ANAEROBIC MICROBIAL DEGRADATION OF ORGANOCHLORINE INSECTICIDES ALDRIN

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Introduction

Aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanon-naphthalene), a cyclodiene organochlorine insecticide, was banned by nations and classified as B2 carcinogen by United States Environmental Protection Agency (EPA). Because of its chemical stability and lipophilicity, aldrin is regarded as a persistent and recalcitrant compound. Aldrin is easily adsorbed to soil and sediment[1] after spreading to the environments, furthermore, it may be accumulated in animal's tissue or milk and then cause adverse effects[2,3] by food-chain.

The dissipation process of aldrin in environments has continuously been paid much attention by researchers. In general, the dissipation of aldrin has been thought as relating to three mechanisms: photo-degradation, chemical hydrolysis, and microbial degradation. And it has been well known that microbial degradation is the most important agent for breakdown of organochlorine pesticides[4]. There has been shown[5] that aldrin could be transformed to its metabolites, such as dieldrin or photo-dieldrin, by microorganisms under aerobic conditions, however, limited information has been shown under anaerobic conditions. For this reason, the degradation potential of aldrin by anaerobic microorganisms obtained from indigenous river sediment was evaluated, and the effect of environmental factors such as temperatures and nutrients on the aldrin degradation was also investigated in this study.

Methods and Materials

Chemicals: Chemical standard aldrin (1,2,3,4,10,10-hexachloro-1,4,4a,5,8,8a-hexahydro-1,4-endo-exo-5,8-dimethanon-naphthalene) with 97% purity was purchased from Riedel-deHaën Co., Ltd, 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: The river sediment was gathered from the Er-Jen River, a serious contaminated river in southern Taiwan. A grab sampler was used to collect the river sediment in a depth of 0 ~ 10 cm. After collecting, the river sediment was stored in a jar and immediately prepared for incubation. Anaerobic mixed 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 in ratio of 85:10:5. The culture

medium was slightly modified from Chang et al[6], which 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 7.0, and added titanium citrate to final concentration of 0.9 mM as a reducing reagent.

Anaerobic batch experiments: For investigating the effects of environmental factors (different incubation temperatures, aldrin concentrations, and different carbon sources) on aldrin degradation, the anaerobic batch degradation experiments of aldrin were performed by adding 5 mL of anaerobic mixed culture into a 125-mL serum bottle containing 45 mL of culture medium (with different carbon sources), and then aldrin was spiked to serum bottle. In the anaerobic batch experiment, in order to avoid oxygen and possible photolysis involving, the serum bottle was sealed with a butyl rubber stopper capped with an aluminum top and incubated in darkness.

Residue analysis: Residues of aldrin in sample culture was 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 ^{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 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 incubation temperature on the microbial degradation of aldrin (2 μg/mL) is shown in figure 1. According to the result, anaerobic microorganisms were able to degrade aldrin in a wide range among 10 to 40□. The microbial degradation of aldrin under anaerobic conditions was most affected by the incubation temperate of microorganisms. During the degradation periods (105 days), the mixed culture incubated at 40□ exhibit the highest aldrin-degrading activity. Meanwhile, we found that the aldrin degradation agreed with the first-order kinetics. In order to calculate the half-life ($t_{1/2}$), the $t_{1/2}$ of aldrin was fitted to the first kinetic equation and the R^2 was higher than 0.9. The half lives ($t_{1/2}$, days) of aldrin at different temperatures were 22 (40□) > 33 (30□) ≈ 33 (20□) > 44 (10□) in orders. There have been reported[7,8] that the incubation temperature did not only affect the degradation rate but also alter the degradation pathway of organic compound. Herein, we could not infer that the incubation temperature affected the degradation pathway of aldrin because there was no metabolite observed. But based on our experimental consequences, 40□ was the optimal degradation temperature for aldrin under anaerobic conditions.

The effect of aldrin concentration on the anaerobic microbial degradation was also evaluated in this study. On the basis of figure 2, the degradation rates were similar when anaerobic mixed culture incubated with 0.5 to 10.0 μg/mL of aldrin (the calculated half-lives were between 34 and 40). However, the degradation was obviously delayed when the anaerobic mixed culture incubated with 100 μg/mL of aldrin. This result indicated that there is a threshold value for aldrin

degradation in this anaerobic mixed culture and the excess concentration of aldrin may be an obstacle to microbial degradation.

