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Anaerobic Degradation of the Organochlorine Pesticides DDT and Heptachlor in River Sediment of Taiwan

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p,p'-DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)-ethane] and heptachlor [1,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetra-hydro-4,7-methanoindene] are of serious concern in some countries because of their chemical stability, estrogen-like characters and potential animal toxicity (Kirby et al. 2001). Several decades ago, DDT and heptachlor were widely used to control insects and environmental pests. Due to high lipophilicity, residues of organochlorine pesticides were still detectable years after use in soils, water, and sediments (Abou-Arab et al. 1995; Sarkar et al. 1997; Yuan et al. 2001). Biodegradation is a very important mechanism for removal of organic chlorinated pesticides from the environment. Under anaerobic conditions, microbial reductive dechlorination was thought to be the predominant process of excluding the chlorine atom from chlorinated hydrocarbons, which are recalcitrant under aerobic conditions. The same characterizations were also found in our previous study on other persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs) (Chen et al. 2001a; Chen et al. 2001b). A variety of organochlorine pesticide residues still exist in the river sediments in Taiwan (Doong et al. 2002). Therefore, a better understanding of microbial degradation of organochlorine pesticides would be quite useful in enabling bioremediation. The present research was designed to improve our knowledge of the anaerobic degradation of DDT and heptachlor in the river sediment by indigenous microorganisms of Taiwan. The effects of environmental factors were also investigated.

MATERIALS AND METHODS

Chemicals *p, p'*-DDT, *p, p'*-DDD [1,1-dichloro-2,2-bis(4-chlorophenyl)-ethane], *p,p'*-DDMU [1-chloro-2,2-bis(4-chlorophenyl)-ethylene], *p,p'*-DDOH [2,2-bis(4-chlorophenyl)-ethanol], heptachlor, and chlordene [4,5,6,7,8,8-hexachloro-3a,4,7,7a-tetrahydro-4,7-methano-1H-indene], with purities of 98%, 97.5%, 99.5%, 99%, 98% and 99.5%, respectively, were purchased from Riedel-deHaën Co, Germany. HPLC-graded solvents used in this experiment, including *n*-hexane and acetone, were purchased from E. Merck Co, Germany. The stock solutions (1 mg/mL) were prepared in acetone and kept in cold storage. Sediment was collected from the Er-Jen River — a seriously contaminated river

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located in southern Taiwan. Each sample was gathered from the sediment surface at a depth of 0 ~ 10 cm with the use of a grab sampler. The collected sample was soaked with river water and sealed in a jar to avoid coming in contact with oxygen, and then rapidly stored at 4℃ for preparation of an anaerobic mixed culture.

The anaerobic mixed culture was prepared in a 1-L serum bottle by adding sediment (100g) to culture medium (400mL), forming a slurry. The culture medium was slightly modified from the study conducted by Chang et al. (2001), and consisted of (in g/L): NH_4Cl (2.7), $MgCl_2 \cdot 6H_2O$ (0.1), $CaCl_2 \cdot 2H_2O$ (0.1), $FeCl_2 \cdot 4H_2O$ (0.02), K_2HPO_4 (0.27), KH_2PO_4 (0.35), yeast extract (1.0), and resazurin (0.001). The medium was neutralized to about pH 7.0 with NaOH. Titanium citrate was then added to a final concentration of 0.9 m*M*, as a reducing reagent. The anaerobic mixed culture was made in a modular atmosphere controller system (Don Whitley Scientific Co, England), with N_2, H_2, and CO_2 gases (85:10:5).

