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REDUCTIVE DECHLORINATION POTENTIAL OF POLYCHLORINATED BIPHENYLS BY STREAM SEDIMENT MICROBES

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Field of Study	Environmental Management
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วิจิตรา สุจริต: ศักยภาพการลดคลอรีนของโพลีคลอริเนเต็ดไบฟีนิลด้วยจุลินทรีย์ตะกอนลำน้ำ (REDUCTIVE DECHLORINATION POTENTIAL OF POLYCHLORINATED BIPHENYLS BY STREAM SEDIMENT MICROBES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รองศาตราจารย์ ดร.จินต์ อโณทัย, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: PROFESSOR I-MING CHEN, Ph.D., 167 หน้า.

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษาการลดคลอรีนของพีซีบีในตะกอนลำน้ำธรรมชาติ 5 แห่ง ผลการศึกษาพบว่าสารอาหารและธาตุอาหารเสริมที่มีอยู่ในตะกอนลำน้ำทั้งหมดเพียงพอต่อการเจริญเติบโตของกลุ่มจุลชีพที่สามารถย่อยสลาย 234-ซีบีพี การเพิ่มสารอินทรีย์และธาตุอาหารเสริมไม่มีผลที่เด่นชัดต่อการลดคลอรีนของ 234-ซีบีพี อย่างไรก็ดีการเติมพาร์มเมอร์ลงในตะกอนลำน้ำที่เก็บไว้ในห้องเย็นเป็นเวลานานสามารถกระตุ้นการทำงานของจุลชีพกลุ่มที่สามารถลดคลอรีนของ 234- ซีบีพีได้ แต่ไม่มีผลที่ชัดเจนต่อการเพิ่มสมรรถนะของการย่อยสลาย 2345-ซีบีพี ซึ่งเกิดขึ้นได้เองอยู่แล้ว ไบโอออกเมนเตชั่นโดยการเพิ่มตะกอนเหลวจากแหล่งที่สามารถย่อยสลายพีซีบีได้ลงไปในตะกอนเหลวและตะกอนเปียกของแหล่งที่ไม่สามารถย่อยสลายพีซีบีได้สามารถเพิ่มประสิทธิภาพในการย่อยสลาย 234-ซีบีพีและ 2345-ซีบีพีได้เป็นอย่างดี การกระตุ้นจุลินทรีย์ด้วยความร้อนเป็นระยะเวลาสั้นๆก่อให้ผลเสียต่อการลดคลอรีนของพีซีบี และเมื่อศึกษาในถังปฏิกรณ์จำลองลำน้ำพบว่าพีซีบีไม่สามารถย่อยสลายได้ในตะกอนลำน้ำภายใต้สภาวะแวดล้อมจริงได้ตลอดระยะเวลา 6 เดือนที่ทำการศึกษา

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WICHIDTRA SUDJARID: REDUCTIVE DECHLORINATION POTENTIAL OF POLYCHLORINATED BIPHENYLS BY STREAM SEDIMENT MICROBES. ADVISOR: ASSOCIATE PROFESSOR JIN ANOTAI, Ph.D., CO-ADVISOR: PROFESSOR I-MING CHEN, Ph.D., 167 pp.

This study aimed to determine the dechlorination of PCB in 5 stream sediments. The results indicated that existing substrate and nutrient minerals in the stream sediments were already sufficient for the growth of microorganisms able to degrade 234-CBp. Addition of organics and nutrients did not provide any significant benefit to 234-CBp dechlorination. However, supplement of primers to stream sediments stored in a cool room for a long period of time could stimulate the activity of 234-CBp dechlorinators; nonetheless, it could not further promote the already active 2345-CBp dechlorinators. Bioaugmentation of sediment slurry containing active PCB dechlorinators to inactive sediment slurry and sediment water could promote the dechlorination efficiencies 234- and 2345-CBps. Temporary heat treatment to the dechlorination consortia had a negative impact on PCB dechlorination. Finally, the dechlorination of 2345-CBp could not happen under simulated system within 6 months of study period.

Field of Study: Environmental Management Student's Signature:.....
Academic Year: 2010 Advisor's Signature:.....
Co-advisor's Signature:.....

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ABBREVIATIONS

4-BZ	Methyl 4-Bromobenzene
14-DBZ	1,4-Dibromobenzene
4-BN	4-Bromobenzonitrile
4-BH	4-Bromobenzoic Hydrazide
BES	Bromoethanesulfonic Acid
BP	Bangplee Industrial Estate
BPK	Bangplakod Canal
CBp	Chlorobenzene
DeCl	Dechlorination
DN	Denitrifiers
E_h	Redox Potential
HLP	Hua Lam Poo Canal
HCB	Hexachlorobenzene
MF	Small Material Recovery Facility
ND	Not Detected
POPs	Persistent Organic Pollutants
PCBs	Polychlorinated Biphenyls
PWP	South Bangkok Power Plant
RAMM	Reductive Anaerobic Mineral Medium
SAB	Simulated Aquarium Boxes
SS	Sediment Slurry
SRB	Sulfate Reducing Bacteria
VAN	Vancomycin

CHAPTER I

INTRODUCTION

1.1 Rationale

As Thailand moving toward industrialization, many parts of the country have been contaminated with several hazardous substances particularly persistent organic pollutants (POPs). Polychlorinated biphenyls (PCBs) are a class of compounds in which the aromatic biphenyls carbon skeleton carries between one to 10 chlorine atoms. They are very persistent in the environment; as a result, they are classified as one of the 12 POPs and are grouped in the industrial chemical POPs, moreover, it is listed as top three of toxic POPs to living organisms (UNEP, 1999).

About 1.5 million tons of PCBs were produced worldwide between the 1930s and the mid-1980 and a substantial fraction of this have entered or will ultimately enter the environment; more than one third of PCBs have been released into the environment by point and non-point sources before being banned globally (Eisenreich, 1987). PCBs are now widely distributed over the Earth and found even in remote parts of the world, such as Antarctica and Northern Greenland (Macdonald et al., 2000).

In Thailand, PCBs can be detected in the surface sediment layers of the Gulf of Thailand after being banned almost a decade (Boonyatumanond et al., 2007). This suggests that some leakages from the storage places or resuspension and remobilization of PCBs accumulated in the sediments of several canals around Bangkok and lower reaches of the Chao Phraya River have occurred continuously. Identification of the sources of PCBs is a difficult task because of lots of unknown PCBs storage locations and exactly quantity. In addition, the past imported volumes and dates of PCBs in Thailand for further assessment have never been recorded either (PCD, 2005).

PCBs and other POPs have very low water solubilities which make them highly lipophilic. These properties make them easy to adsorb onto sediments, especially fatty tissue. For that reason, it tend to be bioaccumulated and biomagnified via the food chain. PCBs can be human carcinogens and are potentially toxic to both human and aquatic lives. Bioremediation techniques were more cost-effective than other ordinary physical and/or chemical processes to remediate excavated soil. Alexander and Bryan, (1988) estimated the treatment cost for contaminated soil by incineration, landfilling, thermal desoption, soil washing, and above-ground bioremediation to be 975, 350, 125, 237, and 95 US$/$m^3$, respectively.

Several literatures revealed that PCBs could be dechlorinated by both acclimated and un-acclimated microbial consortiums. Highly chlorinated PCB congeners could be dechlorinated only under anaerobic condition; whereas, they were recalcitrant under aerobic condition. However, all these researches were performed in a well-establish laboratory environment either under suitable temperature or with modified natural or synthetic nutrient media. Direct investigation of PCBs dechlorination under real or simulated natural environments has not been observed. Moreover according to several researches on the reductive dechlorination of hexachlorobenzene (HCB), another POPs which has a similar but simpler molecular structure than PCBs, it shows that HCB could be dechlorinated potentially in stream sediments under simulated natural environment without any acclimation and enrichment with high dechlorination rates (Anotai et al., 2010; Chen et al., 2010). These results are notably different from other works conducted in developed countries which located in warm or cold climate areas (Chang et al., 2003; Prytula and Pavlostathis, 1996).

Temperature was one of the most important factors controlling the dechlorination process. It might be resulted in important changes in the dechlorination consortiums as well as the dechlorination rate. Wu et al. (1996) investigated the dechlorination of 2346-CBp under various temperatures ranging from 4 to 66°C and found that the optimum temperatures were 12 and 34°C but the highest rate of dechlorination of 2346-CBp to trichlorobiphenyls occurred at 30°C. Additionally, the

HCB dechlorination was optimum under temperature ranging between 30 and 35°C which is the typical range in Thailand and other tropical countries (Chen et al., 2010). It is expected that this temperature range is also suitable for PCBs dechlorination as well. From these information, it is very promising to bioattenuate HCB and PCBs in field practices, which might be proclaimed that HCB may not be a concerned POPs in Thailand. Construction of the PCBs dechlorination systems under tropical climate have not been investigated and direct investigation of PCBs dechlorination under simulated natural environments have not been observed as well.

This experiment was devised to study PCBs degradation in the sediments under anaerobic condition simulating the condition in which the PCBs contaminate in the natural environment in order to characterize the fate of PCBs contaminated in the environment.

1.2 Objectives

The goals of this research were to determine the environmental factors affecting on PCBs dechlorination by anaerobic consortiums from natural stream sediments and fate of PCBs in simulating real environment. To achieve these goals, several objectives were established as follows:

1. To investigate the PCB dechlorination capability of anaerobic consortium.
2. To determine the effect of organic substrates, nutrient supplements and halogenated primers on PCBs dechlorination.
3. To study the effect of temporary heat treatment on PCBs dechlorination.
4. To investigate the fate of PCBs in environmental stimulating system.

