CHAPTER V

EVALUATION OF DEGRADABILITY OF AROCLOR 1242 BY AN ACETICLASTIC METHANOGENIC CONSORTIUM

5.1 ABSTRACT

The rate and extent of degradation of Aroclor 1242 from contaminated surface soil was investigated, using aceticlastic mathanogenic consortia enriched from municipal anaerobic digester. Two different experiments, "intermediate feed" and "starve and feed" were conducted using triplicate 160-mL serum bottle microcosms, containing either 0.1 g, 1 g, 10 g, or 50 g of soil plus 100 mL of the inoculum. The methanogenic consortia in both the experiments were manipulated by feeding them with glacial acetic acid at different time intervals, during 30 days incubation. The fate of Aroclor 1242 in both the experiments was monitored using GC-ECD and GC-MSD analysis. Significant differences were observed between inoculated and uninoculated (control) microcosms, thus suggesting that methanogenic consortium might have been responsible for the degradation of Aroclor 1242. However, GC-MSD results could not confirm that disappearance of PCB was due to anaerobic dehalogenation. Another experiment was performed to evaluate if the removal of Aroclor 1242 was due to its evaporation. It was confirmed that disappearance of Aroclor 1242 was not due to evaporative losses during sample extraction. The actual reason for Aroclor 1242 disappearance from the surface soil microcosms could not be determined from this study.

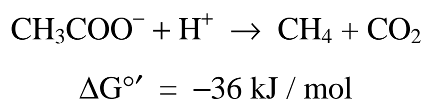
5.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are pollutants that have attracted great concern due to their persistence in the environment and potential health hazards. Because of their chemical inertness, stable molecular structure and hydrophobicity, the breakdown of PCBs is very slow in the environment. Despite of the difficulties to degrade these complex chlorinated compounds, extensive research has been done on the degradation of this group of environmental contaminants. Microbial metabolism of PCBs is one of the major routes of environmental degradation of these widespread pollutants. Aerobic PCB biodegradation by a variety of bacteria

has been documented (Abramowicz, 1990; Abramowicz and Olson, 1995; Bedard et al., 1987; Boyle et al., 1992; Chen et al., 1988; Flanagan and May, 1993; Yadav et al., 1995). Under anaerobic conditions, PCBs can be reductively dechlorinated by a variety of anaerobes (Berkaw et al., 1996; Beurskens and Stortelder, 1995; Kim and Rhee, 1997; Nies and Vogel, 1990; Nies and Vogel, 1991; Quensen III et al., 1990; Rhee et al., 1993; Tiedje et al., 1993; Williams, 1994).

Anaerobic reductive dehalogenation is an important step toward the remediation of PCBs since it reduces the chlorine content of PCB mixture and thus, reducing their bioaccumulation potential and toxicity. Methanogens play an important role in the reductive dechlorination of PCBs (Ye et al., 1995).

Aceticlastic (acetotrophic) methanogens can utilize acetate as growth substrate. These group of microbes ferment acetate to CO_2 and CH_4 as follows:



$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$$
$$\Delta G^{\circ\prime} = -36 \text{ kJ / mol}$$

Only two genera, *Methanosarcina* and *Methanothrix* and a few species can ferment acetate to CO_2 and CH_4 (Ferry, 1993; Ferry, 1995; Fukuzaki et al., 1990; Jones et al., 1987). *Methanosarcina* spp. can utilize acetate at a high rate and has a lower affinity (K_m) for acetate. *Methanothrix* spp. on the other hand are characterized by lower rate of acetate utilization and a higher affinity for acetate (Fukuzaki et al., 1990; Schonheit et al., 1982; Zinder and Mah, 1979). Hence, depending on the concentration of acetate in the environment, either *Methanosarcina* spp. or *Methanothrix* spp., whichever can effectively utilize acetate would outcompete the other for acetate. Thus, by adjusting the acetate concentration to low or high, one predominant species of aceticlastic methanogenic population can be achieved. It is important to monitor the pH of the culture medium since methanogenesis from acetate proceeds maximally between pH 6 and pH 8 (Fukuzaki et al., 1990). If the pH drops below 6, methanogenesis from acetate inhibits, thus accumulating acetate in the environment.

The central enzyme in the pathway of methanogenesis from acetate is a CO dehydrogenase (CODH) complex, which catalyzes the cleavage of acetyl-CoA (Ferry, 1995; Lovley, 1984;

Terlesky et al., 1986). The CODH complex in methanogens consists of vitamin B_{12}, which is known to catalyze reductive dehalogenation of chlorinated phenols (Smith and Woods, 1994). Purified CODH from aceticlastic methanogens have been shown to reductively dechlorinate TCE (Jablonski and Ferry, 1992). Although, no direct evidence has been shown so far on the role of CODH in the reductive dehalogenation of PCBs, it is possible that CODH plays a key role in PCB dechlorination in environments where methanogenic fermentation dominates. Aceticlasic methanogens are very efficient in energy conservation. It may be possible to manipulate methanogens to enhance dechlorination activity. CO dehydrogenase could be very active when the organism has a limited amount of growth substrate. With limited amount of growth substrate, the metabolism would be much faster, which may result in higher rate of PCB dehalogenation.

Bromoethane sulfonic acid (BESA) is a potent inhibitor of methanogenesis (Fathepure and Boyd, 1988; Genthner et al., 1989; Ye et al., 1995). Morris et al (1992), showed the inhibitory effect of BESA on PCB dechlorination. PCB-dechlorinating microorganisms could use the PCBs as terminal electron acceptors in the absence of a more preferred electron acceptor. Ye et al (1995), reported that BESA is a potential electron acceptor for some bacteria, and thus it inhibits PCB dechlorination by non-methanogens by competing with PCBs for electrons.

An extensive amount of research has been done on the biodegradation and dechlorination of PCBs on freshwater, estuarine and marine sediments, but not much work has been done on the degradation of PCBs in soil. Although, estuarine and marine sediments are global sinks for accumulation of PCBs, still a large concentration of PCBs is present in soil.

Bioaugmentation is a process by which microorganisms can be applied to soil under controlled conditions. Bioaugmentation of surface soils with strict anaerobic populations like methanogens is possible. The soil used in this study was an aged, well aerated surface soil. The objective of this study was to analyze the rate and extent of degradation of Aroclor 1242 in a contaminated surface soil, by an aceticlastic methanogenic consortium. This feasibility study dealt with the bioremediation of the contaminated surface soil by bioaugmentation.

5.3 MATERIALS AND METHODS

5.3.1 PCB Contaminated Soil

Aged PCB contaminated surface soil was provided by BioSystems Technology, Inc. (Blacksburg, VA). The soil was taken from a site, which was previously contaminated with Aroclor 1242. The soil batch used for intermediate feed experiment had an Aroclor 1242 concentration of 1739 mg/kg, while the soil used for starve and feed and the BESA experiments had 1521 mg/kg of Aroclor 1242. The soil was sieved through a 2mm sieve, stored in airtight containers and refrigerated at 4° C until use. The soil had 10% moisture content.

5.3.2 Chemicals

The chemicals used to prepare the mineral salt medium were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Yeast extract and BESA were purchased from Sigma Chemical Co. (ST. Louis, MO). Aroclor 1242, and 525, 525.1 PCB Mix standards were purchased from Supelco (Bellefonte, PA). All other chemicals were reagent grade.