A batch experiment was performed by adding 5 mL of anaerobic mixed culture into a 125-mL serum bottle containing 45 mL of culture medium, then either DDT or heptachlor was added to the bottle at a given concentration per temperature. To avoid oxygen and possible photolysis, the serum bottle was sealed with a butyl rubber stopper capped with an aluminum top and incubated in darkness. For the purpose of investigating the effects of temperature, DDT or heptachlor was added to separate batch cultures to a concentration of 2 μg/mL, and incubated under 10, 20, 30 and 40℃ for 15 days. For the purpose of investigating the effects of chemical concentrations on degradation, separate batch culture of five concentrations, 0.5, 2.0, 5.0, 10.0 and 100 μg/mL (1.41, 5.64, 14.10, 28.21 and 282.1 μ*M*, respectively, for DDT or 1.34, 5.35, 15.29, 26.77 and 267.7 μ*M*, respectively, for heptachlor), were incubated at 30℃ for 15 days. To understand the effects of carbon sources on the degradation rate of DDT (2 μg/mL) or heptachlor (2 μg/mL), three substrates, including yeast extract (1 g/L), sodium acetate (1 g/L), or glucose (1 g/L) were added as the only energy source. The effects of an electron acceptor on degradation of DDT (2 μg/mL) or heptachlor (2 μg/mL) were evaluated by adding sodium bicarbonate, sodium sulfate, and sodium nitrate, separately, to batch cultures to a final concentration of 30 m*M*. All experiments mentioned herein were performed in triplicate. At designated times, 2.0 mL of culture from each treatment was then extracted using sterile syringes and analyzed.

Residues of DDT and heptachlor and their metabolites in sample culture were extracted with 2.0 mL of *n*-hexane three times, and after combining the extracts and concentrating, the sample was analyzed via gas chromatography (6890N network series GC system, Agilent Technologies Co., USA) using a ^{63}Ni electron capture detector (ECD) and a HP-1 fused silica capillary column (film thickness, 0.33 mm; inner diameter, 0.25 mm; length, 30 m, Hewlett Packard Co., USA). Nitrogen was used as both carrier and make-up gas. The flow rate of the carrier gas was 3.5 mL / min (20:1 split ratio). The programmatic column temperature

was set at 170℃ initially for 2 min, and then increased to 210℃ by 2.5℃ / min, held for 2 minutes, and then increased to 250℃ by 10℃ / min, and held for 5 minutes. Injection port and detector temperatures were set at 250 and 300℃, respectively. A GC-MS (HP 6890 series GC system, Hewlett Packard Co., USA) equipped with a HP-1-MS fused silica capillary column was used to identify the metabolites. Helium was used as the carrier gas with a flow rate of 1.0 mL / min, splitless. The programmatic column temperature program was set at 50℃, then increased to 220℃ by 5℃ / min and held for 5 minutes. The mass selective detector was programmed to scan over a mass range of 50 to 400 m / z units at 4.1 scan / sec. The method detection limits for *p, p'*-DDT, -DDD, -DDMU, -DDOH, heptachlor, and chlordene were 0.005, 0.0025, 0.005, 0.0125, 0.00125 and 0.00125 μg/mL, respectively. By adding a mixture of sterile slurry and the standard solution of organochlorine pesticide, the recoveries of these insecticides were measured between the range of 87 and 102%.

RESULTS AND DISCUSSIONS

The effect of temperature on degradation of *p,p'*-DDT and heptachlor under anaerobic sediment mixed culture and on formation of the metabolites, *p,p'*-DDD and chlordene, are shown in Fig.1. Figs. 1A and 1B show the dissipation of DDT and heptachlor, respectively. Although temperature was an important factor affecting the degradation rate, heptachlor showed a greater discrepancy on degradation rates between high and low temperatures than DDT did. On the other hand, incubation in sterilized anaerobic mixed culture showed that the degradation rate was much less than that in unsterilized culture. The results indicated that the dissipation of DDT and heptachlor was attributed to microbial degradation. The observed degradation rates of DDT for incubation at 20, 30, and 40 degrees coincided with each other. More than 95% of added DDT (5μM) was degraded after incubation for 15 days (Fig.1A). Complete disappearance of heptachlor at 30 and 40℃ occurred on the twelfth and the sixth day, respectively. Incubation at 10 and 20℃ showed that 31.5 and 5.8 % of heptachlor, respectively, remained after 15 days (Fig. 1B). Almost no DDT was degraded in the sterilized anaerobic mixed culture (Fig. 1A).

Degradation of DDT and heptachlor is accompanied with evolution of their metabolites, DDD (Fig. 1C) and chlordene (Fig. 1D), respectively. Metabolites DDD and chlordene having a retention time of 13.9 min (DDD) and 4.7 min (chlordene) were analyzed from the GC-ECD chromatograms (Fig. 2). DDD and chlordene were found in all temperature treatments within the first day after incubation. DDD was observed at from 1 to 3 μM in the incubated culture under different temperatures through the culture period (Fig. 1C). The additional metabolites DDMU, with retention time of 11.8 min, and DDOH, with retention time of 10.1 min, were also found during the degradation period of DDT but only in trace amounts. Chlordene, the metabolite of heptachlor, was less than 1 μM during the 15 days of incubation period (Fig. 1D).

