DEGRADATION OF DDT AND HEPTACHLOR BY INDIGENOUS MICROORGANISMS FROM RIVER SEDIMENT OF TAIWAN

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Introduction

In recent years, many persistence organic pollutants (POPs) are serious concerned in the world. DDT and heptachlor are the most persistent organochlorine pollutants[1] and distribute into the environment mainly through agricultural processes use as pesticides. In past decades, because the cheap and efficiency, they are widely used for control the pests. But due to the stable and persistence, these kinds of organochlorine pesticides are amount accumulation in environment. Although they have been banned in developed countries, we can still find the residue in many contained sites. And such as DDT, 1990 levels still as much as 10 times higher than the chronic toxicity criterion established by the U.S. Environment Protection Agency[2].

Because organochlorine pesticides are always recalcitrance to degradation, have potential toxicity and maybe partition into soil and sediment[3], so many researches focused on how to reduce them from environment. Microorganisms play a very important role in the degradation of DDT. There are many researches show DDT can be biotransformed to DDD or DDE in anaerobic or aerobic condition[4], but there are seldom reports investigate what kinds of microorganism communities involve in the degradation upon anaerobic environment. In this study, we attempt to understand the degradation of DDT and heptachlor in three different anaerobic microbial conditions (methanogenic and sulfate-reducing and nitrate-reducing conditions), and try to use indigenous microorganisms to degrade these compounds.

Methods and Materials

Chemicals

DDT [1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane] and heptachlor [1,4,5,6,7,8,8–heptachloro -3a,4,7,7a-tetra-hydro-4,7-methanoindene] with 99 % purity were purchased from Aldrich Chemical Co. All other compounds needed in this experiment were purchased from Sigma Co (St. Louis). Sovent used in this experiment was HPLC-graded and purchased from Merck Co.

Sediment

Anaerobic sediment was obtained from Er-Jen River – one of the serious contaminated rivers in southern Taiwan. Sampling method is using an Ekman grab sampler to collect the river sediment below the surface 0 ~ 10 cm in July 2001. After the anaerobic river sediment was collected, it was stored in jars and then used to prepare the anaerobic seed culture.

Anaerobic seed culture

Anaerobic seed culture was established in a 1 L serum bottle with 400 ml anaerobic medium and 300 g anaerobic sediment, the anaerobic medium was modified from Chang[5] and consisted of (all concentrations in g/l) : NH_4Cl, 2.7; $MgCl_26H_2O$, 0.1; $CaCl_22H_2O$, 0.1; $FeCl_24H_2O$, 0.02; K_2HP0_4, 0.27; KH_2P0_4, 0.35; yeast extract, 1.0; resazurin, 0.001. Medium pH was adjusted to 7.0 following

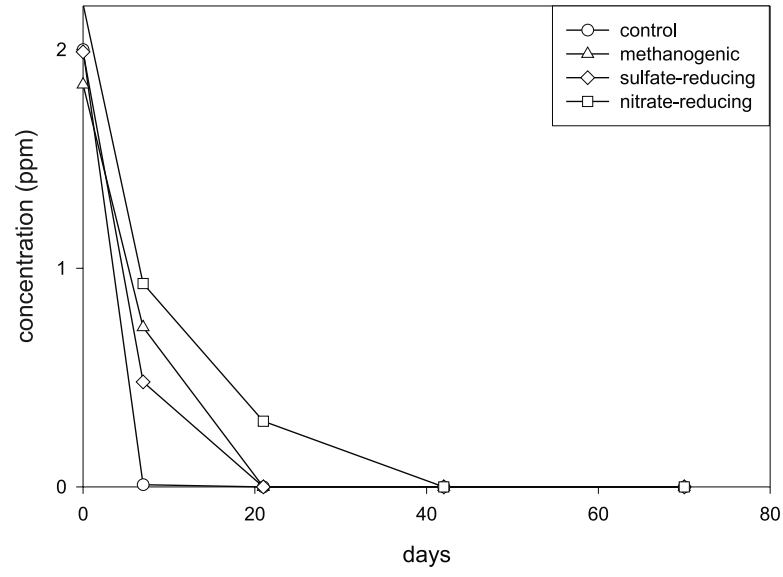

Figure 1. Degradation of heptachlor under anaerobic conditions.

