ANAEROBIC DEGRADATION OF DIOXINS - TECHNOLOGY IN PRACTICE

ANAEROBNÍ DEGRADACE DIOXINŮ – TECHNOLOGIE PRO PRAXI

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Abstract:
Dioxins are one of the most toxic and environmentally stable aromatic compounds. Due to their chemical stability and the lipophilic nature they appear to be very persistent in the environment and are bound to the soil organic matter, sediment and biological tissue, where they accumulate and then pass into the food chain (ECRA, 2002). For the purpose of anaerobic biotransformation of dioxins mixture of soil samples with sewage sludge and potato strips were prepared. The mixtures were placed into a bioreactor without oxygen admission to create anaerobic conditions. During biodegradation selected microbiological and physicochemical parameters were monitored and ecotoxicity tests carried out. Anaerobic conditions in bioreactors were achieved after 5 days of cultivation. The initial concentration of polychlorinated dioxins and dibenzofurans was 380 μg kg^{-1} dw in mixture 1P and up to 1800 μg.kg_{DW}^{-1} in mixture 2P. Concentrations of PCDD/Fs remained unchanged after 6 months of anaerobic incubation in both variants of mixtures. The concentration of pentachlorophenol in a mixture 2P decreased by 75% after 6 months of anaerobic cultivation, while 3-chlorophenol at a high concentration and to a lesser extent 3,4-dichlorophenol were newly detected. This result suggests that reductive dehalogenation took place. The mixture 2P was significantly more toxic than the mixture 1P. The aquatic test with Daphnia was the most sensitive one for mixture 2P. Given that most of dioxins are insoluble or poorly soluble in water, it can be expected that the toxicity of the resulting sample was influenced by accompanying pentachlorophenol contamination rather than by the presence of PCDD/Fs. After dehalogenation of pentachlorophenol to 3-chlorophenol the toxicity significantly decreased.

Keywords:
Dioxins, PCDD/Fs, anaerobic degradation, pentachlorophenol, reductive dehalogenation, ecotoxicity tests.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) are tricyclic aromatic compounds, chemically stable, lipophilic, having the ability to accumulate in the food chain. PCDD/Fs make up a high set of compounds with one to eight chlorine atoms per molecule. 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF) were the most studied ones, due to their high toxicity. PCDD/Fs have never been produced intentionally and have no practical use. They are formed as byproducts in chemical production of chlorinated pesticides or chlorination of phenols in municipal waste incineration, chlorine bleaching of pulp and metal processing. Contamination of soils by PCDD/Fs was caused inter alia by impregnation of wood products with agents containing pentachlorophenol (PCP) that always contains also PCDD/Fs, being generated as a byproduct in the chlorination of phenol (Borden, 2008). In the Nordic countries, Germany, Japan, the Czech Republic and elsewhere old environmental burden caused by contamination by impregnating agents containing PCF exists, causing also contamination by PCDD/Fs in these areas. Reductive dehalogenation of PCDD/Fs by bacteria was already described at the end of the last century (Popat, Deshusses, 2011). Reductive dehalogenation by bacteria is strictly anaerobic process which includes cleavage of chlorine atoms of the aromatic ring. In the case of PCDD/Fs anaerobic reductive dehalogenation is the only biological process applicable to digest congeners with 5 or more chlorine atoms per molecule. It has been shown that microorganisms in sediments are involved in reductive dechlorination of dioxins. Bacteria related to the genus Dehalococcoides are

capable of reductive transformation of dioxins, leading to lower chlorinated dioxins including di- and monochlorinated congeners (Bunge, Lechner, 2009). Less chlorinated congeners (< 5 chlorine atoms per molecule) may further be microbially degraded under aerobic conditions. Bedard et al. (2008) used the addition of other halogenated compounds to enhance the dechlorination of polychlorinated biphenyls. In order to use dehalogenation as a remediation technology for contaminated sediments, in the simplest case, dehalogenic bacteria and biostimulation agents such as electron donors or halogenated co-substrates (e.g. chlorobenzenes, chlorophenols) would be added to the sediment (Cho et al., 2002). The sediments used in this project originated from sites contaminated with PCDD/Fs and chlorophenols, which should contain microorganisms capable of degradation of chlorophenols and / or PCDD/Fs. Sewage sludge was added to the cultivation mixture to provide organic carbon source. During cultivation under anaerobic conditions concentrations of PCDD/Fs and chlorophenols were monitored and ecotoxicity of the substrates studied.