1.3 Hypotheses

1. PCBs could be reductively dechlorinated under proper anaerobic environment.

2. Organic substrates, nutrient supplements and halogenated primers might have a positive effect on PCBs dechlorination.
3. The temporary heat treatment might have a promising effect on PCBs dechlorination.

1.4 Scopes of this Research Study

1. Working at room conditions.
2. Using both lab-scale (serum bottles) and bench-scale (aquarium boxes) reactors.
3. Working with mixed cultures existing in the natural sediments without any pre-treatment.

1.5 Definition of Words in this Research Study

"*Reductive Dechlorination*" means a removal of chlorine atoms from an organic compound under a reductive condition such as anaerobic environment.

"*Bioremediation*" means a spontaneous or managed biological treatment process by using the microorganisms or their enzymes to remove pollutants from environment.

"*Biodegradation*" means the breakdown of a substance into simpler or smaller products caused by microorganisms.

"*Bioaugmentation*" means the addition of non-native microbes into specific biodegradation capabilities to contaminated site.

"*Biostimulation*" means addition of nutrients, electron donors and/or electron acceptors, and some auxiliary substrates to stimulate the growth and activity of microorganisms.

1.6 Expected Results

The outcomes of this study will provide a better understanding on the factors affecting the PCBs dechlorination in natural anaerobic environment as well as the

potential of using external substances, nutrient supplements and chemicals to promote and/or accelerate the dechlorination of PCBs. In addition, the environmental fate of PCBs in stream sediment will be characterized. This information will lead to an effective clean-up process for PCBs contaminated sites both in Thailand and worldwide.

CHAPTER II

BACKGROUND AND LITERATURE REVIEWS

2.1 Properties of Polychlorinated Biphenyls

PCBs are the chlorinated derivatives of a class of aromatic organic compounds called “biphenyls” and are manufactured by direct chlorination of the biphenyl ring system (Brown, Bedard, et al., 1987; Brown et al., 1978). Their empirical formula is $C_{12}H_{10-n}Cl_n$ and the basic structure of PCBs is shown in Figure 2.1. A specific PCB molecule is called “congener” and is named by the number and location of the chlorine atoms. PCBs were sold as commercial mixtures of many PCB molecules or congeners mostly under the name of “Aroclors,” but also “Kenochlor,” “Clorextol,” “Pyroclor,” and other trade names. PCBs occur as liquids or solids, and they are clear to light yellow in color. They have no odor or taste. Because of their thermal stability, they do not easily burn, hence were popularly used as coolants and insulating materials in electrical applications before being banned globally.

PCBs are difficult to be oxidized and reduced. They have very low water solubility and therefore are highly lipophilic. The lipophilic nature and persistence of PCBs also contribute to their high bioaccumulation potential and their biomagnifications in higher tropic levels of the food chain. PCBs are potentially cause human carcinogens and wildlife. PCBs have high electrical resistivity and also low electrical conductance. Commercial PCB mixtures were synthesized by chlorination of biphenyl with chlorine gas. The average degree of chlorination was controlled by the reaction conditions in order to yield the desired chemical and physical properties (Erickson, 1986). The chemical and physical properties of PCBs are shown in Tables 2.1 and 2.2 and average chlorines composition of Aroclor showed in Table 2.3.

Although PCBs have been banned by the Stockholm Convention on Persistent Organic Pollutants (POPs) since 2000, they are found to contaminate in the environment worldwide due to their persistence and bioaccumulated. Tanabe, (1998) suggested that prolonged PCB contamination was due to the continuing emission of PCBs from recently used and/or dumped transformers and capacitors which contain an estimated 65% of the total PCBs produced. In order to predict the future status of PCB pollution, not only on a regional scale but also on a global scale, it is essential to understand the behavior and fate of PCBs at landfill and dumping sites of PCB containing electrical equipment.

In Thailand, the major existing PCB sources are transformers and capacitors imported by the Electricity Generating Authority of Thailand, Metropolitan Electricity Authority (MEA), and Provincial Electricity Authority (PEA). The number of PCBs containing transformers and capacitors were estimated to be 33 and 1,987 for EGAT, 0 and 636 for MEA, 20,190 and 9,810 for PEA, respectively.

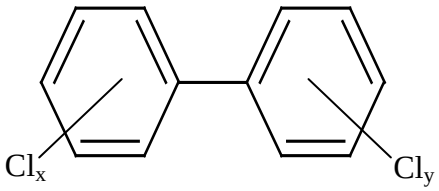


Figure 2.1 Structure formula of PCBs.

Table 2.1 General information of PCBs.

Substance name	Polychlorinated biphenyls (PCB
CASR number	01336-36-3
UN Number	2315
Molecular formula	$C_{12}H_{10-n}Cl_n$
Synonyms	Aroclor, Aroclor-polychlorinated biphenyl, Biphenyl chlorinated, Biphenyl chloro derives, Chlorinated biphenyl, PCBs, Polychlorinated Biphenyls (PCBs)
Physical and Chemical properties	Depend on the number of chlorination (see Table 2.2)

Source: Department of Health, Ministry of Public Health

Table 2.2 Physical and chemical properties of PCBs.

CBs	Molecular weight	Solubility (µg/l)	Vapor pressure (Pa) 20°C	K_{ow}
Mono-	188.7	$1.3x10^3$-$7x10^3$	$2.2\ x10^3$ -$9.2\ x10^2$	4.6-4.7
Di-	233.1	$0.6\ x10^2$-$7.9\ x10^2$	$3.7\ x10^2$ – 7.5 x10	5.2-5.3
Tri-	257.6	$0.1\ x10^2$-$6.4\ x10^2$	$1.1\ x10^2$ – 1.3 x10	5.7-6.1
Tetra-	292.0	$0.2\ x10^2$-$1.7\ x10^2$	1.8-0.4	5.9-6.7
Penta-	326.4	4.5-12	5.3-0.88	6.4-7.5
Hexa-	360.9	0.4-0.9	1.9-0.2	6.4-7.6
Hepta-	395.3	0.5	$0.53\text{-}4.8\ x10^{-2}$	7.0-7.7
Octa-	429.8	0.2-0.3	$7.8\ x10^{-2}$ -$9\ x10^{-3}$	7.0-7.6
Nona-	464.2	0.1	$3.2\ x10^{-2}$ -$1.1\ x10^{-2}$	7.7-7.9
Deca-	498.7	0.02	$5.6\ x10^{-3}$	8.4

Source: Ballschmiter et al. (1988)

Table 2.3 Composition of average molecular (% wt.) of some Aroclors.

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

Source: Brinkman and Dekok (1980)

Table 2.4 Banned of POPs chemicals in Thailand.

Chemicals	Date of banned	Reasons
Aldrin	1988	Persistent, accumulate in living organisms
Chlordane	1995 (PH) 2000 (AG)	Possible carcinogen, persistent, high impact to environment, many alternatives
DDT	1983 (AG) 1994 (PH)	Persistent and accumulate in food chains, possible carcinogen in tested animals
Dieldrin	1988	Persistent, accumulate in living organisms, high acute poisoning, high risk for users
Endrin	1981	Persistent in agricultural products and in food chain, harm to non-target organisms
Heptachlor	1988	Persistent, accumulate in living organisms
HCB	-	Never imported
Mirex	-	Never imported
PCBs	2004	Risk to human health and the environment
Toxaphene	1983	Possible carcinogen in tested animals, persistent

Note: AG = agricultural use, PH: public health use

Source: Department of Agriculture, Adapted from Technical Report: Objectives and Priorities for Persistent Organic Pollutants (POPs), September 2005, PCD (2005).

PCBs in Thailand have been banned several years and a summary for the POPs giving data of ban and its reasons are shown in Table 2.4. However, accidental leakage of PCBs from stored transformers and capacitors was still detected after globally banned (Watanabe et al., 1995).

For minor PCB sources, which being used in small amounts as industrials fluids for hydraulic systems and gas turbines, as lubricating oils, and as plasticizers, no record has been officially reported. Imported volumes and dates for these purposes have never been recorded either (PCD, 2005). Vertical profiles of PCBs in bottom-stream sediment were investigated at many sites. Most studies collected samples from Chao Phraya River which is the main river in the center part of Thailand. It is

flowing through several provinces including Ayutthaya, Nonthaburi, Samutprakharn and Bangkok. There are many industries located along the river and a large number of inhabitants live along the river banks.

Finally, this river reaches to the upper Gulf of Thailand in Samutprakharn Province. The results show concentrations in the surface sediments of the Chao Phraya river in Bangkok area ranged from 0.01 to 0.22 ng/g (dry weight), but could not be detected in surface sediments from Nonthaburi and Ayuttaya Provinces. The estuary of the Chao Phraya River and the upper Gulf of Thailand (in Samutprakran Province) were found to be contaminated with PCBs in the range between 0.02 and 0.05 ng/g (dry weight), and 0 and 0.02 ng/g (dry weight), respectively (Boonyatumanond et al., 2006).

2.2 Sources of PCBs

The origin of the PCBs is exclusively their deliberate manufacture, primarily for the use as dielectric fluids in electrical transformers and capacitors, but also for the use in carbonless copy papers and inks. Other uses of PCBs include: waxes, heat exchange fluids, cutting oils, flame retardant, insulating paper for electric cables, adhesives, dust-removing agents, hydraulic fluids, special lubricants, paints, vacuum pump oil, waterproofing products and certain plastics. Table 2.5 shows PCBs use in the US between 1929 and 1975.

