Reductive Dechlorination of Hexachlorobezenes and polychlorinated biphenyl in Anaerobic Sediments from Tropical Rivers with Enrichment

I-Ming Chen, Fang-Cheng Chang, and Yei-Shung Wang, Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

Abstracts

Reductive dechlorination of hexachlorobenzene (HCB) and polychlorinated biphenyl (PCB) in the sediment culture collecting from tropical rivers was studied. Pentachlorobenzene (QCB) was dechlorinated contemporaneously in three pathways in the enriched sediment culture from Ho-Tsin river. The dechlorinated products, trichlorobenzene (TCB), were 1,3,5-TCB in major, 1,2,4-TCB in minor and 1,2,3-TCB present only in the early short period. The 1,2,4-TCB and 1,2,3-TCB were finally further dechlorinated to 1,3-dichlorobenzene (DCB). Study on dechlorination of PCBs in the sediment culture from Er-Jen river, acclimated sediment culture was amended with 2,3,4-trichlorobiphenyl (2,3,4-CBp), the compound was completely dechlorimated to 2,4-CBp within 10 days, then the culture amended with 3,4,5-CBp, the compound was dechlorinated to 3,5-CBp, but when amended with both chloramphenicol (a protien-synthesis inhibitor) and 2,3,4-CBp, no 2,3,4-CBp was dechlorinated.

Introduction

In recently years, reductive dechlorination under anaerobic conditions thought to be the effective way to metabolize the chlorinated aromatic compounds. Quensen III et al.[1] compared the rate, extent, and pattern of dechlorination of four Aroclors by inocula prepared from two polychlorinated biphenyl (PCB)-contaminated sediments, their results showed that all four Aroclors were dechlorinated with the loss of *meta* plus *para* chlorines ranging from 15 to 85 %. Holliger et al. [2] Studied the enrichment and properties of an aneaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene (1,2,3-TCB) to 1,3-dichlorobenzene (1,3-DCB) in the presence of lactate, glucose, ethanol, or isopropanol as the electron donor. They thought that dechlorinating activity could only be maintained when an electron donor was added. Reductive dechlorination of hexachlorobenzene (HCB) to tri- and di- chlorobenzene in anaerobic sewage sludge had reported by Fathepure et al. [3], their results showed that complete biotransformation of 190 μ M hexachlorobenzene occurred within 3 weeks, and the calculated rate of hexachlorobenzene dechlorination was 13.6 μ mole/liter/day.

The purpose of this study is to clearify the reductive dechlorination of HCB and PCB in sediment culture collecting from torpical rivers, and to compare the dechlorination degree between

in virgin mixed cultures medium and in acclimated culture medium.

Experimental Method

Sediments were collected from Ho-Tsin river and Er-Jen river located in southern Taiwan. Er-Jen river had contaminated slightly by PCBs in the past decade. Sediment from Ho-Tsin river mixed with culture medium (1:2, by volume) was incubated with 1,2,3-TCB (in acetone) in 1-liter screw-cap serum bottle at 30 °C in the dark for acclimation. 1,2,3-TCB was fed at 14 days intervals. The mixed culture showed dechlorination ability after incubation for 30 days. The mixed culture was then enriched by fed with HCB instead of 1,2,3-TCB for another 210 days of further acclimation, and HCB was fed in the same period intervals as the preceding. Sediment from Er-Jen river was directly mixed with the culture medium and the mixed culture was washed out without any further acclimation and enrichment.

Dechlorination of HCB and pentachlorobenzene (QCB) in the enrichment culture medium of sediment from Ho-Tsin river was carried out. HCB or QCB was amended to the enriched culture, when the introduced chlorobenzene compound was completely transformed, the culture medium was amended with the same chlorobenzene congeners and a protien-synthesis inhibitor, chloramphenicol, for continuous incubation. At the dechlorination occurred again and was transformed completely, the chlorobenzene congeners to study was added to the culture for dechlorination test. Dechlorination of 2,3,4-trichlorobiphenyl (2,3,4-CBp) and 3,4,5-trichlorobiphenyl (3,4,5-CBp) in the mixed culture medium eluting from sediment of Er-Jen river were also studied in like manner with a slight modification. After PCB transformed completely due to dechlorination, the incubated culture medium was transfered to a fresh culture medium and amended with the same PCB congener for further incubation, when the PCB was completely transformed again, the chloramphenicol with the same or different PCB congeners to study were added for further dechlorilnation test.