Methods

Anaerobic cultivation in a bioreactor:

Anaerobic cultivation was performed in a bioreactor which was made from a plastic container (HDPE) with sealing removable lid of a volume of 120 l. The bottom of the reactor was sprinkled with coarse gravel up to 0.25 m high layer. Onto the geotextile covering the gravel the mixture of soil for anaerobic cultivation was placed (about 55 kg). In the layer of soil wire temperature sensor and pressure hose for sampling of soil air were installed. Mixtures of soils contained two basic soil samples from localities with less contamination (1) and with higher contamination (2). Both versions (1P, 2P) were mixed with the addition of mineral nutrients and support organic material (sewage sludge and potato strips) and placed in an incubator. Sampling was carried out every 3 months.

Determination of PCDD/Fs:

After mixing, the entire content of the bioreactor was sampled in duplicates. Overdried and homogenized sample was analyzed by high resolution gas chromatography / high resolution mass spectrometry (HRGC / HRMS) in the laboratory ALS Czech Republic, Prague.

Determination of chlorophenols:

After mixing, the entire content of the bioreactor was sampled in duplicates. Overdried and homogenized sample was determined using gas chromatography-mass spectrometry (GC-MS) in a laboratory ALS Czech Republic, Prague.

Preparation of water extract for ecotoxicity tests:

The water extract was prepared according to ČSN EN 12457-2 (2003) at water to solid ratio 10 : 1(based on dry mass). After 24 hours of shaking on an end-over-end shaker (Reax 20, Heidolph, 7 to 8 rpm), liquid and solid phases were separated by centrifuging the suspension for 20 min at 4000 g followed by filtration using filter paper Munktell Filtrak 1289.

Determination of the inhibition of root elongation of lettuce - aquatic test:

The method was performed according to the modified Methodical Guideline (ME, 2007) . The test was performed at temperature (22±2) °C in three replicates of 15 seeds of lettuce *Lactuca sativa* var. capitata, Safir (Sempra Veleliby, Ltd.) with an exposure time (96±2) h, in the dark. After incubation, the mean value of the root length was calculated and compared with the mean root length of non-toxic control. If a seed did not create rootlet, it was counted as a zero to the mean value. For sample and control, Arithmetic Average (for homogenous data with a normal distribution) or Median Value (for data where the normality of data distribution and / or homogeneity of variances were not confirmed) was calculated. From the obtained mean root length, root growth inhibition of lettuce (I) was calculated or EC_{50} value was calculated by nonlinear regression analysis using the software Prism 4.0.

Determination of freshwater algae growth inhibition:

Test of freshwater algae growth inhibition was performed according to ČSN EN ISO 8692 (2012). Culture of exponentially growing algal cells *Desmodesmus subspicatus* (Institut of Botany AS CR, Dukelská 135, Treboň) was incubated with the tested water extract of the sample for 72 hours at

temperature (23±2) °C under continuous illumination with white light. Algal cells were maintained in suspension by stirring on a rotary shaker IKA KS 260 Basic (IKA, Germany). After 72 hours, cell concentration was measured by counting apparatus Cellometr Auto T4 Plus using counting chambers CHT4 (Nexcelom Bioscience LLC). The calculated growth rate of three replicates was used to calculate the growth rate inhibition of algae (I), or to calculate the EC_{50} value by nonlinear regression analysis using the software Prism 4.0.

Determination of the inhibition of the mobility of Daphnia:
Daphnia mobility inhibition test was performed according to ČSN EN ISO 6341 (2013). The test is based on determination of the inhibitory effect of the substances contained in the water to Daphnia. For the test female *Daphnia magna* Straus from own breeding were used, at least 2h and most 24 hours old, obtained under defined breeding conditions according to ČSN EN ISO 6341. The test was carried out at a temperature of (20±1) °C, without aeration, without feeding and in the dark, using 4 - 5 replicates. After 24 h and 48 h exposure number of immobilized daphnids in the test sample and control was recorded. Inhibition was determined as percentage of immobilized daphnids in relation to the total number of individuals, or the EC_{50} value was calculated by nonlinear regression analysis using the software Prism 4.0.

Results
For the purpose of biotransformation of dioxins 2 mixtures of original soil samples (a mixture of 1P and 2P) with sewage sludge and potato strips were prepared. After mixing of soil mixtures the first sampling was held. Both reactors achieved anaerobic conditions in very short time after the closure of the bioreactor (< 5 days). Anaerobic conditions in bioreactors remained constant throughout cultivation period. The temperature ranged from 20 °C (in winter months) to 26 °C (in summer months).