In this study, microbial degradation of DDT under anaerobic conditions occurs

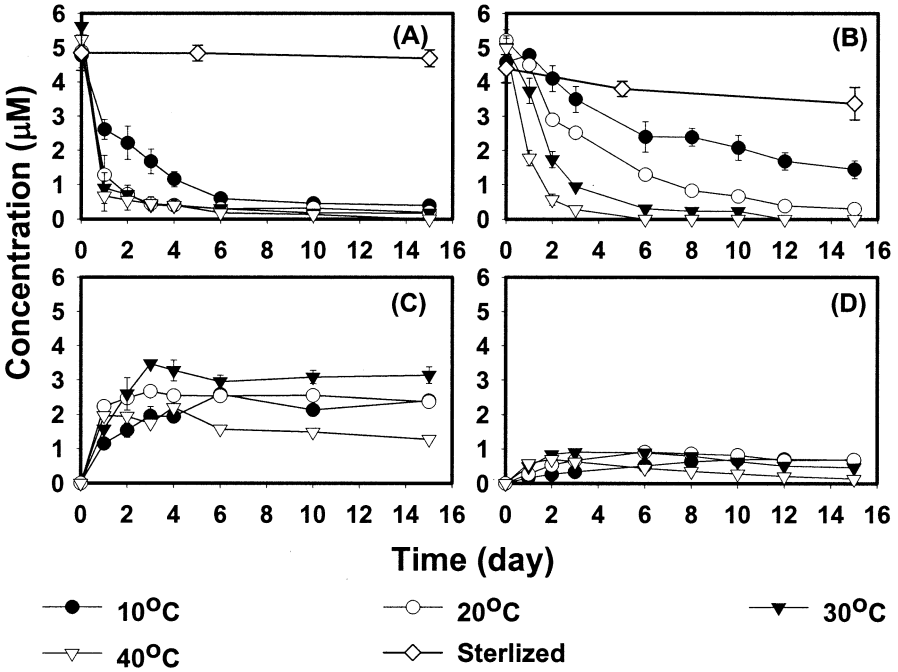

Figure 1. Effect of temperature on degradation of *p,p'*-DDT (A) and heptachlor (B), and on formation of the metabolites *p,p'*-DDD (C) and chlordene (D) under anaerobic mixed culture.

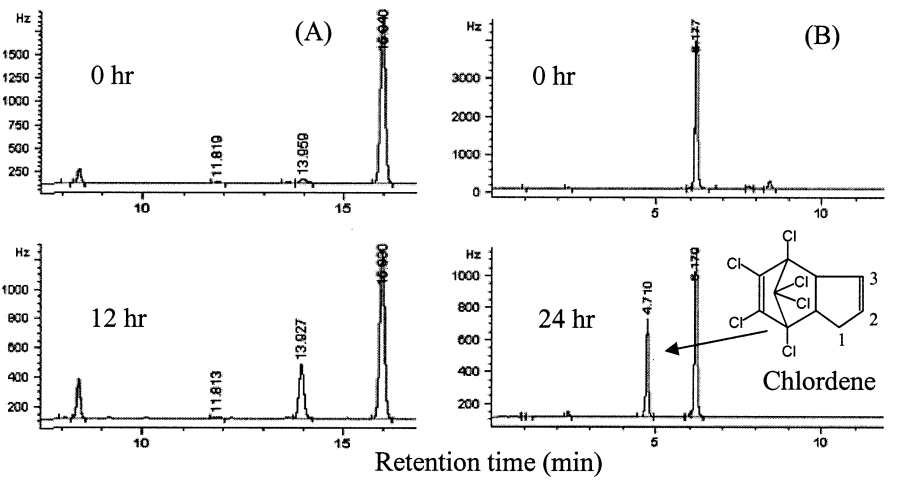

Figure 2. Gas chromatogram of *p,p'*-DDT (A) and heptachlor (B) and incubated for 12 and 24 hours, respectively, in sediment microorganisms under anaerobic condition.

mainly by substituting chlorine on the aliphatic moiety with hydrogen; the mechanism is reductive dechlorination. Our results showed the major product of DDT by dechlorination was DDD although the other metabolites DDMU and DDOH were infrequently observed. Meanwhile, heptachlor was metabolized to chlordene by removing chlorine on C_1, the sole chlorine on the cyclopentene ring, by way of reductive dechlorination. Some literature has reported that heptachlor can be transformed to different metabolites by epoxylation, hydroxylation, and reduction (Carter and Stringer 1970; Miles et al. 1971). The present results under anaerobic conditions indicate that only reductive dechlorination happened and only chlordene was observed.