5.3.3 Culture Conditions

Sewage sludge was collected from the Peppers Ferry Regional Wastewater Treatment Plant (Radford, VA) and stored at 4° C, prior to use. The mineral salts anaerobic media used in this study were prepared in three 2-liter Erlenmeyer flasks. The medium in each flask consisted of 0.54 g of KH_2PO_4, 0.7 g K_2HPO_4, 1.06 g NH_4Cl, 0.2 g $MgCl_2.6H_2O$, 0.146 g $CaCl_2.2H_2O$, 0.04 g $FeCl_2.4H_2O$, 2 mL of trace metal solution and 1 mL of 0.1% resazurin redox indicator in 2 liter of deionized water. To the medium in each flask, 24 g of $NaCH_3CO_2$ was added as substrate. The media were autoclaved for 15 minutes to remove O_2 and then cooled by maintaining under a positive pressure of N_2 that was previously passed through hot (300° C) copper fillings to remove traces of O_2. When the media reached room temperature, 2.4 g $NaHCO_3$, 0.24 g $Na_2S.9H_2O$ and 0.4 g of 0.02% yeast extract was added to each flask and pH of the media was adjusted to 7.0. From each flask, 1600 mL of media was taken out in 2-liter Erlenmeyer flasks

and 400 mL of sludge was added to each flask. The flasks were sealed tightly with rubber stoppers and were degassed every few days to release the gas pressure build up.

In an effort to manipulate methanogens to enhance dechlorination activity, the culture flasks were treated differently. One flask containing the culture (designated Reactor 1, started with sludge collected 06/24/96) was amended with 2 mL of 17 mM acetic acid every 5 days whereas the second flask (Reactor 2, started with sludge collected 07/18/96) was amended with 2 mL of 17 mM acetic acid every 9-10 days (assuming that all the acetate was used up by the culture within this time period, based on the complete cessation of gas production in the microcosms). The third culture (Reactor 3, started with sludge collected 07/18/96) was amended with 0.2 mL of 17 mM acetic acid every 5-6 days. Reactor 1 was setup to enrich for *Methanosarcina* spp., which could utilize acetate at high rates. On the other hand, Reactor 3 was setup to enrich for *Methanothrix* spp., which could utilize acetate only in lower concentrations. After 30 days of incubation, aceticlastic methanogenic cultures were established in the Reactors, which was confirmed by the detection of methane in the headspace of the flask.

5.3.4 Microcosms and Controls

Intermediate Feed Microcosms

Various amounts (triplicate sets of 0.1 g, 1g, 10g and 50 g) of the PCB contaminated soil were transferred into 160-mL serum bottles. One hundred mL of the methanogenic inoculum from Reactor 1 was dispensed into each serum bottle while sparging with deoxygenated N_2 using a Hungate gassing apparatus. All the microcosms were sealed with thick butyl rubber stoppers and aluminum crimp seals, shaken thoroughly and incubated at 31° C for one month. After degassing the bottles every 3-4 days, the microcosms were amended with 0.1 ml of 17 mM glacial acetic acid, shaken well and incubated.

Starve and Feed Microcosms

Triplicate sets of microcosms, consisting of 0.1 g, 1 g, 10 g and 50 g of PCB contaminated soil and 100 mL of the methanogenic inoculum from Reactor 2 were setup in 160-mL serum bottles,

while flushing with oxygen-depleted N_2. Another triplicate set of microcosms containing 10 g of soil and 100 mL of inoculum from Reactor 2 were also setup. These bottles were incubated and sacrificed periodically (7, 14 and 21 days) to study the pattern of PCB degradation. Triplicate set of microcosms containing 10 g of soil and 100 mL of inoculum from Reactor 3 were also setup. All the microcosms were sealed with thick butyl rubber stoppers and aluminum crimp seals, shaken thoroughly, and incubated at 31° C for one month. Each of the microcosms, setup from Reactor 2 and from Reactor 3 were degassed every 8-9 days, and amended with 0.1 mL and 0.01 mL of 17 mM glacial acetic acid, respectively

BESA Experiment

Triplicate set of microcosms consisting of 10 g of PCB contaminated soil and 100 mL of inoculum from Reactor 2 were setup by flushing with O_2-free N_2. They were added with 0.2 mmol L^{-1} of BESA inhibitor, prepared in distilled water. Microcosms were sealed with thick butyl rubber stopper and aluminum crimp seals, and incubated at 31° C for one month. They were treated similar to the microcosms (from Reactor 3) from starve and feed experiment.

Controls

Two triplicate set of controls were prepared by adding 100 mL of freshly prepared mineral salts media to 10 g of PCB contaminated soil in 160-mL serum bottles. No inoculum was added to these bottles. The bottles were sealed and incubated like the microcosms. One set of controls were treated similar to the microcosms from intermediate feed experiment while the other set were treated as the ones from starve and feed experiment.

5.3.5 Extraction of Aroclor 1242 from Soil

Following incubation, the bottles were stored at 4° C prior to soil extraction. In preparation for extraction, the microcosms were well shaken, and the contents transferred to beakers. The soil was dried with low heat (40° C) on a hot plate (Thermolyne) to evaporate any water layer. Methylene chloride : acetone (1:1 v:v) (100 mL) was added to the dried soil in the beaker, and

mixed thoroughly with a spatula. Aroclor 1242 was extracted from the soil by Sonication extraction procedure (EPA Method 3550).

An Ultrasonic Cell Disrupter (Ultrasonic, Inc., Model W-385; 475 watt) Sonicator with a Tapped Disrupter Horn (No. 207 3/4″), equipped with a titanium tip was used for the extraction. The bottom surface of the tip of the #207 3/4″ disrupter horn was placed about 1/2″ below the surface of the solvent in the beaker, but above the soil layer. The soil slurry extraction mixture was sonicated for 3 minutes, with output control knob set at 50%. Extracts were then decanted and filtered through Whatman No. 41 filter paper into a clean flask. The extraction was repeated with another 100 mL of 1:1 methylene chloride : acetone. This time, the entire sample was poured into the Buchner funnel, rinsed with extraction solvent, and filtered into the flask. Sonication extraction was performed for all the samples and the extracted solvent (containing Aroclor 1242) were collected in different flasks.

A Kuderna-Danish (K-D) apparatus was assembled by attaching a 10-mL graduated concentrator tube (Kontes K-570050-1025) to a 500-mL evaporator flask (Kontes K-570001-0500). The extracted solvent was poured into the K-D concentrator. The extractor flask was rinsed with some more of the extraction solvent to make sure that all of the extracted PCB was transferred into the K-D concentrator. To the evaporative flask, 2-3 clean boiling chips (solvent extracted, approximately 10/40 mesh) were added. A three-ball macro Snyder column (Kontes K-503000-0121) was attached to the evaporator flask. The Snyder column was prewetted by adding about 1 mL methylene chloride to the top. The K-D apparatus was placed on a hot water bath (80-90° C) with the concentrator tube partially immersed in hot water and the entire lower rounded surface of the flask bathed with hot vapor. The vertical position of the apparatus and the water temperature was adjusted accordingly, to complete the concentration in 15-20 minutes. When the apparent volume of liquid reached 1 mL, the Snyder column was removed and 100 mL of hexane (exchange solvent) and a new boiling chip was added to the K-D concentrator, and the Snyder column was reattached. The extract was concentrated by raising the temperature of the water bath, to maintain proper distillation. When the liquid reached an apparent volume of approximately 5 mL, the apparatus was removed from the water bath, and allowed to drain and cool for 10 minutes. The Snyder column was removed and its lower joint was rinsed into the

concentrator tube with approximately 0.5 mL of hexane. The final volume of the solvent was adjusted to 10 mL with hexane. The concentrated extract was then transferred to clean screw-cap vial. This procedure was repeated for all the samples and the extracts were collected in vials. The vials were labeled and stored in the dark at 4° C, until they were analyzed.

To check if any PCB was lost during the extraction process due to evaporation or adsorption into the glassware, a small experiment was conducted. In 6 flasks, 10 ppm of Aroclor 1242 and 2 mL of methylene chloride were taken. No soil was added. The contents were shaken well and evenly dispersed in the sides of 500-mL Erlenmeyer flasks as thin film. To 3 out of the above 6 flasks (dispersed with Aroclor 1242), 100 mL of freshly prepared anaerobic media was added and these samples were ready for GC analysis. To the remaining three flasks, 100 ml of the media was added and it was evaporated up to 10 mL using a K-D concentrator. Then, it was exchanged with 100 ml of hexane, and the contents were boiled until only 10 ml of the solvent was left. These concentrated extracts were diluted with hexane, and were injected into a GC to determine the final PCB concentration.

