THE SEQUENTIAL ANAEROBIC-AEROBIC BIODEGRADATION OF PCBS IN PHYTOREMEDIATION CUTTINGS

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Abstract

The sequential anaerobic-aerobic process was applied for PCB biodegradation from plant cuttings. The PCBs were absorbed by plant roots called phytoextraction. The anaerobic process dechlorinated highly chlorinated PCBs (tetra-, penta-, hexa-) to lower chlorinated PCBs (mono-, di-, tri-). Then, the aerobic cultures degraded the low chlorinated PCBs. In the experiment, Aroclor 1254, the commercial PCB mixtures, were used. The culures were fed by methanol (CH_4) as a sole carbon source. The temperature was an essential parameter to control the dechlorinated state. Produced gas from the anaerobic process was collected to examine the anaerobic condition. The highly chlorinated PCBs were dechlorinated with different pathways dependent upon the dominant cultures in the process. The PCB dechloriantion was detected by gas chromatography and reported by chromatograms. The phytoabsorption showed difficulty of PCB biodegradation. The PCB removal efficiency was compared with the PCB removal from contaminated soil of the previous study.

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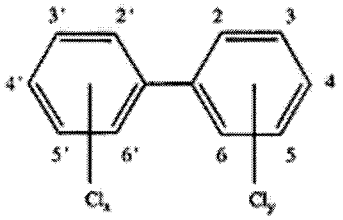
Chapter 1

Introduction

Overview of PCB properties

Polychlorinated biphenyls (PCBs) are a set of 209 distinct chemical compounds, called congeners. PCBs are categorized as nonpolar, chlorinated hydrocarbons with a biphenyl nucleus ($C_{12}H_{10}$) on which one to ten of the hydrogen has been substituted by chlorine. A general formula of PCBs is $C_{12}H_{10-n}Cl_n$, where n = 1-10, that is monochlorobiphenyl through decachlorobiphenyl and a general structure of PCBs is shown in Fig. 1



x + y = 10

Fig. 1: General Structure of PCBs

The entire group of 209 PCBs is subdivided by degree of chlorination and the term *homolog* is used to describe the family of compounds with the same number of chlorine atoms. In a homolog, each PCB has different chlorine replacement position, called *isomers*; for example, 2,3,4-trichlorobiphenyl and 3,3',5-trichlorobiphenyl are two of the twelve trichlorobiphenyl isomers (Table 1; Erickson, 1997). The distribution of chlorine atoms in the two rings of biphenyl is shown in Table 2 (Erickson, 1997). General

characteristics of most PCBs congeners are colorless, odorless crystals, low water solubilities, and low vapor pressures. However, the PCBs are soluble in most organic solvents (e.g. methanol, isooctane, and hexane), oils, and fats. In addition, the commercial mixtures of PCB congeners are clear viscous liquids especially highly chlorinated commercial PCBs. For example, Aroclor 1260 (i.e., the most highly chlorinated mixture) is a highly viscous liquid.

Table 1: Composition of Chlorinated Biphenyls by Homolog

Empirical formula	Molecular weight		Chlorine (%)	No. of isomers
	Base[a]	Mean[b]		
$C_{12}H_{10}$	154.1	154.2	0	1
$C_{12}H_9Cl$	188	188.7	19	3
$C_{12}H_8Cl_2$	222	223.1	32	12
$C_{12}H_7Cl_3$	256	257.6	41	24
$C_{12}H_6Cl_4$	289.9	292	49	42
$C_{12}H_5Cl_5$	323.9	326.4	54	46
$C_{12}H_4Cl_6$	357.8	360.9	59	42
$C_{12}H_3Cl_7$	391.8	395.3	63	24
$C_{12}H_2Cl_8$	425.8	429.8	66	12
$C_{12}H_1Cl_9$	459.7	464.2	69	3
$C_{12}Cl_{10}$	493.7	498.7	71	1

[a] Based on ^{35}Cl, ^{12}C, and ^{1}H. [b] Based on natural isotopic abundance of C, Cl, and H

Table 2: Distribution of Chlorine Atoms in the Two Rings of Biphenyl

Chlorine Atoms on Ring

Ring y

Ring x	0	1	2	3	4	5
0	1	3	6	6	3	1
1		6	18	18	9	3
2			21	36	18	6
3				21	18	6
4					6	3
5						1

Table 3: Physical Properties of PCB Homologs[a]

PCB isomer group	Melting point (°C)[b]	Boiling point (°C)[b,c]	Vapor pressure (Pa) 25°C[c-e]	Water Solubility 25°C (g/m3)[e,f]	Log-octanol-water partition coefficient[c,f]	Approximate bioconcentration factor in fish[c,f]	Approximate evaporation rate 25°C (g/m2h)[c,f]
Biphenyl	71	256	4.9	9.3	4.3	1000	0.92
MonoCB	25-77.9	285	1.1	4	4.7	2500	0.25
DiCB	24.4-149	312	0.24	1.6	5.1	6300	0.065
TriCB	28-87	337	0.054	0.65	5.5	1.60E+04	0.017
TetraCB	47-180	360	0.012	0.26	5.9	4.00E+04	4.20E-03
PentaCB	76.5-124	381	2.60E-03	0.099	6.3	1.00E+05	1.00E-03
HexaCB	77-150	400	5.80E-04	0.038	6.7	2.50E+05	2.50E-04
HeptaCB	122.4-149	417	1.30E-04	0.014	7.1	6.30E+05	6.20E-05
OctaCB	159-162	432	2.80E-05	5.50E-03	7.5	1.60E+06	1.50E-05
NonaCB	182.8-206	445	6.30E-06	2.00E-03	7.9	4.00E+06	3.50E-06
DecaCB	305.9	456	1.40E-06	7.60E-04	8.3	1.00E+07	8.50E-07

[a] Many values are approximations of the range across the isomers.

[b] Average properties of all isomers in group.

[c] Shiu and Mackay, 1986

[d] Mackay, 1986

[e] Mean value for liquid

[f] Mackay, 1986

As mentioned above, it seems that the physical properties of PCBs are similar. In fact, the physical and chemical properties of PCBs are quite varied. For example, shown in Table 1 are the molecular weights of PCB homologs. In addition, physical constants are important to consider such as, melting point, boiling point, vapor pressure, water solubility, octanol-water partition coefficient, bioconcentration factor in fish, and evaporation rate (Table 3; Metcalfe et. al., 1988). In the case of commercial mixtures (PCBs were commercially manufactured as complex mixtures), the physical and chemical properties including homolog composition, physical state, pour point, vapor pressure, specific gravity, viscosity, fluid density, water solubility, log K_{ow}, bioconcentration factor in fish, evaporation rate, flash point, fire point, askarel composition, and properties are reviewed (Table 4; Brinkman, 1980).

Production and Use of Commercial PCBs

PCBs consist of 209 individual chemical compounds. Commercially, PCBs were produced as complex mixtures for diversified uses; for example, transformers (dielectric fluids), capacitors, printing inks, paints, dedusting agents, pesticides, and other applications (Durfee et. al., 1976). The principal manufacturer is Monsanto Corporation located in St. Louis, MO which marketed PCBs under the trade name Aloclor® from 1930 to 1977. Because of their chemical and physical stability, and their electrical insulating properties, PCBs became widely used. There are various procedures for synthesis of PCB mixtures. The commercial PCB mixtures were synthesized by chlorination of biphenyl with chlorine gas (NRC, 1979; Durfee et al., 1976). In reverse, congener standards can be prepared by catalytic dechlorination, followed by separation of the resultant mixtures

Table 4: Characteristics of Aroclor Mixtures

Aroclor	Density at 20°C	Viscosity (Saybolt Univ. sec) at 98.9°C	n_D^{20}	Flash point °C	Fire point °C	Pour point °C	Distillation range (°C)	Vaporization rate (g/cm2/h)*106,at 250°C	Dielectric const.		Solubility in water at 25°C (µg/l)	CAS registry no.[a]
									At 20°C	At 100°C		
1221	1.18	30-31	1.62	141-150	176	1	275-320	1,740			15,000[b]	11104-28-2
1232	1.26	31-32	1.62	152-154	238	-35	270-325	874	5.7	4.6	1,450[b]	11141-16-5
1016	1.37		1.62	170	ntb		323-356				420	12674-11-2
1242	1.38	34-35	1.63	176-180	ntb	-19	325-366	338	5.8	4.9	240	53469-21-9
1248	1.44	36-36	1.63	193-196	ntb	-7	340-375	152	5.6	4.6	52	12672-29-6
1254	1.54	44-58	1.64	Ntb[c]	ntb	10	365-390	53	5	4.3	12	11097-69-1
1260	1.62	72-78	1.65	ntb	ntb	31	385-420	13	4.3	3.7	3	11096-82-5
1262	1.64	86-100	1.65	ntb	ntb	35-38	390-425	9				37324-23-5
1268	1.81			ntb	ntb		435-450		2.5			11100-14-4
1270	1.95			ntb	ntb		450-460					

[a] The registry number for Aroclor (number unspecified) is 12767-79-2.

[b] Estimated.

[c] ntb = None to boiling.

(Kozloski, 1985). In a process of methylthio-substituted PCBs synthesis, γ irradiation of the parent PCB in dimethyl disulfide displaces the Cl- substituent by a CH_3S- group (Buser, 1985). Then, the methylthio-PCB can be transformed to the mehtylsulfone derivative (CH_3SO_2-PCB) by oxidation (Buser, 1992). The Aroclors 1248 and 1254 (used in this research), are prepared by neutron irradiation of the natural abundance materials (Stalling and Huckins, 1971). Aroclor 1254 contains 54% chlorine.

As mentioned, the Monsanto PCB mixtures were sold under the registered trademark of Aroclor®. As can be seen in Table 5 (Brinkman and De Kok, 1980), are lists of commercial PCB mixtures were manufactured by several different companies under different tradenames. PCB mixtures were utilized in various applications including dielectric fluids in capacitors and transformers; heat transfer fluids; hydraulic fluids; lubricating and cutting oils; and as ingredients in pesticides, paints, copy paper, carbonless copy (NCR) paper, adhesives, sealants, and plastics (Fig. 2, Durfee et.al., 1976). PCB use can be categorized by World Health Organization (WHO) (1993) into three classifications:

- **Completely closed systems**: electrical equipment such as capacitors and transformers.
- **Nominally closed systems**: hydraulic and heat transfer systems, vacuum pumps.
- **Open-ended applications**: plasticizer in PVC, neoprene, and other chlorinated rubbers, surface coatings, paints, inks, adhesives, pesticide extenders, microencapsulation of dyes, and carbonless copy paper.

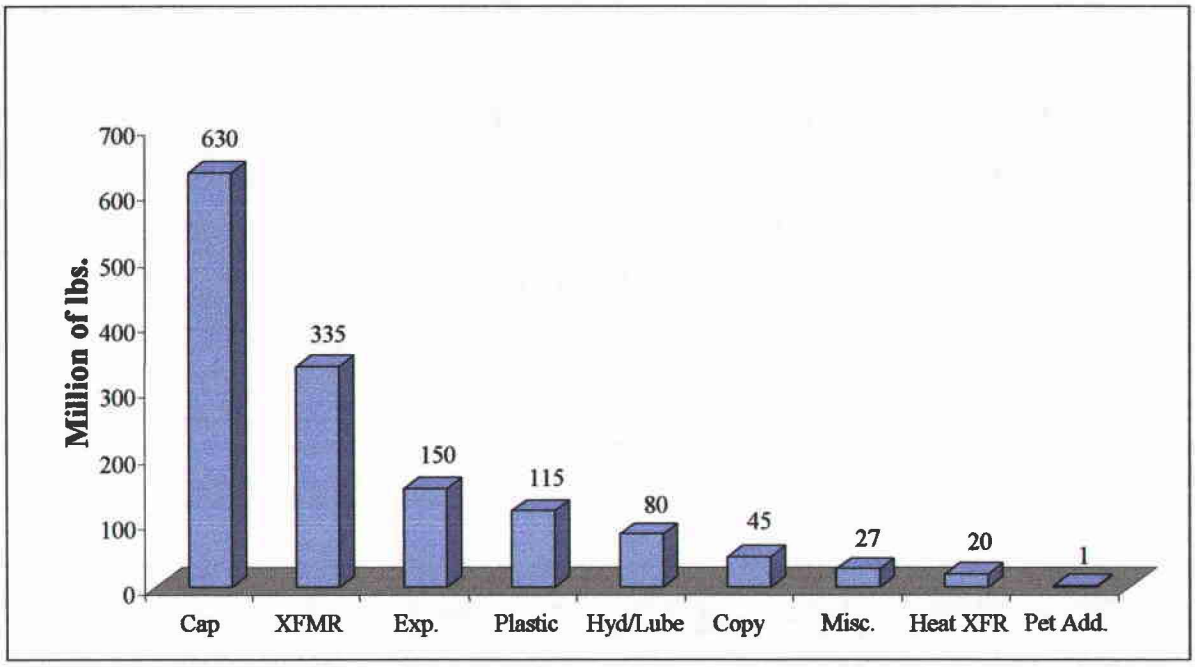


Fig. 2: Applications of PCB in the U.S. Based on Sales Records for 1930-1975

Table 5: Comparison of Commercial PCB Mixtures

Tradenames										
Aroclor	Clophen	Phenoclor	Pyralene	Kanechlor	Fenchlor	Delor		Av. No. Cl/molecule	Approx. wt.% Cl	Approx. mol. wt.
1221								1.15	21	193.7
1232			2000	200				2	32-33	223.0
			1500					2.5	38	240.3
1242, 1016	A30	DP-3	3000	300	42	2	Sovol	3	40-42	257.5
1248	A40	DP-4		400		3		4	48	291.9
1254	A50	DP-5		500	54	4; 5	Trichlorodiphenyl	5	52-54	326.4
1260	A60	DP-6		600	64			6-6.3	60	366.0
1262								6.8	62	388.4
					70			7.7	65	419.4
1268								8.7	68	453.8
								9.5	70	481.4
1270					DK			10	71	498.6