2.3 Toxicology of PCBs

Commercially uses of PCBs were raised up, due to their excellent of chemicals properties. Therefore, PCBs were toxic and bioaccumulated in environments. The evidence for their toxicity in case of risk to human health and aquatic lives were adequate for special illustration by the Toxic Substances Control Act, U.S. congress before being banned in U.S. and contributed to worldwide. Tables 2.6 and 2.7 presented a summary of effects of PCBs and pathological affected, respectively. Generally, studies of both occupational or environmental exposure and

laboratory exposure are subject to unclear because of the type and purity of PCBs were used. Most of occupational, environmental studies and laboratory studies have been performed with commercial mixtures. Either effect of more than 70 congeners were tested simultaneously, or the possibility presence of contaminants; for example, polychlorinated terphenyls, quaterphenyls, PCDDs, PCDFs are makes obligation of the observed effects to PCBs subject to criticism (Erickson, 1986).

The indicial of PCB single congeners have been conducted. These studies indicate that PCBs toxicity is dependent, both degree of chlorination and isomer. PCB congeners which did not have *ortho*-chlorines substitution that are heavily substituted at the *meta* and *para* positions, are capable of assuming a planar conformation which can interact with the same receptor as TCDD. 343′4′-CBp, 3453′4′-CBp, 3453′4′5′-CBp were a cytrochrome P-448 inducers or 3-MC inducers or aryl hydrocarbon hydroxylate (AHH) inducers. These congeners are not major components of the Aroclors. The other active group of congeners is the phenolbarbital-type or "PB-type" inducers or cytochrome P-450 inducers as represented in Table 2.8. Thus, many congeners exhibit mixed responses or no observable response (Erickson, 1986).

Table 2.5 Industrial use of PCBs.

PCBs use	Percentage
Capacitors	50.3
Transformers	26.7
Plasticizer uses	9.2
Hydraulics and lubricants	6.4
Carbon-less copy paper	3.6
Heat transfer fluids	1.6
Petroleum additives	0.1
Miscellaneous industrial uses	2.2

Source: US.EPA., 1994

Table 2.6 The chronic toxic effects of PCBs.

Test	Effects
Chronic exposures	
Aquatic species	-Threshold effects in egg hatchability of vertebrates and invertebrates at levels of 2-5 μg/l -Embryo toxicity evident at 50 μg/l
Terrestrial species	-Mouse- some liver change with exposure to high chlorine containing products, 300-500 μg/g -Rat: some liver changes, minimal reproductive effects, 100-500 μg/g -Monkey: Yusho symptoms, altered reproduction cycles, hyperplastic gastritis and ulceration, 2.5-5 μg/g -Chicken: some morphologic deformity, reproduction decline, subcutaneous edema, 20-50 μg/g -Mink: dose response relationship in growth and reproduction 10 μg/g -Pelican: some hepatocellular changes, 100 μg -Dogs: reduced growth, some liver changes, 100 μg -Wildfowl: some reproduction changes, varies with species 50-200 μg/g
Teratogenicity	-Effects seen in avian species 50-200 μg/g
Mutagenicity	-Chromosomal abnormalities negative results -Dominant lethal mutations- negative results -Ames test- 1221, 4 chlorobiphenyl significantly mutagenic
Oncogenicity	-High chlorinated compounds produced tumors in rats and mice, relationship with PCBs not always clear

Source: National Research Council (1979)

2.4 Occupational Exposure and Its effects

The exposure of highly concentration of PCBs can be entry to ours body by both dermal contracting and inhalation of vapors (Table 2.9). Power plants provided the highest reported exposure potential. The effects of occupational exposure of PCBs had been reported since 1936, when chlorance was associated. The symptoms

of highly PCBs exposure included: burning of eyes, face and skin, moreover, general clinical symptoms are indicative of PCBs-related liver injury, including elevated serum triglycerides and induction of mixed function oxidases. Moreover, there also has some evidence of potentially be a cancer. The occupational exposure may be widespread among workers handling PCBs in use; i.e. mechanics handing lubricating and hydraulic oils, office workers handing carbonless copy paper, analytical laboratory workers, and electrical component handlers.

Table 2.7 The pathologic disease regarding to toxicity of PCBs.

Pathologic Disease	Rat	Mouse	Guinea Pig	Chicken	Monkey	Human
Decreased body weight	+	+	+	+	+	+
Acne/ Alopecia	**(-)**	**(-)**	**(-)**	**(-)**	+	+
Edema	**(-)**	+	**(-)**	+	+	+
Lymphoid atrophy						
Thymus	+	+	+	+	+	**n.r.**
Spleen	+	+	+	+	+	**n.r.**
Hepatomegaly	+	+	+	+	+	+
Hepatomegalocytosis	+	+	**(-)**	**(-)**	+	+
Multinycleated giant cell	+	**(-)**	**(-)**	**(-)**	**(-)**	**(-)**
Necrosis, Degeneration	+	+	+	+	+	+
Fat	+	+	+	+	+	+
Bile duct hypertrophy	+	**(-)**	**(-)**	**(-)**	**(-)**	**(-)**
Porphyria	+	+	**(-)**	+	**(-)**	+
Urinary bladder hypertrophy	**(-)**	**(-)**	+	**(-)**	**(-)**	**(-)**
Gastropathy	**(-)**	**(-)**	**(-)**	+	+	**(-)**
Tumor induction	+	+	**(-)**	**(-)**	**(-)**	**(-)**

Source: McConell, 1980; n.r.: not related

Table 2.8 Summarization of PCB congeners which Cytochrome P-450 inducers.

PCB congeners	Cytochrome P-450 inducers		
	Strong	**Weak**	**Weak-Inactive**
23452′3′4′5′-CBp	O		
2452′4′5′-CBp	O		
2352′3′5′-CBp	O		
2342′3′4′-CBp	O		
242′4′-CBp	O		
2462′4′6′-CBp		O	
232′3′-CBp		O	
353′5′-CBp		O	
44′-CBp		O	
33′-CBp		O	
2362′3′6′- CBp			O
252′5′-CBp			O
262′6′- CBp			O
22′-CBp			O

Source: Erickson, 1995

2.5 PCBs Removal Processes

Significant removal of PCBs from the mobile environmental reservoir can occur through natural processes. One process in the aquatic system is simple physical removal due to sediment burial. However, permanent removal by degradation is the preferred method for ultimate protection of the environment. Chemical degradation in natural systems is minimal, except for photolysis (Callahan et al., 1979). Anderson and Hites (1996) measured hydroxyl radical degradation as it occurs during photolytic exposure.

They estimated half-lives ranging from 2 to 34 days for several PCB congeners. Atkinson (1996) reviewed the literatures on the atmospheric chemistry of

PCBs and verified that losses due to photolysis can be high. Tropospheric half-lives due to hydroxyl radical removal were listed as follows: mono-, 3.5 to 7.6 days; di-, 5.5 to 11.8 days; tri-, 9.7 to 20.8 days; tetra-, 17.3 to 41.6 days; and penta-, 41.6 to 83.2 days.

Biological degradation is slow, according to most studies performed over a wide range of organisms including higher animal, plants, and several microbial systems. PCBs are very resistant to biological transformation which is one of the very important features of the group that made them useful in commerce.

Table 2.9 PCBs concentration in the occupational environment and in blood of workers exposed to PCBs.

Duration of PCBs exposure	**PCB levels**		**Effects reported**
	Environmental (mg/m^3)	**Blood (ppb)**	
Not known	10	-	Unbearable irritation Chlorance
4-8 months	5-7	-	Chlorance,
< 1-20 years	0.2-1.6	370, (avg.)	hyperpigmentation, liver injury
2.5 years (avg.)	Not reported	820, (avg.)	Chlorance
2.5-18 years	0.013-0.27	36-286	Irritation, liver injury
14 months	0.1	-	Chlorance, liver injury
2-23 years	0.32-1.44	>200	Chlorance, liver injury
Up to 15 years	Not reported	7-300	Chlorance, elevated triglycerides
	<1	74-1,900	No effect

Source: Erickson, 1995

In spite of the abundant evidence for PCBs' ability to resist biological degradation, much study has gone into attempting to isolate consortium of microbes that will magically clean up the numerous hot sports of PCB know to exist in the environment.

2.6 Biological Transformation of PCBs

Centeno et al. (2003) reviewed the physical and chemical treatment options for PCB degradation and found that the organisms might modify organic pollutants such as PCBs in such a way that the negative effects were minimized. Microorganisms participated in the biodegradation by producing enzymes which modified the organic pollutant into simpler compounds. Biodegradation is of two forms, mineralization and co-metabolism (Dobbins, 1995). In mineralization, competent organisms use the organic pollutant as a source of carbon and energy resulting in the reduction of the pollutant to its constituent elements. Co-metabolism, on the other hand, requires a second substance as a source of carbon and energy for the microorganisms but the target pollutant is transformed at the same time. If the products of co-metabolism are amenable to further degradation, they can be mineralized, otherwise incomplete degradation occurs. This may result in the formation and accumulation of metabolites that are more toxic than the parent molecule requiring a consortium of microorganisms which can utilize the new substance as source of nutrients. The effectiveness of biodegradation depends on many environmental factors.

Biodegradation rates vary depending on the conditions present in the environment. These factors include the structure of the compound, the presence of exotic substituent and their position in the molecule, solubility of the compound and concentration of the pollutant. For aromatic halogenated compounds, a high degree of halogenation requires high energy by the microorganisms to break the stable carbon–halogen bonds (Dobbins, 1995).

Chlorine as the substituent alters the resonant properties of the aromatic substance as well as the electron density of specific sites. This may result in the

deactivation of the primary oxidation of the compound by microorganisms. Additionally, the positions occupied by substituted chlorines have stereochemical effects on the affinity between enzymes and their substrate molecule (Furukawa and Fujihara, 2008; Sylvestre and Sandossi, 1994).