5.3.6 Analysis by GC-ECD

The concentrated extracts were diluted with hexane to reach a concentration of one part per million (ppm). Different dilution factors were used for different amount of soil (0.1 g, 1 g, 10 g and 50 g), to reach an Aroclor 1242 concentration of 1 ppm in each sample. In preparation for GC-ECD analysis, the extracted PCB samples were then transferred to small auto sampler glass vials capped with Teflon-lined lids. Two µL of each sample was injected into a Hewlett-Packard Model 5890 Series II gas chromatograph (BioSystems Technology, Inc.) equipped with an automatic sampler, an electron capture detector (ECD), and fitted with a HP-5 capillary column (30 mm by 0.53 mm internal diameter, 0.88 µm film). Gas chromatographic conditions were based on EPA Method 8080. The injector and detector temperatures were maintained at 200°C and 290°C, respectively. The oven program consisted of an initial temperature of 150°C, an increase rate of 5°C/min to 280°C, and maintenance at that temperature for 10 minutes. The flow rate of the carrier gas, helium, was 60 mL min^{-1}. Final Aroclor 1242 concentration in the samples (after 30 days of incubation) was determined by comparing the total area under the

chromatograms of the samples with the total area of a 1 ppm Aroclor 1242 standard and multiplying by the appropriate multiplication factor.

5.3.7 Analysis by GC-MSD

Samples were also analyzed by GC-mass spectrometry (GC-MS) with a Hewlett-Packard 5890A GC (Virginia Tech) equipped with an automatic sampler and HP-5MS column (30 mm by 0.25 mm internal diameter, 0.25 μm film), connected to a Hewlett-Packard 5890 mass selective detector (MSD). The injector and GC interface detector temperatures were maintained at 250°C and 285°C, respectively. The oven program consisted of an initial temperature of 70°C for 2 min, an increase rate of 20°C/min to 210°C, and maintenance at that temperature for 1 minute. The flow rate of the carrier gas, helium, was 1 mL min^{-1}. The mass spectrometer was operated at electron energy of 70 eV. The ion source pressure was maintained at 2.3×10^{-5} Torr.

5.4 RESULTS AND DISCUSSION

Following a 30 day incubation, decrease in the amount of extractable Aroclor 1242 was observed in the serum bottle microcosms. The extent of removal varied between intermediate feed and starve and feed treatments. For the intermediate feed treatments, average Aroclor 1242 removal of up to 53.2% of was observed in 30 days (Table 5.1), while for starve and feed treatments, average removal, up to 66.5% was observed (Table 5.2).

The percent Aroclor 1242 removal decreased as the amount of soil per microorganism increased. For both the experiments, the maximum percent removal or reduction in extractable PCB was observed for microcosms with low Aroclor 1242 content (Tables 5.1 and 5.2). Microcosms with higher Aroclor 1242 content showed a minimum difference in extractable PCB. It might be possible that Aroclor 1242 was toxic to the methanogenic consortium in microcosms containing the greater amount of PCB contaminated soil. It is also possible that efforts to remove oxygen from the soil prior to inoculum addition was incomplete, which could have resulted in an oxygen toxicity problem in the initial phase of microcosm start up.

Table 5.1. Aroclor 1242 content in intermediate feed soil microcosms.

Microcosm treatment	mg Aroclor 1242 per microcosm at day 0	mg Aroclor 1242 in microcosms at day 30 Average ± SD	Average amount (mg) of Aroclor 1242 removed at day 30	% Aroclor 1242 removed in 30 days Average ± SD
0.1g/100mL	0.17	0.08 ± 0.01	0.09	53.45 ± 8.68
1g/100mL	1.74	1.17 ± 0.03	0.57	32.43 ± 1.81
10g/100mL	17.39	9.68 ± 1.6	7.71	44.34 ± 9.23
50g/100mL	86.95	61.18 ± 3.95	25.77	29.64 ± 4.54
Control	17.39	16.78 ± 0.29	0.61	3.56 ± 1.65

Table 5.2. Aroclor 1242 content in starve and feed soil microcosms.

Microcosm treatment	mg Aroclor 1242 per microcosm at day 0	mg Aroclor 1242 in microcosms at day 30 Average $\pm$ SD	Average amount (mg) of Aroclor 1242 removed at day 30	% Aroclor 1242 removed in 30 days Average $\pm$ SD
0.1g/100 mL	0.15	0.06 $\pm$ 0.03	0.09	58.36 $\pm$ 17.66
1.0g/100mL	1.52	0.51 $\pm$ 0.06	1.01	66.58 $\pm$ 4.08
10g/100mL	15.21	7.57 $\pm$ 3.1	7.64	50.26 $\pm$ 20.39
50g/100mL	76.06	63.88 $\pm$ 8.36	12.18	16.03 $\pm$ 10.99
Control	15.21	14.01 $\pm$ 1.64	1.20	7.88 $\pm$ 10.77

The extent of Aroclor 1242 extractable was greater for most of the starve and feed treatment compared to the intermediate feeding regime. This could be due to the fact that the soils used for the intermediate feed and starve and feed experiments had a different amount of Aroclor 1242 to start with, and also the methanogenic cultures were inoculated from sludge collected at different times and were cultured under different treatment conditions. Another possibility was that the starve and feed enhanced methanogenic activity and thus degradation activity. The starve and feed treatment was established by providing the methanogens with a limited amount of growth substrate (acetate). Acetate limitation, could have resulted in stimulation of cometabolism activity. It was suspected that CO dehydrogenase, present in acetotrophic methanogens, played a significant role in Aroclor 1242 degradation in the starve and feed treatments.

The treatments containing the inoculum from Reactor 3 were treated with a low rate of acetate feeding to enrich *Methanothrix* spp. Only up to 21.66 ± 7.46% Aroclor 1242 removal was observed in microcosms containing 10 g of the soil. The extent of Aroclor 1242 removal was much smaller in these treatments as compared to the percent removal of the other microcosms containing the same amount of soil (10 g/100 mL treatments from intermediate and starve and feed experiments).

Loss of PCBs due to evaporation or adsorption to the inoculation vessel has often been mistaken as biodegradation. The physical loss of PCB due to evaporation often causes false positive results in biodegradation experiments (Dercova et al., 1996). From this study, it was confirmed that evaporation or adsorption into the glassware was not the cause of disappearance of Aroclor 1242 from the contaminated soil. I did not observe any PCB loss during the extraction process. Possible losses of PCB during the extraction process was evaluated by starting with 10 ppm of Aroclor 1242 and ending with the same amount, even after the extraction process. Aroclor 1242 are non-volatile to semi-volatile compounds with boiling points between 325-366° C.

From Tables 5.1 and 5.2, it can be seen that the extent of Aroclor 1242 removal for the uninoculated control microcosms was much less compared to the removal in the inoculated microcosms. No methane production was observed in the controls. This observed differences in Aroclor 1242 content between the controls and the inoculated microcosms suggested that the

methanogenic consortium played a role in Aroclor 1242 removal. It was suspected that the methanogenic consortium reductively dehalogenated Aroclor 1242.