At the beginning of cultivation, concentrations of higher-chlorinated congeners were analyzed on which basis the TEQ (toxic equivalent) was calculated. TEQ of dioxins in sample was 5.2 $\mu g\ kg^{-1}$ dw for the mixture 1P and 14.1 $\mu g\ kg^{-1}$ dw for the mixture 2P. Mixture 2P contained 10 times more 1,2,3,4,6,7,8-heptachlorodibenzodioxin (1,2,3,4,6,7,8-HpCDD) and 6.4 times more oktachlorodibenzodioxin (OCDD) compared to variant 1P. Also the concentration of furans were higher in variant 2P than in variant 1P, 3.7 times more 1,2,3,4,6,7,8-HpCDF and 6.2 times more OCDF (Fig. 1). Within 6 months of cultivation, the concentration of PCDD/Fs remained unchanged in both variants. Variations in the assay results were due to measurement uncertainty, which is 30% and the heterogeneity of the matrix.

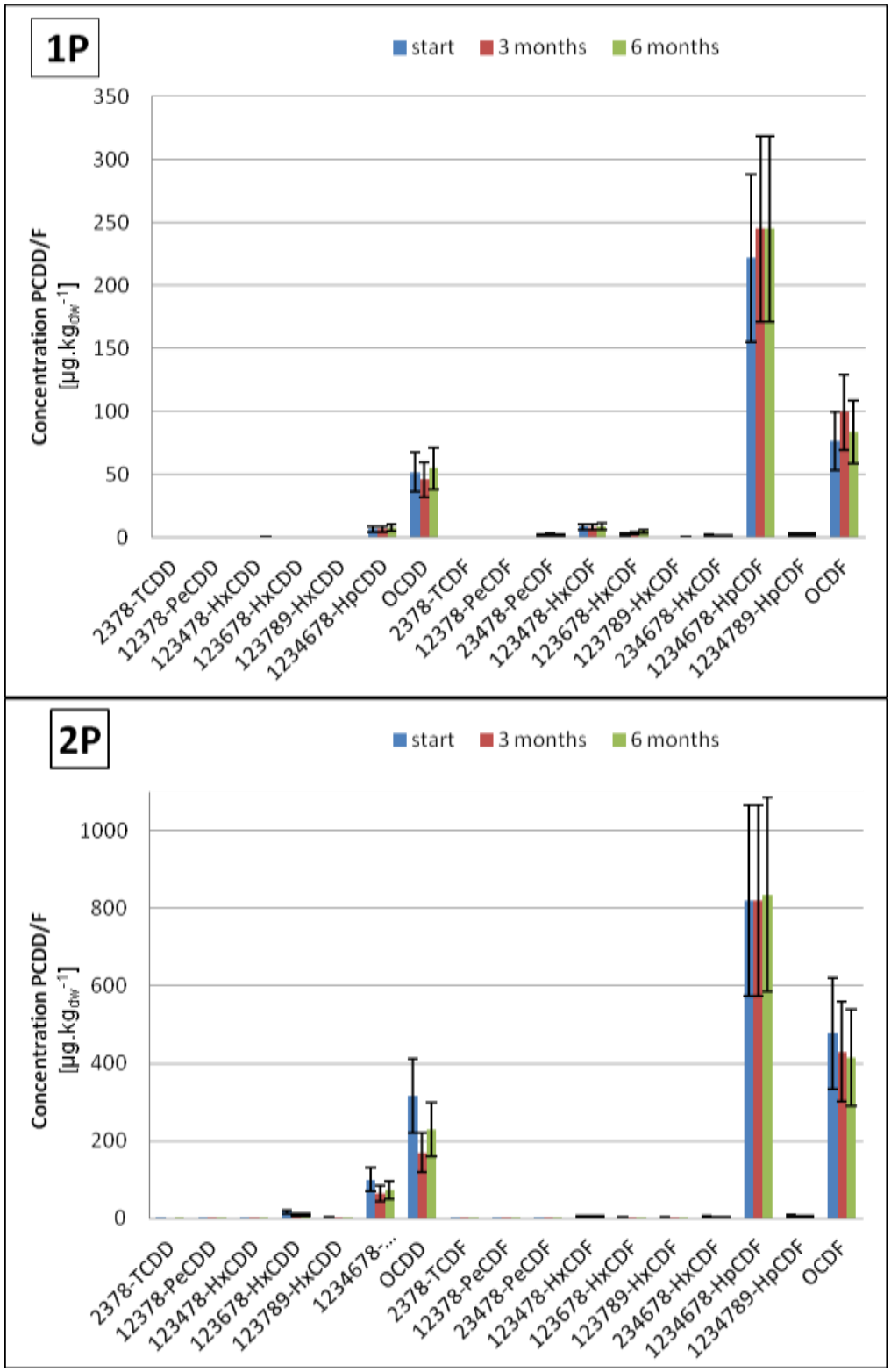

Fig. 1: Graphs showing concentration of individual higher-chlorinated congeners of PCDD/Fs in two variants of the mixture of contaminated soils from various locations in bioreactors (1P, 2P) determined at the beginning, after 3 months and after 6 months of anaerobic incubation. Error bars for 30% measurement uncertainty.

