Chapter IV

EVALUATION OF TOXICITY OF PCBs (AROCLOR 1242) ON AN ACETICLASTIC METHANOGENIC CONSORTIUM

4.1 ABSTRACT

The toxicity of an aged Aroclor 1242 contaminated soil was determined for a methanogenic consortium enriched from a municipal anaerobic digester, using acetate as the primary substrate. Triplicate 160-mL serum bottle microcosms containing either 1 g, 10 g or 50 g of soil plus 100 mL or 50 mL of the inoculum were set up. Glacial acetic acid was added every 3 days, and methane production was monitored using gas chromatography (GC). The microcosms were incubated for 30 days. Toxicity of Aroclor 1242 contaminated soil was determined based on the decreased rate of methane production in the microcosms relative to non-soil containing controls. The methane production for the microcosms with different amounts of soil followed a particular pattern. Total gas production in the microcosms decreased with increasing amount of soil. Slight reduction in gas (methane) production was observed for the 1 g and 10 g soil microcosm treatments, however, a significant reduction was observed for the 50 g soil microcosm treatment. Whether toxicity was due to Aroclor 1242 or due to trapped oxygen in the soil could not be determined. The rate of acetate metabolism (total gas production) for most of the treatment types was linear. Aroclor 1242 contaminated soil did not show significant methanogenic inhibition in 30 days, at most of the concentrations examined.

4.2 INTRODUCTION

Polychlorinated biphenyls (PCBs) are members of the halogenated aromatic group of environmental pollutants that have been identified worldwide in diverse environmental matrices. Due to their chemical inertness and hydrophobicity, PCBs do not degrade easily and are persistent in the environment. PCBs have well known toxic effects and can pose a serious threat to mammalian systems, laboratory animals and wildlife (WHO, 1993). PCBs are known to bioaccumulate in aquatic food chains (Borlakoglu and Haegele, 1991; Clarkson, 1995). PCB mixtures can elicit a spectrum of toxic responses including lethality, loss of body weight, dermal

toxicity, liver damage, decreased reproduction, neurotoxicity, diminished immune response, induction of diverse enzyme systems, teratogenic effects and carcinogenic effects (Borlakoglu and Haegele, 1991; Crine, 1988; Mousa et al., 1996; Safe and Hutzinger, 1987).

PCBs are also known to be toxic to some microorganisms, including the freshwater and marine algae and soil fungi (WHO, 1993). Ruben et al. (1990) studied the influence of light intensity and photoadaptation on the toxicity of PCBs to marine diatom *Ditylum brightwellii*. Chu et al. (1997), determined acute toxicity of PCBs to *Photobacterium phosphoreum*. However, very little work has been done to determine the toxicity of PCBs to bacteria.

Xenobiotic compounds are known to be toxic to many microorganisms. Phenol is a simple aromatic chemical, but is toxic to most microorganisms (Fang and Chan, 1997; Huang and Tseng, 1996). Cenci et al. (1987) evaluated the toxicity of halogenated phenolic compounds by screening for effects on the specific growth rates (SGR) and the dehydrogenase activity (DHA) of *Escherichia coli*. Sierra-Alvarez and Lettinga (1991) evaluated the toxicity of aromatic compounds, including phenolic compounds to aceticlastic methanogens in unacclimated methanogenic granular sludge.

Due to the concern with environmental contamination, much attention has been given to the carcinogenic and toxic properties of chlorinated pollutants. Halogenated compounds are toxic to methanogens and often inhibit methanogenesis (Madsen and Aamand, 1992). As the number of chlorine atoms on the phenolic compounds increase, toxicity increases, thus increasing inhibition. Fang and Chan (1997) studied the effect of phenol on methanogens and concluded that phenol is toxic to methanogens and methanogenic activity of the biogranules decreased progressively with the increase of phenol concentration. Hydrophobicity is also an important factor contributing to the toxicity of chlorophenols to aceticlastic methanogens (Schuurmann et al., 1997; Sierra-Alvarez and Lettinga, 1991).

Oxygen, which is essential to aerobic microorganisms is lethal to strict anaerobes, including methanogens. Methanogens have the reputation of being the strictest of anaerobes. When oxygen comes in contact with reduced flavoproteins or iron-sulfur proteins, two toxic

compounds, hydrogen peroxide and superoxide radical can be formed. Anaerobes usually die in the presence of oxygen because of the deterious effects of the superoxide radical. According to Ferry (1993), there is considerable variability in the sensitivity of methanogens to oxygen.