In an effort to determine if dechlorination was the cause of disappearance of Aroclor 1242 from the microcosms, the hexane extracts were evaluated using GC-MSD. Aroclor 1242 consists of 42% chlorine by weight and an average of 3-4 chlorines per molecule. From Fig 5.1 (chromatogram of Aroclor 1242 standard), it can be seen that the first peak in the PCB standard mixture eluted at retention time (t_r) 7.55 minutes and was identified as 2,3-dichlorobiphenyl (MW 222) by matching its mass spectrum and fragmentation pattern (Fig 5.2 A) with that of a 2,3-dichlorobiphenyl standard (Fig 5.2 B) [(525, 525.1 PCB Mix, Supelco (Bellefonte, PA)]. Based on the library search (Libraries: HPPEST. L, NBS75K. L, and PESTLAB. L), the peak that eluted at 8.58 min (Fig. 5.1) could be either 2,4,5-trichloro- or 2,3′,5-trichloro- (99% match) or 2,2′,5-trichlorobiphenyl (98% match). The peak eluting at 9.23 min was the most abundant peak. Based on the library search (Libraries: NBS75K. L, and PESTLAB. L), the spectra of this peak showed 99% matching to 2,3,6-trichloro-, 2,4,6-trichloro- and 3,4,4′-trichlorobiphenyls. The relative abundance of peak at 8.58 min (Fig. 5.1) was 99%.

If Aroclor 1242 were being reductively dehalogenated, then I expected to see some dichlorobiphenyl. However, GC-MSD analysis of the hexane extract from the 50 g/100 mL microcosm (Fig 5.3), revealed that the first peak eluted at the retention time (t_r) of 8.55. The mass spectrum and fragmentation pattern for this peak (Fig 5.4) closely matched that of a 2,4,5-trichlorobiphenyl (MW 256) standard (525, 525.1 PCB Mix, Supelco). I did not observe a peak with $t_r < 8.55$ (like appears in Aroclor 1242 standard) indicating that either dichlorobiphenyl was not present or it was present, but not at detectable levels.

The GC-MSD analysis of a hexane extract from a 10 g soil sample is shown in Fig 5.5. Many of the peaks and the peak pattern from the chromatogram (Fig 5.5), matched the peaks of Aroclor 1242 standard (Fig 5.3). This soil was contaminated in the early 1970’s and has been exposed to weathering since that time.

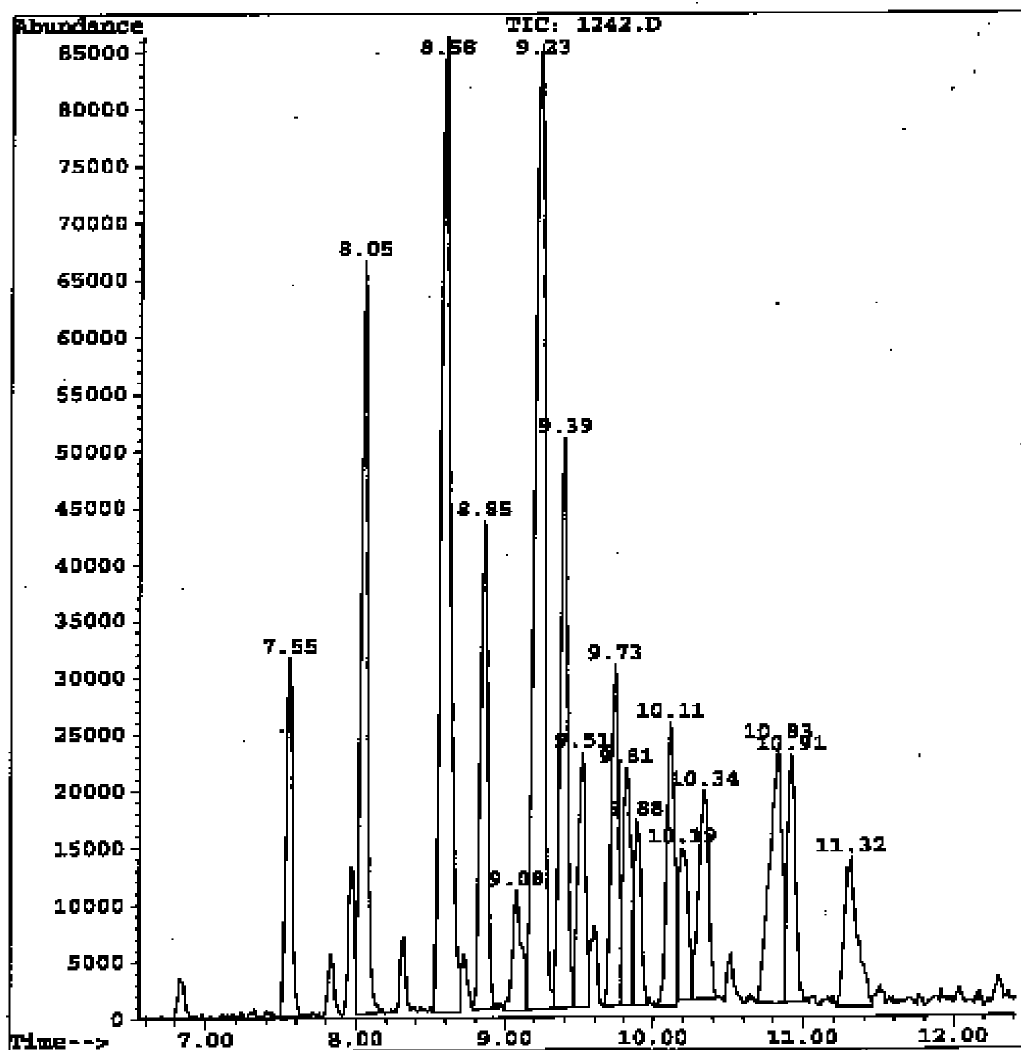


Figure 5.1. Chromatogram of Aroclor 1242 standard (GC-MSD analysis).