Monsanto Industrial Chemicals Company, St. Louis, MO.
Bayer, Germany.
Caffaro, Italy
Kanegafuchi Chemical Company, Japan.
Prodelec, France.
Chemko, Czechoslovokia
Manufactured in the former USSR.
The two-digit number should indicate the wt.% Cl; however, this does not fit in with the manufacturer's specifications.

Table 6: Relative retention time ranges and weight percent distribution of homologs in Aroclors.

		Chlorinated Biphenyls by Homolog									
		Cl1	Cl2	Cl3	Cl4	Cl5	Cl6	Cl7	Cl8	Cl9	Cl10
Aroclor 1221	% of weight	99.4	0.6								
	Retention time	0.15-0.2	0.22								
Aroclor 1232	% of weight	31.5	28.2	21.5	18.7	0.4					
	Retention time	0.15-0.2	0.22-0.35	0.31-0.54	0.44-0.65	0.75					
Aroclor 1016	% of weight	0.9	24.3	47.1	27.3	0.4					
	Retention time	0.15-0.2	0.22-0.35	0.31-0.54	0.44-0.62	0.61					
Aroclor 1242	% of weight	0.6	19.5	39.1	36.6	4.2					
	Retention time	0.15-0.2	0.22-0.35	0.31-0.54	0.44-0.65	0.61-0.87					
Aroclor 1248	% of weight		0.7	22.0	61.3	16.0					
	Retention time		0.28-0.33	0.31-0.54	0.44-0.72	0.61-0.87					
Aroclor 1254	% of weight				16.0	59.9	23.8	0.4			
	Retention time				0.49-0.65	0.61-0.87	0.76-1.04	1.04			
Aroclor 1260	% of weight				0.3	12.2	50.3	31.4	5.9		
	Retention time				0.49	0.61-0.82	0.76-1.4	0.99-1.11	1.16-1.23		
Aroclor 1262	% of weight					3.8	37.2	42.4	14.5	1.4	
	Retention time					0.61-0.75	0.76-0.9	0.99-1.11	1.16-1.23	1.28	
Aroclor 1268	% of weight							4.0	36.3	52.3	7.4
	Retention time							0.97-1.08	1.04-1.23	1.2-1.28	1.3

Retention times are relative to chysene-d12. (From Alford-Stevens, A.L., et al., Anal. Chem., 58(9):2014, 1986a.)

It was estimated by Trench (1990) that there were 2.6 million mineral oil transformers in the U.S. containing PCB concentrations between 50 and 500 ppm and another 260,000 mineral oil transformers holding PCB concentrations more than 500 ppm. As mentioned, each Aroclor has different properties depending on weight percent distribution of homologs. Table 6 demonstrates a general breakdown of Aroclor applications (Alford-Stevens, et al., 1986). As can be seen from Table 6, the relative retention time of Aroclor 1254 is in range of 0.49-1.04 hours (Cl_4-Cl_7). For Aroclor 1254, the highest percentage by weight of chlorinated biphenyls is Cl_5. Table 7 (Brinkman and Dekok, 1980) shows the average molecular composition (wt %) of several Aroclors.

Table 7: Average Molecular Composition (wt.%) of Some Aroclors

Homolog (Chlorines)	Aroclor						
	1221	1232[a]	1016	1242	1248	1254	1260
0	10						
1	50	26	2	1			
2	35	29	19	13	1		
3	4	24	57	45	22	1	
4	1	15	22	31	49	15	
5				10	27	53	12
6					2	26	42
7						4	38
8							7
9							1

[a]Six percent unidentified.

Not only are PCB species mixed in Aroclors, but impurities such as polychlorinated dibenzofurans (PCDFs), are also amalgamated with the commercial mixtures. Mostly, the impuities in the marketing PCBs are approximately <0.01% (NRC, 1979). The concentration of the impurities is shown in Table 8 (Veceta et.al., 1983) and Table 9

(Wakimoto et.al., 1988). It can be seen in Table 9 that chlorinated homologs of PCDFs can be identified, while Polychlornated dibenzo-p-dioxins (PCDDs) are not detected. Some compounds such as polychloroquaterphenyls (PCQs) are produced during the use of PCBs.

Table 8: Levels (μg/g) of PCDFs in Commercial PCBs

Mixture	Tri	Tetra	Penta	Hexa	Hepta	Total
Aroclor 1248 (1969)	-	0.5	1.2	0.3	-	2.0
Aroclor 1242	-	0.07	0.03	0.003	-	0.15
Aroclor 1242	-	2.3	2.2	N.D.[a]	-	4.5
Aroclor 1242	0.1	0.25	0.7	0.81	-	1.9
Aroclor 1254 (1969)	-	0.1	0.2	1.4	-	1.7
Aroclor 1254 (1970)	-	0.2	0.4	0.9	-	1.5
Aroclor 1254	-	0.02	0.2	0.4-0.6	-	0.8
Aroclor 1254 (KK 602)	-	0.05	0.1	0.02	-	0.2
Aroclor 1254	-	0.1	3.6	1.9	-	5.6
Aroclor 1260	0.06	0.3	1	1.10	1.35	3.8
Aroclor 1260 (1972)	-	0.1	0.4	0.5	-	1.0
Aroclor 1260	-	0.8	0.9	0.5	-	2.2
Aroclor 1260 (AK 3)	-	0.2	0.3	0.3	-	0.8
Aroclor 1016 (1972)	-	N.D.	N.D.	N.D.	-	-
Clophen A60	-	1.4	5	2.2	-	8.4
Clophen T64	0.1	0.3	1.73	2.45	0.82	5.4
Phenoclor DP-6	-	0.7	10	2.9	-	13.6
Prodelec 3010	0.41	1.08[b]	0.35	0.07	-	2.0
Kanechlor 400	-	-	-	-	-	20.0
Mitsubishi (used)	2.13	4.00	3.30	0.53	-	10.0

[a] N.D. = none detected.

[b] Major isomer 2,3,7,8-tetraCDF.

Table 9: Levels (ug/g) of PCDFs and PCDDs in Commercial PCBs

Mixture	Tetra-CDF	Penta-CDF	Hexa-CDF	Hepta-CDF	Octa-CDF	Total PCDFs	Total PCDDs
Aroclor 1242	0.5	0.09	0.02	-	<0.01	0.6	<0.002
Aroclor 1248	2.6	1.1	0.07	-	<0.02	3.7	<0.002
Aroclor 1254	0.7	2.0	1.3	0.03	<0.02	4.2	<0.002
Aroclor 1260	0.5	1.1	1.9	2.0	2.0	7.5	<0.002
Kanechlor 300	4.4	1.6	1.1	0.3	0.2	7.5	<0.002
Kanechlor 400	18.8	5.4	1.3	0.3	0.2	26	<0.002
Kanechlor 500	1.5	2.8	2.1	0.6	0.2	7.2	<0.002
Kanechlor 600	0.6	0.9	1.0	1.1	1.9	5.4	<0.002

Individual congeners or coeluting combinations, including several "toxic" 2,3,7,8-substituted congeners, were reported in original article.

- = None detected (quantitation limits given for individual isomers, but not for aggregate).

Aroclors are the names of one brand of commercial PCB mixtures. However, it has been found that PCBs can be produced as byproducts by chemical processes and thermal degradation comprised of chlorine and hydrocarbon sources (Erickson, 1997). The processes include the production of chlorinated benzenes, vinyl chloride, chlorinated solvents (e.g., chloroform), chlorinated alkanes, chlorophenylsiloxane adhesives, organosilicone drugs, organic intermediates (e.g., 3,3'-dichlo-robenzidine salts), and pigments (e.g., phthalocyanine green). Because the chemical means for PCB byproduct synthesis and commercial mixtures are dissimilar, the compounds in each PCB mixtures are not alike. However, both types can affect the environment. PCBs byproducts are also regulated, if their concentration is over the practical limit of quantitation (LOQ). Therefore, U.S. regulation not only applies to the commercial PCB mixture usage, but also to any chemical process which forms PCBs as byproducts.

Environmental Occurrence of PCBs

Not only mercury, lead, and certain pesticides but also PCBs and PCB byproducts are toxic to animals and plants. Their presence in the environment is a major concern. PCB environmental occurrence is indicated to environmental sources and distribution. The potential environmental sources include past open and uncontrolled uses, past disposal practices, illegal disposal, and accidental releases. PCBs can be found in natural resources (i.e., soil, water, and air), plants (i.e., marine plants), and animals (i.e., fish, birds, mammals, and wildlife). Table 10 (Wassermann et.al., 1979) shows the occurrence of PCBs in animals and Table 11 shows the PCBs appearance in nature in significant-level places. Furthermore, PCBs are found in food such as fish 1,870 mg/kg, fish

byproducts 1,170 mg/kg, cheese 250 mg/kg, milk 2,270 mg/kg, and shell eggs 550 mg/kg (Finlay et. al., 1976).

Table 10: Occurrence of PCBs in Animals

Organism	Concentration range (mg/kg)
Marine organisms	
Zooplankton	<0.003-1.055
Shellfish	<0.003-7
Seals	3-212
Whales and dolphins	0.012-147
Fish	
Fresh water (U.S.)	0.1-15
Marine	0.03-190
Birds	
North America	0.1-14,000
Europe	0.5-9,570
Eggs	0.1-434
Terrestrial animals	
Humans	
Adipose (general population)	0.3-10
Plasma (general population)	0.001-0.029
Plasma (occupational exposure)	0.036-1.9
Adipose (Yusho)	0.7-75.5
Plasma (Yusho)	0.002-0.015
Milk	0.01-0.39
Milk-extracted lipids	0.01-18.6

The toxic effect of PCBs was noted in 1968 in western Japan following the discover of PCB contamination in cooking oil. Once PCBs became known as toxicants, scientists started studying the destruction or degradation of PCBs.