The effects of varying the initial concentration on degradation of DDT and heptachlor are shown in Fig. 3. The results revealed that both DDT and heptachlor could be degraded by anaerobic microorganisms at 0.5 ~ 10 μg/mL, but not at 100 μg/mL. With 100 μg/mL of DDT or heptachlor, the activity of microorganisms was completely inhibited, and no metabolite was found. This suggests that there is a limiting concentration value for DDT and heptachlor dechlorination, and the dechlorination activity of microorganisms may be inhibited when the concentration exceeds this value.

Metabolism of halogenated hydrocarbons in diverse microbial communities by using alternative electron acceptors to establish different anaerobic conditions has been reported by Häggblom et al. (2000). Using bicarbonate ($NaHCO_3$), sulfate (Na_2SO_4) and nitrate ($NaNO_3$) as the alternative electron acceptors in anaerobic incubation culture can results in the state of methanogenesis, sulfidogenesis and denitrification, respectively (Ghiorse and Wilson 1988). In general, the presence of one of these alternative electron acceptors is thought to enrich specific microbial communities and may affect the degradation rate. The effect of electron acceptors on microbial degradation of DDT and heptachlor was studied, with half-lives shown in Table 1. The half-life of DDT or heptachlor under different anaerobic conditions was fitted to a first order kinetic equation. Therefore, $-dc/dt = KC$, or represented by $\ln(C/C_0) = -kt$, in which C is the concentration of chemicals at time t versus the original concentration C_0, and k is the dissipation coefficient. The half-life ($T_{1/2}$) was calculated from the equation $T_{1/2} = \ln 2 / K$, when $C/C_0 = 1/2$ (Yen et al. 2000). From the results it was learned that the half-life of DDT was much greater with nitrate amendment than with bicarbonate or sulfate amendment. Degradation of heptachlor was retarded after amendment with alternative electron acceptors, not only for $NaHCO_3$ or Na_2SO_4 but also for $NaNO_3$. The degradation rates for heptachlor were inoculated > sulfate-reducing > methanogenic > denitrifying conditions. Both DDT and heptachlor displayed tardy degradation in nitrate-reducing conditions. In the anaerobic incubation culture, electron acceptor retardation of DDT or heptachlor degradation may be attributed to the competition between alternative electron acceptors and DDT or heptachlor (Häggblom et al. 2000).

The effects of carbon source (yeast extract, sodium acetate, or glucose) on the degradation of DDT or heptachlor are shown in Fig. 4. Fig. 4A showed that

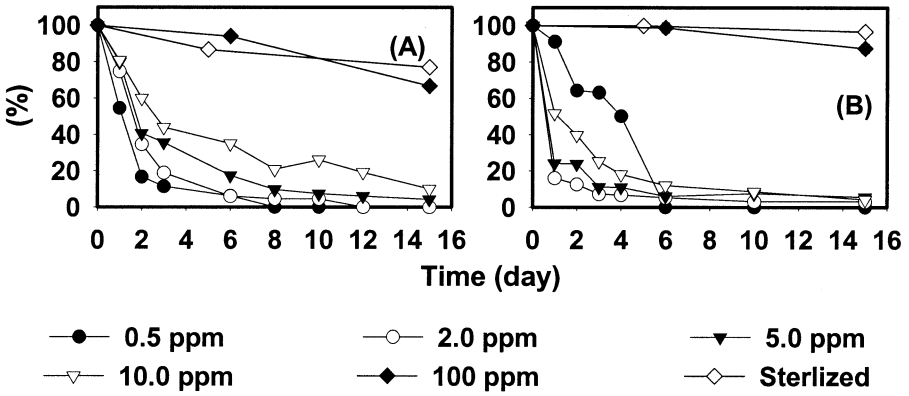

Figure 3. Degradation of *p,p'*-DDT (A) and heptachlor (B) under different initial concentrations at 30℃.