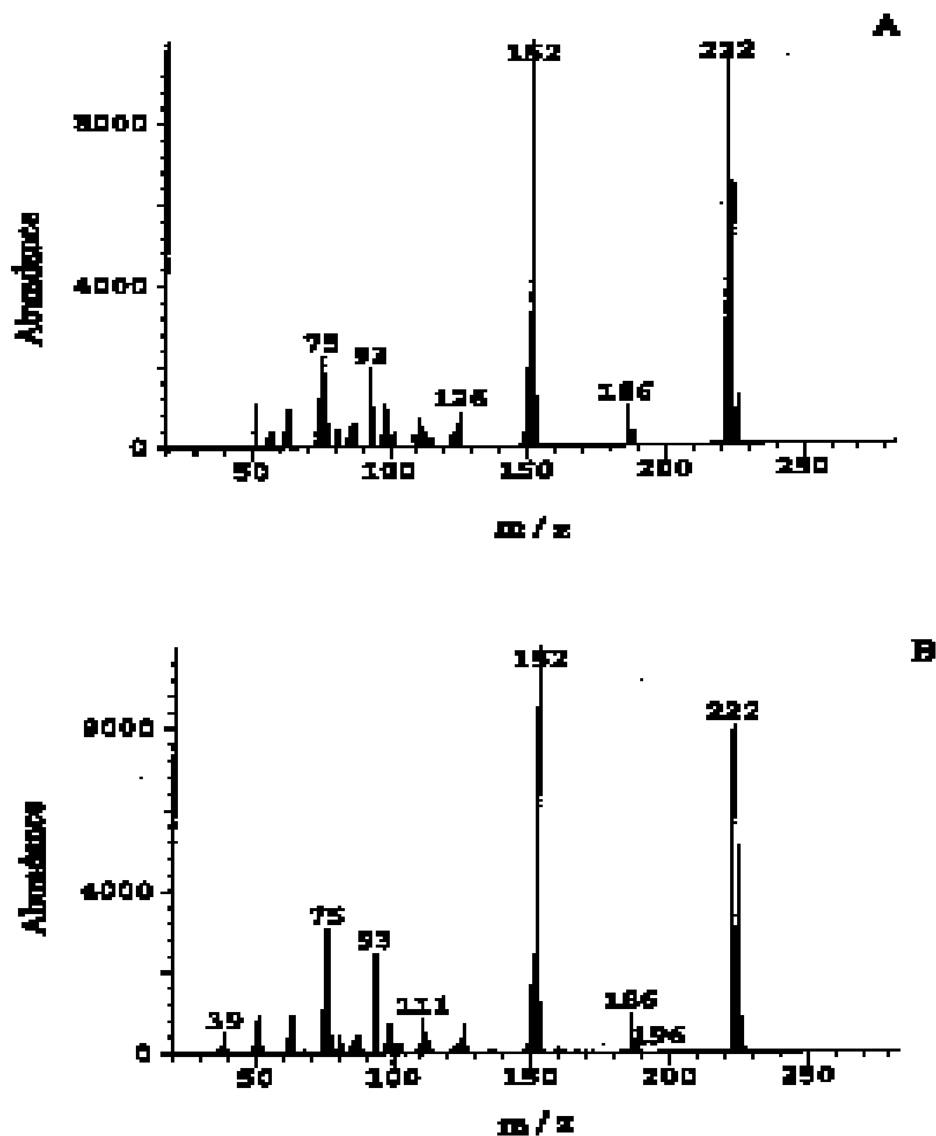


Figure 5.2. Mass spectrum of 2,3-dichlorobiphenyl. (A) Mass spectrum obtained from Aroclor 1242 standard. (B) Mass spectrum of 2,3-dichlorobiphenyl standard.

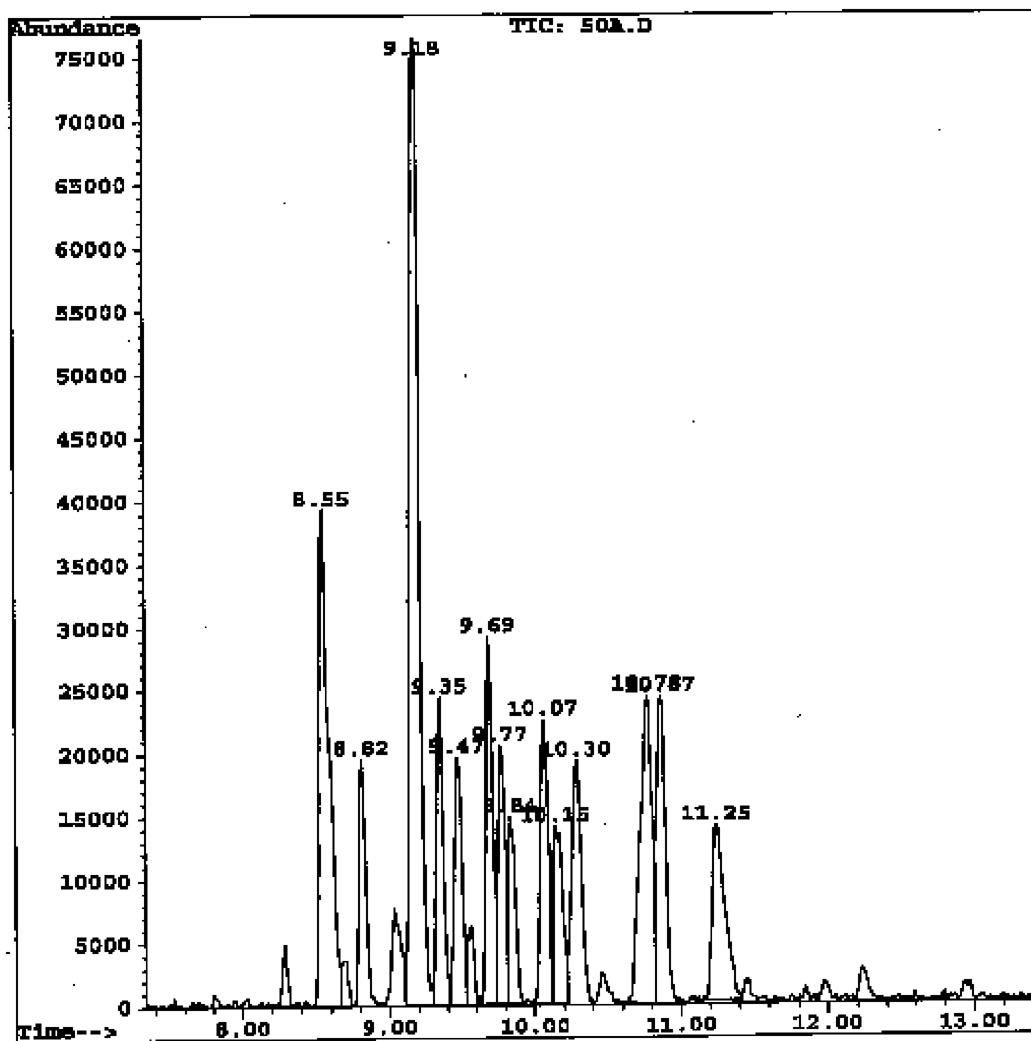


Figure 5.3. GC-MSD analysis of a hexane extract obtained from a 50 g soil microcosm.

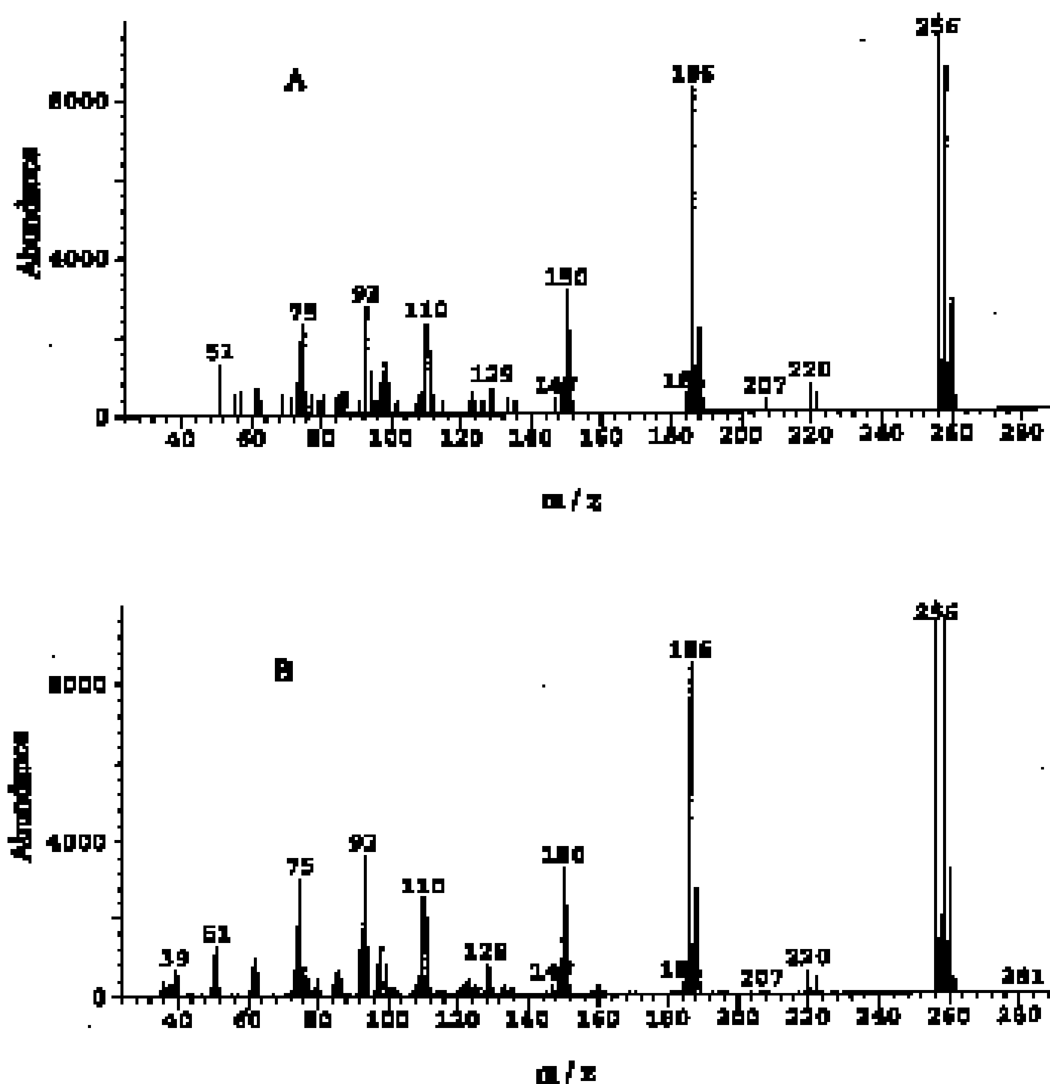


Figure 5.4. Mass spectrum of 2,4,5-trichlorobiphenyl. (A) Mass spectrum obtained from 50g/100 mL sample. (B) Mass spectrum of 2,4,5-trichlorobiphenyl standard.