Little is known about the toxicity of PCBs to methanogens and other bacteria. The toxicity of chemicals may prevent the biodegradation of pollutants and inhibit anaerobic digestion processes. Since microbial biodegradation of PCBs is a potential means of remediation of these environmental contaminants, the effect of these contaminants on the microorganisms themselves, need to be evaluated. Because of the toxicity of similar xenobiotic compounds, such as chlorophenols to most microorganisms, including methanogens, the toxicity of PCBs to these organisms needs to be determined. Therefore, the objective of this study was to evaluate the toxicity of an Aroclor 1242 contaminated soil to an acetotrophic methanogenic consortium. This contaminated surface soil was well aerated.

4.3 MATERIALS AND METHODS

4.3.1 PCB Contaminated Soil

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. It was an aged soil and had an initial Aroclor 1242 concentration of 1739 mg/kg. 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.

4.3.2 Chemicals

The chemicals used to prepare the mineral salt medium were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Yeast extract was purchased from Sigma Chemical Co. (ST. Louis, MO). Methane standard gas was obtained from Matheson Gas Products (Twinsburg, OH). All other chemicals were reagent grade.

4.3.3 Enrichment Culture Procedure

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 medium used in this study was prepared in a 2-liter Erlenmeyer flask. The medium 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 liters of deionized water. To the medium, 24 g of $NaCH_3CO_2$ was also added as substrate. The medium was 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 medium 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 it and pH of the medium was adjusted to 7.0. Four hundred milliliters of sludge was added to 1600 mL of media in a 2-liter Erlenmeyer flask. The flask was sealed tightly with a rubber stopper and was degassed every few days to release the excess gas pressure buildup. The flask containing the culture was amended with 2 mL of 17 mM acetic acid, every 7-8 days. After one month of incubation, an aceticlastic methanogenic consortium was established in the flask, which was confirmed by the detection of methane in the headspace of the flask.

For experiment number one, various amounts (triplicate sets of 1g, 10g and 50 g) of the PCB contaminated soil was transferred into 160-mL serum bottles. To each serum bottle, 100 mL of the methanogenic inoculum was dispensed (resulting in treatment types: 1g/100mL, 10g/100mL and 50g/100mL), while sparging with deoxygenated N_2 using a Hungate gassing apparatus. For a second experiment, 50 g of the contaminated soil was added to three 160-mL serum bottles, followed by 50 mL of the inoculum (50g/50mL treatment). Triplicate sets of controls were also prepared by dispensing 100 mL of inoculum to 160-mL serum bottles while flushing with O_2-free N_2. No soil was added to these controls. All the microcosms and the controls were sealed with thick butyl rubber stoppers and aluminum crimp seals. The serum bottles were shaken thoroughly and incubated at 31° C for 30 days.

Gas production in the serum bottles was measured every 3 days using the syringe technique (section 4.3.4). Following the gas measurement, the microcosms and the controls were amended

with 0.1 mL glacial acetic acid, shaken well and incubated. The gas production in the bottles was monitored during one month of incubation period.

4.3.4 Total Gas Monitoring (Syringe Technique)

Total gas production in the incubated serum bottles was measured by inserting the needle of a Micro-Mate Interchangeable Hypodermic 50-mL glass syringe (Popper and Sons, Inc. New Hyde Park, NY) through the rubber stoppers to determine the gas volume. The syringe was held horizontal for measurement. Volume determinations were made by allowing the syringe plunger to move (gently twirling to provide freedom of movement) and equilibrate between the headspace pressure inside the bottle and atmospheric pressure so that the pressure inside the serum bottle was maintained at 1 atmosphere. The total amount of gas produced in each serum bottle was measured this way during a 30 day incubation period.

4.3.5 CH_4 Analysis

Quantification of methane produced in the serum bottles was accomplished by injecting 10-µL of the headspace gas (subsampled from the 50 mL syringe used to measure total gas) into a gas chromatograph (5890 Hewlett-Packard Co.) equipped with a thermal conductivity detector and fitted with a Porapak N column (1.8 m by 2.1 mm internal diameter, 80/100 mesh). The column temperature was maintained at 50° C and the detector temperature was maintained at 150° C. Helium was used as the carrier gas with a flow rate of 20 mL min^{-1}.

