THE EFFECT OF BESA AND MOLYBDATE ON DEGRADATION OF ORGANOCHLORINE PESTICIDES ALDRIN DIELDRIN

Tzu-Chuan Chiu, Jui-Hung Yen and Yei-Shung Wang

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

Introduction

Organochlorine pesticide aldrin [1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo- 5,8-dimethanon-naphthalene] and dieldrin [1,2,3,4,10,10-Hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4,5-endo-exo-5,8-dimetha-nonaphthalene] was extensively used in the past. Because of their chemical stability and lipophilicity, aldrin and dieldrin were persistent and recalcitrant in the environment, especially in soil or sediment[1]. Aldrin and dieldrin may be accumulated in animal's tissue or milk and cause some advise effects[2,3]. Therefore, they were classified as category B2 carcinogens by the US Environmental Protection Agency (EPA) in 1987. Up to nowadays, aldrin and dieldrin were still detectable in aquatic environment[4]. To investigate the degradation of organochlorine pesticide under anaerobic conditions is contributed to comprehend the fate of organic chlorinated compounds. There have been reported anaerobic microorganisms play an important role in eliminating organochlrine pesticides[5,6]. But fewer papers have reported the microbial populations involved in degradation of aldrin and dieldrin. Under anaerobic conditions, methanogen and sulfate-reducing bacteria have been regard as involved in degrade chlorinated compounds such as PCB or chloroform[7,8]. In this study, we attempt to investigate the degradation of aldrin and dieldrin by using indigenous microorganisms from river sediment in Taiwan. Besides, in order to understand the roles of methanogen and sulfate-reducing bacteria in degradation of aldrin and dieldrin, microbial inhibitors BESA (2-bromoethanesulfonate) and molybdate were chosen to inhibit the methanogen and sulfate-reducing bacteria, respectively, so as to explain which group of bacteria population is involved in degradation.

Methods and Materials

Chemicals

Aldrin and Dieldrin with 97% and 99%, respectively, pure standards were purchased from Riedel-deHaën Co, Germany. HPLC-graded solvents used in this experiment such as *n*-hexane and acetone were purchased from E. Merck Co, Germany. The stock solutions (1 mg / mL) were prepared in acetone and then stored at 4 .

Preparation of anaerobic mixed stock culture

River sediment was gathered from Er-Jen River, one of a serious contaminated river in southern Taiwan. An Ekman grab sampler is used to collect the river sediment in depth of 0 ~ 10 cm. After collected, the river sediment was stored in jars and readily preparing for the anaerobic stock culture. Anaerobic mixed stock culture was prepared in a 1-L serum bottle by adding sediment (100g) to culture medium (400mL) in a modular atmosphere controller system (dwscientific Co, England) filling with N_2, H_2, and CO_2 gases (85:10:5). The culture medium was slightly modified from Chang

et al[9], which is consisted of (in g/L): NH_4Cl (2.7), $MgCl_2$ $6H_2O$ (0.1), $CaCl_2$ $2H_2O$ (0.1), $FeCl_2$ $4H_2O$ (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 7.0, and added titanium citrate to final concentration of 0.9 mM as a reducing reagent.

Batch experiments

The batch degradation experiments of aldrin and dieldrin were performed by adding 5 mL of anaerobic mixed culture into a 125-mL serum bottle containing 45 mL of culture medium, then 10 μg/mL of aldrin and dieldrin were spiked to serum bottles, respectively. To avoid oxygen involved and possible photolysis, serum bottle was sealed with a butyl rubber stopper capped with an aluminum top and incubated in darkness. In the inhibitor study, BESA and molybdate were additional added to bottles in final concentrations of 5 mM and or 50 mM, respectively. BESA is a structure analog of Coenzyme M (CoM; $HSCH_2CH_2SO_3^-$) which is a cofactor involved in methane biosynthesis, and BESA has been regard as a methanogen inhibitor. Molybdate is an inhibitor which impedes the synthesis of ATP surfurylase, and it was regard as an inhibitor of sufate-reducing bacteria. Therefore, Addition of BESA and molybdate specifically inhibit methanogen and sulfate-reducing bacteria, respectively.

Residue analysis

Residues of aldrin and dieldrin in sample culture were extracted by 2.0 mL of *n*-hexane for three times, and measured with gas chromatography (Agilent technologies 6890N network series GC system, Agilent technologies Co., USA) was equipped with a 63Ni 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 carrier gas was 3.5 mL / min (20:1 split ratio). The column temperature program was set at 170 in initial for 2 min, then increased to 210 by 2.5 / min, held for 2 minutes, and then increased to 250 by 10 / min, held for 5 minutes. Injection port and detector temperatures were set at 250 and 300 , respectively.

