THE PCBS DEGRADING ABILIY MICROORGANISMS ISOLATED FROM ER-JEN RIVER IN TAIWAN

Fang-Cheng Chang, Jui-Hung Yen and Yei-Shung Wang

Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan

Introduction

Polychlorinated biphenyls (PCBs) are industrial pollutants that have become ubiquitous in nearly all environmental compartments, including air, soil, water, sediments and vegetation. In most countries PCBs productions and uses were phased out and banned by the 1970s. In 50-year period, approximately 1.4 billion pounds of PCBs were produced. Such extensive application of these compounds has resulted in widespread contamination. It has been estimated that several hundred million pounds have been released to the environment. The persistence of PCBs in river and harbor sediments worldwide has become a focus for environmental regulation because PCBs accumulate in biota and are potentially toxic to wildlife and human. It is possible for PCBs to be oxidatively degraded under aerobic conditions, but such processes generally occur only with congeners having five or fewer chlorines[1]. Sequential anaerobic-aerobic degradation schemes have been suggested as a mean of taking advantage of the different characteristics of anaerobic and aerobic microorganisms—the former is better for attacking more highly chlorinated biphenyls and the latter is better for oxidizing less chlorinated biphenyls[2, 3]. The anaerobic microbial communities most often found in river sediments show various effectiveness in reductively dechlorinating commercial PCB mixtures, typically those of accumulating less chlorinated *ortho*- and *ortho*- plus *para*-chlorinated congeners. Biological treatments including aerobic degradation and anaerobic dechlorination that has been well documented[4, 5, 6, 7, 8, 9]. Microorganisms being able to grow on biphenyl usually have the ability to co-metabolize various PCB congeners, and it is generally assumed that biphenyl-oxidizing enzymes with a wide substrate range are responsible for this action. The ability of co-metabolizing PCB congener varies substantially from species to species[10]. Although complete mineralization of monochlorinated biphenyls by single strains has been reported, no culture with the ability either to completely mineralize more highly chlorinated biphenyls or to grow on any congener that has one or more chlorines on each ring has been unequivocally described yet. Aerobic microorganisms attack and occasionally grow on major congeners that are the products of anaerobic dechlorination. In this investigation, we also try to find the existence of different materials in the cultures that accelerate the degradation of PCB congeners, and to isolate the microbes with PCBs—degrading ability from the mixed cultures.

Materials and methods

Standard PCB congeners (99% purity), including five 2-chloride (Cl) substituted congeners [2,2'-DiCB (PCB-4), 2,3-DiCB (PCB-5), 2,3'-DiCB (PCB-6), 2,4'-DiCB (PCB-8), 2,6-DiCB (PCB-10)], five 3-Cl substituted congeners [2,4,2'-TriCB (PCB-17), 2,5,2'-TriCB (PCB-18), 2,6,2'-TriCB (PCB-19), 2,6,3'-TriCB (PCB-27), 3,5,2'-TriCB (PCB-34)], eight 4-Cl substituted congeners [2,4,2',4'-TetraCB (PCB-47), 2,4,2',5'-TetraCB (PCB-49), 2,4,6,2'-TetraCB (PCB-50), 2,4,2',6'-TetraCB (PCB-51), 2,5,2',5'-TetraCB (PCB-52), 2,5,2',6'-TetraCB (PCB-53), 2,3,4,6-TetraCB (PCB-62), 2,6,3',5'-TetraCB (PCB-73)], and four 5-Cl substituted congeners [2,4,6,2',6'-PentaCB (PCB-104), 2,4,5,3',4'-PentaCB (PCB-118), 2,4,6,3',5'-PentaCB (PCB-121), 3,4,5,3',4'-PentaCB (PCB-126)] for contrast,