The gas sample was identified and quantified by comparing the chromatograms (from the GC run) with the chromatogram of an external methane standard (10 % CH_4, balance He) (Matheson Gas Products, Twinsburg, OH), on the basis of retention time and peak area respectively. The concentration of methane in 10 µL of the CH_4 standard was 4.09×10^{-8} moles.

4.4 RESULTS AND DISCUSSION

For microcosms containing 100 mL inoculum, I observed a decrease in total gas production with an increase in the amount of soil. The average cumulative amount of gas produced (in triplicate treatments) was plotted over time (Fig. 4.1) to determine relative rates of metabolism of acetate among the treatments. The rate of gas production for the first 4 treatments, control, 1 g, 10 g, and 50 g with 100 mL of the inoculum appear to exhibit linearity. Total gas production for microcosms containing 50 mL inoculum exhibited curvilinear behavior.

Aceticlastic methanogens ferment acetate to CO_2 and CH_4 as follows:

$$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$$

$$\Delta G^{\circ\prime} = -36 \text{ kJ / mol}$$

Assuming that half the amount of total gas produced by the methanogenic consortium is methane, methane production would decrease with the decrease in total gas production. Based on the assumption that all the acetate added went to CH_4 and CO_2, I expected the total methane production to be 0.085 moles.

Based on the results of GC analysis, the level of CH_4 production decreased as the amount of soil per microcosm increased (Fig. 4.2). The total methane production in the microcosms containing different amounts of soil followed a particular pattern (Fig. 4.2). The observed decrease in methane production could result from a decrease in acetotrophic metabolism. It could be possible that the soil was toxic to the methanogenic consortium, thus decreasing metabolism and total gas production, including CH_4.

In order to determine if the soil was inhibitory to the methanogenic consortium, the level of methane production in the soil-containing microcosms was compared to non-soil containing controls (for the 100 mL inoculum only). The level of methane production was higher for the non-soil containing samples as compared to inoculated microcosms, thus suggesting that the contaminated soil probably was responsible for decrease in methane production. It is possible that the contaminated soil at different amounts, showed different levels of inhibition. Microcosms containing 1 g and 10 g PCB contaminated soil did not really exhibit methanogenic inhibition, while microcosms containing 50 g PCB experienced inhibition and possibly toxicity.