Table 11: Occurrence of PCBs in nature

air			
Matrix	Location	Concentration (ng/m3)	Source
Outdoor air	U.S. transformer manufacturer	17-5,900	MacLeod, 1979
	U.S. spill site	10-10,800	MacLeod, 1979
	U.S. electrical substations	1-47	MacLeod, 1979
	U.S. landfills	2-18	MacLeod, 1979
	Germany	5-10	Benthe et al., 1992
	Tokyo, Japan	20	Kimbrough, 1980b
Indoor air	U.S. after light ballast burnout	5,860	MacLeod, 1979
	Germany Clophen in building sealant	40-1,200	Balfanz et al., 1993
	Office bldgs. In MN with PCB transformers; no fire	457	Oatman and Roy, 1986
Stack gas	ENSCO, EI Dorado, AR	12,000-58,000	Erickson et al., 1984a
	Rollins, Deer Park, TX	9.8	Erickson et al., 1984a
water			
Matrix	Location	Concentration (ng/L)	Source
Water	U.S., Lake Michigan, spill site	100-450	WHO, 1976
	General, highly polluted rivers	<500	WHO, 1976
	Rain and snow, Croatia	<1-203	Fingler et al., 1994
	Ocean water	0.3-4,200	Bidleman et al., 1983a
	Japan, tap	1-100	WHO, 1976
Soil			
Matrix	Location	Concentration (μg/g)	Source
Soil	Japan - near electrical component factory	510,000	WHO, 1976
	U.S. - transformer manufacturer	17-17,800	MacLeod, 1979
	Japan - agricultural	<1,000	WHO, 1976
Sediment	Spill site	1,400-61,000	WHO, 1976

Destruction and Degradation of PCBs

Because PCB contamination tends to be stable, complex, heterogeneous, and refractory, it is not easy to destroy or degrade in general. Nevertheless, under certain conditions, PCBs can be degraded by chemical, thermal, and biochemical processes. For thermal destruction technologies, incinerators are regulated under the Toxic Substances Control Act (TSCA) to ensure that PCBs are destroyed. In the U.S., incineration under TSCA requires 99.9999% destruction and removal efficiency. Moreover, TSCA regulations also require a package of high heat, a long retention time, and surplus oxygen (e.g., 1,200±100°C for 2 seconds with 3% O_2 or 1,600±100°C for 1.5 seconds with 2% O_2) and the ash must include less than 2 ppm PCBs (TSCA, 40 CFR 761.70) . One concern with PCB destruction by incineration is incomplete combustion that can produce PCDFs in the emissions of incinerators. There are chemical techniques to remove PCBs from contaminated matter including chlorinolysis, catalytic dehydrochlorination, microwave plasma, ozonation, photolytic, wet air oxidation, reaction with sodium naphthalide, reaction with molten sodium, and reaction with a sodium salt in an amine solvent (Erickson, 1997). One effective process uses potassium hydroxide in polyethylene glycol (KPEG) to extract chlorine from the biphenyl ring. The KPEG process has been used to remove PCBs from contaminated transformer oil used for electrical insulation purposes (Ruggeni et. al., 1993; Brunelle etl al., 1985). Electrochemical catalysis has been investigated for elimination of PCBs using zinc phthalocynine as the catalyst and a lead cathode (Ahang and Rusling, 1993). Finally, the biological destruction of PCBs is directly associated with this research.

Biologically, PCBs can be degraded by both aerobic (oxidizing) and anaerobic (reducing) systems. Anaerobic microorganisms can partially dechlorinate the highly chlorinated PCBs congeners (e.g., tetra-, penta-, and hexa- PCBs), while aerobic cultures can deal with the lower chlorinated PCBs congeners (e.g., mono-, di-, and tri- PCBs) and produce carbon dioxide (CO_2), water, and chloride ions (Cl^-) through a chlorinated benzoic acid intermediate. In nature, microorganisms prefer to consume and attack familiar molecules; thus, it is necessary to acclimate them to PCBs by various strategies; for instance, cometabolism.

Reviews of Previous PCB Biodegradation Studies

Researchers have been investigating PCB biodegradation for several decades. Not only have PCBs been studied, but otherchlorinated recalcitrant compounds have also been examined. An early report revealed that two species of *Achromobacter* were able to degrade mono- and dichlorobiphenyls (Ahmed and Focht, 1973). Early studies discovered that highly chlorinated PCBs were not degraded under aerobic conditions (Zitomer, and Speece, 1993). However, they are dechlorinated under anaerobic conditions (Abramowicz, 1990, Mohn and Tiedje, 1992). In nature, the two-step sequence of dechlorination was affected by anaerobic bacteria from aquatic sediments and aerobic cultures from water (Brown, 1987). Each chlorinated compound will be dechlorinated by various types of microorganisms. It was revealed that *Acinetobacter* sp. Strain 4CB1 can use 4-chlorobenzoates as a sole source of carbon and energy under aerobic and anaerobic conditions (Adriaens, 1989). PCB biodegradation can be accomplished by exceptional bacterial strains. For example, *Pseudomonas putida* attacks

every tetra, and pentachlorobiphenyl congener (Unterman et al., 1998). Moreover, with a noval technology, upflow anaerobic sludge bed (UASB), *Pseudomonas*, *Xanthomonas*, and *Rhodococcus* app. were dominant in the system when PCB dechlorination was studied (Guiot, 1998). PCB biodegradation is slow. The process can take approximately 5-24 months (Rhee, 1993; Natarajan, 1998). In the long term study of Aroclor 1254, the greatest dechlorination activity took place in the first 5 months and the activity was substantially slow in the 5^{th} to 24^{th} months (Rhee, 1993). In the process of dechlorination, different PCB congeners are degraded and this transforms the composition. Generally, highly chlorinated PCBs are dechlorinated by anaerobic bacteria to low chlorinated PCBs, and then the low chlorinated PCBs are dechlorinated by aerobic bacteria to biphenyl compounds or one ring compounds. However, the differences in congener specificity provide distinct pathways of PCB degradation (Unterman, 1998). In addition, the dechlorination of one isomer of PCB can produce several types of PCBs. For example, 2,3,5,6-CB can be transformed to 2,3,6-CB or 2,3,5-CB (Heidi, 1991). Some researches have studied the enhancement of PCB dechlorination by adding catalysts or co-oxidation compounds. Vitamin B12 can be used as a catalyst in PCB dechlorination (Anid, Nies, and Vogel, 1991). Significant sites at which PCB biodegradation has been studied include the Hudson River (Anid, Nies, and Vogel, 1991; Morris et. al., 1992; Quensen III, Boyd, and Tiedje, 1990), and Lake Michigan (Natarajan et. al., 1998; Rhee et. al., 1993). PCBs mostly contaminate soil and sediment. Recently, it was found that phytoextraction might occur; thus, PCBs can be transferred from soil to harvestable plants.

The Current Situation of PCB Contamination in Hawai'i

It was found that PCBs contaminate the soil at the former Navy facility in Haiku Valley, Oahu, Hawai'i. It is also reported that a small amount of phytoextraction of PCBs has occurred at the site. The quantity of PCBs in the vicinity of plant roots was higher than that in the surrounding soil and some plant leave tissue contained PCBs (Spengler, 2002). Therefore, the transfer of PCBs into the plant tissue via phytoextraction is considered as the primary stage of PCB treatment. Treatment of harvested contaminated plant tissue must be a subsequent step. Incineration burial of the plants in approved facilities, and biological treatment are potentially viable approaches. Of these, the most cost-effective might be PCB biodegradation. With this in mind, this study was conceived to investigate the sequential anaerobic-aerobic biodegradation of PCBs in phytoremediation cuttings.

Chapter 2

Materials and Methods

Materials

Polychlorinated biphenyls (PCBs) disposed in a certain area in Hawaii caused contamination in soil. The phytoextraction of PCBs has occurred. PCBs were transferred from soil to plant roots, and then to plant tissue. Therefore, the thesis experiment was to simulate the phytoextraction of PCBs and the sequential anaerobic/aerobic biodegradation of PCBs in the plant tissue.

Preparation of PCBs Standard

Since it was not possible to obtain sample of PCB contaminated plants, commercial-grade Aroclor 1254 PCB standards were used for this research. A 1,000 mg/L solution of Aroclor 1254 PCBs in methanol was created and stored in two 40 mL blue cap vials at room temperature.

Preparation of grass

In the field remediation system, the PCBs are adsorbed by the plants and stored inside tissue of the plants. Therefore, the laboratory test system needed to add plant tissue for the PCBs to attach to. California grass was selected for this experiment because it was readily available, it was easy to chop and grind, and it was durable. The California grass would be cut into tiny pieces and then ground by a grinder. The ground grass was stored in a beaker sealed with plastic film in a refrigerator set at 20°C.

Preparation of media solution

The concentrated media solution is a mixture of mineral salt. It needed for the basic nutrition of the microorganisms in the experiment. The concentrated mineral media composition was as follows:

Mineral	mg/L	Mineral	mg/L
$(NH_4)_2HPO_4$	80	$CuCl_2 \cdot 2H_2O$	0.2
NH_4Cl	1,000	$Na_2MoO_4 \cdot H_2O$	0.23
K_2HPO_4	200	$ZnCl_2$	0.19
NaCl	10	$NiSO_4 \cdot 6H_2O$	0.2
$CaCl_2$	10	$FeSO_4 \cdot 7H_2O$	1
$MgCl_2$	50	$AlCl_3 \cdot 6H_2O$	0.4
$CoCl_2 \cdot 6H_2O$	1.5	H_3BO_3	0.38

The mineral media was added in reactors as supplementary nutrients in order to enhance efficiency of degradation when microorganisms consume food (methanol) and dechlorinate PCBs.

Methods

The experiment aimed to study the process of commercial PCB biodegradation by sequential anaerobic-aerobic process. Two methods were applied for the experiment including the blue-cap vial method, and the two liter-flask reactor method. Both methods were based on the batch reactor process. The blue-cap vial method was run first and followed by the two liter-flask reactor method. The major reason that the two methods were utilized was because the results of the blue-cap vial method seemed not to be sufficiently effective to demonstrate the PCBs biodegradation. Therefore, the second method was set up to improve the experiment and to achieve more reliable results.

The Blue-cap Vial Method

Various samples of the blue-cap vial method were created. The vial size was 45 mL with the containing a Teflon-lined rubber septa blue cap (Fig.3). One set of the experiment consisted of five different samples including blank samples, blank with grass samples, anaerobic with grass samples, anaerobic without grass samples, and anaerobic from wastewater treatment plant samples. Ten sets were prepared for six periods of time (duplicate samples for each period of time). The steps to prepare the samples were as follows:

1. Add 1 g of ground grass into a 45 mL blue cap vial (for the samples with grass).
2. Add 3 mL of concentrated mineral media.
3. Add 10 mL of culture (i.e., anaerobic).
4. Add distilled water to obtain 30 mL total volume.
5. Spike 30 μL of PCB standard (will get 1 ppm of PCBs).
6. Bubble nitrogen gas into the vial for 15 min to remove oxygen and create anaerobic conditions using a nitrogen evaporator, N-EVAP^{TM} 111 (Fig.4) (anaerobic process samples).
7. Seal the cap with paraffin film and tape to prevent air coming inside the vial for anaerobic samples.
8. Put samples in G24 environmental incubator shaker at 280 rpm and 30°C (Fig.5).

1. Blank samples and blank with grass samples.

These two samples were prepared as controls. The blank samples were used to examine the PCB change without the addition of microbial cultures. The blank with grass

samples were created to investigate the effect of the grass on the spiked PCBs and the extraction process.

2. *Anaerobic Process samples with and without grass.*

The samples were the principal samples of this work. The objective was to investigate the biodegradation of commercial PCBs (Aroclor 1254) by anaerobic microbes in the presence of plants. The anaerobic bacteria were expected to dechlorinate highly chlorinated biphenyls to lower chlorinated biphenyls. However, the potential of biodegradation of PCBs inside plant tissues was unknown. In the experiment, the aerobic samples were designed for three conditions including anaerobic with grass samples, anaerobic without grass samples, and anaerobic from wastewater treatment plant with grass samples. The first two types of anaerobic samples used anaerobic cultures from the department of Bioengineering, University of Hawai'i at Manoa,

Fig.3: Blue-cap Vials

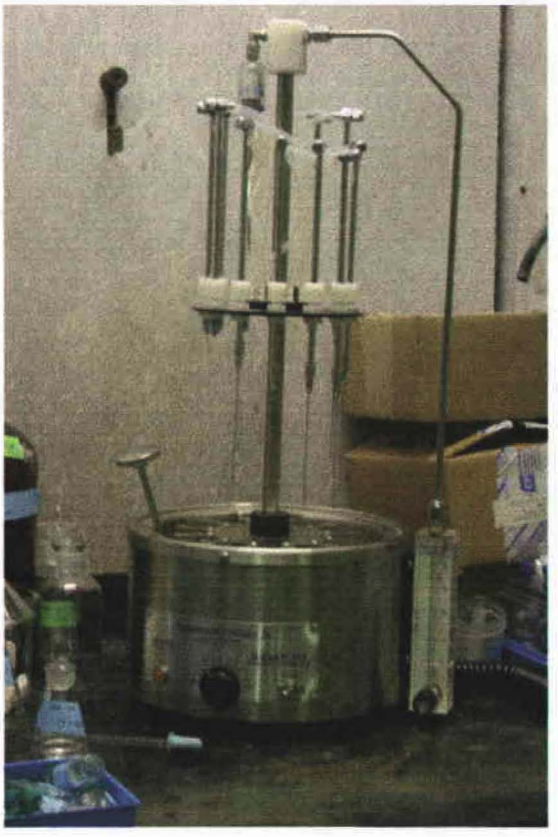


Fig. 4: Nitrogen Evaporator

Fig.5: G24 Environment Incubator Shaker

while the third type of sample used anaerobic bacteria from the Hawai'i Kai Wastewater Treatment Plant. The results would compare the difference of PCBs biodegradation between with/without grass as well as the difference of PCBs biodegradation efficiency between two sources of anaerobic microorganisms.