were obtained from AccuStandard Co. (New Haven, CT., USA). The stock solution (1 mg/mL) were prepared in *n*-hexane and stored at -20?. HPLC grade *n*-hexane was purchased from E. Merck, Germany. Sand and chitin were purchased from Sigma, USA. 100 mL medium (contained 2.7 g NH_4Cl, 0.1 g $MgCl_2$, 0.1 g $CaCl_2$, 0.27 g K_2HPO_4, 0.35 g KH_2PO_4 and yeast extract 5 g per liter) and 100 g sediment were mixed in a 250 mL-scale flask, and then shaken for 10 days (120 rpm) at 30 °C. The 10 mL soil suspension was transferred to 100 mL fresh medium and incubated under the same condition for 10 days, and then the suspension medium was transferred to the medium, which yeast extract has being replaced by biphenyl (1 g/L). The single strains were separated from mixed cultures on LB medium petri dish by four-way streak plate inoculation. About $8´10^7$ cells/mL of single strains (5 mL) was added to 50 mL serum bottle containing 15 mL of cultural medium, 2 mg L^{-1} of PCB-8 and 2 mg L^{-1} of biphenyl individually. All the treatments were incubated at 30°C in darkness. Treated samples were collected at 0, 1, 3, 5, 9, 13, 26, 38, 50, and 60 days after treatments. After incubation, a mixture solvent (n-hexane: acetone =9:1 by volume, 10 mL) was added into the serum bottle and put it into ultrasonic bath for 15 min. Then the vessel was shaken intensively for 2 min. The hexane layer was collected in a 10 mL volumetric flask. The hexane layer was added to the volumetric flask that was then filled with n-hexane to 10 mL. The procedure was repeated twice. The extract solutions of these treatments were analyzed by gas chromatography. The irradiated samples (2 mL) were applied directly to the gas chromatograph for analysis. The half-lives of individual PCB congeners were calculated by the pseudo first order reaction. The PCBs concentrations were analyzed by GC (Varian 3600, Walnut Creek, CA) with nitrogen as a carrier gas (80 psi, 2.7 mL min^{-1}), using an electron capture detector (300 °C, make up gas N_2 at 34.33 mL min^{-1}), and a DB-5 fused silica capillary column (30 m´0.53 mm i.d.). The injector temperature was 280 °C. The column was held at 170 °C for 2 min, then increased to 260 °C at the rate of 3 °C min^{-1}, and finally held at 260 °C for 30 min.

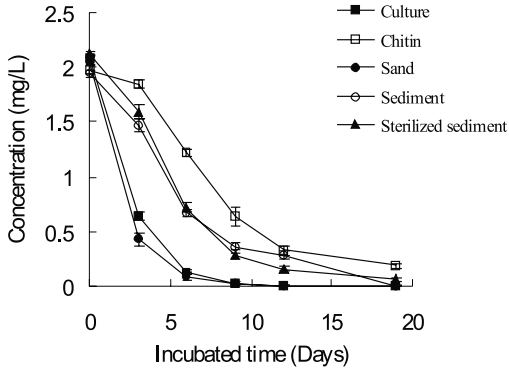
Table 1. After 12 days, the degradation percentage of PCB-8 (2,4'-DiCB) by these 28 strains

Strains	Degradation (%)	Strains	Degradation (%)	Strains	Degradation (%)
S01	62.10	S11	31.52	S21	10.06
S02	52.75	S12	50.52	S22	29.89
S03	41.79	S13	94.98	S23	28.41
S04	13.23	S14	69.90	S24	30.03
S05	44.36	S15	43.94	S25	53.38
S06	53.02	S16	33.35	S26	51.36
S07	43.64	S17	25.35	S27	94.89
S08	25.15	S18	61.49	S28	60.55
S09	33.18	S19	55.58	Control	2.64
S10	42.40	S20	15.45		

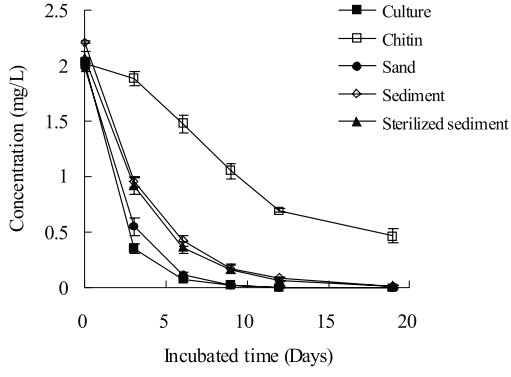
Results and Discussion

Twenty-eight single strains were obtained in our experiment. The PCBs degradation abilities of these 28 strains were tested by PCB-8 (2,4'-DiCB). The degradation of PCB-8 by these 28 strains after 12 days was shown in Table 1. In Table 1., it was found the degradation percentages of PCB-8 by these 28 single strains were ranged from 10.06 to 94.98%. From the result, we found S13 and S27 have more