At designed sampling time, 2 ml of incubation culture was removed by syringe and extracted with *n*-hexane for three times and analyzed with gas chromatography. At analysis of chlorobenzene, the ECD gas chromatograph (Hewlett Packard 5890) was equipped with an Ultra-1 fused silica capillary column (0.2 mm ID x 25 m, film thickness 0.33 μ m). Oven temperature was held at 80 °C for 5 min, then programmed to 120 °C at 5 °C/min and kept for 2 min, then raised in 5 °C/min to the final temperature of 200 °C and held for 5 min. The injector and detector temperatures were set at 240 °C and 300 °C, respectively. Nitrogen gas was used as both the carrier and make-up geases, the linear velocity was 16 cm/s and the split ratio was kept at 10:1. At analysis of PCBs, the ECD gas chromatograph (Varian 3600) was equipped with a DB-5 (J & W Scientific) fused silica capillary column (0.53 mm ID x 30 m, film thickness 1.5 μ m). The temperature programmed was held at 170 °C for 2 min, then raised to 260 °C at a rate of 3 °C/min and held for 18 min. The injector and detector temperatures were 280 °C and 300 °C, respectively. Using nitrogen as carrier and make-up gases, the linear velocity was 27.3 cm/s and split ratio was 15:1.

Results and Discussion

Incubation in 1,2,3-TCB for 30 days and without further acclimation with HCB, the HCB or

QCB was dechlorinated in major to 1,3,5-TCB *via* 1,2,3,5-TeCB and in minor to 1,2,4-TCB *via* 1,2,4,5-TeCB in the mixed culture medium of sediment from Ho-Tsin river. The 1,2,4-TCB was then further dechloronation to 1,3-DCB. In addition, a pathway for dechlorination of QCB to 1,2,3,4-TeCB in the early incubation period was found in this study. The 1,2,3,4-TeCB was further to 1,2,4-TCB and 1,2,3-TCB. Two congeners, 1,2,3,4-TeCB and 1,2,3-TCB, disappeared rapidly with the time elapse. Fig. 1 show the diagram of dechlorination of QCB in the sediment mixed cultures on day 11 after acclimation with 1,2,3-TCB for 30 days. A further acclimation in HCB for 210 days, the mixture cultures could not showed the dechlorination of HCB and QCB *via* the pathway of producing 1,2,3-TCB as described above, the 1,3,5-TCB was the only dechlorinated product. The transformation was the most popular pathway similar to historical researches *via* HCB → QCB → 1,2,3,5-TeCB → 1,3,5-TCB.

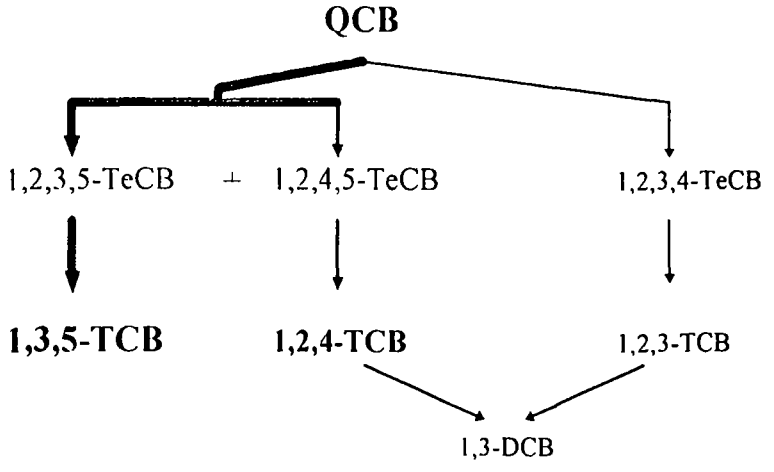

Fig. 1 Diagram of transformation pattern for QCB dechlorination in sediment mixed culture on day 11 after the culture was acclimated with 1,2,3-TCB for 30 days. Bold handwriting indicate higher amounts.