Fig. 5.6 shows the chromatogram generated from a GC-MSD analysis of a hexane extract of a 10g/100mL microcosm that underwent a starve and feed regiment. Like the other microcosm treatment, the peak eluting at 9.24 min is the most abundant. Comparison of Figures 5.5 and 5.6 reveal that a change had occurred in the relative abundance (i.e., abundance of the peaks in terms of the most abundant peak) and the ratio of the relative abundance (relative abundance of peaks in Figure 5.5 vs. 5.6) in the peaks. Table 5.3 lists the retention time of some of the peaks from Figures 5.5 and 5.6, and Aroclor 1242 structure of those peaks based on the library search (Libraries: HPPEST. L, NBS75K. L, and PESTLAB. L). Table 5.4 lists the relative abundance, and ratio of relative abundance of the peaks from Table 5.3. The relative abundance of the peak, with a t_r = 8.6 (Fig 5.6) was 0.26, while the relative abundance for the same peak, under the chromatogram of 10 g soil sample (Fig 5.5) was 0.46. The relative abundance of peaks, eluted at 10.84 and 10.94 minutes (Fig 5.6) were 0.8 and 0.78, respectively, while the relative abundance for the same peaks in the 10 g soil sample (Fig 5.5) were 0.3 and 0.34, respectively. The ratio of relative abundance for the first peak (t_r = 8.6) was 1.77, while for the last peak (t_r = 11.32), the ratio was 0.42. So, a change in ratio was observed between the early and later eluting peaks. Comparison of the peak ratios between Figures 5.5 and 5.6 shows that while several of the peak ratios change, at least one does not. The ratio of the peaks at 10.84 and 10.94 minutes are consistent between Figures 5.5 and 5.6.

Significant change in relative abundance and ratio was observed particularly in this 10g/100mL microcosm sample. Fig. 5.7 shows the chromatogram of a sister sample, which underwent the same treatment. No such significant difference in abundance can be seen in the peaks of this chromatogram. This difference in peak pattern suggested that there might be something happening in the sample microcosms. This was an unusual observation since during reductive dehalogenation, anaerobes attack the higher chlorinated species first. But, close observation of chromatograms of my samples suggested that relative abundance of higher chlorinated species seemed to increase and lower chlorinated species seem to decrease. I did not observe any higher chlorinated congener decreasing in size or any lower chlorinated congener either appearing or increasing in size. This resulted in the suspicion of dechlorination not being the reason of Aroclor 1242 removal in my microcosms.

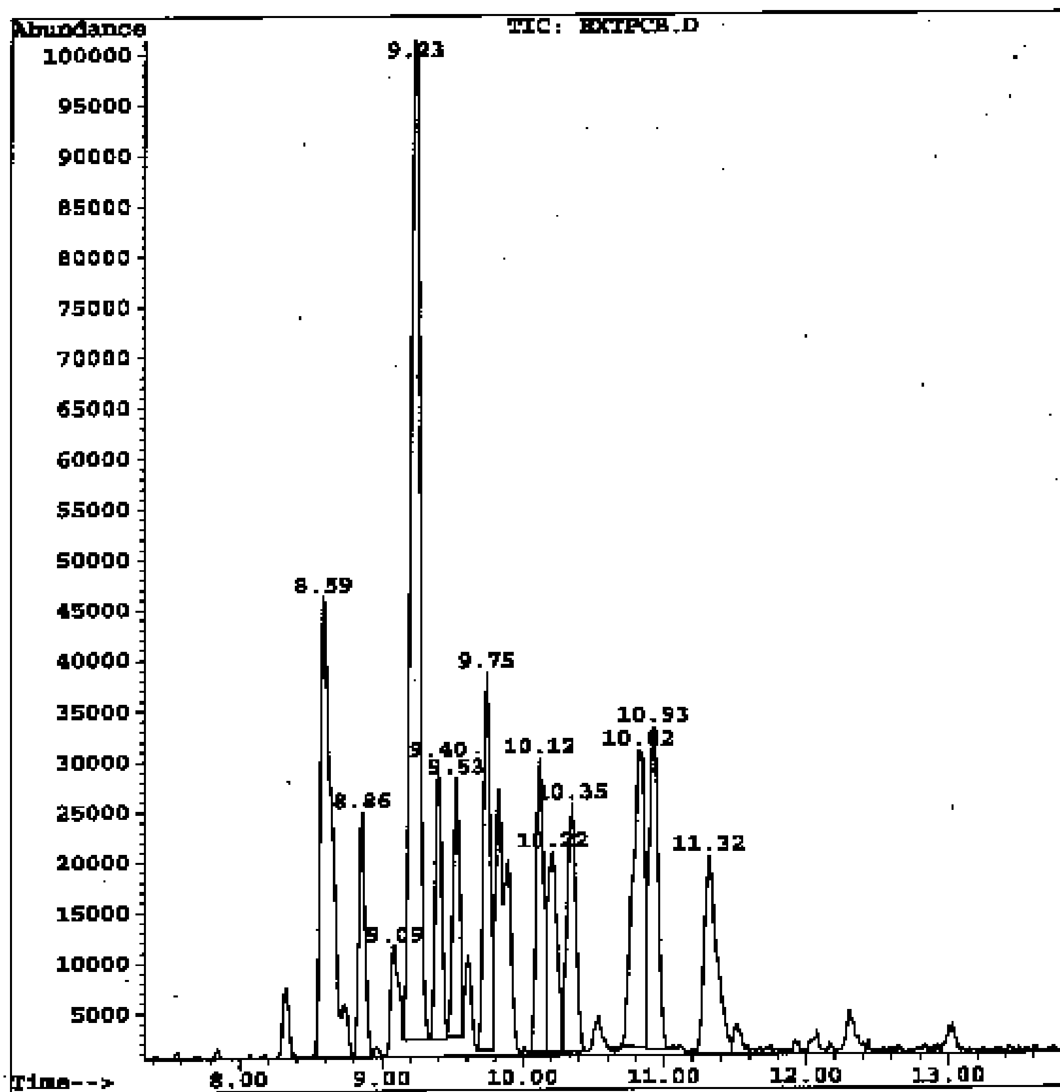


Figure 5.5. GC-MSD analysis of a hexane extract obtained from a 10g soil sample.