To feed food for anaerobic cultures, 1 mL of methanol was added to the vials weekly. The anaerobic samples needed to release the gas production out of the vials weekly. The samples of each set were taken by period of time. The existing PCBs would be extracted by a solvent.

Reactor Method

As mentioned above, the method was designed to improve the experiment of the PCBs biodegradation from the blue-cap vial method. The main idea of the reactor method was to create one large reactor for taking samples over time. The system was separated into two sequential systems which were comprised of the anaerobic system following by the aerobic system.

Anaerobic System

The concept of the PCBs biodegradation was as same as that of the blue-cap vial method. The steps to start-up the anaerobic system were as follows:

1. Add 40 g of ground grass into a 2,000 mL flask.
2. Add 120 mL of concentrated mineral media.
3. Add 680 mL of distilled water to reach 1,200 mL total volume.
4. Spike 1.2 mL of PCB standard (will get 1 ppm of PCBs).
5. Add 400 mL of anaerobic bacteria (from WW).

6. Bubble nitrogen gas into the vial for one hour at 2 PSI to create anaerobic conditions.
7. The reactor was placed in a small basin containing water (in order to distribute the temperature to the whole system) and both the reactor and the basin were placed on a stirring hotplate (see Fig.6). One magnet was put in the reactor as a stirrer to completely mix the system. Temperature was maintained at 30-35 °C.

Substrate addition was accomplished by opening the rubber stopper of the flask, injecting 1.2 mL of the substrate, and closing the rubber stopper. Since the stopper was opened, the reactor lost the anaerobic condition. Nitrogen gas was again bubbled for 15 min to remove oxygen. The substrate (methanol) was added weekly. A gas collector was connected to the reactor to measure the produced gas from the system. Samples were regularly taken by a pump drive that removed the sample from the reactor without opening the stopper. Parameters measured when taking samples were PCB concentration, pH and oxidation-reduction potential (ORP). The pH and ORP were measured in order to monitor the anaerobic condition of the system.

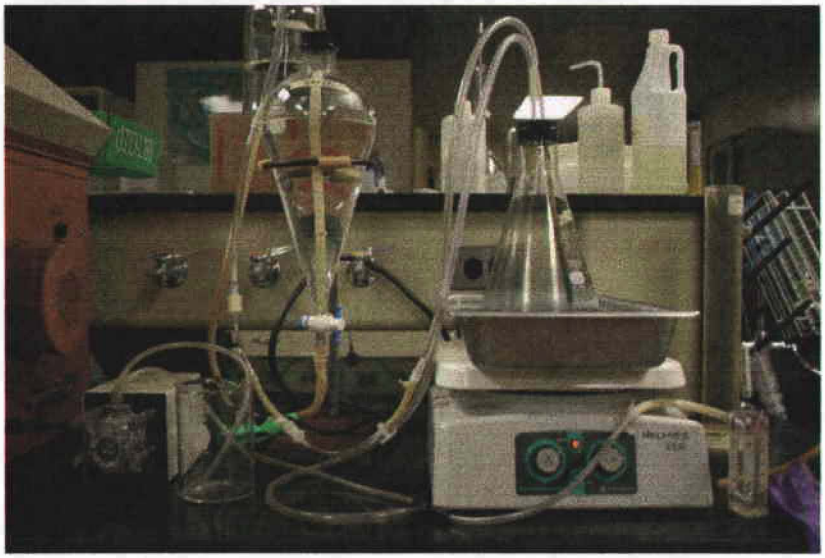


Fig.6: Anaerobic Reactor with a Gas Collector

Aerobic System

Some of the mixed contents of the anaerobic reactor containing both highly chlorinated PCBs and low chlorinated PCBs were added to the aerobic reactor in order to continue the low chlorinated PCB aerobic biodegradation as well as investigate the aerobic biodegradation of highly chlorinated PCBs. The steps for set-up of the aerobic system were as follows:

1. Take 450 mL of the mixed contents of the anaerobic system and add into a 1,000 mL flask.
2. Add 90 mL of concentrated mineral media.
3. Add 300 mL of aerobic culture from bioengineering department.
4. Add 60 mL of distilled water to reach 900 mL total volume.

5. The reactor was placed on the stirring plate and located in a fume hood at 25°C. One magnet was put in the reactor as a stirrer to completely mix the system. Compressed air to diffuse air was used into the system.

In addition to continuous diffused air, the rubber stopper was also opened daily for sufficient oxygen. The substrate, 1 mL of methanol, was spiked in the flask weekly. Samples were regularly taken.

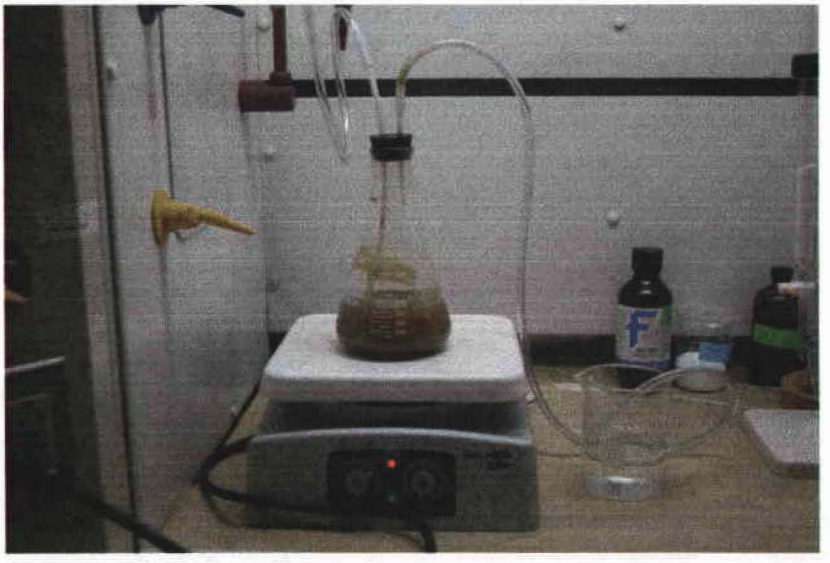


Fig.7: Aerobic Reactor System

Extraction procedure

PCBs are insoluble in water; however, they are able to dissolve in organic solvents. To investigate the amount of PCBs in the samples, it was necessary to use a solvent to extract the PCBs from the samples. The extracted PCBs were then quantified by gas chromatography (GC).

1. *The selection of the solvent.*

There are several solvents that can be used for PCB extraction; for instance, hexane, isooctane, acetone, and other solvents. For this study, hexane and isooctane were tested and compared. It was concluded that isooctane was slightly more effective to extract PCBs and it was selected for this study.

2. *The physical mechanism of the extraction.*

This is one of the most important aspects of the extraction. Inclusion of grass tissue with adsorbed PCBs in this study caused challenges with effective extraction. Therefore, vigorous shaking was conducted for three minutes. However, the vigorous shaking alone was insufficient for extraction. It was found that the ultrasonic water bath (Fig.8), with high frequency vibration, could improve the efficiency of the extraction. Samples were placed in the ultrasonic water bath for 15-20 minutes causing some of the absorbed PCBs in the grass to be released and extracted by the solvent.

3. *Separation of the solvent from the samples.*

After adding 10 mL of isooctane solvent to the samples to extract PCBs, the solvent needed to be separated. The solvent will mostly separate from the samples on its own because of the different fluid specific weights. However, there were some colloidal particles and water in the solvent. They must be removed since; they can damage the gas chromatograph. To separate those contaminants, the samples were centrifuged for 15 minutes.

4. *Preparation of the solvent for the gas chromatograph (GC).*

Even though, the particles were removed from the solvent by the centrifuge, some part of water was still mixed with the solvent. Water was removed from the solvent by

passing it through sodium sulfate (Na_2SO_4) when filling the solvent into the gas chromatograph vials (Fig.9).

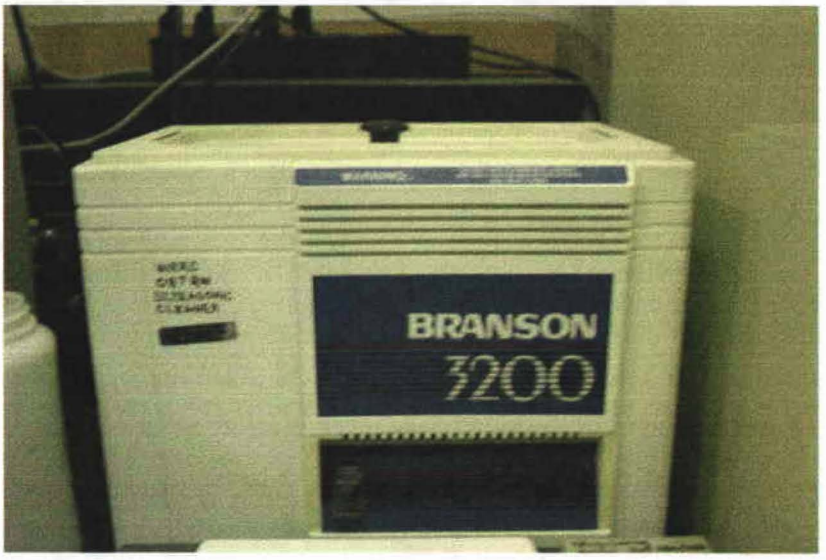


Fig.8: Ultrasonic Water Bath

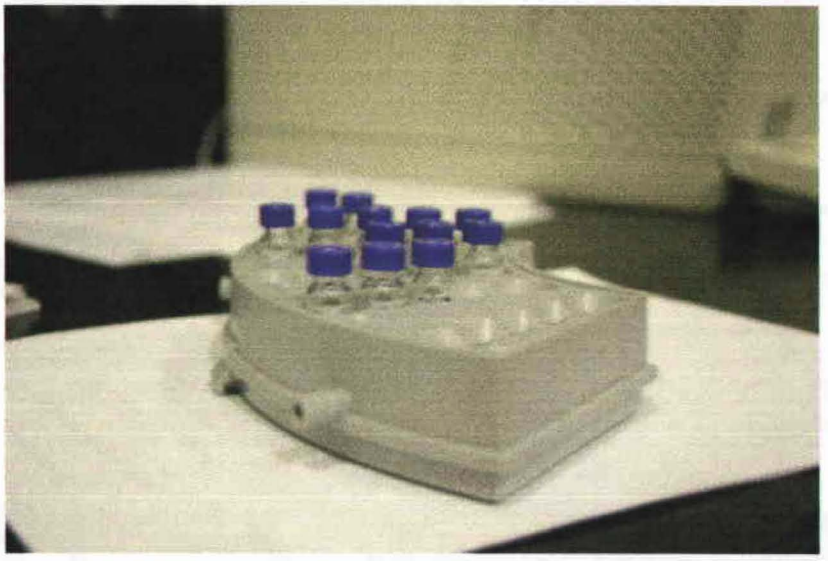


Fig.9: Gas Chromatography Containers

5. *Gas chromatography.*

The PCB analysis instrument for this study was the gas chromatograph (GC), Hewlett Packard 5890 with Electron Capture Detector (ECD) (Fig.10). The column used was a J&W Scientific DB-XLB capillary column, 30m x 0.53mm ID x 1.5µm film at 5 mL/min flow rate (Helium carrier gas). The GC oven temperature program was 110°C, hold 1 min, to 140°C at 20°/min, to 280°C at 11°/min, hold 10 min, to 300°C at 20°/min, hold 4.27 min. The total running time was 30.5 minutes. The injector temperature was 250°C and the detector temperature was 300°C. The sample injection volume was 2µL in isooctane or hexane. The result from the GC was reported to chromatograph. Fig.11 shows an example of the chromatograph of 1 ppm PCB standard.