2,3,4-CBp and 3,4,5-CBp were completely transformed to 2,4-CBp and3,5-CBp, rspectively, after incubation in the mixed culture eluting from sediment of Er-Jen river for 160 days. Transfer the part of the incubation cultures to a fresh culture medium and re-incubation again, the same PCB congener were dechlorinated within a short lag phase of about 20 days. When 2,3,4-CBp and 3,4,5-CBp were amended to the transfered culture on day 47 after transfer (0 day in Fig. 2), the PCBs were rapidly metabolized (Completely transformed within 10 days) to 2,4-CBp and 3,5-CBp, respectiveely. But when the PCB congener was cross amended, 2,3,4-CBp adding to the 3,4,5-CBp vessel or the 3,4,5-CBp adding to the 2,3,4-CBp vessel, both PCB congeners were contineous transformed to the degraded PCB congeners. When amended with 2,3,4,5-CBp, the 2,3,5-CBp was produced (Fig. 2). When amended with HCB, a single benzene ring compound,

no dechlorination occurred. (Fig. 2). The incubation cultures amended with both PCB congeners and chloramphenicol, no dechlorination of PCB congener was found. The enzyme system for PCB congeners transformation may be the same.

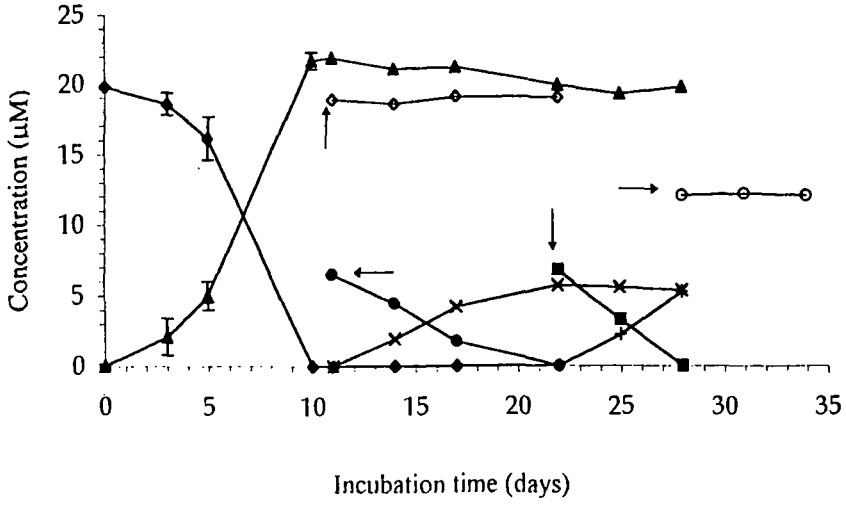

Fig. 2. Dechlorination of 234-CBp and other PCB congeners by continuous feds in transfered cultures. Error bars represent the standard deviation of duplicate samples. Treatment of 3,4,5-CBp (←) and of 2,3,4-CBp plus chloramphenicol (↑) on day 11, 3,5-CBp was produced from 3,4,5-CBp and no dchlorination was formd by 2,3,4-CBp at treating chloramphenicol. ◆: 2,3,4-CBp; ◇: 2,3,4-CBp plus chloramphenicol; ▲: 2,4-CBp; ●: 3,4,5-CBp; ×: 3,5-CBp; ■: 2,3,4,5-CBp; ⊦: 2,3,5-CBp; ○: HCB.

Acknowledgments

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