Water solubility of PCBs has a vital role in their degradation. Congeners with high aqueous solubility are easily accessed by microorganisms than those with low solubility. Highly chlorinated congeners of PCBs are very insoluble in water. This could account for the resistance of highly chlorinated PCB congeners to biodegradation.

Pollutant concentration is also a major factor affecting biodegradation. In general, a low pollutant concentration may be insufficient for the induction of degradative enzymes or to sustain growth of competent organisms. On the other hand, a very high concentration may render the compound toxic to the organisms (Guilbeault et al., 1994). At low concentrations, degradation increases linearly with increase in concentration until such time that the rate essentially becomes constant regardless of further increase in pollutant concentration (Dobbins, 1995).

Other environmental factors affecting degradation are temperature, pH, presence of toxic or inhibitory substance and competing substrates, availability of suitable electron acceptors, and interactions among microorganisms. All these factors interplay and make the rates of biodegradation unpredictable.

The use of microorganisms, both anaerobic and aerobic, is the only known process to degrade PCBs in soil systems or aquatic environments. Aerobic microorganisms can dechlorinate low chlorinated PCBs whereas anaerobic dechlorination is known as the only effective degradation way for highly chlorinated PCBs. Anaerobic bacteria possess characteristics that are well adapted to pollutants with high carbon concentration because of the diffusional limitation of oxygen in high concentration systems (Dobbins, 1995).

The environment of anaerobes is conductive to reductive transformations where chlorine is displaced by hydrogen (McEldowney et al., 1993). The dechlorinated compound is suitable for the oxidative attack of the aerobic bacteria. Aerobic bacteria grow faster than anaerobes and can sustain high degradation rates resulting in mineralization of the compound. Theoretically, the biological degradation of PCBs should result in CO_2, chloride, and water. This process involves the removal of chlorine from the biphenyl ring followed by cleavage and oxidation of the resulting compounds (Boyle et al., 1992).

2.7 Anaerobic Degradation Process

The biological conversion of the organic matter can be divided in three steps (see in Figure 2.2). The first step in the process involves the enzyme-mediated transformation (hydrolysis) of higher-molecular-mass compounds into compounds suitable for use as a source of energy and cell carbon. One group of organisms is responsible for hydrolyzing organic polymers and lipids to basic structural building blocks such as monosaccharides, amino acids, and related compounds.

The second step (acidogenesis) involves the bacterial conversion of the intermediate compounds. Second group of anaerobic bacteria ferments the breakdown products to simple organic acids, the most common of which in anaerobic digester is acetic acid. This group of microorganisms, described as non-methanogenic bacteria, consists of facultative and obligate anaerobic bacteria. Collectively, these microorganisms are often identified in the literature as “acidogen,” or “acid formers.”

The third step (methanogenesis) involves the bacterial conversion of the intermediate compounds formed in the second step into simpler end products, principally methane and carbon dioxide. Microorganisms of a third group convert the hydrogen and acetic acid formed by the acid formers to methane gas and carbon dioxide. The bacteria responsible for this conversion are strict anaerobes and are called “methanogenic bacteria”. They are identified as “methanogens” or “methane

formers." It is important to note that methanogenic bacteria can only use a limited number of substrates for the formation of methane.

Currently, it is known that methanogens use the following substrates: CO_2+H_2, formate, acetate, methanol, methylamines, and carbon monoxide for their growth. The methanogens are able to utilize the hydrogen produced by the acidogens because of their efficient hydrogenase.

2.8 Mechanism of Anaerobic Dechlorination

The rate and occurrence of dehalogenation are strongly dependent on the environmental conditions and chlorinated compounds present. Reductive dechlorination of aryl halides is thought to involve two one-electron reduction steps, resulting in the removal of halogen substituent and the formation of an intermediate aryl halide radical, which abstracts a proton from water to complete the reaction.

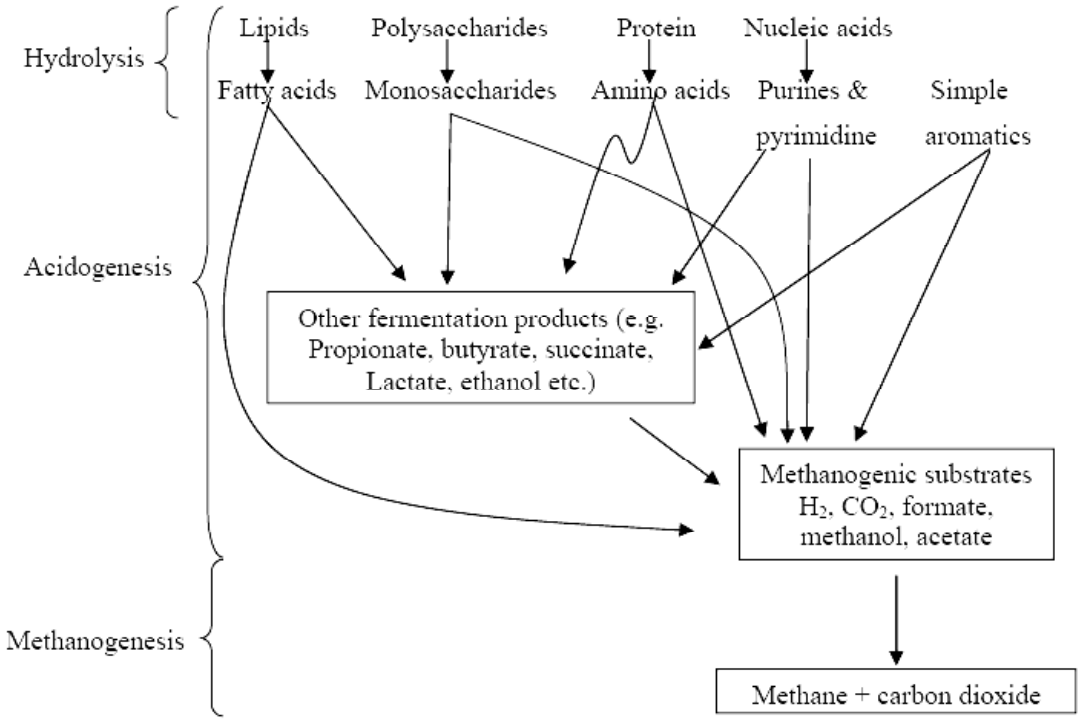


Figure 2.2 Schematic diagram of the patterns of carbon flow in anaerobic digestion (Tchobanoglous and Burton, 1991).

In all reported examples of biologically mediated reductive dehalogenation, the halogen is released as a halide. Dehalogenation appears to be the rate-limiting step during anaerobic aryl halide biotransformation, as the aromatic ring can only be further degraded once all the halogen substituents are removed. Reductive dehalogenation has been observed with chlorinated single ring structures (e.g., benzenes, benzoates, phenols), fused rings (e.g., naphthalenes) and carbon-carbon linked (e.g., PCBs and ether-linked (e.g., furans, dioxins)) rings. Depending on the system, chlorines have been removed, resulting in the accumulation of up to monochlorinated aromatic compounds when the compound becomes too reduced for further reductive dechlorination. Steric factors have also been shown to affect the dechlorination of some compounds; generally, the ortho-substituted isomers are removed at the lowest rate.

As mentioned previously that the transformations resulting in the degradation of organic materials can be classified into two broad categories, i.e., mineralization and co-metabolism. Whether a compound is mineralized or co-metabolized has an implication for the development of a waste treatment process for environmental remediation, if the compound can be directly taken up and utilize by the microorganisms as their growth substrate and energy source, complete mineralization will occur. On the other hand, for the compound that cannot be directly used by microorganisms, it can be degraded via nonspecific enzymatically mediated transformations (co-metabolism). Hence, the co-metabolism does not result in the increase in cell biomass or energy. Consequently, the ability to co-metabolize a compound is not benefit to the microorganisms. Halogenated compounds can be utilized as a growth substrate or co-metabolized by anaerobic and aerobic microorganism and consortia.

Degradation of halogenated compounds can occur through the combined activities of fortuitously acting enzymes present in one or more microorganisms. Reductive dechlorination is generally accepted to be a co-metabolic process, except in a few isolated cases. Even highly chlorinated and poorly water soluble aromatic

hydrocarbons that do not contain polar functional groups can also undergo reductive dehalogenation (Judith et al., 1991).

2.9 Growth–Linked Biodegradation

Microorganisms require proper environment and many synthetic chemicals for supported their growth. Normally, they can use several chemicals as a source of C, energy, N, P, S, or another element to sustain growth of cells. The most attention has been emphasized on the gaining of C and energy to maintain the growth of bacteria and fungi. For synthetic substrates that are extensively degraded, whereas, the molecule can be used as other simple organic substrates, which consortium can gain the elements and/or the energy for biosynthesis reactions.

The enrichment culture technique is a commonly research procedure that relies on the ability of microorganisms which used organic compounds as sources of C and energy to promote their growth. This method is based on the selective advantage gained by an organisms that can be used a particular measurement compound as a C and energy source in medium; which contained inorganic nutrients but no other sources of C and energy. Under these conditions a species that is able to grow by utilizing that chemicals will increase. Few of others bacteria and fungi will proliferate in this medium. However, the populations that use products expelled by the species acting on the added organic nutrient will also thrive, and thus the final isolating of a microorganism in pure culture requires plating on an agar medium so individual colonies can be selected. This agar medium is also made a selective by using a single source of C and energy. Repeated transfer of the enrichment culture through solutions that contain the measurement chemicals/compounds and organic nutrients further increase the degree of selectivity before plating because organic materials and uninvited species from original environmental sample are diluted by the serial transfer (Alexander, 1994).

