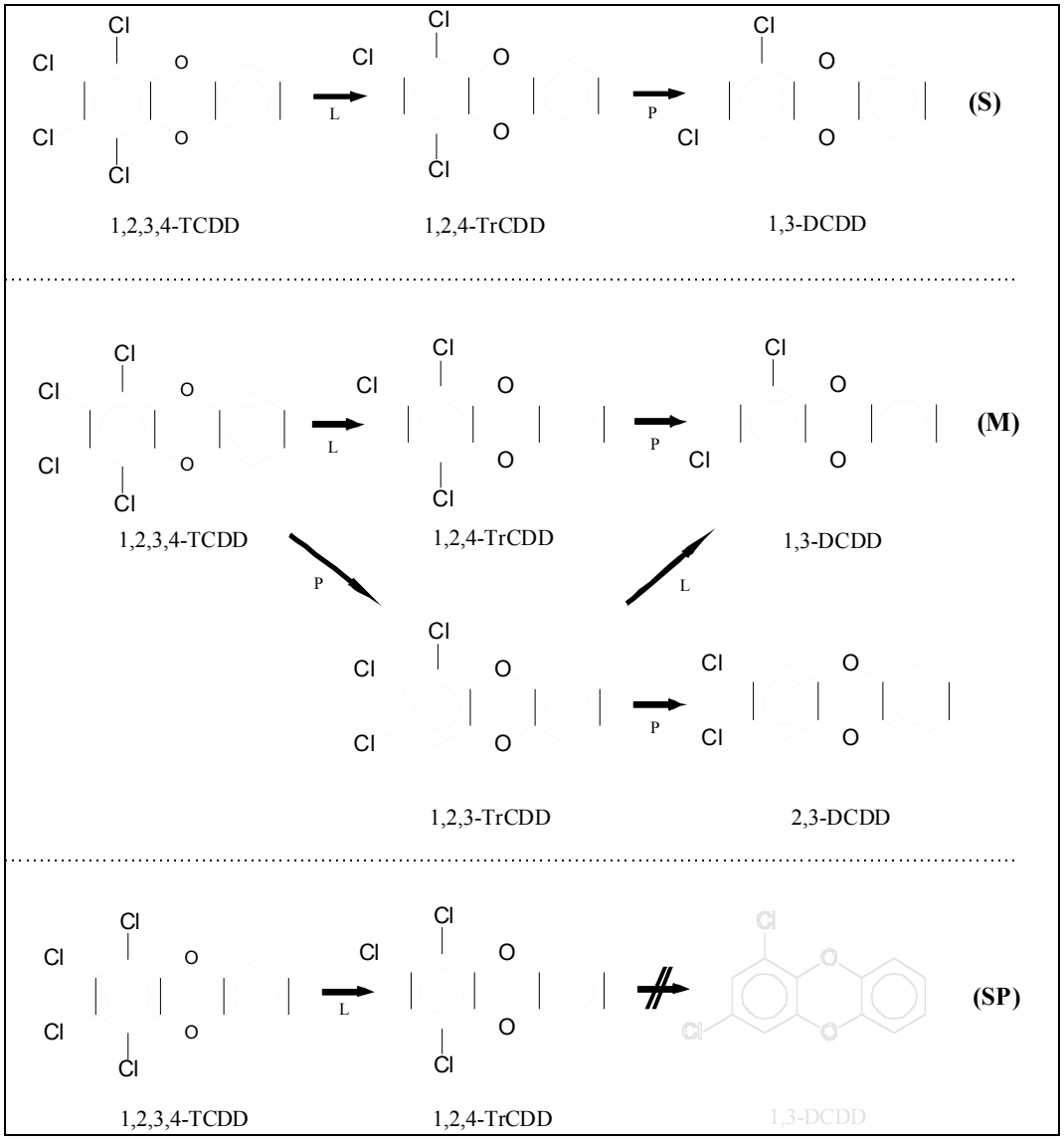

Fig. 1: Proposed dechlorination pathways of 1,2,3,4-TCDD in enrichment cultures from river sediments. S: lateral(L)-*peri*(P) dechlorination, M: simultaneous lateral and *peri*-dechlorination, SP: lateral dechlorination.

Table 1: Observed dechlorination patterns and occurrence of organisms of the *Sulfurospirillum* group and members of the genus D*esulfitobacterium* within the enrichment cultures.

Process	Origin	Sample Site	Depth (cm)	Occurrence of bacteria hybridizing with the specific 16S rRNA probes
S	Mulde	1	surface	DSO1a
M	Mulde Leine Spittelwasser Spittelwasser	3 B B	surface surface 20-40 surface	SUG2b, DSO1a, DSO2a SUG2b, DSO1a, DSO2a, SUG2b -
SP	Spittelwasser Spittelwasser	A B	20-40 10-20	- DSO1a

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