S13



S27



Mixed cultures

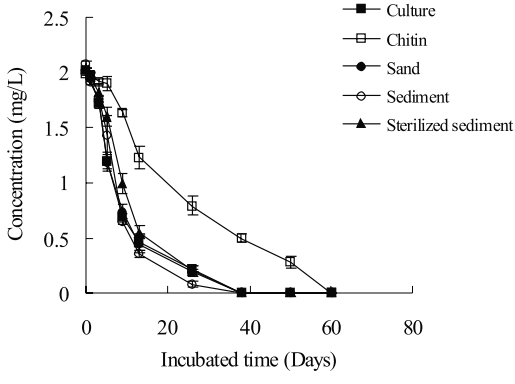


Figure 1. Amounts for biodegradation of PCB-8 (2,4'-DiCB) by single strains, S13 and S27 in different treatments.

efficient than other strains. In this study, the single strains S13 and S27 were selected for our experiment. To identify the two single strains by Gram's stain. We found both of them are Gram-negative bacteria, and the biodegradation of PCB-8 by S13, S27 and mixed cultures in five treatments has shown in Figure 1.

The degradation of PCB-8 in five treatments by the single strain, S13 or S27 is similar, and the degradation abilities of S13 and S27 are more efficient than mixed cultures. The degradation abilities of S13 or S27 in our experiments are in the order of Chitin >Sediment >Sterilized sediment >Sand >Culture and Chitin >Sediment ≲Sterilized sediment >Sand ≲Culture, respectively. The degradation abilities of single strains (S13 or S27) are predominate in treatment of mixed cultures among these five treatments.

In summary, the degrading abilities of mixed microorganisms and single strains we obtained are according to the presence of various added materials pronouncedly. The degraded abilities of single strains (S13 or S27) are more excelled than the mixed cultures. Thus, choosing an appropriate added material is important to the biodegradation of PCB congeners.

References

1. Bedard, D. L., Wagner, R., E. Brennan, M. J., Haberl, M. L. and Brown Jr, J. F. (1987) Appl. Environ. Microbiol. 53: 1094-1102.
2. Brown Jr., J.F., Wagner, R. E., Feng, H., Bedard, D. L., Brennan, M. J., Carnahan, J. C. and May, R. J. (1987) Environ. Toxicol. Chem. 6: 579-593.
3. Brown, J. F., Bedard, D. L., Brennan, M. J., Carnahan, J. C., Feng, H. and Wagner, R. E. (1987) Science. 236: 709-712.
4. Chen, I. M., Chang, F. C. and Wang, Y. S. (1997) In proceedings of the 17th Symposium on Chlorinated Dioxins and Related Compounds, Indianapolis Indiana. Vol. 33, pp. 189-192.
5. Chen, I. M., Chang, F. C and., Wang, Y. S. (1999) In proceedings of the 19th Symposium on Chlorinated Dioxins and Related Compounds, Venice Italy. Vol. 41, pp. 463-466.
6. Chen, I. M., Chang, F. C., Chang, B. V. and Wang, Y. S. (2000) Water Environment Research 72, 675-679.
7. Chen, I. M., Chang, F. C. and Wang, Y. S. (2001) Chemosphere 43, 649-654.
8. Chen, I. M., Chang, F. C. and Wang, Y. S. (2001) Chemosphere 45, 223-229.
9. Chang, F. C., Yen, J. H. and Wang, Y. S. (2000) In proceedings of the 20th International Symposium on Halogenated Environmental Organic Pollutants &POPS, Monterey California. Vol. 45, pp. 392-395.
10. Mcleese, D. W., Metcalfe, C. D. and Pezzak, D. S. (1980) Arch. Environ. Contam. Toxicol. 3: 507-518.