The enrichment-culture technique is basically for the isolation of pure cultures of bacteria and fungi that are capable to use an enormous number of organic

molecules as C and energy sources. However, to attempt and obtain microorganisms that are able to grow on a variety of other organic compounds have not been successful. Certainly, many of failures can be attributed to misuse of the technique or errors in the approach of the investigator: for example, sometimes the concentration of the organic nutrients may be too less to give detectable turbidity in the enrichment solution or too high so that microorganisms fail to develop because of the toxicity. In other instances, the failure results from the absence from the selectivity medium of the growth factors essential for the organisms degrading the compound. Nevertheless, when the failure to isolate a microorganism by enrichment culture coupled with the prolonged persistence of the chemicals in nature, it is likely that the compound is not used by microorganisms as a source of C and energy (Alexander and Bryan, 1988).

An immense of a large number of genera of bacteria and fungi have been isolated that grow on one or more synthetic compounds. Much of research literature works with sugars, amino acids, other organic acids, and other cellular or tissue constituents of living organisms, whereas, a variety of pesticides have also been evidenced to support the growth of one or another bacterium or fungus. Under these conditions, bacteria increase in number and fungi increase in biomass in culture media. At that time, the chemical disappears, typically at a rate that parallels the increase in cell number or biomass. Since the concentration of the C source declines, the rate of cell or biomass increase diminishes until, when all the substrate in consumed and then the population rise ends (Alexander, 1974).

Mineralization of organic compounds is characteristic of growth-linked biodegradation, in which the organism converts the substrate to CO_2, cell components, and products typical of the usual catabolic pathways. The mineralization in natural occasionally may not be linked to growth but instead results from nonproliferating populations. In contrast, some species growing at the expense of a C compound may still not mineralize and produce CO_2 from the substrate; however, if O_2 is present, the organic products excreted by one species and probably will be converted to CO_2 by another species, so that even if the initial population does not produce CO_2, the

second species will. Whereas, the totally effect is still one of mineralization (Alexander, 1994).

The environmental pollutants that represented as novel C and energy source for a particular population, is transformed by the metabolic pathways that are characteristic of heterotrophic bacteria. For the microorganisms to grow on the compound, it must be converted to the intermediates, which can be characterized it to major metabolic sequences. If the compound cannot be modified enzymatically to yield such intermediates, it will not serve as a C and energy source because the energy-yielding and biosynthetic processes cannot be a function. The initial phases of the biodegradation thus involve modification of the novel substrate to yield product that is itself an intermediate or, following further metabolism, is converted to an intermediate in these ubiquitous metabolic sequences. This need to convert the synthetic molecule to intermediate is characteristic of both aerobes and anaerobes, which derived C and energy from the substrate. An organic compound need not be a substrate for growth in order for it to be metabolized by microorganisms (Alexander, 1965). Two pathways of transformations are including: first, the biodegradation provides C and energy to support growth, the process therefore the growth-linked; second, the biodegradation is not linked to multiplication, the reasons will be considered in the following.

Several studies have demonstrated that increasing of number of microbial cells or the biomass of the species is response on the chemicals interested; called as degradation proceeds. During a typical growth-linked mineralization brought about by bacteria, the cells use some of the energy and C for their organic substrates to make a new cell, and this also increasingly large population causes an increasingly rapid mineralization. In these situations, the mineralization has an affected to the population change. During decomposition of 2-, 3-, or 4-chlorobenzoate or 34-dichlorobenzoate in sewage; for example, bacteria acting on these compounds multiply and the increase in cell numbers parallels with the decrease of the interested chemicals that serve as their source of C (Alexander, 1994).

2.10 Treatment Strategies: Problems and Potential Solutions

2.10.1 Activating or Accelerating Anaerobic Dechlorination

Two approaches techniques have been used to activate or accelerate the dechlorination of PCBs are including: Biostimulation (adding some chemicals or compounds to stimulate the indigenous microorganisms) and Bioaugmentation (adding non-native microorganisms). Up to date approach, the microorganisms may be enrich cultures from the same site or may be from a different sources.

2.10.1.1 Biostimulation Technique

PCB-dechlorinating microorganisms are presented at that sites, but the site shows no potential or no evidence of in-situ dechlorination activity. It is possibly to stimulate the activity of the indigenous PCB-dechlorinating microorganisms in some sediment. Recently studies on additive of carbon/electron source or nutrients have been investigated, in order to promote the dechlorination ability of PCBs; hence, PCBs was assumed as selective electron acceptor. The result shows promising outcomes to enhance the dechlorination ability under anaerobic condition; either, acclimated or pre-acclimated consortiums as observed in chlorine loss (Winchell and Novak, 2008; Nollet et al., 2005; Chang et al., 2001; Hendrik et al., 2005).

Additionally, the source of electrons for the dechlorination reaction might reduce the substrates required for microbial growth. Investigation of effects of organic substrates on dechlorination of Aroclor 1242 has been observed (Nies and Vogel, 1990). The sediment samples were collected from PCBs contaminated sites and incubated anaerobically with acetate, acetone, methanol, or glucose. The pattern of dechlorination was similar for each substrate-fed batch; although, the extents and rates of dechlorination were different. The extent of dechlorination was the greatest for methanol, glucose, and acetone but was the least for acetate. Dechlorination was not occurred in un-additional organic substrate sets.

Moreover, the additional of a halogenated aromatic substrates which used in term of “priming” to stimulate PCBs dechlorination activity by indigenous microbes also been explored. This process based on the hypothesized that the high concentration of appropriate dehalogenation substrates could promote the growth of dehalogenating microorganisms; which used these compounds as electron acceptors. And then the dehalogenators population could also dechlorinated PCBs in sediment (DeWeerd and Bedard, 1999; Wu et al., 1999; VanDort et al., 1997). Early study used sediment from Housatonic River (Lenox, MA), which is contaminated with Aroclor 1260.

The PCBs in this sediment showed clear evidence of past dechlorination (Bedard and May, 1996) and the laboratory experiments have been confirmed that the PCBs dechlorinators still exist at these sites (Bedard et al., 1997; VanDort et al., 1997; Wu et al., 1997c; Bedard and May, 1996); whereas, not yet the dechlorination could be observed in un-amended sediment incubated more than a year under anaerobic condition (Wu et al., 1997c; Bedard et al., 1995). However, a variety of halogenated aromatics have been indentified that can specifically stimulating Process N or Process P dechlorination in this sediment (DeWeerd and Bedard, 1999; Bedard et al., 1998; VanDort et al., 1997). Adding brominated biphenyl congeners has been showed highly effective substrate for stimulating Process N in Housatonic River Sediments. For example, 26-dibromobiphenyls (26-BB) primes rapid dechlorination of hexa-trough nonachlorobiphenyls, converting them to mainly *ortho*-, *para*-substituted tetrachlrobiphenyls (Bedard et al., 1998). Furthermore, 26-BB could prime PCB dechlorination in this sediment at temperatures as low as 8^{o}C (Wu et al., 1999).

During 1992 to 1995 a field practices was conducted in Woods Pond, Housatonic River to determine a potential of priming PCB dechlorination in-situ. The experiment was conducted at ambient temperatures in 6-foot diameter caissons driven into the sediment and the result showed very promising. A single addition of 26-BB (350μM) activated the indigenous PCB-dechlorinating microorganisms. The sediment contaminated with six or more chlorines were 62% decrease within three months and 74% decrease in a year (Bedard et al., 1995). The dechlorination activity

was follow Process N and the products were primarily *ortho*-, and *para*-substituted tetrachlorobiphenyls, especially 242′4′-CBp and 242′6′-CBp. This result clearly notifies that it is possible to activate the PCB dechlorination in field practices.

Another promising approach to stimulation involves the addition of ferrous sulfate or other inexpensive and environmental friendly compounds, to promote nearly complete *meta* and *para* dechlorination of Aroclor 1242 (Zwiernik et al., 1998). The experiment had been provided an influential evidence that the microorganisms responsible for dechlorination Processes M and Q in Hudson River sediment are sulfate-reducing bacteria (Zwiernik et al., 1998). These dechlorination processes are not active in the presence of sulfate, but are promoted after the sulfate disappeared. Additive ferrous iron or another divalent metal could precipitate sulfide and thus reduce its toxicity. Clear evidence showed that sulfate stimulated the growth of bacteria responsible for dechlorination Processes M and Q, and these bacteria uses PCBs as alternative electron acceptors after absence of sulfate. This experiment was tested with sediment spiked with Aroclor 1242, and incubated in anaerobic mineral medium. For prolongs test in the future which is applying in contaminated river sediment under stimulated field condition, and the similarly results, this finding could be a major revived for PCBs remediation site (Zwiernik et al., 1998).

2.10.1.2 Bioaugmentation Technique

An alternative potentially to bioremediation is augmentation with PCB-dechlorinating organisms enriched from the same site, or from other sites. In this case, if PCB dechlorinators do not exist in the given site, it is possibly to introduce the microorganisms enriched for another site in order to enhance the dechlorination activity. However, the microbial ecology and biogeochemistry of the site have to be concerned. For example, similar temperature, pH, electron donors and acceptors, organic components, mineral composition and sediment composition and co-contaminants.

There have been several laboratory attempts to simulate PCB dechlorination in sediment from the Housatonic River by inoculating with PCB-dechlorinating enrichments consortiums from the same site; but did not succeed (Bedard et al., 1997; Wu and Wiegel, 1997). Another approach technique of bioaugmentation is introducing a stable anaerobic consortium with PCB-dechlorinating ability derived from other sources. Early study was investigated the dechlorination of 23456-pentachlorobiphenyls by using a consortium in form methanogenic granules (Natarajan et al., 1996). The granules are predominantly composed of syntrophic, hydrolytic, acetogenic, fermentative, and methanogenic bacteria.