Fig.10: Gas Chromatography

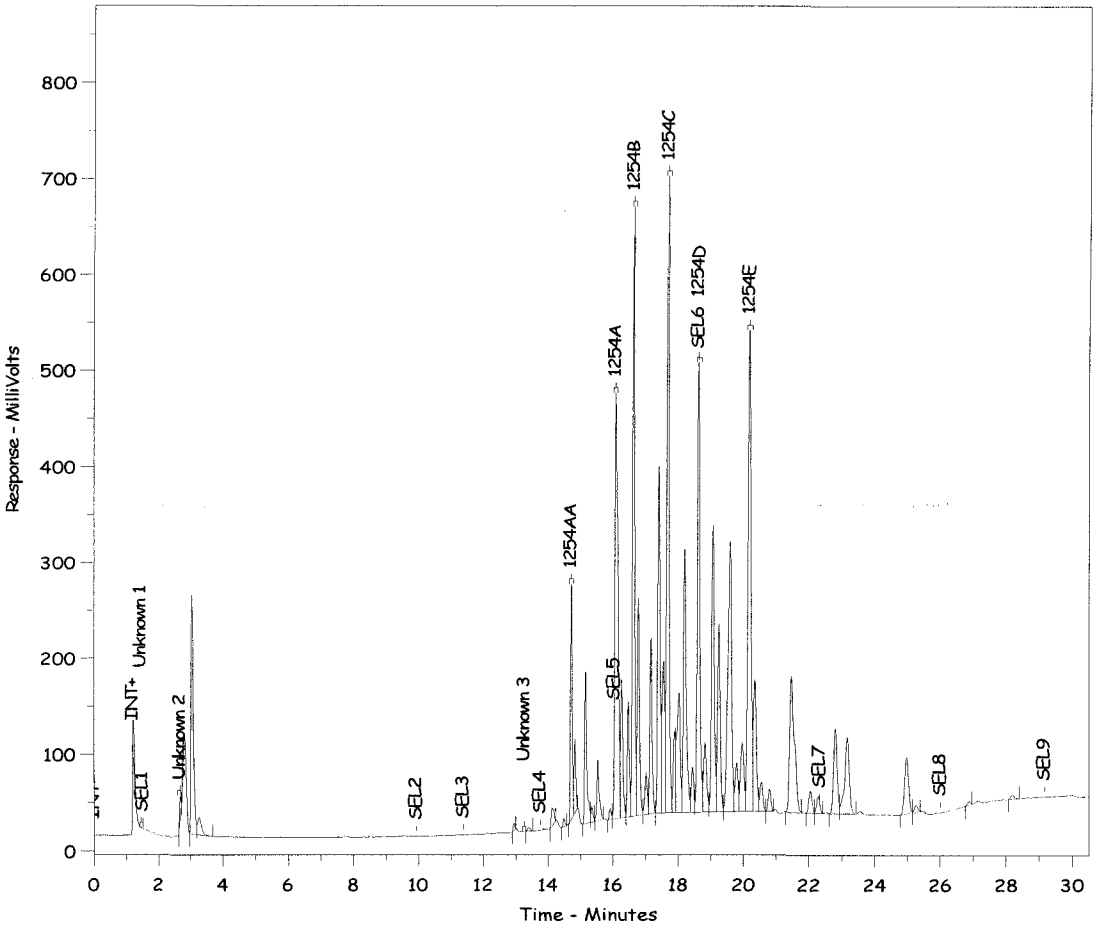


Fig.11: Chromatograph of 1 ppm PCB Standard

Chapter 3

Results and Discussion

The experimental results are disclosed separately: first the blue-cap vial method, and second the reactor method.

Blue-cap vial method

The result of the grass control (Fig.13) showed that the PCB concentration was in the range of 0.25-0.35 ppm and seemed to be steady during the 16-week experiment. In spite of 1 ppm (spike 1 ppm of PCBs), the concentration of PCBs dropped around 0.65-0.75 ppm. It can be explained that phytoabsorption occurred in the system. Therefore, the extracted PCBs were only some PCBs adsorbed on the plant's surface. The absorbed PCBs were not extracted (please note that the ultrasonic bath had not been used in the blue-cap vial method). The chromatograms look the same shape for grass control (Fig.12). Next, three types of anaerobic samples were analyzed. It can be observed from Fig.14, Fig.15, and Fig.16 that the tendencies of the PCB concentration were similar. The PCB concentration tended to decrease in the first 8 weeks and increase after 8 weeks. For example, the anaerobic with grass (Fig.14) showed that the PCB concentration of each peak began approximately 0.28-0.372 ppm. at the first extraction (first 2 weeks) and tended to slightly increase in next 2 weeks. During 6-8 weeks, the PCB concentration declined again. After 8 weeks, the PCBs concentration increased. This fluctuation can be explained step by step. For the first extraction that PCB concentration showed 0.28-0.372 ppm instead of 1 ppm, it resulted from three effects 1. the biodegradation process occurrence in the system that anaerobic cultures dechlorinated some highly chlorinated PCBs to low chlorinated PCBs; 2. phytoabsorption that a certain amount of PCBs were

absorbed by the tissue of the grass, and 3. biosorption that some PCBs were absorbed by bacteria. The second step that PCB concentration seemed to be steady can be analyzed that some highly chlorinated PCBs were dechlorinated; whereas, some highly chlorinated PCBs from the phytoabsorption or biosorption were concordantly released. Thus, the high chlorinated PCB concentration was maintained. The third step, the week of 6^{th}-8^{th}, the PCB concentration declined again. This was caused by PCB biodegradation. The last step, over 8 weeks, the PCB concentration tended to be higher. It can be assumed that the PCBs were released from the grass and the cultures. The grass tissue might be broken with time. Moreover, the cultures in the sample might die, so they might release PCBs out. Focusing on the pathway of PCB transformation, it can be notice from Fig.14 and Fig.16 that the pathways of two different sources of the cultures were different. Overall of the blue-cap vial method did not provide the evident potential of the anaerobic process in the PCB biodegradation. In addition, two chromatographs of anaerobic samples (Fig.17, and Fig.18) show no PCB biodegradation (no peak of low chlorinated PCBs and small compounds occurred in Fig.18). This may have been caused by several defects during the experiment.

