Influence of the Substitution Pattern on the Microbial Degradation of Mono- to Tetrachlorinated Dibenzofurans and Dibenzodioxins

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1. Introduction

Microbial degradation of hazardous compounds provides an effective method for the bioremedation of contaminated soil and ground water [1]. Polychlorinated dibenzofurans (PCDF) and dibenzodioxins (PCDD) resist microbial mineralization because of their haloaromatic structure [2]. Even the nonhalogenated carbon skeletons are difficult substrates for microorganisms to degrade. Recently bacterial strains were isolated, which utilize dibenzofuran as the sole source of carbon and energy [3]. Degradation of PCDF/PCDD has yet been reported only as cometabolic transformation [4]. The degradation yield depends on the number of chlorosubstituents, but also on the substitution pattern. 2,3,7,8-substituted PCDF/PCDD are of special interest, because of their extreme toxicity and their bioaccumulation [5].
This paper describes biotransformation experiments with two bacteria strains and a mixture of all 210 PCDF/PCDD. One of the two bacteria strains (Sphingomonas species [3]) was able to grow on dibenzofuran, whereas the other one (Pseudomonas species [6]) grew on 1,2,4,5-tetrachlorobenzene as sole source of carbon and energy.

2. Experimental

Bacteria cultures were grown in a mineral salts medium with dibenzofuran and 1,2,4,5-tetrachlorobenzene, respectively, as substrate. Synthesized mono- to trichloro-DD were added to a fly-ash extract, to receive an all 210 PCDF/PCDD containing solution. 200 µl of the completed fly-ash extract was spiked on a small glassfiber-filter, which was given into the bacteria suspension. The resulting concentration of PCDF/PCDD differed between 200 ng/l and 50 µg/l, or $1 \cdot 10^{-9}$ mol/l and $100 \cdot 10^{-9}$ mol/l, respectively. The batch cultures were left for 25 days at 28 °C on a rotary shaker (175 rpm). The degradation experiments were stopped by 10 minutes of sonification. Afterwards toluene, methoxyethanol and hydrochloric acid were added to the suspension and the mixture was refluxed for 24 hours. The organic layer was submitted to several clean-up steps. Finally the concentrations of the non-

transformed PCDF/PCDD were determined by HRGC-MS on a SP2331 capillary-column [7]. For determination of degradation yields, results were compared to results of a sterile kept suspension, which was treated in exactly the same way as the bacteria batch cultures. Coelution and other chromatographic interferences did not allow the evaluation of all 210 PCDF/PCDD congeners.

3. Results

Table 1 lists some of the degradation yields of mono- to tetrachlorinated DF/DD for both applied strains. Degradation of penta- and higher chlorinated DF/DD was not observed.

Table 1:

PCDF/PCDD congener	Substitution pattern	Degradation yield Sphingomonas strain	Degradation yield Pseudomonas strain
Furans			
F8	1,6	0 %	100 %
F13	2,4	48 %	100 %
F28	1,3,6	0 %	29 %
F29	1,3,7	0 %	82 %
F40	2,3,6	19 %	85 %
F43	2,4,6	0 %	9 %
F61	1,2,7,8	0 %	49 %
F67	1,3,4,9	0 %	0 %
F75	1,4,6,9	10 %	0 %
F80	2,3,4,8	38 %	24 %
F81	2,3,6,7	0 %	86 %
F83	2,3,7,8	0 %	38 %
Dioxins			
D6	1,6	8 %	28 %
D9	1,9	0 %	29 %
D12	2,8	32 %	95 %
D13	1,2,3	35 %	84 %
D21	1,3,8	0 %	93 %
D23	1,4,6	0 %	7 %
D26	2,3,7	12 %	100 %
D31	1,2,3,9	8 %	7 %
D44	1,3,7,8	0 %	40 %
D47	1,4,7,8	0 %	0 %
D48	2,3,7,8	0 %	76 %

Monochlorinated DF/DD were completely transformed by both strains. The degradation of dichlorinated dibenzofurans by the Sphingomonas strain shows structure dependency (highest degradation yield for 2,4-Cl_2DF (F13), lowest degradation yield for 1,6-Cl_2DF (F8)), whereas the Pseudomonas strain totally degrades all examined dichlorinated dibenzofurans. Concerning the trichlorinated dibenzofurans, both strains show different selectivities in degradation. In opposite to Pseudomonas strain, Sphingomonas strain is hardly able to degrade tetrachlorinated dibenzofurans. Remarkable high is the degradation yield of the 2,3,7,8-Cl_4DF (F83) by Pseudomonas strain (38 %).
Degradation yields of PCDD are generally smaller than degradation yields of PCDF of the same chlorination degree. Similar to the microbial transformation of PCDF, degradation yields decrease with increasing chlorination degree. Within one degree of chlorination both bacteria strains show different selectivities in transformation. General spoken, in case of the Pseudomonas strain, higher degradation yields are observed. It reduces e.g. 2,3,7,8-Cl_4DD (D48) by 76 %, the 1,4,7,8-Cl_4DD (D47) however to 0 %.

4. Conclusion

The recalcitrance of PCDD against microbial transformation is generally higher than the recalcitrance of PCDF of the same chlorination degree. Pseudomonas strain shows a higher potential for transformation of PCDF/PCDD than Sphingomonas strain. Obviously the ability to grow on chlorinated arens is more suitable for the degradation of PCDF/PCDD than the ability to grow on the nonhalogenated carbon skeletons. For microbial transformation Sphingomonas strain is dependent from two vicinal hydrogen atoms and a nonchloro-substituted 1, 4, 6 or 9 position of the carbon skeleton. In both rings chlorinated DF/DD with (1,3-), (2,4-), (2,3-) or (7,8)-substitution in one of them are hardly degraded by Sphingomonas strain (vicinal C-H-groups are missing). Dioxygenation is supposed to be the first degradation step. In case of Peudomonas strain, chlorosubstitution in 1, 4, 6 or 9 position also prevents PCDF/PCDD from microbial transformation. For example for 1,3,4,9-Cl_4DF (F67) and 1,4,6,9-Cl_4DF (F75) no degradation was observed. Whereas in 2, 3, 7 or 8 position chlorosubstituted congeners are degraded relative easily. A possible explanation therefor is the structural similarity of substrate, 1,2,4,5-tetrachlorobenzene, and cosubstrate, for example 2,3,7,8-Cl_4DD (D48). The metabolism of 1,2,4,5-tetrachlorobenzene involves dioxygenation, followed by dehydrochlorination as first degradation steps [6].

5. References

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