The results suggested that they have an ability to remove *ortho, meta* and *para* chlorines from 23456-penachlorophenyls (Natarajan et al., 1997; Natarajan et al., 1996). These granules were acclimated with PCB contaminated (Aroclor 1242 and 1248) sediment from Raisin River; the experiment was conducted by using an anaerobic upflow reactor fed 0.01% poplar wood to sustain the granules (Natarajan et al., 1997). Unamended sediment showed very modest but dechlorination still occurred. In contrast, the sediment treated with the granules showed strong decreases of tri- through pentachlorobiphenyls, and the acclimation of primarily *ortho*-substituted *mono*- and *di*-chlorobiphenyls. Furthermore, it also implied that there is a potential to dechlorinate 23456-CBp neither absence nor presence of sediments (Natarajan et al., 1998).

Augmentation of PCB dechlorinators could also be evaluated by introducing enrichment or pure cultures of PCB dechlorinators (Bedard et al., 2007; Hartkamp-Commandeur et al., 1996). The enriched cultures were obtained by using Aroclor or individual PCB congeners, thus they has an evidenced that it not matter cultures were isolated from enriched with Aroclor or single PCB congeners, the dechlorinators consortiums were quite similarly (Cutter et al., 2001; Pulliam Holoman et al., 1998; Williams, 1997; Zanaroli et al., 2010).

Although it has been practiced in agriculture and in wastewater treatment for many years, bioaugmentation is still experimental. Several strategies have been currently explored to make bioaugmentation a successful technology in sites that lack significant populations of biodegrading microorganisms. However, the most successful cases of bioaugmentation occur in confined systems, such as bioreactors in which the conditions can be controlled to favor survival and prolonged activity of the exogenous microbial population (*El* Fantroussi and Agathos, 2005).

2.11 Literature Reviews

There are enormous of literatures studied on PCBs dechlorination in natural environment. Two distinct biological processes capable of biotransforming PCBs are aerobic oxidative processes and anaerobic reductive processes. Anaerobic PCBs degradation transforms highly chlorinated PCBs to lightly chlorinated *ortho*-enriched congeners. The intermediate products from anaerobic degradation processes are readily degradable by a wide range of aerobic bacteria, thus the attentions of these reviews are direct on anaerobic condition. Anaerobic dechlorination of PCBs was observed in many cases under different environmental conditions as summarized in Table 2.10.

2.11.1 Dechlorination of Single PCBs congeners

Experiments involving the addition of specific single PCB congeners to anaerobic sediment slurries have been verified to determine: first, the dechlorination pathways; second, the effect of chlorines substitution patterns on reactivity; and third, the dechlorination capabilities of microbial population and the end products of PCB dechlorination. Sediment from Hudson River, Silver Lake, and Woods Pond have been widely tested. The Aroclor studies revealed that each of these sediments exhibits several distinct dechlorination processes that are presumably mediated by different microbial populations and eight dechlorination processes described to date have been given latter names and are briefly summarized in Table 2.11.

Table 2.10 Sites containing PCB dechlorinating microorganisms.

Location	PCB-contaminated site	Type of valuation	References
Canada			
British Columbia	Esquimalt Harbor	lab	(Kuipers et al., 1999)
Ontario	Otanabee River/ Rice Lake	*in situ*	(Bedard and Quensen III, 1995)
Japan	Lake Shinji	*in situ*	(Bedard and Quensen III, 1995)
The Netherlands			
	Lake Ketelmeer	*in situ*	(Beurskens et al., 1993; Beurskens et al., 1995)
	Rhine River	*in situ*	(Beurskens and Stortelder, 1995)
United States			
Florida	Escambia Bay	*in situ*	(Brown, Bedard, et al., 1987)
Georgia	LCP Superfund Site, Brunswick	lab	(Maruya et al., 1999)
Illinois	Waukegan Harbor	*in situ*	(Brown, Bedard, et al., 1987)
Maryland	Baltimore Harbor	*in situ*	(Wu et al., 1998a)
Massachusetts	Hoosic River	*in situ*	(Brown, Bedard, et al., 1987)
	Housatonic River/ Woods Pond	*in situ*, lab	(Bedard and May, 1996; Bedard et al., 1996)
	New Bedford Harbor Silver Lake	*in situ*, lab *in situ*, lab	(Alder et al., 1993; Brown and Wagner, 1990) (Alder et al., 1993; Bedard and Quensen III, 1995; Brown, Bedard, et al., 1987; Brown, Wangner, et al., 1987)
Michigan	River Raisin	lab	(Quensen III and Tiedie, 1997)
New York	Hudson River	*in situ*, lab	(Alder et al., 1993; Brown, Bedard, et al., 1987; Brown, Wangner, et al., 1987; Fish and Principe, 1994)
	St. Lawrence River	*in situ*, lab	(Cho et al., 2000; Sokol et al., 1998; Sokol, Kwon, et al., 1994)
South Carolina	Charleston Horbor	lab	(Wu et al., 2000)
	Lake Hartwell	*in situ*	(Farley et al., 1994)
Washington	Puget Sound	lab	(Æfjord et al., 1994)
Wisconsin	Fox River	*in situ*	(Imamoglu and Christensen, 2002)
	Sheboygan River&Harbor	*in situ*	(Quensen III and Tiedie, 1997)

Source: Adapted from Bedard et al. (2003)

Note: *In situ* means that its clear evidence of microbial dechlorination of PCBs in sediments form these locations; lab means that laboratory studies have been investigated that sediments containing PCB dechlorinating microbes.

Table 2.11 Comparison of microbial dechlorination activities.

Dechlorination activity	Targeted chlorine	Homolog substrate range	Reactive chlorophenyl groups[a]	Primary chlorophenyl products
P	Flanked *para*	4-6	3**4**, 23**4**, 2**4**5, 23**4**5, 23**4**56	(23), 25, 235, 2356
H	Flanked *para* [b]	4-7	3**4**, 23**4**, 2**4**5, 23**4**5	3, 24, 25, 235
H′	Flanked *para* [b,c]	3-5	2**3**, 3**4**, 23**4**, 2**4**5, 23**4**5	2, 3, 24, 25, 235
N	Flanked *meta*	5-9	2**3**4, 2**3**6, 24**5**, 2**3**4**5**, 2**3**46, 2**3**4**5**6	24, 25, 26, 246
M	Flanked&unflanked *meta*	2-4	**3**, 2**3**, 2**5**, **3**4, 2**3**4, 2**3**6	2, 4, 24, 26
Q	Flanked&unflanked *para* [b,c]	2-4	**4**, 2**3**, 2**4**, 3**4**, 2**3**4, 2**4**5, 2**4**6	2, 3, 25, 26
LP	Flanked&unflanked *para* [d]	3-6	2**4**, 2**4**5, 2**4**6	2, 25, 26
T	Doubly flanked *meta* [e]	7-8	2**3**45	245

Source: Bedard, D.L. (1997)

[a] The target chlorines are bold and underlined.

[b] The doubly flanked *meta* chlorine of 234-CBp is also targeted.

[c] The *meta* chlorine of 23-CBp is also targeted.

[d] The substrate range of this dechlorination process has not been completely characterized.

[e] This dechlorination process has been explored only at 50 to 60^{o}C (Wu et al., 1997c).

Single PCB congeners have been usefulness for screening environmental samples for dechlorination activity as well as identifying unusual PCB dechlorination activities such as an *ortho* dechlorination.

2.11.2 Mass Balance and Quantitation

Aroclor dechlorination studies have been showed the decreases in highly chlorinated PCBs and increases in less chlorinated congeners; however, it was

appeared to correspond on a molar basis to the decreases of more chlorinated congeners.

Alder et al., (1993) investigated PCBs concentration in dechlorination experiments by using sediments from New Bedford Harbor, Silver Lake, and Hudson River, to determine the total molar PCBs concentration in each sediment remained constant for 17 months of incubation, thus confirming that dechlorination was only spontaneously significant transformation. Moreover, some researchers also reported that a mass balance of the transformation showed the total molar concentration of PCBs remained unchanged during seven months using sediment from Hudson River and Stated. All transformation during this period involved dechlorination without any disappearance of PCB molecules (Rhee, Bush, et al., 1993b; Rhee, Sokol, Bush, et al., 1993).

In contrast to the mass balance that observed in the Aroclor studies, the total molar PCBs concentration decreased 35%-90% in studies of dechlorination of 23456-CBp, 2342′4′5′-CBp, 343′4′-CBp, and 245-CBp by microorganisms from the same sediments (Rhee, Sokol, Bethoney, et al., 1993a; Rhee, Sokol, Bethoney, et al., 1993b). The explanation for their failure to obtain mass balance in these experiments with single congeners because anaerobic transformation may included mechanisms either than dechlorination and that mechanisms are congener dependent (Rhee, Sokol, Bethoney, et al., 1993b). However, another explanation might be because of their inability to obtain mass balance with these PCB congeners due to the analytical problems and sensitivity issues.

Rhee, Sokol, Bethoney, et al., (1993b) could recover only 50% of 2345-CBp after 15 months of incubation. Furthermore, during first 3 months about two-thirds of the 23456-CBp was dechlorinated to 2356-CBp and 246-CBp (in ratio 2:1) with no decrease in total PCB concentration. After 3 months another 30% of the 23456-CBp was dechlorinated, and a corresponding increase of 2356-CBp was observed. In the same time period the concentration of 246-CBp decreased by 75%, but did not detect 26-CBp, which is the expected products, whereas, 236-CBp and 26-CBp were not

observed; hence, total recovery was only 63% of expected value. At 15 months, when the experiment was ended, a significant concentration of 26-CBp was detected for the first time, but not insufficient amounts to account for the decreases in the higher chlorinated congeners, and the concentration of PCBs was decreased only 50% of beginning. The lack of correspondence in time between the disappearance of 2356-CBp and 246-CBp and the occurrence of the expected products, 26-CBp, suggested that 26-CBp was not detected until high level of concentration had been accumulated.

