STIMULATION OF PCDD DECHLORINATION IN HISTORICALLY-CONTAMINATED SEDIMENTS

Iris D. Albrecht, Andrei L. Barkovskii and Peter Adriaens

Department of Civil and Environmental Engineering, The University of Michigan, 1351 Beal ave, Ann Arbor, Mi, 48109-2125, USA

INTRODUCTION

Many industrial waterways and near-coastal regions in large metropolitan areas are contaminated with metals, aromatic hydrocarbons, petroleum hydrocarbons, and chlorinated organic compounds. The presence of PCDD is of particular concern, due to their suspected adverse human and ecological effects after biomagnification in the trophic food chain. Environmentally relevant concentrations of PCDD/F were recently demonstrated to undergo dechlorination in contaminated reduced freshwater sediments[1] and by estuarine and freshwater sediment-derived microorganisms, when incubated under reduced anaerobic conditions[2]. Moreover, it was also shown that 2,3,7,8- tetraCDD residues which had partitioned to sediment-derived microorganisms could be rapidly dechlorinated to nontoxic mono-, di- and triCDD[3], which indicates the potential for environmental production of these isomers in sediments as the result of microbial activity.

Stimulated dechlorination of historical PCB contamination in freshwater using both chloro- and bromobiphenyls, as well as observed hydrogen-stimulated reductive alkyl halide and aryl halide dechlorination, provided us with the impetus to investigate the use of hydrogen and 2-bromo-dibenzo-p-dioxin as possible agents for the enhancement of natural PCDD dechlorination processes in historically contaminated sediments[4-7].

In order to show that 2,3,7,8-tetraCDD is dechlorinated, the selective appearance of 2,3,7-triCDD, 2,3-, 2,7-, and 2,8-diCDD, or 2-monoCDD, must be established. Since mono- to triCDD are not included in standard methods, we needed to develop a method to incorporate these lower chlorinated congeners within typical PCDD enrichment and isolation procedures. EPA method 1613 [8] provided the basis of our analytical procedure. However, some modifications to the bulk matrix cleanup and carbopak column procedures, either adopted from others [9-11], or developed in our labs [12,13] were necessary and are described here.

EXPERIMENTAL METHODS

A. Sediment treatment/incubations

Four kilograms of historically contaminated sediment obtained from the Passaic River (Diamond Alkali site) were homogenized and acclimated for a period of 3 months under anaerobic estuarine conditions. Fifteen x 200g subsamples of this acclimated sample were then placed in wide mouthed jars, treated as necessary and sealed. The treatments listed in Table 1 were performed in triplicate. Two timepoints (time zero, time three months) were established.

Table 1: Summary of treatments

Description	Treatment
Unamended (UA)	Substrate (Acetate, Propionate and Butyrate, 100 mg/kg
Bromodioxin (Br)	Substrate+ 20 ppb 2-monobromodibenzo-p-dioxin
Hydrogen (H_2)	Substrate+ H_2 gas in headspace with daily manual mixing
Sterilized control (STC)	autoclaved+ substrate+ 20 ppb 2-monobromodibenzo-p-dioxin

B. Sample preparation and analysis:

(i) Reagents, columns and standards:
All solvents were purchased from Fischer Scientific, OPTIMA grade. Reagent grade potassium hydroxide, sodium hydroxide, silver nitrate, instrument grade mercury metal and chromatographic silica gel 100-200 mesh, were all purchased from Fischer Scientific. Copper powder -40 mesh was purchased from the Aldrich Chemical Company. Celite 545 was purchased from J.T. Baker. Carbopak C 80/100 mesh was purchased from Supelco, and Alumina Basic super 1 50-200 mesh from Alltech. The HRGC column and standard solutions containing all native PCDD congeners and all ^{13}C-labelled PCDD were purchased from J&K Environmental, Canada. A solution of 50µg/mL ^{13}C-labelled 2,3,7,8-T4CDD was purchased from CIL.

(ii) Preparation of cleanup reagents:
Cu-Hg amalgam: To 100g copper powder, cleaned by rinsing with 20% HNO_3 followed by 5 rinsings of distilled deionized water, 82g of Hg and 20 mL of 30% HNO_3 was added. Once the reaction started, 60 mL of water was added and the mixture was extensively stirred. After settling overnight, the liquid was decanted and the amalgam rinsed 5 times with acetone, followed by 5 rinses with hexane. ***Activated silica gel, 33% 1N NaOH, 30% H_2SO_4, 44% H_2SO_4, and Basic Alumina:*** As described in EPA method 1613 [8] ***10% $AgNO_3$:*** As described by Lamparski and Nestrick[9] ***Potassium Silicate:*** As described by Smith et. al. [11] ***Carbopak 32%:*** 8g of Carbopak C combined with 17 g Celite 545 was activated at 130°C.