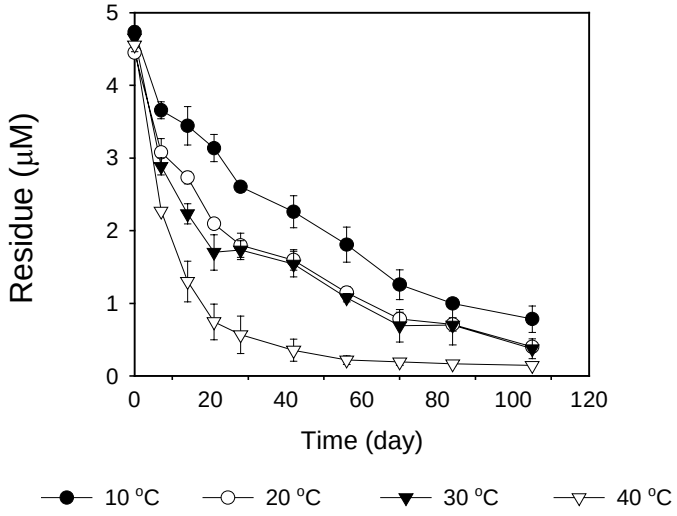

Figure 1: The effect of incubation temperature on the anaerobic microbial degradation of aldrin.

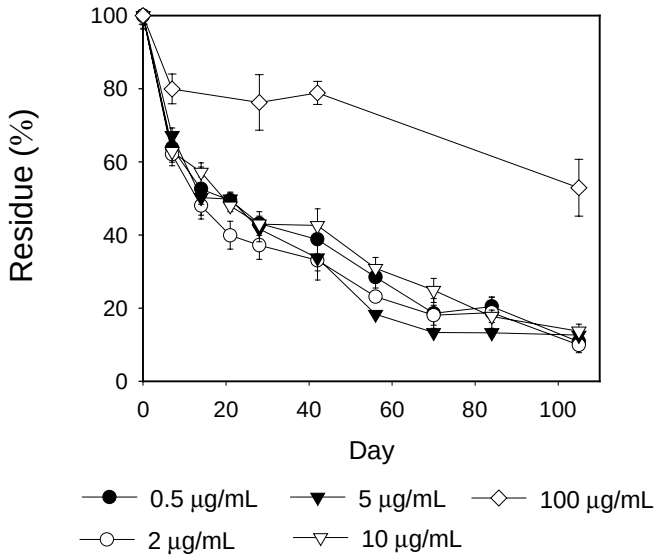

Figure 2: The effect of initial concentration on the anaerobic microbial degradation of aldrin.

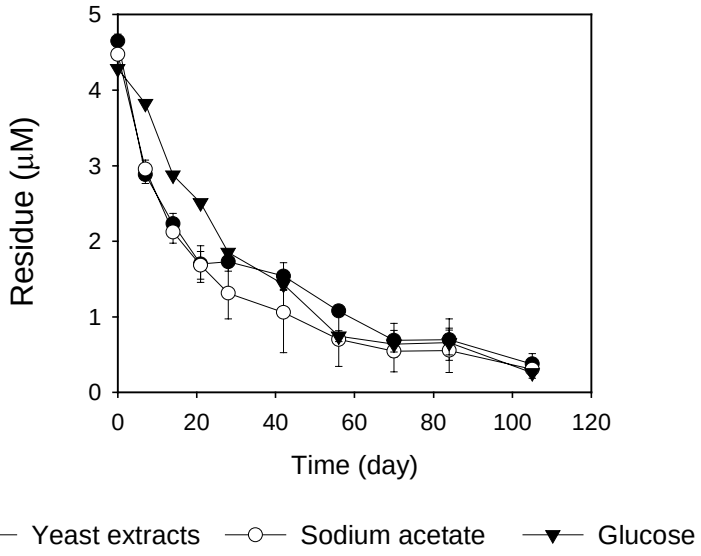

Figure 3: The effect of carbon source on the anaerobic microbial degradation of aldrin.

In this study, yeast extract was used as the major carbon source for microorganisms. For the purpose of revealing the influence of carbon sources on degradation, yeast extract was replaced by alternative carbon source such as sodium acetate or glucose. There has been reported[9] that the degradation rate of organochlorinated compounds might be increased if the favorable carbon source for degrader existed. However, based on the result of figure 3, the degradation rates of aldrin were not significantly affected by using different compounds as major carbon source after 105 days of incubation periods.

References

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