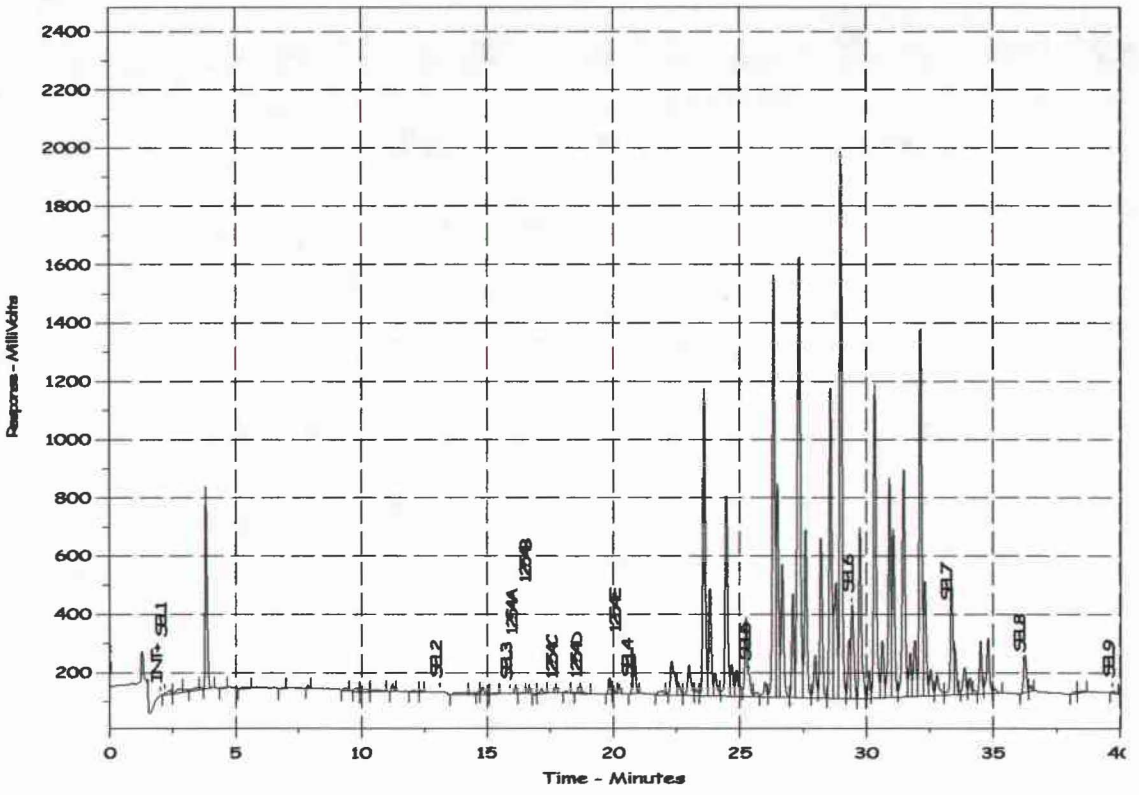


Fig.12: Chromatograph of Grass Control

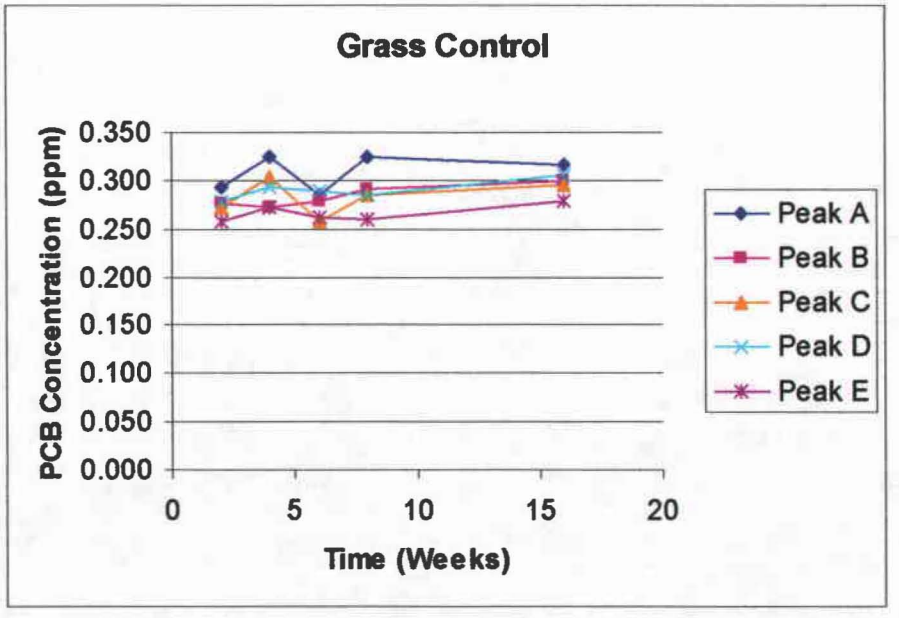


Fig.13: Blue-cap Vial Method: Grass Control Graph

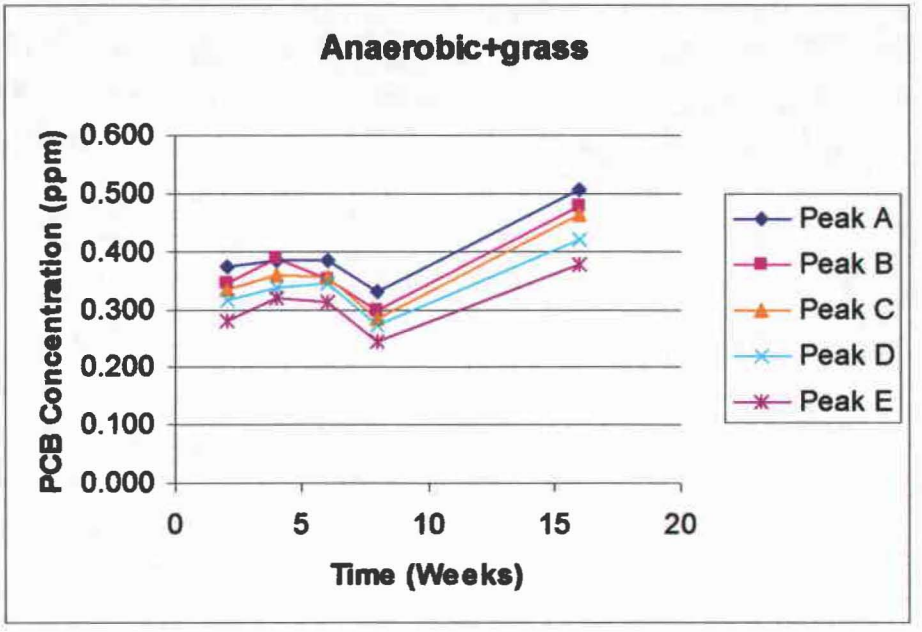


Fig.14: Blue-cap Vial Method: Anaerobic with Grass Graph

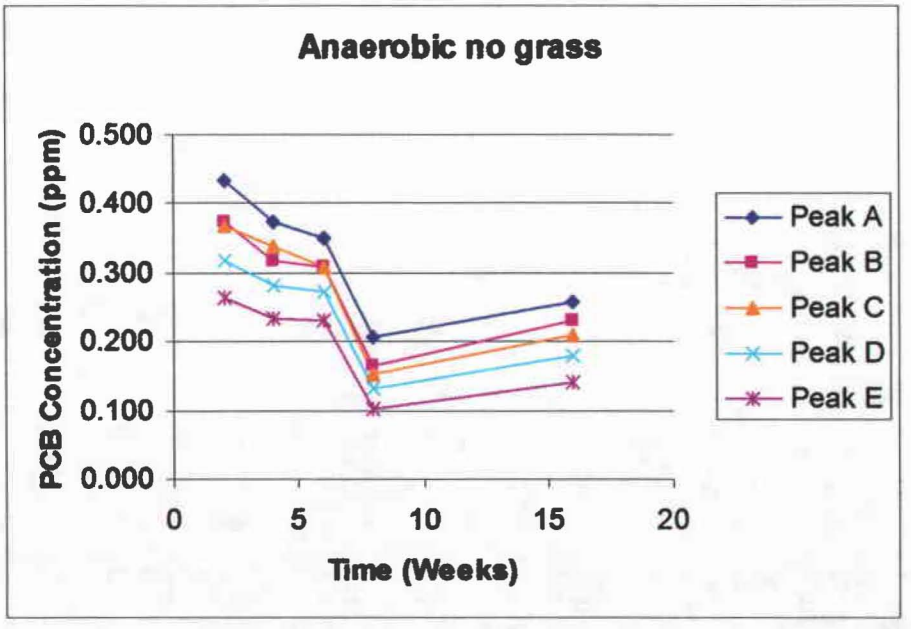


Fig.15: Blue-cap Vial Method: Anaerobic without Grass Graph

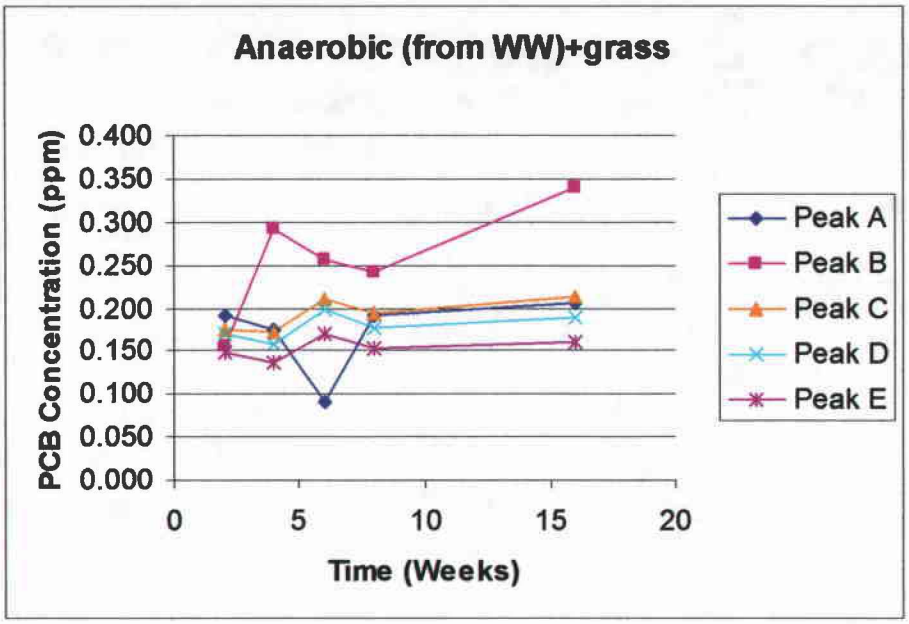


Fig.16: Blue-cap Vial Method: Anaerobic (from WW) with Grass Graph

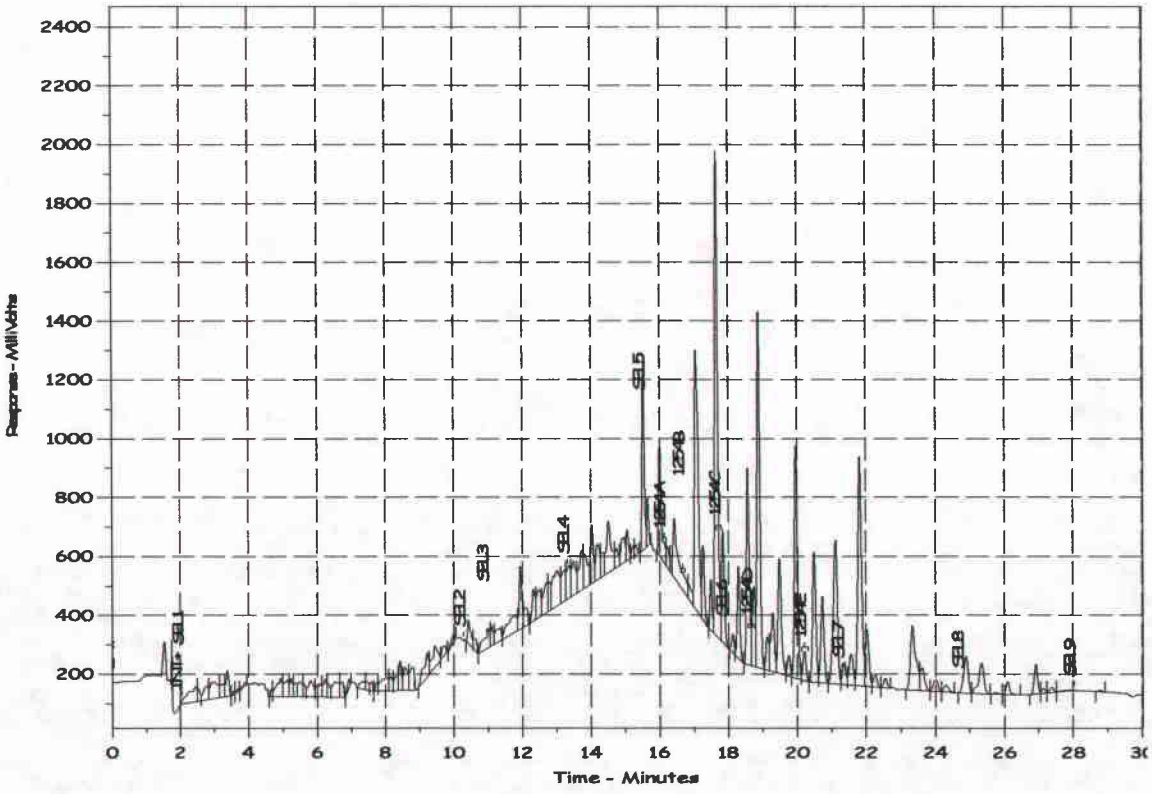


Fig.17: Chromatograph of 2-week Sample of Anaerobic Blue-cap Vial Method

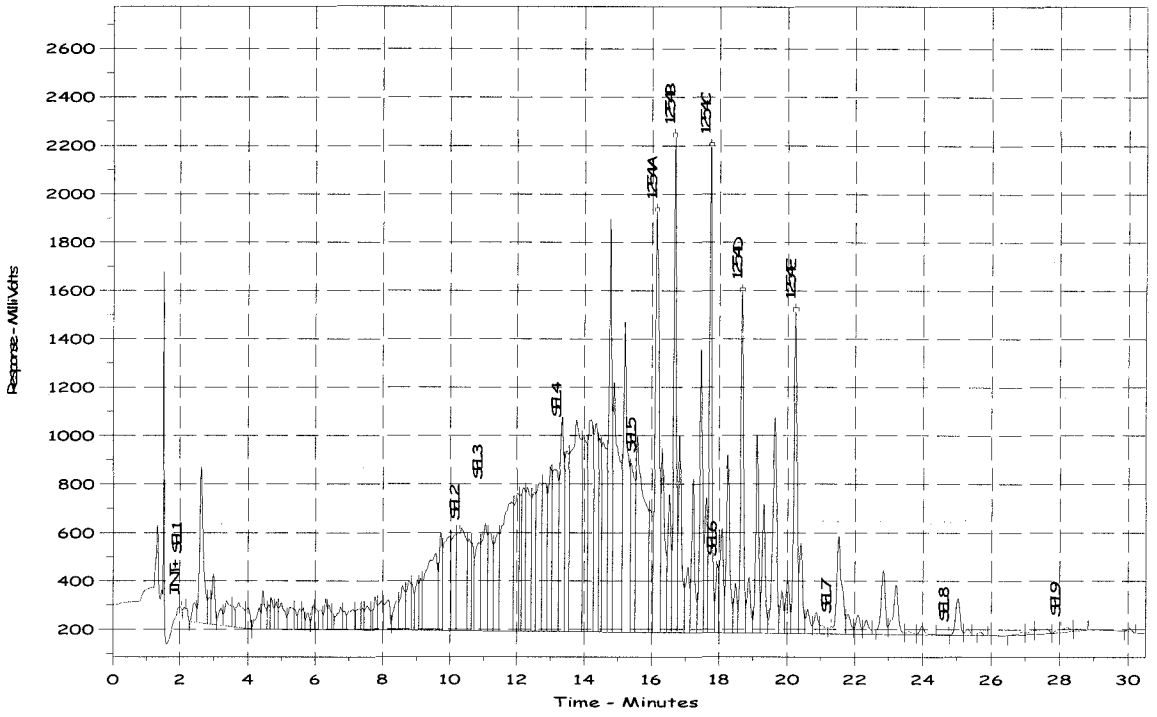


Fig.18: Chromatograph of 8-week Sample of Anaerobic Blue-cap Vial Method

In the experiment, there were some deficiencies which caused inconsistent results. First of all, it was necessary to maintain the anaerobic condition for the anaerobic samples. The action to create the anaerobic condition was bubbling nitrogen gas to remove oxygen gas from the vials. The pressure and time of nitrogen gas induction were monitored to confirm that oxygen gas could be completely replaced. During the process, a cap of each vial would be perforated. Therefore, it needed to be sealed with paraffin to prevent oxygen from environment. The properties of paraffin were elastic, and smooth; thus, it was suitable for this use. The paraffin was changed weekly when the caps were opened. Secondly, sufficient substrate was required for cultures for growth. The substrate used for this study was methanol (CH_3OH) by spiking 1 mL. of the methanol weekly. The common idea was while the cultures were dealing with the substrate, they would also

dechlorinate the PCB. The closer the optimum amount of the substrate was added, the more effective the biodegradation of PCBs. Thirdly, one of the most important parameters was temperature of the system. Normally, anaerobic bacteria are classified as mesosphillic bacteria which were active at 30-35 °C. Controlling in the range of optimum temperature was needed; otherwise, the cultures were inactive or no longer living. Fortunately, the samples were placed in an incubator where the temperature could be managed. Although all aspects were carefully controlled, the problem still unpredictably happened. Several anaerobic samples lost their anaerobic condition. It can be noticed that the anaerobic bacteria were dead by the color of the cultures, and gas production from the process. With the obscure results from the blue-cap vial experiment, the reactor method was arranged to study the PCB biodegradation process.

Reactor method

It can be said that the reactor method was able to depict the PCB biodegradation better than the blue-cap method by comparing the chromatograph of anaerobic reactor. Fig.19 shows the chromatograms of 4-week sample and Fig.20 shows the chromatograms of 17-week. The sizes of highly chlorinated PCB chromatograms decreased with time; whereas, several unidentified chromatograms occurred. Fig.21 clearly shows the declination of PCB concentration in the anaerobic reactor over more than 40 week experiment. When considering each type of PCB, their concentrations were different; however, all declined. It can be noticed from Fig.21 that the concentration of PCB 1254AA exhibited the largest decrease. The reactor was connected to a gas collector. Gas production could be a detector to examine the microbial activity. The produced gas could consist of methane (CH_4), carbon dioxide (CO_2), and small amounts of nitrogen (N_2),

hydrogen (H_2), hydrogen sulfide (H_2S), water vapor, and other gases. Unfortunately, collected gas was not analyzed; however, the volume of gas was measured and shown in Table 12. Therefore, the rate of methane production could be calculated. Theoretically, gas production from anaerobic system contains about 65 to 70 percent CH_4 by totally gas production volume (Metcalf&Eddy, 2003). The estimation of CH_4 volume is shown in Table 12 (use 65 percent). The anaerobic reactor developed a leak at the 30^{th} week. That resulted in no gas production in the gas collector. Instead of unchanged PCB concentration, the concentration increased. A possible explanation is that oxygen, a toxicant for the anaerobic cultures, contaminated the system; thus, some groups of bacteria were killed and then PCBs were released out of the cells. As a result, the PCB concentration increased. After reconditioning (N_2 purge), the system returned to normal and the PCB concentration started decreasing again.