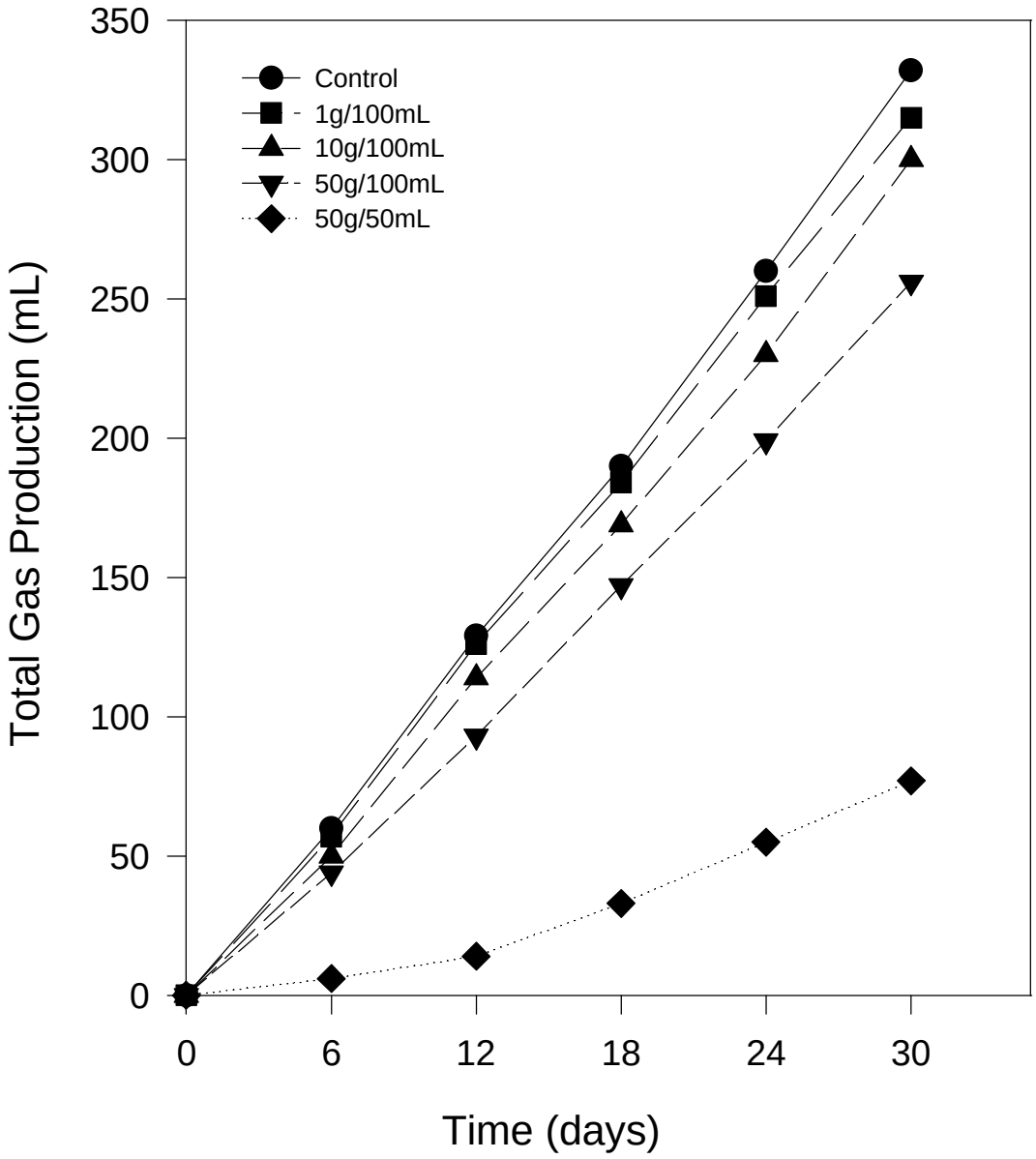

Figure 4.1. Total gas production during 30 days incubation in microcosms. The error bars for standard deviation are too small to notice.