Results and discussion

The effect of microbial inhibitor on anaerobic degradation of aldrin and dieldrin was studied and the results are presented in table 1 and 2. The half-life ($T_{1/2}$) of aldrin and dieldrin was fitted to the first kinetic equation and the R^2 was higher than 0.9. The $T_{1/2}$ of aldrin and dieldrin was 39.74 and 115 days, respectively. Degradation of aldrin was obviously delayed by spiking of BESA and molybdate. Table 1 shows the half life of aldrin incubated with or without inhibitors. The degradation of aldrin incubated with inhibitor was obviously slower than inoculated control. Among them, addition of BESA leads delay in aldrin degradation, but concentrations of BESA do not cause any difference in degradation rate. In molybdate-treated cultured, higher concentration of molybdate could slower the degradation rate of aldrin. Table 2 represents the degradation percentage of dieldrin after 140 days of incubation periods, in inoculated control 61.2 % of dieldrin was degraded after 140 days of incubation periods. Both of BESA and molybdate impede significantly the degradation of dieldrin, and the degrees of inhibition were similar between BESA and molybdate-treated culture.

The results of methane production were shown in Fig. 1. Large quantities of methane were produced in inoculated control. In comparison, production of methane was inhibited by treating with BESA and molybdate. The result presented here indicates that methanogenesis was

Table 1. The half-life of aldrin by treating with microbial inhibitors.

Treatment	Aldrin		
	k	$T_{1/2}$ (days)	R^2
Inoculated control	0.017	39.74	0.99
5 mM BESA	0.010	67.66	0.97
50 mM BESA	0.011	63.10	0.97
5 mM Molybdate	0.011.	65.44	0.95
50 mM Molybdate	0.008	83.39	0.95
50 mM BESA+Molybdate	0.009	79.14	0.90

Table 2. The degradation percentage of dieldrin after 140 days of incubation periods by treating with microbial inhibitors.

Treatment	Degradation percentage of dieldrin after 140 days of incubation periods (%)
Inoculated control	61.20
5 mM BESA	25.75
50 mM BESA	25.19
5 mM Molybdate	16.98
50 mM Molybdate	21.14
50 mM BESA+Molybdate	29.05

suppressed regardless of addition of BESA or molybdate. It suggests that molybdate may have inhibited production of electron donors for methanogenesis[10]. Nonetheless, according to our observation in this study, we could deduce methanogens and sulfate-reducing bacterial may involve in the degradation of cyclodiene pesticides, aldrin and dieldrin.

Refernces

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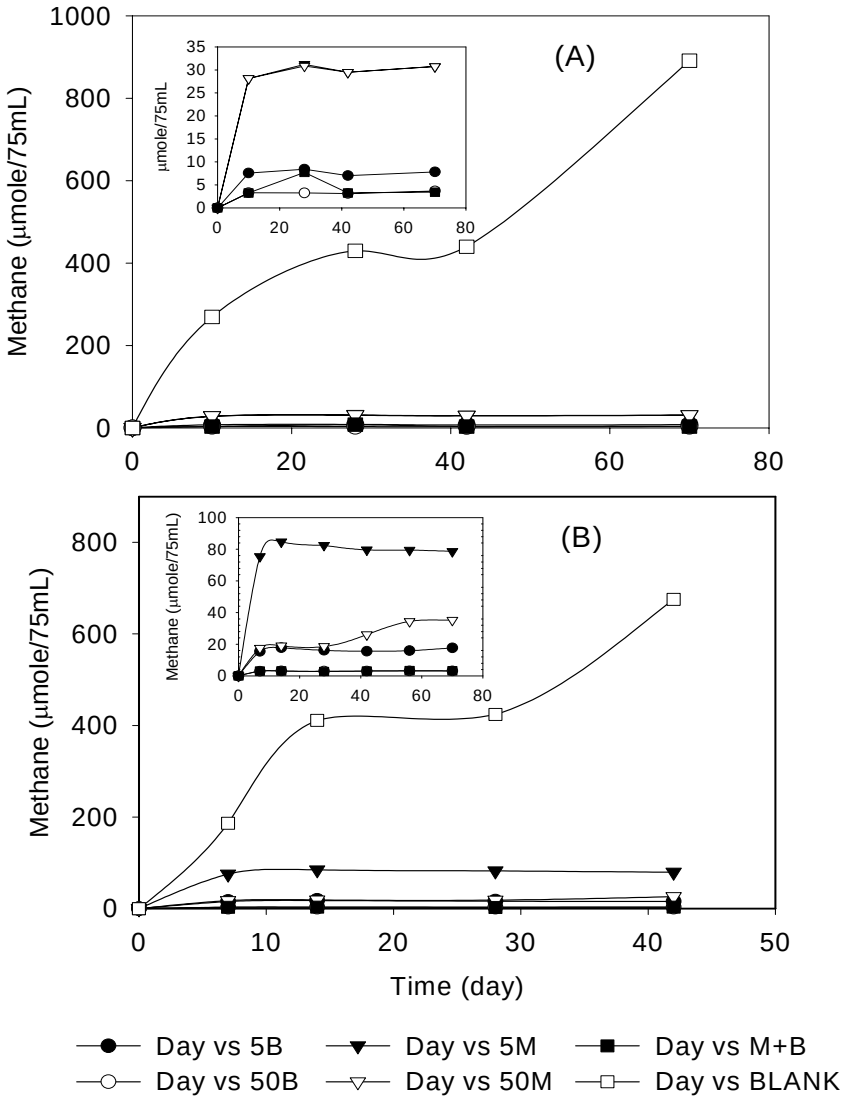

Fig. 1. Methane production by inhibitors-treated culture during the degradation process of alrin (A), and dieldrin (B).