Methane production in anaerobic degradation of DDT and heptachlor in sediment culture under different conditions

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Introduction

Chlorinated insecticides *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] were extensively used for controlling pests in the agricultural field and human-being living environments in the past several decades. Due to high lipophilicity, they were still detectable many years after applying to the soils.[1, 2] Biodegradation is a very important mechanism for removal of chlorinated pesticides from environments. Under anaerobic conditions, reductive dechlorination of microbes was thought to be the predominant process of excluding the chlorine atom from chlorinated hydrocarbons. In the anaerobic incubation, reduction of substrates such as carbon dioxide, methanol and acetate by methanogenic bacterial through the mode of electron transfer linked phosphorylation to catch energy and formation of methane.[3] This metabolic pathway was processed specifically by methanogenic bacteria, hence, methane production in the anaerobic culture is then become the index of anaerobic toxicity analysis of organic compounds.[4] In order to understand the effect of microorganisms on dechlorination of *p,p'*-DDT and heptachlor, activities of methanogenic bacteria under different concentrations and temperatures was chosen to investigate the potential toxicity of *p,p'*-DDT and heptachlor to microorganisms.

Materials and Methods

p,p'-DDT and heptachlor, both with purities of 98 %, were purchased from Riedel-deHaen Co., Germany. Sediment samples were collected from Er-Jen River, a seriously contaminated river located in southern Taiwan, in a depth of 0 to 10 cm. The collected sediment sample was placed in a jar and soaked with river water, and the jar was sealed and stored at 4°C for preparation of anaerobic mixed culture. The anaerobic mixed culture was prepared in a 1-L serum bottle by adding sediment (100 g) to culture medium (400 mL) in the bottle. Constitutes and prepared method of the culture medium as well as the made of anaerobic mixed culture are described in the previous paper.[5]

The batch experiment was performed by adding 5 mL of anaerobic mixed culture into a 125-mL serum bottle containing 45 mL of culture medium, the either *p,p'*-DDT or heptachlor was added to the bottle at 0, 0.5, 2.0, 5.0, 10 and 100 *m*g/mL of concentrations and incubated for 70 days. For investigating the effects of temperature on methane production, *p,p'*-DDT or heptachlor was added to separate batch cultures to a concentration of 2 *m*g/mL and incubated under 10, 20, 30 and 40°C for 70 days. All experiments were performed in triplicate. Methane production was measured at designed intervals during the incubation of *p,p'*-DDT and heptachlor. Headspace gas of 0.5 mL was removed from the serum bottle using a sterilized syringe at the designed time, and then analyzed by GC-FID (GC-9800 series, China Chromatography Co., Taiwan). In the GC-FID analysis, the temperatures of injector, column, and detector were 100, 80 and 120°C, respectively. Using a flame ionized detector (FID) and a Propark Q column with inner diameter of 0.5 cm and length of 2 m. Nitrogen was used as carrier gas.

Results and Discussion

Amounts of methane occurring in the degradation of different concentrations of *p,p'*-DDT and heptachlor under anaerobic conditions for 70 days are shown in Figure 1. Effect of *p,p'*-DDT on methane production by methanogenic bacteria in anaerobic mixed culture is obvious. Total 59.5 *mM* of methane was produced during 70 days of incubation period in the culture of free *p,p'*-DDT, products of methane decreased obviously by amending 2, 5, 10 and 100 *m*g/mL of *p,p'*-DDT, although no obviously decreased was found in amending with 0.5 *m*g/mL (Figure 1A). Figure 1A also showed the higher concentration of *p,p'*-DDT added the lower methane occurred. The result indicated that presence of *p,p'*-DDT would inhibit methanogenic bacteria to producing methane. In the other hand, amending

with 0.5 and 2 *m*g/mL of heptachlor, the production of methane was decreased than that of control at the early stage, but obviously, more methane was produced than that of control until 70 days of incubation (Figure 1B). Maximum methane product (92.5 *mM*) was showed at amending with 2 *m*g/mL of heptachlor. The ability of methane production was inhibited when the concentration of heptachlor higher than 5 *m*g/mL, however, normal physiological function of methanogenic bacteria was not affected when concentration lower than 2 *m*g/mL. This result indicated that the toxicity of heptachlor to methanogenic bacteria is lower than that of *p,p'*-DDT.