(iii) Sediment sample preparation, extraction and cleanup:
Air dried samples (25g), homogenized with a mortar and pestle, were placed in Soxhlet thimbles each charged with 2g preextracted silica gel. After spiking with the required labelled PCDD internal standard and/or isotope dilution standard, a 48 hr toluene Soxhlet extraction was implemented. Ten grams each of Cu-Hg amalgam and 30% H_2SO_4 were added to each extract. The mixtures were stirred for 1 hour, filtered through soxhlet thimbles with 5 x 20 mL hexane rinsings each, and rotovapped to 3-5 mL. Further cleanup was carried out using layered silica columns consisting of (from bottom to top): 1g silica gel, 1g 44% H_2SO_4, 4g silica gel, 5g potassium silicate, 1g silica gel, 5g 10% $AgNO_3$, 1g silica gel, 5g 33% 1 N NaOH, 1 g Silica gel, 14g 44% H_2SO_4, 6g silica gel, 5g Na_2SO_4, eluting with 600 mL hexane. After rotovapping to 2 mL, the extracts were applied to columns each consisting of 6g activated basic alumina. Elution was carried out with 100 mL hexane (discarded) followed by 25 mL of 50% CH_2Cl_2 in hexane. In each case, the target fraction was rotovapped to 1 mL and applied to further cleanup using a column of 0.55g carbopak 32% which had been prewashed as described in EPA method 1613 [8]. Elution program as follows: Fraction 1: Application of extract+ 2x 1ml hexane rinsings, Fraction 2: 3ml hexane, Fraction 3: 2ml CH_2Cl_2/cyclohexane + 2mL CH_2Cl_2/methanol/toluene, Fraction 4: 25 mL Toluene in reverse direction. As it was previously found that this cleanup step was highly variable with respect to congener discrimination and overall recoveries [12,13], all fractions were collected and saved. Fractions 3 and 4 were prepared for analysis by volume reduction to 50-100µL under N_2.

(iv) Instrumental analysis:
Hewlett-Packard HP5890/5972A GC-MSD using split- splitless injection at 280°C. The column was 30m x 0.25mm id x 0.25µm ICB-5. Temperature program : 100°C (2min), 5°C/min, 230°C, 10°C/min, 300°C(10min). Interface temperature: 300°C. EI-SIM mode was used, monitoring 5 ions per congener group (3 for monoCDD). Identification of PCDD required that the following criteria be met: (a) a given peak retention time correspond to one generated by a standard containing all PCDD, (b) the peak is present in mass chromatograms corresponding to at least 2 signature ions, (3) the ratios of the peak areas correspond to the expected values based on the natural isotopic abundance of chlorine. Quantitation was based on internal standard calculations using labeled 2378-T4CDD. One out of each triplicate was also spiked with a labeled mixture consisting of all PCDD congeners in order to aid in the confirmation of peak identification, and to provide a basis for comparison of results by using isotope dilution. It was found that with the exception of OCDD, for which the recoveries differed substantially from the other congeners, there was reasonable consistency between results obtained by internal standard versus isotope dilution.

RESULTS AND DISCUSSION

Whereas no trends could be discerned for OCDD due to spatial variability and site heterogeneity, the dioxin congener distribution shows some indicators for dechlorination. Figure 1 shows the comparison of PCDD distributions resulting from the various treatments. The results clearly demonstrate the occurrence of dechlorination reactions in all amendments including the sterilized sediment core sample. This control exhibited a two-fold increase in heptaCDD isomers, with no apparent bias towards either isomer, and a three-fold increase in 2,3,7,8-TCDD. Abiotic dechlorination of PCDD mediated by heavy metals, organometal complexes and sediment organic fractions has been demonstrated earlier and was shown to cease at the tetraCDD level [14, 15].

Stimulation of microbial dechlorination using electron donor cocktails resulting in the production of heptaCDD totalling approximately 15% of octaCDD. In addition, 2,3,7,8-TCDD production amounted to less than 50% of that observed in the control systems, indicating that competitive electron fluxes between biotic and abiotic systems affect the efficiency of electron transport to co-metabolic reactions [6]. Indeed, combined microbial/model humic constituent-mediated PCDD dechlorination was previously shown to reduce the yield of 2,3,7,8-TCDD[15].

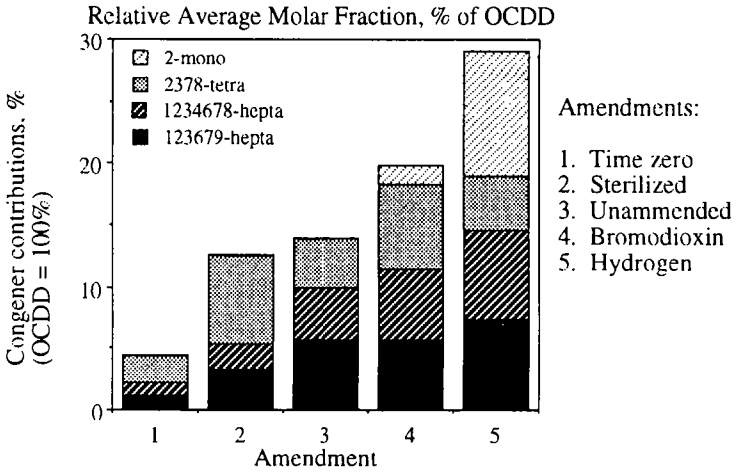

Figure 1: Dioxin congener distribution in response to various enhancement strategies (presented as relative average molar fraction of OCDD).