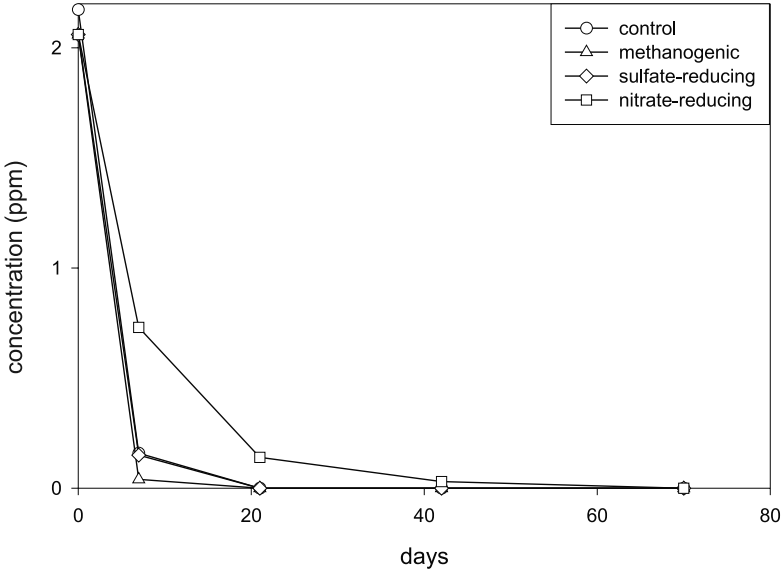

Figure 2. Degradation of DDT under anaerobic conditions.

autoclaving; 0.9 mM titanium citrate was added as a reducing reagent. Three defined conditions including: methanogenic conditions (20 mM $NaHCO_3$ added), sulfate-reducing conditions (20 mM $NaSO_4$ added), nitrate-reducing conditions (20 mM $NaNO_3$ added) and inoculated control (absence of sodium sulfate, sodium nitrate or sodium hydrogen carbonate). All operations were progressed in an anaerobic glove box filled with N_2 (85 %), H_2 (10 %), and CO_2 (5 %) gases.

Procedures

DDT and heptachlor degradation test were performed using 125 ml serum bottles containing 45 ml medium, 5 ml anaerobic seed culture, and spiked 2 ppm DDT or heptachlor. Serum bottles were sealed with butyl rubber stoppers and capped with aluminum foil. To prevent photolysis, all the bottles were incubated without shaking at 30? in darkness. All experiments were performed in triplicate.

Extraction and measurement

Extraction was performed in 15 mL tubes, by adding 2 mL *n*-hexane and shaking for 1 minute, after the initial *n*-hexane layer was removed, culture was extracted with two additional *n*-hexane treatments. The concentration of DDT and heptachlor were measured with a gas chromatograph (Hewlett-Packard 6890 series N) equipped with an 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). Nitrogen was used as both a carrier and make-up gas with flow rates of 2.5 mL/min (20:1 split ratio). The column temperature program was set at 170^o in initial for 2 min, then increased by 2.5 ml/min to 210^o, where it was maintained for 2 min before being increased by 10 ml/min to 250^o, where it was maintained for 5 min. Injector and detector temperatures were set at 250 and 300^o, respectively.

Result and discussion

The degradation of heptachlor is shown in Fig 1, after adding different compounds (NaHCO3, Na_2SO4, NaNO3) , we can enrich three different major microbial communities and an inoculated control without adding above compounds, from the result; the degradation kinetic of heptachlor was fitted the first order reaction, then the degradation rate was fastest in sulfate-reducing culture, but slowest in nitrate-reducing culture, and the half life of control, methanogenic, sulfate-reducing and nitrate-reducing were 7, 5.25, 3.41 and 7.44 days, respectively. The degradation of DDT is shown in Fig 2. In anaerobic condition, all of three treatments including inoculated control can transform DDT to DDD, and DDD was accumulated in the medium culture (data not shown). The degradation of DDT was fitted the psedo first order reaction, and calculated to be the half life (in days) of control, methanogenic, sulfate-reducing and nitrate-reducing were 1.85, 1.23, 1.85 and 1.98 days, respectively. From the experiment, the results provided not only the anaerobic microorganisms from river sediment in Taiwan have the potential to degrade heptachlor and DDT, but also showed the major microbial community related to the anaerobic degradation of DDT and heptachlor. The results can also used to further define operating parameters for river sediment bioremediation.

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