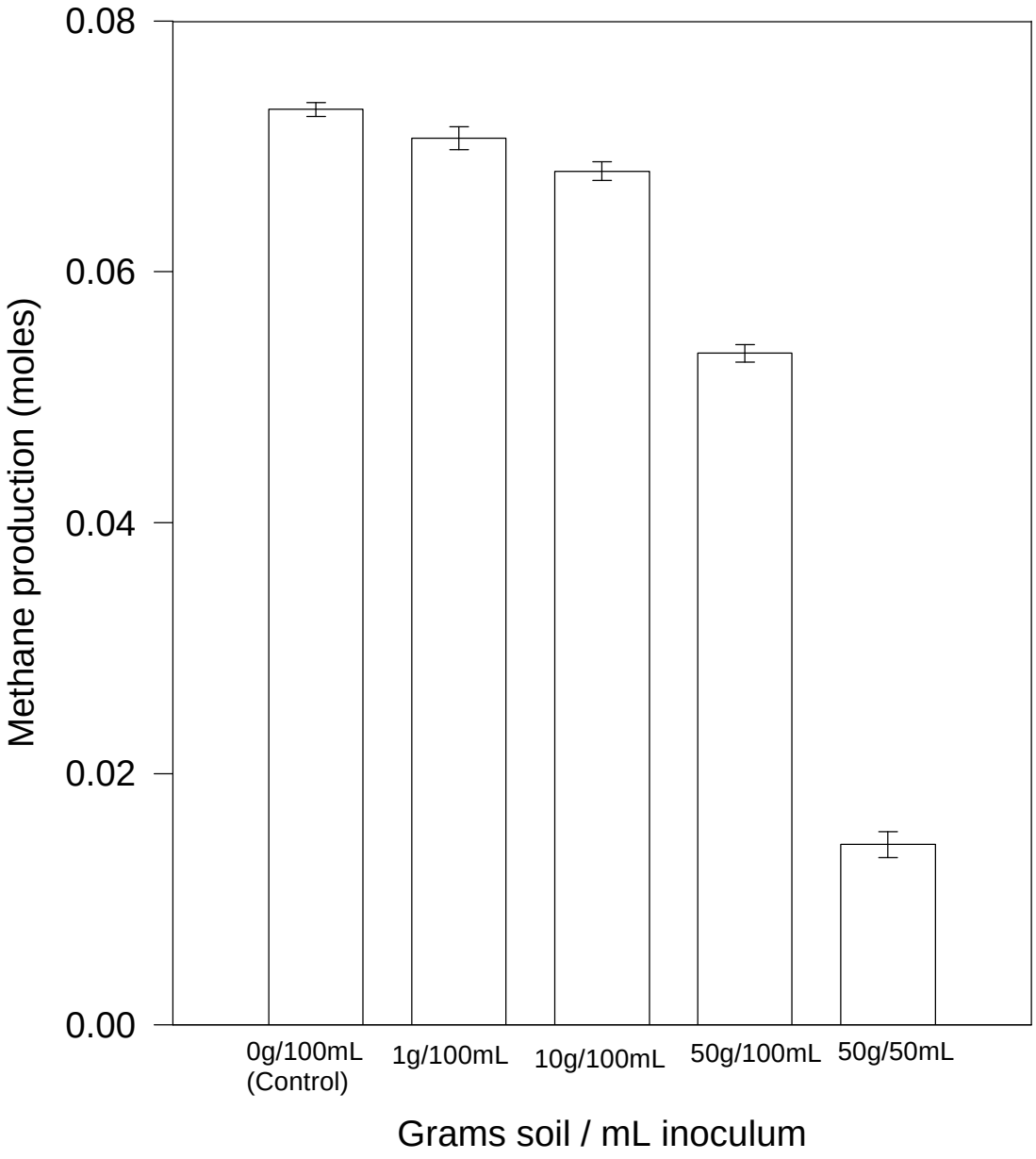

Figure 4.2. Total amount of methane produced (moles) in microcosms containing different amounts of soil and inoculum. The error bars indicate the standard deviation.

Significant decrease in CH_4 production was noticed for samples containing 50 g of the contaminated soil. Both the treatments, 50g/100mL and 50g/50mL contained the same amount of soil, but produced different amounts of gas. Total methane production was amount of soil, but produced different amounts of gas. Total methane production was quantitated to be 0.053 ± 0.0007 moles for 50g/100mL treatment, while it was monitored to be 0.014 ± 0.001 moles for 50g/50mL sample microcosms. This could be due to the difference in size of the methanogenic population (due to difference in amount of inoculum).

The decreased methane production with increasing soil amount might have been due to the increase in content of Aroclor 1242 (Table 4.1). For different treatment types, different amount of soil was used, and thus the microcosms had different amounts of Aroclor 1242 (Table 4.1). Methanogenic activity was not inhibited in microcosms containing 1.7 and 17 mg Aroclor 1242. However, inhibition of methanogenesis was observed in microcosms containing 85 mg Aroclor 1242.

Methanogens are strict anaerobes and extremely sensitive to oxygen which is toxic to them. The Aroclor 1242 contaminated soil used in this study was an aged surface soil and must have had a high amount of oxygen trapped in it. Before addition of inoculum, the soil was flushed with oxygen free N_2. But, due to the slow rate of diffusion of gases, there might have been traces of O_2 present in the soil, especially in the bottom part of the serum bottles. Trapped oxygen in the Aroclor 1242 contaminated surface soil could also have been responsible for the reduction in methanogenic activity.

Increase in toxicity with increasing soil amount could have been due to the increase in oxygen concentration. Methanogens are very sensitive to oxygen and even a low concentration of oxygen could be lethal to them and could inhibit methanogenesis. Analysis of the GC chromatograms (Fig. 4.3) confirmed the headspace gas, produced in the microcosms, as methane. The retention time (2.31 min) of the gas sample matched the retention time of the methane standard.

Table 4.1. Initial amount of Aroclor 1242 in microcosm cultures.

Microcosm treatment	**mg Aroclor 1242 per microcosm**
Control	0
1 gram	1.7
10 grams	17
50 grams	85