Continuing study of Rhee, Sokol, Bethoney, et al., (1993b), it has been reported that only 25% of the beginning concentration of 2342′4′5′-CBp could account after 15 months of incubation time. About half of 2342′4′5′-CBp could dechlorinated to 2452′4′-CBp, 242′4′-CBp and 242′5′-CBp in the first 3 months. Within 9 months, 90% of 2342′4′5′-CBp was decreased, 2452′4′-CBp, 242′4′-CBp showed net decreases, and the total molar PCB concentration had decreased to 78% of that expected. 242′-CBp was detected only sole product but sufficient amounts to account for losses of the more chlorinated congeners. The further decrease in total molar PCB concentration to 25% at 15 months occurred concomitantly with net losses of the intermediates 242′-CBp and 242′5′-CBp. 22′-CBp was an expected terminal product but was not detected; probably due to a very low response factor, its response is only 4.5%-9% of 242′-CBp and 242′5′-CBp, and 2342′4′5′-CBp, and therefore 22′-CBp would be harder to detect that its precursors (Mullin et al., 1984).

In contrast, mass balance was obtained for the dechlorination of 234- to 24-CBp; of 245- to 24-CBp; and 2452′4′5′- to 242′4′-, 242′5′-, and 252′5′-CBp (Rhee, Sokol, Bethoney, et al., 1993b; Rhee, Sokol, Bethoney, et al., 1993a). Whereas, tetrachlorobiphenyl products were not further dechlorinated, hence, the detection of products with low response factor was not an issue. It could be proposed that biotransformation of PCB may include mechanisms other than dechlorination and that mechanisms are congeners dependent because the aromatic compounds that lack of aliphatic or polar substitutents are extremely difficult to metabolize anaerobically (Schink, 1988).

Rhee, Sokol, Bethoney, et al., (1993b) conducted an experiment to confirm fully recovery of products in their experiments and used authentic pure congeners standard to indentify products by matching GC retention times. These information shows all PCB concentrations were determined in the linear range of the ECD response and the calibration curves were used a single concentration of mixture Aroclors 1221, 1016, 1254, and 1260 (Rhee, Bush, et al., 1993b). However, Aroclor standards were not well suited for quantitative analysis of pure congeners and it were not necessary, since all PCB congeners are commercially available now (Bedard and Quensen III, 1995). The detection level for each PCB congener should be different because the ECD response factors vary over several orders of magnitude (Erickson, 1986).

2.11.3 Factors Affecting Rate and Extent of PCBs Dechlorination

It has been hypothesized that unique responsible for the various PCBs dechlorinating patterns was exhibited because of each microorganisms has a distinct dehalogenating enzymes; which observed in both laboratory and in the environment (Bedard and Quensen III, 1995; VanDort et al., 1997). The growth and metabolic activities of different microorganisms has an effected by environmental factors and conditions; furthermore, it influence divergently the extent and rate of differ PCB dechlorinating activities. A study of factors that can influence PCBs dechlorination is important for obtaining and understanding of the diversity of PCB dechlorination as well as suited and/or un-suited condition which able to be dechlorinated. Moreover, this knowledge will be useful to forecast the potential of environmental degrading PCBs and will lay in developing of the bioremediation issues. The environmental factors have been evidenced to influence on PCBs dechlorination including; temperature, pH, available of carbon source, H_2 as electron donor and the presence of electron acceptors rather than PCBs.

A significantly factors in the biotransformation and biodegradation which play important roles on the bioavailabilities of PCBs is adsorption. The rates of desorption are different among PCB congeners, the most hydrophobic congeners (with high K_{ow}

values) behaved roughly as predicted from models; whereas, less hydrophobic congeners couples with low K_{ow} values. Unfortunately, the bioavailability are a function of PCBs contaminated periods, which leads over time to stronger adsorption and lower recovery rates of PCBs.

2.11.4 Temperature

Temperature has a significantly effect on the growth and the physiological activity including uptake and enzymatic dehalogenation of PCB congeners. Several studies have been conducted at room temperature (around 25^{o}C). However, the PCB-contaminated sediments in the environment typically experience a different and wider range of temperatures. The range of temperatures depends on climate and the depth of water and sediments itself. The influences of temperature are comprehensive and can be affected to adsorption and desorption kinetics of PCBs from soil particles (Jota and Hasset, 1991), hydrolytic dehalogenation and availability of PCBs for microbial transformation. Thus, these effects are probably minor in comparison with the effect of temperature on the growth of microorganisms and the catalytic activity of enzymes.

Wu and colleagues investigated the dechlorination of residual Aroclor 1260 in Wood Pond sediments by adding 2346-CBp, and stored at around 7^{o}C, then incubated at 18 temperature points from 4 to 66^{o}C; fluctuation of temperature was less than ± 1^{o}C (Wu et al., 1997a; Wu et al., 1997c; Wu et al., 1996). The residual of Aroclor 1260 had been only slightly dechlorinated in the environment in contrast to the microcosms incubated with 350 μM of 2346-CBp as a primer where the dechlorination occurred at 8-34^{o}C and at thermophilic temperatures of 50-60^{o}C. The optimal temperatures for overall chlorines removal from 2346-CBp and from residual PCBs were 18-30^{o}C and 20-27^{o}C, respectively.

Flanked *meta*-dechlorination occurred between 8 and 34^{o}C and 50-60^{o}C, whereas, flanked *para*-dechlorination was observed only between 18 and 34^{o}C. The dechlorination of doubly unflanked *para*- chlorines occurred only in the temperature range of 18-30^{o}C, thus, unflaked *ortho*- dechlorination of 246-CBp and 24-CBp was

observed at 8-30^{o}C. These results suggest that temperatures have an intense influence on the rate, extent and products of PCBs dechlorination.

The typical average temperature in Wood Pond ranging from 15^{o}C at 45 cm depth to between 18 and 20^{o}C at a depth of 10-15 cm, and the winter temperature for these depths has been decreased to 1 and 4^{o}C, respectively. This result notifies that during about half year, the dechlorination cannot occur. However, during summer period, the temperature at a 10-15 cm depth is in the range at which the modified *N*-dechlorination pattern changed to the simple *N*-pattern, i.e. unflanked *para* chlorine substituents will not be removed by this dechlorination pattern. This is in concurrence with the distribution of PCBs in Woods Pond sediment. It also suggests an important point that around ~18^{o}C which ubiquitous in summer temperature, given the highest variability between repeated experiments and among the triplicate incubations was observed.

Furthermore, the highest dechlorination rates were observed at higher temperatures rather than normal summer temperatures in the upper 45 cm of the sediment. Whereas, incubated at room temperatures (around 20-25^{o}C) would not provide an applicable estimation of dehalogenation potential. Besides that without highly concentration of priming congeners or Aroclor 1260 (above 500 ppm), the dehalogenation could not be happened in Woods Pond sediment at any incubation temperatures (Wu et al., 1997b). This repeatedly investigated effect of consistent with the lack of continuing in situ dehalogenation at measureable rates in Woods Pond. It seems that high concentrations are required to activate the dehalogenating bacteria and this study was the first detailed on the occurrence of PCB dehalogenation patterns by temperature affected.

Two different systems was measured, uncontaminated (Sandy Creek Nature Park in Athens) and contaminated pond (Woods Pond), the results imply a similarly effects to chlorophenol degradation; it can be speculated that these effects may also be observed for PCB dehalogenation in sediment form other locations as well (Wu et al., 1997a; Wu et al., 1996). Moreover, the observations on temperature effects of the

dehalogenation of Aroclor 1242 in Hudson River are also in agreement with this report. (Tiedje et al., 1993) notified that samples incubated at 12, 25, 37, 45 and 60^{o}C showed reductive dechlorination of Aroclor 1242 at 12 and 25 but not at 37^{o}C or above.

Different from the temperature dependence studies in laboratory in which the samples were incubated at constant temperature, the sediments in nature are mattered to temperature variations. The temperature is fluctuations not only seasonal basis but also from day and night time, as well as strong rain or hot spells. Although, daily fluctuations are less impact in water sediments covered with several depths of water. Recently, it is unclear of impact on the seasonal temperature changes on the PCB dechlorination pathways after introduced into provided sediment.

2.11.5 pH

Sediments are normally well-buffered systems, but in contrast to strictly aerobic processes, anaerobic microbial processes may lead to an increase in acidic fermentation products, that caused local changes in pH. The effect of pH has an effect like temperature and carbon sources; however, the effect of pH on the dechlorination of PCBs in sediments is more complicated because it has several interactions between different dehalogenating and non-dehalogenating microbial populations. Furthermore, pH also has an effect on the availability of PCBs in soil which involving the adsorption equilibrium between PCBs that are dissolved and/or adsorbed to organic matters (Jota and Hasset, 1991).

Dechlorination of 2346-CBp added as a primer and of residual of PCBs in Woods Pond sediments was investigated under pH ranging between 5.0 and 8.0, and incubated at different temperatures of 15, 18, 25 and 34^{o}C. The results revealed that the dechlorination processes could happen. The pH of each slurry was adjusted and maintained by periodically (2~10 days) adding sterile 2N NaOH or HCl. Except for 34^{o}C at pH 5.0, PCB dechlorination was observed at all pH and temperature examined. The optimum pH for overall removal of chlorines was around 7.0-7.5.