The measured concentrations of chlorophenols at the beginning of cultivation for both variants of soil mixtures also varied considerably. The mixture 1P contained chlorophenols of maximum concentration of pentachlorophenol 0.2 mg kg^{-1} dw, being the most abundant. Concentration of

chlorophenols in the mixture 1P was not further monitored, due to its low value. Mixture 2P initially contained high concentrations of 2,3,4,6-tetrachlorophenol and pentachlorophenol (8.6 and 53 mg kg^{-1} dw). After 3 months of anaerobic incubation higher concentration of 3,5-dichlorophenol, 3,4,5-trichlorophenol, 2,3,4,5-tetrachlorophenol, 2,3,4,6-tetrachlorophenol and pentachlorophenol were measured than at the beginning of experiment (Fig. 2). After 6 months, the concentration of tri-, tetra- and pentachlorophenol decreased to 0.87 mg kg^{-1} dw, 5.4 mg kg^{-1} dw and 7.9 mg kg^{-1} dw, respectively, whereas the concentration of 3-chlorophenol and 3 4-dichlorophenol increased (107 mg kg^{-1} dw and 21 mg kg^{-1} dw) (Fig. 2).

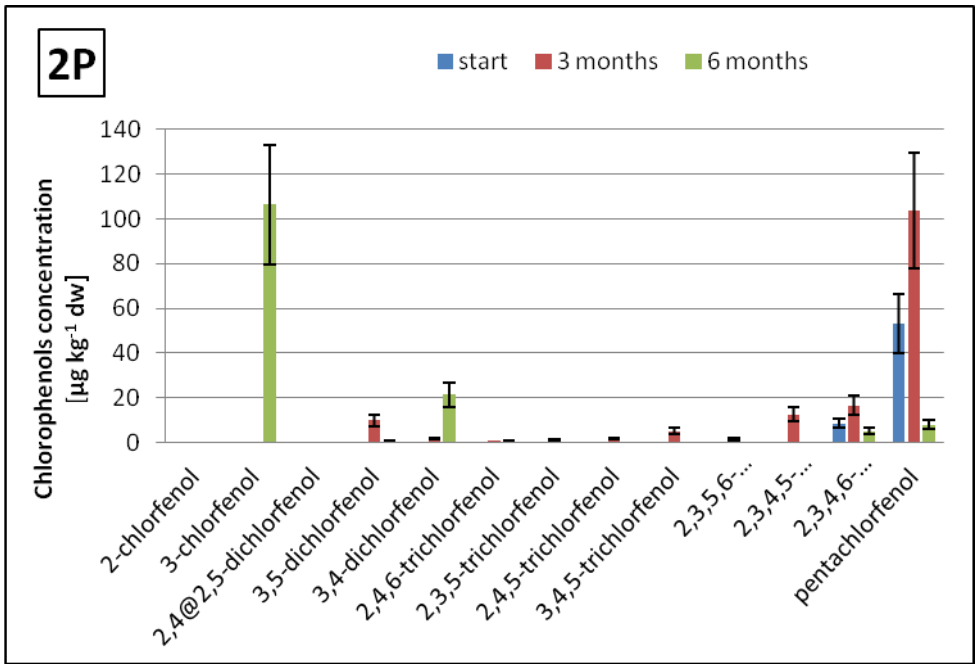

Fig. 2: Graph showing concentration of individual congeners of chlorphenols for variant 2P determined at the beginning, after 3 months and after 6 months of anaerobic incubation. Error bars for 25% measurement uncertainty.