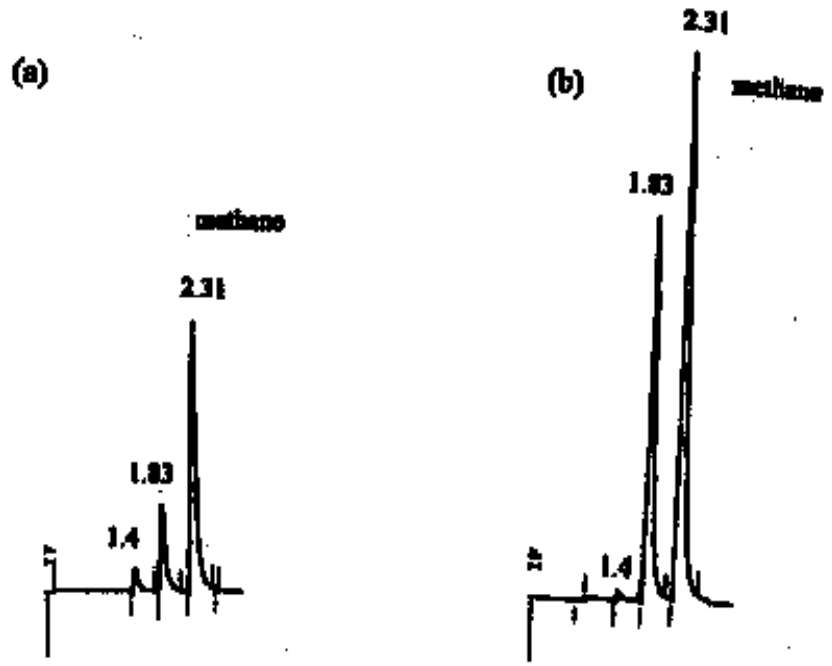

Figure 4.3. Comparison of GC chromatograms of methane standard (a) and sample (b) confirming the presence of methane (tr 2.31 min) in the sample.

PCBs may be dissolved in the organic phase of soils and are also protected under waxy layers of aged organic matter and do not reach biodegrading organisms (Tiedje et al., 1993). Within anaerobic digesters, organic chemical contaminants that are poorly soluble can sorb to the surfaces of sludge solids, resulting in a high concentration suspension phase (Madsen and Rasmussen, 1996). It is possible that a portion of the soil-bound PCB could have been transferred to the sludge flocs. If this process was occurring, it is difficult to know how bioavailability (or biodegradability) might have been influenced. It is also not clear to what extent (if any at all) sorption of PCB to flocs influenced extractability.

Immobilization of microorganisms results in their protection from toxic chemicals like chlorinated phenols (Keweloh et al., 1989). Apajalahti and Salkinoja-Salonen (1984) have shown that reversible absorption of PCP to bark chips detoxified the surroundings of the PCP-degrading bacteria. In this study, Aroclor 1242 could have been adsorbed to the sludge flocs and therefore, did not show significant toxicity in most of the treatment types.

In this study, the inhibitory effect of an aged Aroclor 1242 contaminated surface soil on an acetotrophic methanogenic consortium was investigated. Anaerobes were exposed to different amount of PCB contaminated soil and the microbial activity monitored via gas production. Total gas production in the microcosms decreased with the increase in amount of PCB contaminated soil. Inhibition was determined by comparing the amount of methane production in the samples to a non-soil containing control. Whether decrease in methane production was as a result of toxicity of Aroclor 1242 or due the toxicity of trapped oxygen in the soil or due to any other reason could not be determined from this study. If using a surface soil, any amount of oxygen present in the soil should be removed.

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Appendix A

Data of total gas production and total methane production by the serum bottle microcosms during 30 days incubation.

Table A.1. Total gas production during 30 days incubation.

TREATMENT	----------GAS PRODUCTION DURING 30 DAYS INCUBATION (mL)----------						
	6 days	12 days	18 days	24 days	30 days	Total	Average ± SD
Control	58	70	61	66	69	324	332 ± 7
	61	68	66	71	72	338	
	60	71	55	74	73	333	
1g/100mL	56	67	59	65	62	309	315 ± 6
	62	68	60	64	66	320	
	53	71	55	73	64	316	
10g/100mL	52	66	59	63	65	305	300 ± 5
	49	61	52	61	73	296	
	50	63	56	58	72	299	
50g/100mL	45	49	52	53	59	258	256 ± 9
	47	48	56	52	60	263	
	40	51	53	50	52	246	
50g/50mL	6	8	20	21	23	78	77 ± 5
	5	6	16	20	25	72	
	8	10	19	25	20	82	

Table A.2. Total methane production (moles) during 30 days incubation

TREATMENT	----------METHANE PRODUCTION (MOLES)----------		
	Triplicate Samples	Average	Std. Dev.
Control	0.0724 0.0735 0.0729	0.072933	0.000551
1g/100mL	0.0698 0.0716 0.0705	0.070633	0.000907
10g/100mL	0.0687 0.0672 0.0681	0.068	0.000755
50g/100mL	0.0535 0.0528 0.0542	0.0535	0.0007
50g/50mL	0.014 0.0135 0.0155	0.014333	0.001041