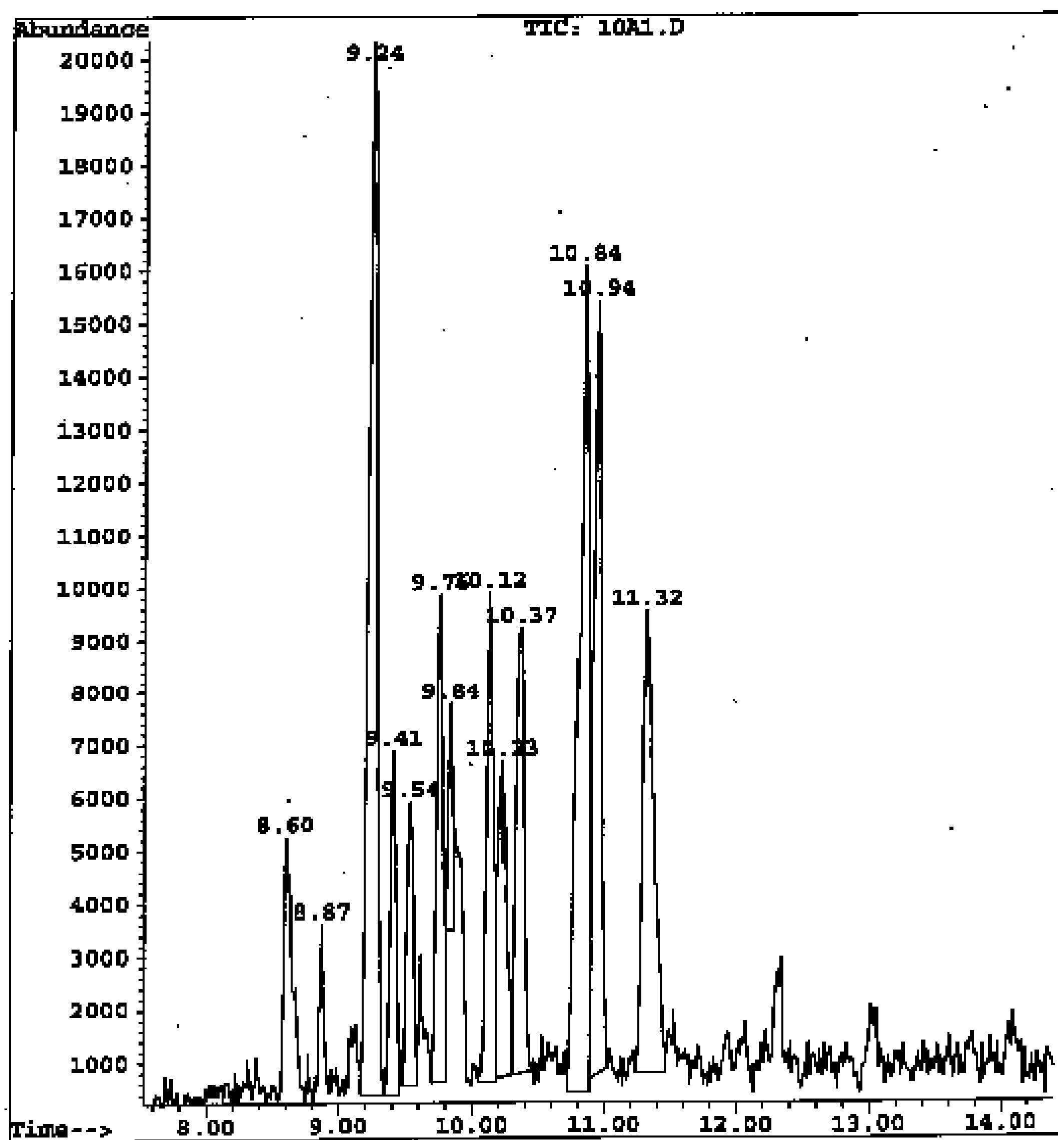


Figure 5.6. GC-MSD analysis of a hexane extract obtained from a 10g soil microcosm.

Table 5.3. Retention time (t_r) and Aroclor 1242 structure (based on the library search) in chromatographic peaks from Fig. 5.5 and 5.6.

t_r in min (± indicate difference in t_r in peaks between Fig. 5.5 and 5.6)	Aroclor 1242 structure
8.6 ± 0.01	2,4',5-trichlorobiphenyl 2,3,4'-trichlorobiphenyl 2,2',5-trichlorobiphenyl
9.24 ± 0.01	2',3,4-trichlorobiphenyl 2,4,6-trichlorobiphenyl 3,4,4'-trichlorobiphenyl
9.76 ± 0.01	2,2',3,4-tetrachlorobiphenyl 2,2',4,5'-tetrachlorobiphenyl 2,2',5,6-tetrachlorobiphenyl
10.84 ± 0.02	2,3',5,5'-tetrachlorobiphenyl 2,3',5,5'-tetrachlorobiphenyl 2,3',4,4'-tetrachlorobiphenyl
10.94 ± 0.01	2,3,4',6-tetrachlorobiphenyl 2,3,5,6-tetrachlorobiphenyl 3,3',4,4'-tetrachlorobiphenyl
11.32	2,4,4',5-tetrachlorobiphenyl 3,3',5,5'-tetrachlorobiphenyl 2,4,4',6-tetrachlorobiphenyl

Table 5.4. Peak ratio, relative abundance (RA), and ratio in RA factor in chromatographic peaks from Fig. 5.5 and 5.6.

Peak ratio	Figure 5.5	Figure 5.6	Ratio in factors
RA_{tr} 8.6 / 9.24	0.46	0.26	1.77
RA_{tr} 9.24 / 9.24	1	1	1.00
RA_{tr} 9.76 / 9.24	0.38	0.5	0.76
RA_{tr} 10.84 / 9.24	0.3	0.8	0.38
RA_{tr} 10.94 / 9.24	0.34	0.78	0.44
RA_{tr} 11.32 / 9.24	0.2	0.48	0.42

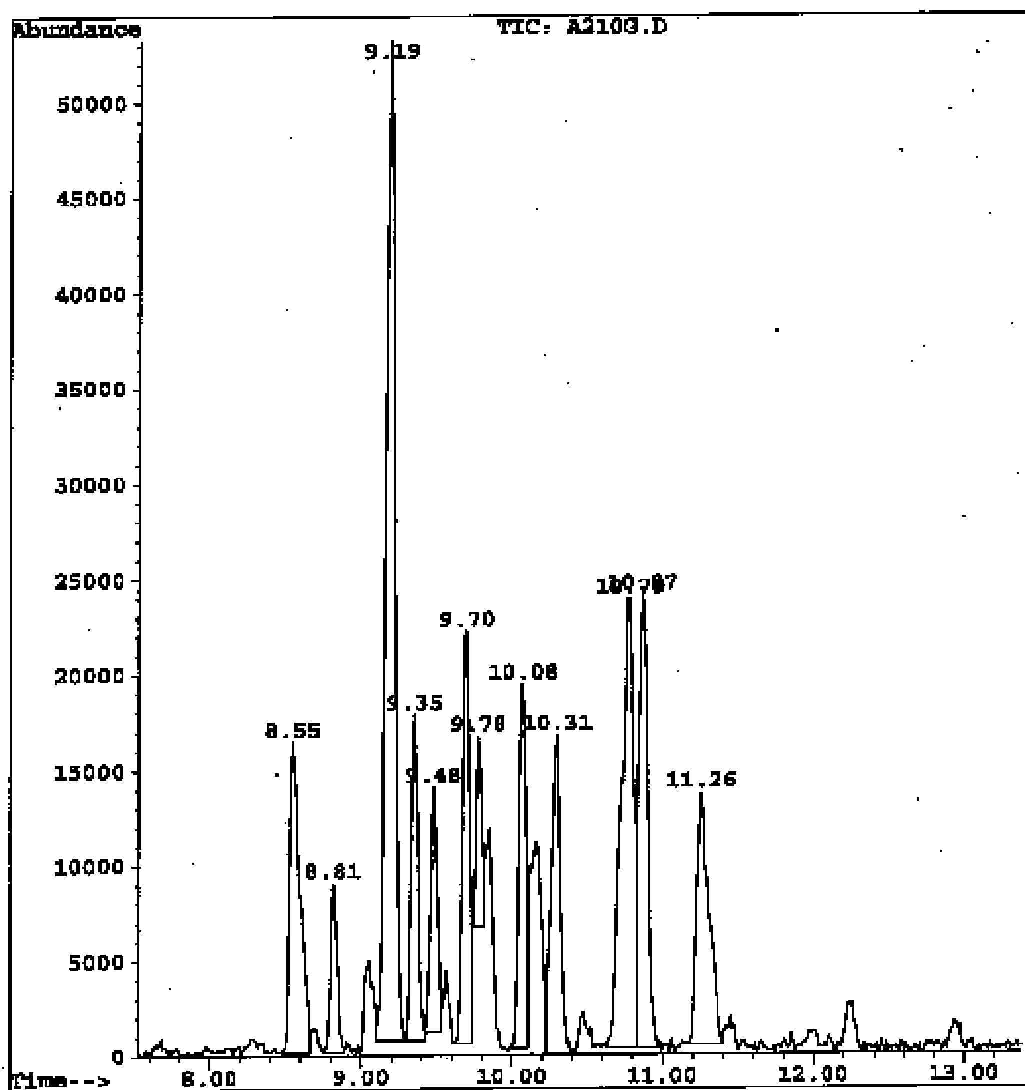


Figure 5.7. GC-MSD analysis of 10g soil microcosm sample. This sample is a sister sample of 10g soil microcosm sample from Fig 5.6.