The bromodioxin- and hydrogen- amended systems exhibit the most extensive degree of dechlorination, resulting in the appearance of differential amounts of 2-monoCDD, totalling 2 and 10% of octaCDD, for both treatments. Stimulations of intrinsic dechlorination activity by brominated compounds has been demonstrated earlier in the case of PCB bioremediation[4]. The mechanism has been attributed to the derepression of expression of dehalogenating enzymes by structurally similar compounds[7]. In the experiment presented here, 2,3,7,8-TCDD accumulates to the same extent as in the sterile control, and the extent of heptaCDD accumulation is similar to that observed in the substrate-amended control. However, the appearance of 2-monoCDD indicates that the accumulation of the tetraCDD isomer is transient. The production of 2-monoCDD, as linked to 2,3,7,8-TCDD appearance was demonstrated earlier using sediment-derived microbial cells[3]. The surprising result of the hydrogen amendment is likely attributable to microbial hydrogenase activity. The enzyme is widely distributed among anaerobic microbial populations, and has been implicated in the reduction of heavy metals. These results indicate that while the unamended and sterile (abiotic) systems undergo some extent of OCDD dechlorination relative to the time zero PCDD distribution, the two treatments, in particular the hydrogen amendment, effected extensive dechlorination resulting in the accumulation of 2-monoCDD.

ACKNOWLEDGEMENTS

This research was supported by the Office of Naval Research, The U.S. Army Corps of Engineers Waterways Experiment Station, and the U.S. EPA Region II Superfund Office.

REFERENCES

1. Adriaens, P., Fu, Q. and Grbic'-Galic', D.; *Environ. Sci. Technol.* **1995**, 29, 2252-61.
2. Beurskens, J.E.M., Toussaint, M., de Wolf, J., van der Steen, J.M.D., Slot, P.C., Commandeur, L.C.M. and Parsons, H.H.; *Environ. Toxicol. Chem.* **1995**, 14, 939-43.
3. Barkovskii, A.L. and Adriaens, P.; *Appl. Environ. Microbiol.* **1996**, 62, 4556-62.
4. Bedard, D.L., Van Dort, H.M., Brunnel, S.C., Principe, J.M., DeWeerd, K.A., May, R.J. and Smullen, L.A.; *Abstr. Am. Soc. Microbiol. Conf.*, Athens, GA: Office of Research and Development, U.S.EPA **1993**, pp. 19-21
5. Petrovskis, E. A., Vogel, T.M. and Adriaens, P.; *FEMS Microbiol. Lett.* **1994**, 121: 357-64.
6. Petrovskis, E.A., Vogel, T.M., Saffarini,D.A., Nealson, K.H. and Adriaens, P.; *Appl. Environ. Microbiol.*, **1998**, In Review.
7. Sullita, J.M. and Townsend, G.T. ; In Young, L.Y. and C.E. Cerniglia (Eds.), *Microbial Transformation and Degradation of Toxic Organic Chemicals*, **1995**, pp. 243-269. John Wiley and Sons, Inc., New York, N.Y.
8. EPA method 1613: Tetra- through Octa Chlorinated dioxins and furans by isotope dilution HRGC/HRMS. EPA Office of Water Engineering and Analysis Division (4303) Washington, DC 20460.
9. Lamparski, L.L. and Nestrick, T.J.; *Anal. Chem.*, **1980**, 52, 2045-54.
10. Nestrick, T.J. and Lamparski, L.L.; *Anal. Chem.*, **1982**, 54, 2292-99.
11. Smith, L.M., Stalling, D.L. and Johnson, J.J.; *Anal. Chem.* **1984**, 56, 1830-42.
12. Albrecht, I.D. and Adriaens, P.; In H. Verachtert and W. Verstraete, eds., *International Symposium for Environmental Biotechnology*, Technological Institute, Antwerp, Belgium, **1997**, pp. 405-408.
13. Albrecht, I.D. and Adriaens, P.; *Forum On Environmental Remediation And Environmental Toxicology*, Sept. 19, **1996**, Lansing, MI. pp. 219-21.
14. Adriaens, P., Chang, P.R.-L. and Barkovskii, A.L. ; *Chemosphere* , **1996**, 32: 433-41.
15. Barkovskii, A.L., and Adriaens, P.; *Environ. Toxicol. Chem.* **1998**, In Press.