Effects of 2 *m*g/mL of *p,p'*-DDT and heptachlor on the methane production under anaerobic mixed culture at different incubation temperatures are shown in Figure 2. From the results, methane production was very low under 10°C of incubation temperature, and at the higher temperature (20 to 40°C) made the methane production increased. Temperature affects the activity of microorganisms in the anaerobic mixed culture was obvious.

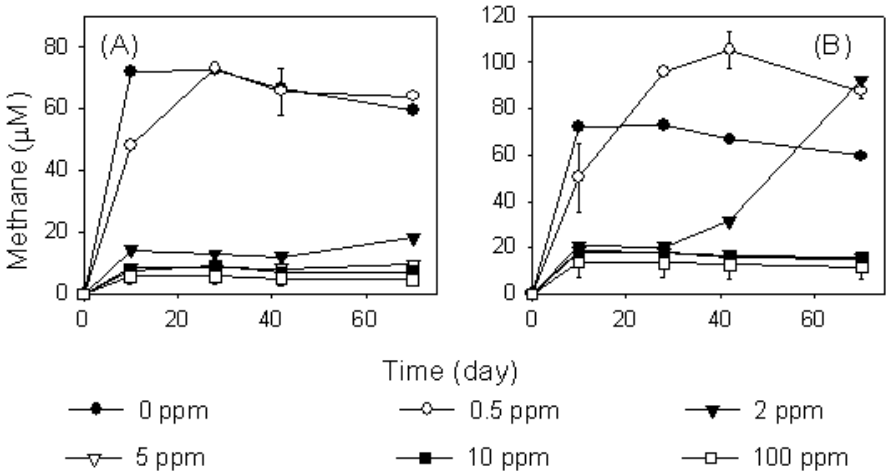

Figure 1. Effects of the different initial concentrations of the organochlorine pesticide *p,p'*-DDT (A) and heptachlor (B) on the methane production under anaerobic mixed culture.

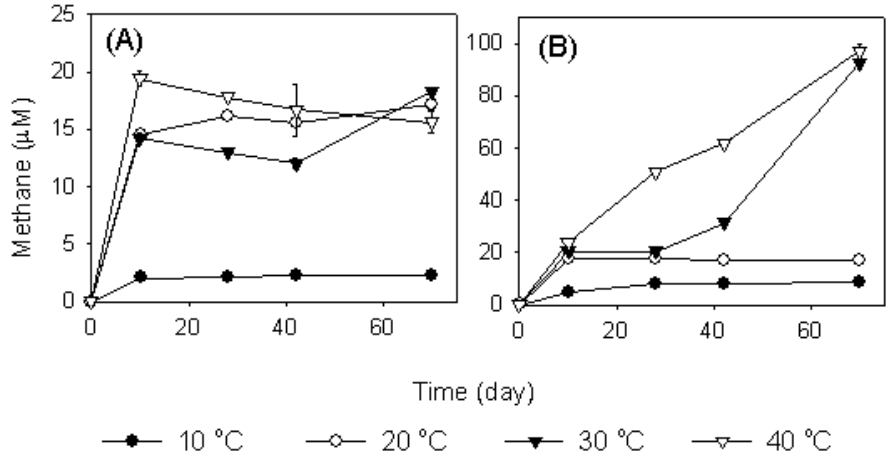

Figure 2. Effects of 2 g/mL of organochlorine pesticides *p,p'*-DDT (A) and heptachlor (B) on the methane production under anaerobic mixed culture at different incubation temperatures.

Acknowledgements

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