The sequential step after the anaerobic process was the aerobic process. 450 mL from the anaerobic reactor was placed in the aerobic reactor at week 42. In addition, the anaerobic reactor was spiked with the new PCBs and new anaerobic cultures to examine and confirm the efficiency of the anaerobic biodegradation. It can be seen from Fig.22 that the PCB concentration tended to decrease again. In addition, the rate of PCB decrease of the new spiked PCBs was higher than that of the first spike. It implied that the cultures to dechlorinate PCBs were acclimated. In case of the aerobic system, the objective was to dechlorinate the low chlorinated PCBs as well as some highly chlorinated PCBs. To identify the low chlorinated PCBs, the standard of the low chlorinated PCBs was needed for the gas chromatograph. Unfortunately, the standard was not supplied for this study; therefore, only the high PCB concentration was monitored.

Fig.23 shows that the concentration of the highly chlorinated PCBs decreased with time. Moreover, two chromatographs of the aerobic reactor (Fig.24 and Fig.25) also demonstrate highly chlorinated PCB dechlorination. Therefore, it can be predicted that aerobic cultures would dechlorinate the low chlorinated PCBs. Overall from the reactor method; the sequential batch system was able to perform the PCB biodegradation from the phytoextraction cuttings.

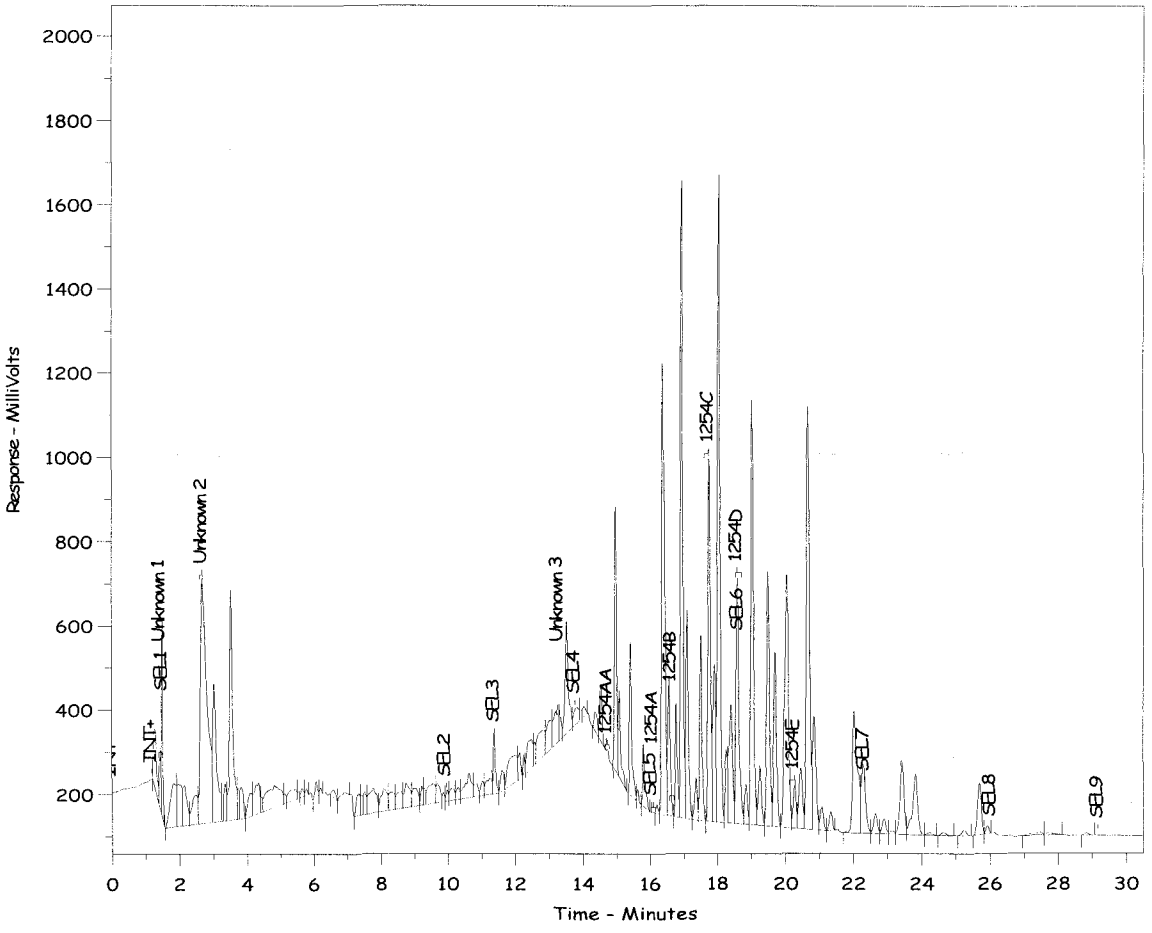


Fig.19: Chromatograph of 4-week Sample of Anaerobic Reactor

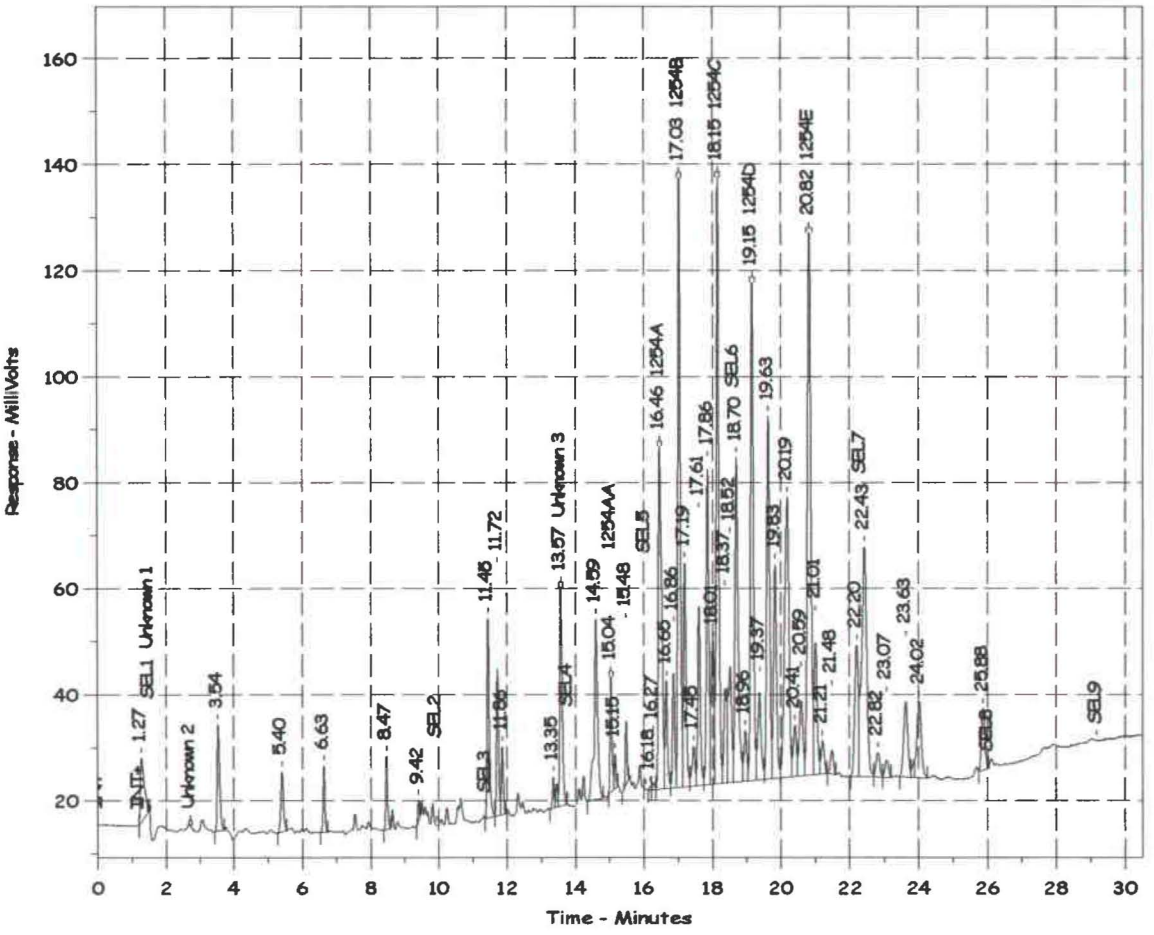


Fig.20: Chromatograph of 17-week Sample of Anaerobic Reactor

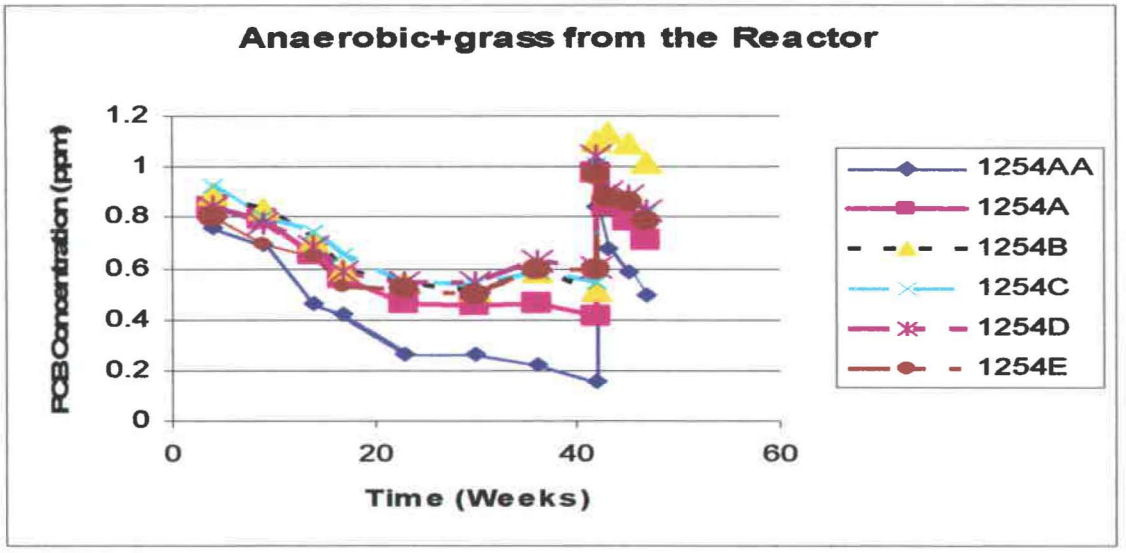


Fig.21: Reactor Method: Anaerobic with Grass Graph

Table12: Produced Gas Amount of Anaerobic Reactor

Week	Produced Gas Amount (mL)	Comment	Methane Amount (mL)	Week	Produced Gas Amount (mL)	Comment	Methane Amount (mL)
1st	820		533	27th	500		325
2nd	600		390	28th	600		390
3rd	1,270		825.5	29th	350		227.5
4th	1,030		669.5	30th	N/A	system leaked	N/A
5th	800		520	31st	500		325
6th	775		503.75	32nd	590		383.5
7th	N/A	no measured	N/A	33rd	500		325
8th	760		494	34th	550		357.5
9th	50*	gas line clogged	32.5	35th	500		325
10th	1,200		780	36th	550		357.5
11th	650		422.5	37th	600		390
12th	600		390	38th	580		377
13th	550		357.5	39th	600		390
14th	600		390	40th	580		377
15th	420		273	41st	N/A	prepared new spike	N/A
16th	500		325	42nd	N/A	prepared new spike	N/A
17th	400		260	43rd	1,000*	new spike	650
18th	400		260	44th	460		299
19th	230		149.5	45th	500		325
20th	400		260	46th	575		373.75
21st	0*	no food fed	0	47th	400		260
22nd	450		292.5	48th	200		130
23rd	350		227.5	49th	700		455
24th	500		325	50th	300		195
25th	400		260	51st	600		390
26th	450		292.5				