DDT completely disappeared after 15 days of incubation. The degradation rates ($T_{1/2}$) were yeast extract (1.6) > sodium acetate (1.9) > glucose (2.2). A similar result was observed for heptachlor. Heptachlor was almost totally degraded in 12 days (Fig. 4B). The degradation rate ($T_{1/2}$) order was yeast extract (1.4) > sodium acetate (1.7) > glucose (2.2). No metabolites (for both DDT and heptachlor) were found in the sterile control. For anaerobic microbial dechlorination, it is thought that microorganisms utilize organochlorine compounds as electron acceptors and do not cleave the carbon bonds of compounds. Therefore, the dechlorinators need other compounds as the energy source. Dechlorination of organochlorine compounds in the environment usually involves cooperation of several kinds of microorganisms. The data presented herein implied that supplementation of yeast extract might be beneficial to those dechlorinators, or to non-dechlorinating bacteria, by providing nutrients (Wiegel and Wu, 2000), which results in increasing the rate of anaerobic microbial dechlorination.

The literature indicates that DDT and heptachlor still remain in the environment despite having been banned for decades. Many aerobic bacteria have been reported to degrade DDT (Rajkumar and Manonmani 2002; Nadeau et al. 1994). However, only a few studies have focused on the metabolic behavior of anaerobic

Table 1. The half-life of *p,p'*-DDT and heptachlor upon treatment with different electron acceptors

Electron acceptors amended	$T_{1/2}$ (day)	
	DDT	Heptachlor
Inoculated Control	1.86	1.62
Sodium bicarbonate (methanogenesis)	1.63	5.25
Sodium sulfate (sulfidogenesis)	1.85	3.41
Sodium nitrate (denitrification)	6.98	7.45

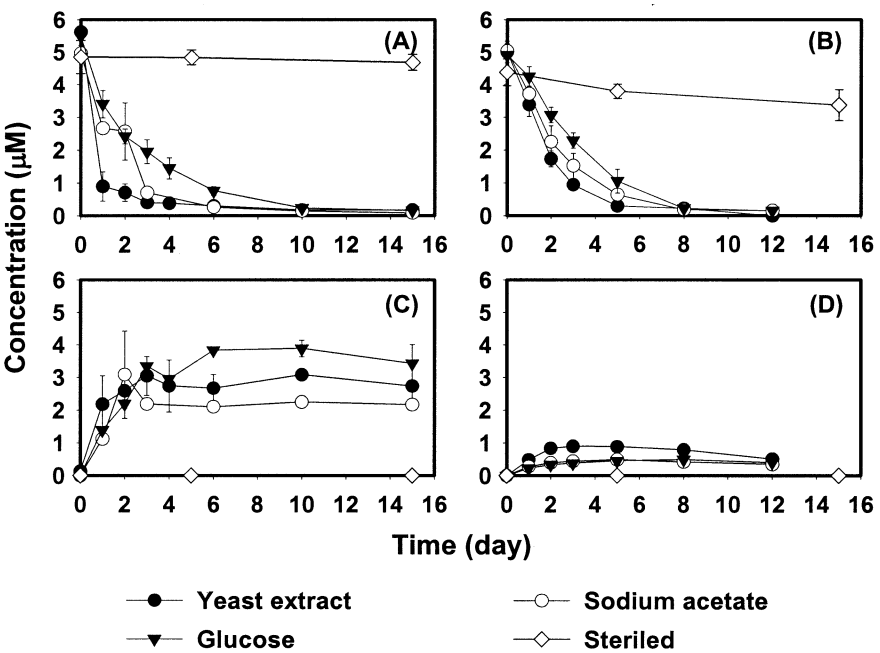

Figure 4. Effect of carbon sources on microbial degradation of *p,p'*-DDT (A) and heptachlor (B), and production of their metabolites, *p,p'*-DDD (C) and chlordene (D) at 30℃.

microorganisms (Huang et al. 2001). Moreover, assessments of biodegradation by indigenous microorganisms are helpful in understanding the dissipation of chemicals in situ. Experimental evidence in this study indicates that factors such as incubation temperature, concentrations of pesticide, presence of utilizable carbon sources, and alternative electron acceptors would affect the dechlorination rate under anaerobic conditions.

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