For both variants of soil mixtures the ecotoxicity was evaluated by the test with Daphnia, the test with the growth of lettuce roots that evaluate the acute toxicity of substances and the test with algae, which assesses the chronic toxicity of substances. Mixture 2P was significantly more toxic than the mixture 1P throughout the anaerobic cultivation. After three months, toxicity in both variants of mixtures increased significantly. For a mixture 2P there was even 100% inhibition at all three tests (Tab. 1). After 6 months, ecotoxicity dropped again with the exception of tests with Daphnia, showing 100% inhibition of the mobility again.

Tab. 1: Ecotoxicity tests for two variants of soil mixtures (1P, 2P) determined in the beginning, after 3 months and after 6 months of anaerobic incubation. I - inhibition EC_{50} - concentration of a sample which induces 50% inhibition.

Measured parameter	**I [%]**	**EC_{50}** [ml/l]	**I [%]**	**EC_{50}** [ml/l]	**I [%]**	**EC_{50}** [ml/l]
Sample	**1P - start**		**1P - 3 months**		**1P - 6 months**	
lettuce	14	-	61	806	36	-
algae	11	-	37	-	5,9	-
daphnia	4,0	-	84	750	25	-
Sample	**2P - start**		**2P - 3 months**		**2P - 6 months**	
lettuce	79	280	100	67	36	-
algae	>47	256	>100	183	28	-
daphnia	100	110	100	27	100	350

Discussion

In both bioreactors, anaerobic conditions were achieved after a very short time from mixing the soil mixtures, additional organic material and mineral nutrients. Anaerobic conditions managed to maintain stable without further interventions to the reactors. Therefore the required conditions were achieved for the biological transformation of higher-chlorinated dioxins such HxCDD/F HpCDD/F OCDD/F, and pentachlorophenol. During six months of cultivation no loss of PCDD/Fs was observed, however, there was 85% reduction of PCF. The reason for preferential dehalogenation of pentachlorophenol was 30 times higher concentration of PCF than of the PCDD/Fs in the soil mixture 2P. Other studies have also shown that the concentration of the contaminant has a great effect on its biodegradation rate. They are in an agreement that contaminants of high concentration biodegrade faster than contaminants of low concentration (D'Angelo, Reddy, 2000). The availability of contaminant for biodegradation is particularly affected by its solubility in water, which for PCF is 14 mg l^{-1} (Czaplicki, 2004) and for OCPP 0,074-2 ng l^{-1} (Persson et al., 2008). Although high water solubility increases the bioavailability of the contaminant, also more soluble contaminants are more toxic to aquatic organisms. Ecotoxicity of the contaminant can be estimated using two approaches: chemical approach and an approach based on the determination of toxicity. In the first case, the toxic potential of a mixture is determined on the basis of chemical analysis. In the second case, the toxicity is determined directly by ecotoxicity tests. Estimation based on chemical analysis may be seriously underestimated or overestimated, because it is not possible to capture the interactions between the different substances. Example of toxicity evaluation based on the calculation is TEQ, which is based on the assumption that substances like dioxins have a common mechanism of action - binding to Ah-receptor. When using TEQ concept the toxicity of different congeners is determined in comparison to 2,3,7,8-TCDD (ECRA, 2002). Under German law, the TEQ limit for soil (regardless of location) is 10 μg kg^{-1} dw. In the case of residential areas the limit is much lower. Therefore, soil mixture 2P would have not met the required criteria. Czech legislation has no limits based on TEQ for PCDD/Fs, but evaluates waste disposal on the surface by ecotoxicity tests in accordance with Decree no. 294/2005 Sb. (2005). Ecotoxicity tests are sensitive to the bioavailable fraction of contaminants and include the effects of all contaminants, even those that were not detected by chemical analysis (CEN/TR 16110, 2010). The tested soil mixtures would not be considered a hazardous waste according to Decree No. 376/2001 Coll. based on the ecotoxicity results, but in accordance with Decree No. 294/2005 Coll. could not be used on the surface of the ground. Aquatic test with Daphnia was the most sensitive to the present contamination, which can be explained by the presence of PCF, which, due to its higher solubility, leached into the solution. Toxicity of the mixture 2P significantly decreased after dehalogenation of pentachlorophenol to 3-chlorophenol.

Conclusion

The period of six months proved unsatisfactory for the anaerobic degradation of PCDD/Fs. However, conversion of PCF to 3-chlorophenol and 3,4-dichlorophenol demonstrated that microorganisms capable of reductive dehalogenation are present in the soil. Anaerobic cultivation will continue, since it can be assumed that the next stage of soil transformation will be dehalogenation of PCDD/Fs.

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