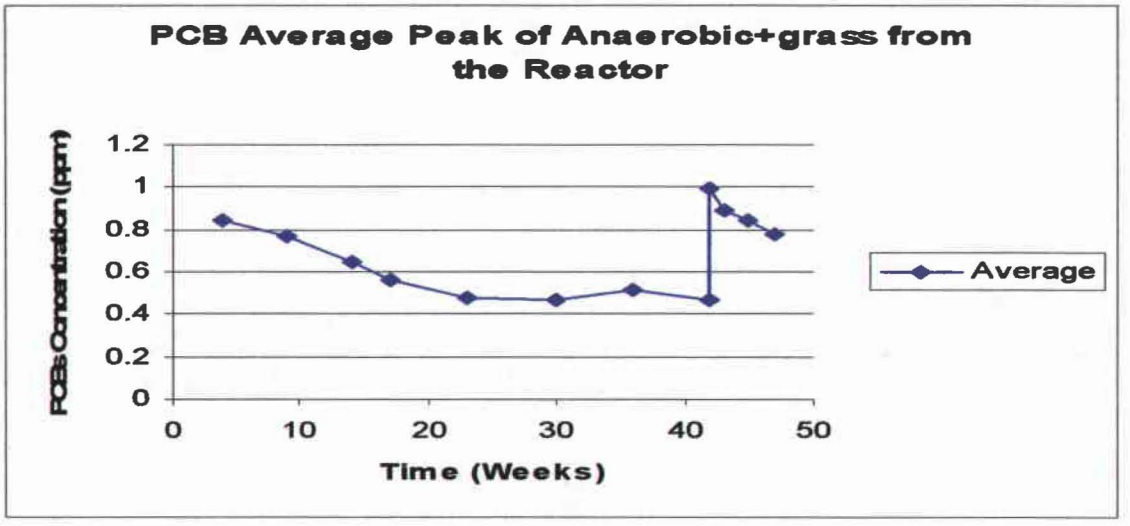


Fig.22: Reactor Method: Anaerobic with Grass Average Peak Graph

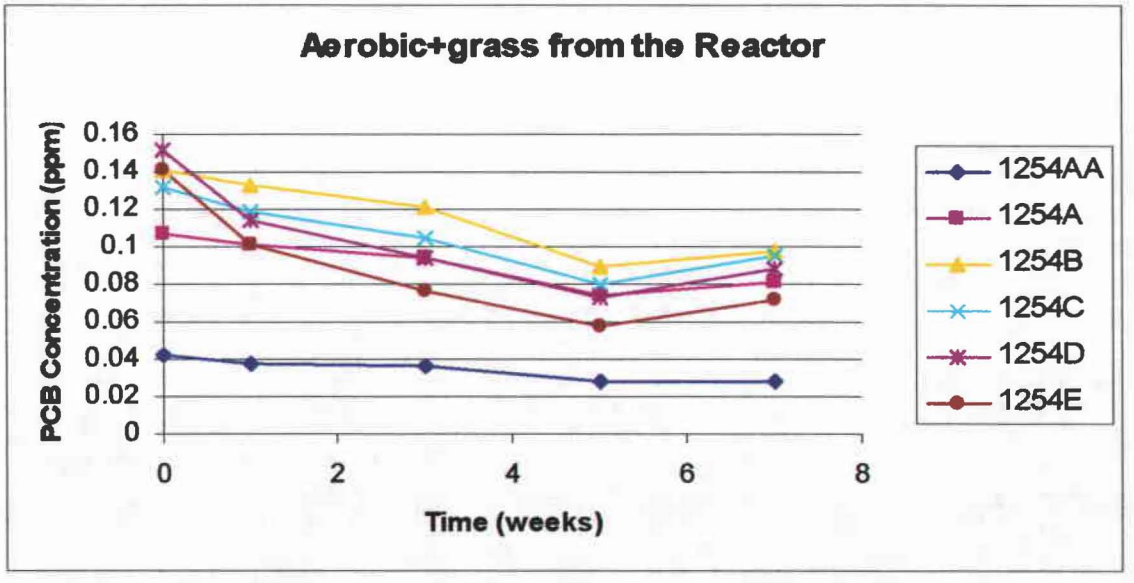


Fig.23: Reactor Method: Aerobic with Grass Graph

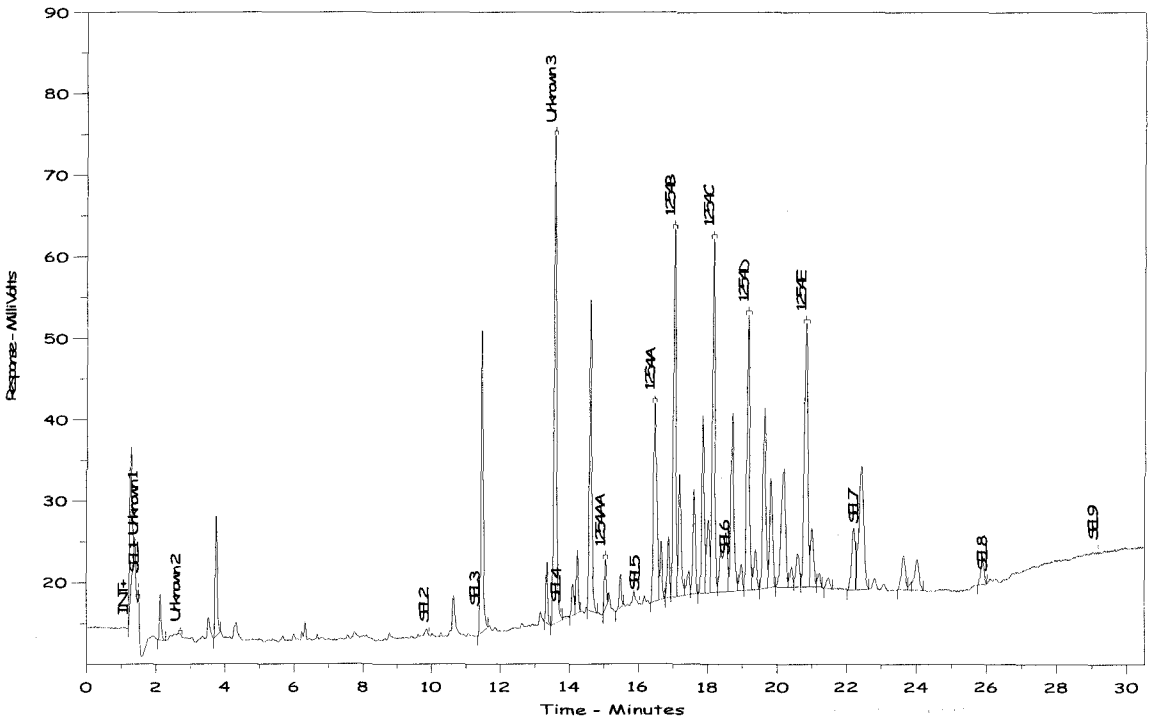


Fig.24: Chromatograph of 0-week Sample of Aerobic Reactor

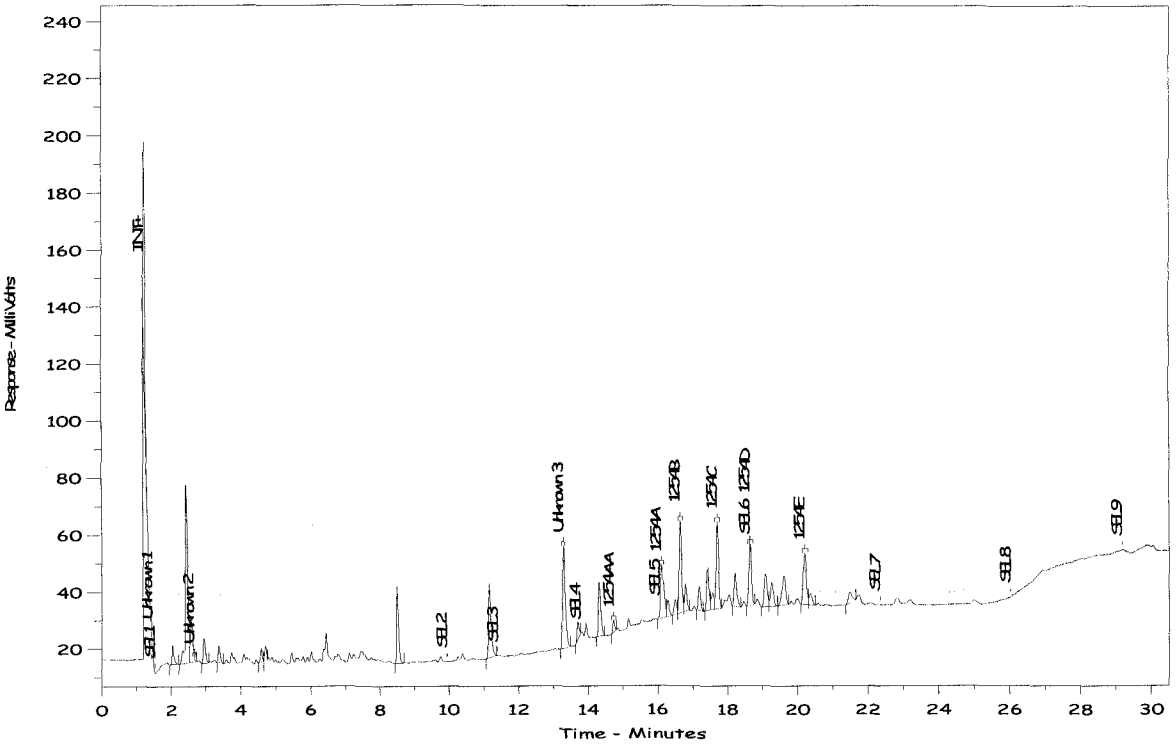


Fig.25: Chromatograph of 5-week Sample of Aerobic Reactor

The result of the experiment could be summarized that the anaerobic reactor method showed the PCB biodegradation. The efficiency of the PCB removal was approximately 60%. Compared with the efficiency of PCB removal from soil examined in previous studies, PCB removal in this research was comparable. The normal range of the PCB removal from soil was 70-90%. It also showed that the period of PCB biodegradation from soil was roughly 5-24 months; while, the period of PCB biodegradation from the phytoextraction was approximately 42 weeks (10 months, and 2 weeks). In addition, PCBs can be also degraded or destroyed by incineration. With high efficiency and taking short time, the incineration could be a practical fashion for the PCB removal. However, the operational cost is high. For the long term PCB removal, the PCB biodegradation would be more suitable and cost effective.

Chapter 4

Conclusions

1. The sequential anaerobic-aerobic batch reactor demonstrated PCB biodegradation in the presence of plant tissue. In the process of PCB biodegradation, the highly chlorinated PCBs were dechlorinated by anaerobic cultures to the low chlorinated PCBs, and the low chlorinated PCBs were dechlorinated by aerobic cultures.
2. The plant tissue affected the rate of PCB biodegradation. Some PCBs were adsorbed into plant tissue.
3. The anaerobic activity can be monitored by gas production from the gas collector. Although gas production from the reactor was monitored, the composition of produced gas was not determined. In fact, produced gas consisted of methane (CH_4), carbon dioxide (CO_2), and small amount of nitrogen (N_2), hydrogen (H_2), hydrogen sulfide (H_2S), water vapor, and other gases.
4. In the process of dechlorination, PCBs can be transformed by several pathways dependent on the dominant microorganisms. The evidence can be seen from the result of blue-cap vial method comparing between anaerobic with grass samples and anaerobic from the wastewater treatment plant with grass samples. These two samples used different sources of the cultures; thus, the concentration of each peak was different.
5. An ultrasonic water bath can be applied for improving the efficiency of PCB extraction. High frequency vibration is effective to bring absorbed PCBs out of the tissue. This equipment could be used in a pretreatment process by vibrating the phytoextraction cuttings before adding them to the anaerobic reactor.

6. The results obtained herein for phytoextraction cutting biodegradation are comparable with those published by others for soil remediation. It can be noticed that the PCB removal efficiencies and operation time of both alternatives are similar.
7. The PCB destruction by incineration can provide higher efficiency of destruction and shorter operation time than that of biodegradation. However, the operation and maintenance cost are also very high and there air pollution issues. For the long term treatment, PCB biodegradation using sequential anaerobic-aerobic reactor should be considered.

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