Since dechlorination apparently did not seem to play a significant role in Aroclor 1242 disappearance, and since I did not have any lower chlorinated congeners (mono- or dichloro-) in my samples, initially it was thought that all the Aroclor 1242 that disappeared might have been converted to biphenyl. The chromatogram (GC-MSD) of the biphenyl standard (Fig 5.8) was compared with the chromatogram from the samples. Since biphenyl is lighter in weight (MW 154.21) and does not have any chlorine molecule, it eluted early, and the t_r of the biphenyl (6.13 min) did not match the t_r of any of the peaks, thus suggesting that there was either no biphenyl or the levels of biphenyl were such that it was not detectable. No test was done to see if biphenyl could have just evaporated during the extraction of Aroclor 1242 from soil. However, biphenyl has a boiling point of 255° C and it is very unlikely that it evaporated during the extraction procedure. In this study, I have observed that evaporation was not the cause of Aroclor 1242 disappearance since it had a high boiling point.

Bromoethane sulfonic acid (BESA) is known to be a potent inhibitor of methanogenesis (Fathepure and Boyd, 1988; Genther et al., 1989). For the treatments containing BESA, an average of 31.64 ± 32.66 % Aroclor 1242 was removed by the methanogenic consortium in 30 days. This treatment had 17.39 mg of Aroclor 1242 initially. BESA at the concentration of 0.2×10^{-3} M did not inhibit methanogenesis. I probably should have used a higher concentration for BESA with a more frequent addition of the inhibitor.

The microcosms set up to study the pattern of PCB degradation with time showed very inconsistent results. Microcosms sacrificed on 7, 14, and 21 days showed 22.85%, 2.78%, and 16.88% Aroclor 1242 removal, respectively. Sacrificing the microcosms periodically may not be the right way to study the PCB degradation pattern. Despite being treated equally, the microcosms could differ and could contain different consortia. Removal of soil sample periodically, from one particular microcosm, might be a better way to evaluate a time course study. It could be very difficult to remove any soil sample from the serum bottle microcosm without exposing the methanogenic consortium to oxygen.

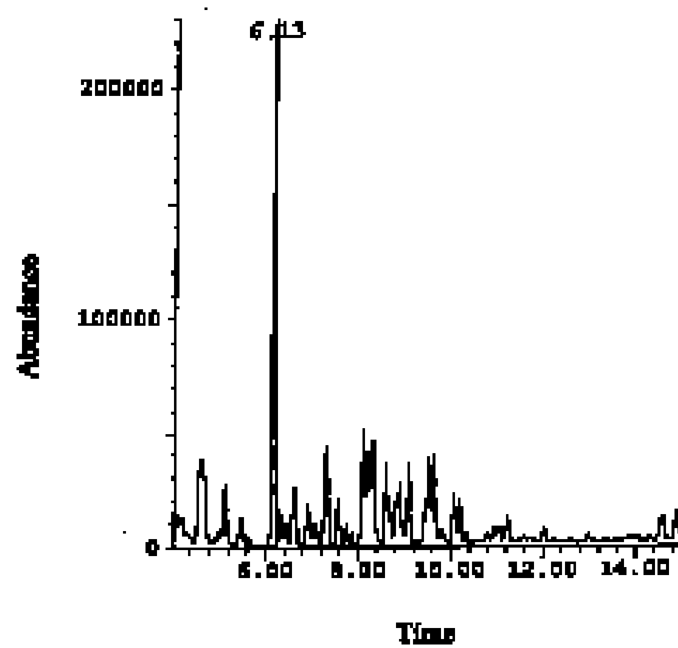


Figure 5.8. Chromatogram of biphenyl standard (GC-MSD analysis)

In this study, the rate and extent of degradation of Aroclor 1242 from a contaminated surface soil by acetotrophic methanogenic consortia was investigated. Two different experiments were conducted to manipulate the methanogens to enhance dechlorination of Aroclor 1242. Soil samples containing different concentration of the contaminant were chosen. Significant differences in Aroclor 1242 removal between the inoculated microcosms and the uninoculated controls suggested that Aroclor 1242 removal was due to the methanogenic consortium. However, GC-MS results suggested that disappearance of Aroclor 1242 was not due to dehalogenation. It is not clear from this investigation whether the disappearance of Aroclor 1242 in the microcosm was due to degradation or some other process (e.g., incomplete extraction). It is a possibility that if this study were performed for a longer time period, different results could have been seen. Also this study was an effort to evaluate bioaugmentation of surface soils using an oxygen sensitive consortium. I added a methanogenic consortium to a surface soil, possibly containing some residual intrapped oxygen. Further research should emphasize a longer term study to evaluate dehalogenation of PCB contaminated surface soil by a methanogenic consortium. Special attention should be given to remove traces oxygen, present in the soil.

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Appendix B

Data from intermediate feed and starve and feed experiments showing
the disappearance of Aroclor 1242 in contaminated surface soil.

Table B.1. Data from intermediate feed experiment showing the degradation of Aroclor 1242 in contaminated surface soil.

Microcosm treatment	mg Aroclor 1242 per microcosm at day 0	mg Aroclor 1242 per microcosm at day 30 (triplicate samples)	% Aroclor 1242 removed in 30 days
	0.174	0.095	45.4
0.1g/100mL	0.174	0.065	62.64
	0.174	0.083	52.3
	1.739	1.141	34.39
1g/100mL	1.739	1.203	30.82
	1.739	1.181	32.09
	17.39	7.87	54.74
10g/100mL	17.39	10.24	41.12
	17.39	10.93	37.15
	86.95	57.34	34.05
50g/100mL	86.95	60.97	29.88
	86.95	65.23	24.98
	17.39	16.82	3.38
Control	17.39	16.47	5.29
(0g/100mL)	17.39	17.04	2.01

Table B.2. Data from starve and feed experiment showing the degradation of Aroclor 1242 in contaminated surface soil.

Microcosm treatment	mg Aroclor 1242 per microcosm at day 0	mg Aroclor 1242 per microcosm at day 30 (triplicate samples)	% Aroclor 1242 removed in 30 days
	0.152	0.041	73.03
0.1g/100mL	0.152	0.056	63.29
	0.152	0.093	38.75
	1.521	0.447	70.61
1g/100mL	1.521	0.507	66.67
	1.521	0.571	62.46
	15.211	6.180	59.37
10g/100mL	15.211	5.400	64.5
	15.211	11.120	26.9
	76.055	67.550	1.18
50g/100mL	76.055	69.750	8.29
	76.055	54.300	28.6
	15.211	12.130	20.26
Control	15.211	14.810	2.64
(0g/100mL)	15.211	15.100	0.73
	15.211	12.840	15.59
10g/100mL	15.211	10.650	29.98
(Reactor 3)	15.211	12.260	19.4