However, the specificity of dechlorination varied, e.g., for 2346-CBp, flanked *meta* dechlorination occurred at pH 5.0-8.0, un-flanked *para*- dechlorination at pH 6.0-8.0 and *ortho*- dechlorination at pH 6.0-7.5. However, at pH 7.0 and 15^{o}C, *ortho*-dechlorination was dominated, whereas, un-flanked dehalogenation was occurred at 18 and 25^{o}C, which evidences an outpaced of dehalogenation reactions. These results indicate that the pH of the incubation also strongly influences not only the rate and extent of dechlorination but also the route of dechlorination of 2346-CBp and the residual of Aroclor 1260 (Wiegel and Wu, 2000).

2.11.6 Supplementation of Carbon Sources

Reductive dechlorination of PCBs under anaerobic condition is presumably that PCBs are used as electron acceptors, but does not cleave the rings. Thus, the PCB dechlorinators should need other compound as a source of carbon and electron for support their growth. Up to date, study on PCBs dechlorination under anaerobic condition was conducted in the presence of sediment which provided a variety of organic matter. Besides that all experiments were performed with indigenous microcosms or enrichment cultures which contained both PCB dechlorinators and non-PCB dechlorinators. Addition of a specific carbon source to culture, which could enhance the dechlorination of PCBs by providing a desirable carbon sources and electron sources to the PCB dechlorinators or to non-PCB dechlorinators, might provided a suitable electron donors, vitamins or carbon sources to support growth of PCB dechlorinating microorganisms. Moreover, the addition could stimulate the utilization of substrates that inhibit the PCB dechlorination. In contrast, the addition could also inhibit PCB dechlorination by supplying a carbon sources to non-PCB dechlorinating and/or competitive microorganisms so that these nuisance microbes could out-complete the PCB dechlorinators for electron donors and/or acceptors. Thus it will inhibit the dechlorination. Apparently, both effects could simultaneously occur to different dehalogenation populations. However, all of these possibilities are difficult to differentiate in mixed cultures.

Alder et al., (1993) demonstrated that repeated addition of fatty acid (acetate, propionate, butyrate and hexanoic acid) stimulated dechlorination of added PCBs in carbon-limited sediment slurries, but not in sediment slurries which had higher organic carbon contents. The addition of 0.1% (v/v) thioglycolate medium with beef extract or acetate could enhance the dechlorination in term of shorter lag phase (Abramowicz et al., 1993) and also increasing the observed overall dechlorination rate (Tiedje et al., 1991). The addition of 0.06% pyruvate and malate substantially increased the extent of PCB dechlorination in Aroclor 1248-contaminated soil (Klasson et al., 1996), whereas, malate and primers were added together in Woods Pond sediment contaminated with Aroclor 1248, showed shortened only in lag time before the onset of the reductive dehalogenation of PCBs (Bedard et al., 1998; Bedard and Van Dort, 1998). The stimulation of the dechlorination was highly dependent on the incubation temperature and pH; i.e. at pH 7.5, the addition of 10 mM malate to sediment led to a significantly shortened lag phase and the half-life for the removal of the first chlorine (2346-CBp to 246-CBp) at 15, 18 and 25^{o}C and to an increase in the maximal observed dechlorination rate at 15^{o}C, except 18-34^{o}C. At pH 6.0, malate had no effect on the half-life and dechlorination rate. For another detail experiments was studied on priming sediment with 26-bromobiphenyls (26-BB) and malate, to verify the influence of malate on hydrogen utilization (Wu et al., 1999). It proposed that the dechlorination was happened by stimulating of growth of specific microbes, which could be used malate under particular conditions.

Compared to sediments, enrich cultures provide more defined conditions. Nies and Vogel, (1990) found some dependence of carbon sources used for enrichment cultures. Addition of glucose, methanol, acetate or acetone could enhance the rate and extent the dechlorination of PCBs. The addition of 20 mM pyruvate and 10 mM malate enhanced *meta*- dechlorination of 2346-CBp by 2346-CBp enrich cultures, while addition of 20 mM pyruvate creased the rate of *para*- dechlorination of 246-CBp enrichment cultures. Two PCB-dechlorinating enrichment consortia were derived from estuarine sediment (Charleston Harbor, SC): a 2356-CBp *meta* dechlorinating enrichment culture (Wu et al., 1998b). After the cultures had been transferred eight times into estuarine medium containing only 0.1% (wet w/v) of

sediment and 173 μM of 2356-CBp or 2345-CBp, the addition of the potential electron donor formate (10 μM) resulted in an increase in the extent of the 2356-CBp dehalogenation to 236-CBp from 17% to 75% chlorine removed. The dechlorination of PCBs under various conditions have been investigated by Change et al., (2001), it found that the dechlorination was fastest under methanogenesis, sulfate reducing and lowest under nitrate reducing condition. Additionally, under methanogenesis and sulfate reducing condition coupled with organic substrate (lactate, pyruvate and acetate) could significantly enhance the rates of dechlorination.

Furthermore, the extension of dechlorination rates for low concentration of PCBs (2 mg/kg dry weight) was observed in sediment microcosms undergoing biostimulation and bioaugmentation, it notified that the degradation of low concentration was not difference to highly concentration of PCBs (Krumins et al., 2009). The preliminary determinations of the dechlorination ability by indigenous microbes around Samuthparakarn Province, Thailand have been investigated.

Recently evaluation of the degradation potential of chlorinated aromatic compounds, likes hexachlorobenzene (HCB) has been tested at Hua Lam Poo Canal, Thailand under the very promising effected results as HCB could be dechlorinated effectively by indigenous microbes without any nourishing and enrichments (Anotai et al., 2010; Chen, Wanitchapichat, Jirakittayakorn, Sanohniti, et al., 2010). These results given a piece of evidence that HCB might not be concerned as persistent organic pollutants (POPs) in Thai Canal. It notified impressive and unique results from other developing country which has been found it could be dechlorinated by modifying medium and/or well control conditions.

Winchell and Novak, (2008) investigated the effect of biostimulation (supplement of H_2 via elemental iron (Fe^0)) and bioaugmentation (amend of PCB-dechlorinating enrichment culture) spiked with 2345-CBp. Insignificantly dechlorination of 2345-CBp were occurred in both sediment from Raisin River (PCBs contaminated site) and Duluth Harbor (non PCBs contaminated site) under stimulating condition. However, the extensive dechlorination was observed after

augmenting microcosms, whereas these microcosms was enriched and grown on acetate (20 mM) under a headspace of 3% H_2 to 97% N_2. Moreover, the investigation of dechlorination of PCBs in Ohio River sediment under field condition were not accomplish, thus PCBs concentration were stable under elevated redox status and low temperature conditions (D'Angelo and Nunez, 2010).

2.11.7 Supplementation of H_2 as Electron Donor

Reductive dechlorination is a two electron transfer reaction, which H_2 is assumed to be direct and indirect electron donor (DeWeerd et al., 1991; Zhang and Wiegel, 1990) and the proton source is from water (Griffith et al., 1992; Nie and Vogel, 1991). Lake sediments contain H_2 utilizers with different affinities for H_2. Microorganisms can successfully competition for H_2 which is depending on the partial pressure, affinity of the microorganism and the presence of utilizable carbon sources and electron acceptors; whereas, the partial pressure of H_2 can stimulate or inhibit this microbial dechlorination processes (DeWeerd et al., 1991; Madsen and Aamand, 1991; Zhang and Wiegel, 1990; Linkfield and Tiedje, 1990). A test with frequently replenished the headspace gas with H_2 at 0, 1 or 10% (v/v) under shaking condition to the contaminated sediment from Woods Pond incubated with Aroclor 1260 at 15, 25 or 34^{o}C, and at pH 6.2 or 7.2 revealed that no significant differences in the rate and extent of dechlorination neither supplement with nor without 1% H_2 gas. It might be because of this amount did not change considerably to the available amount of H_2 compared to the microbially produced H_2. Lower dechlorination rates and a lower extent of dechlorination of 2346-CBp and sediment PCBs were found in the samples incubated under 10% H_2, whereas, highly inhibition was investigated at pH 7.2. Ten percent of H_2 in the headspace gas inhibited or changed the dechlorination reactions of 2346-CBp and residual PCBs depending on the incubation temperature and pH. The provided H_2 distorted the pathway and products of reductive dechlorination of 234-CBp by the Hudson River sediment microorganisms (Sokol, Bethoney, et al., 1994). Under H_2/CO_2, 234-CBp was dechlorinated to 24- and 23-CBp and dechlorinated further to 2-CBp. In contrast to under N_2 or N_2/CO_2, 234-CBp was converted to 24-CBp only.

2.11.8 Electron Acceptors

PCBs are used as electron acceptors by reductive dechlorinating microorganisms. Kim and Rhee (1997) reported that spiked of 300 ppm Aroclor 1248 to enrich PCB cultures in anaerobic sediment could increase 188 folds in the number of PCB dechlorinators. In contrast, the number decreased by 93% from initial value in the samples without addition of Aroclor 1248. It can conclude that the growth of PCB dechlorinatiors requires the presence of PCBs. It also observed the microorganisms in Woods Pond sediment capable dehalogenating of 26-BB and PCBs increased nearly 1000 folds after priming with 26-BB (1050 μM) couple with 10 mM of malate (Wu et al., 1999). These results demonstrate that halogenated biphenyls prime PCB dechlorination primarily by stimulating the growth of PCB-dechlorinating microorganism; furthermore, it could stimulate the induction of dehalogenation enzymes.

Several investigations of PCB dechlorination were constructed in the presence of common electron acceptors for anaerobic microorganisms, mostly observed under methanogenic condition (Alder et al., 1993; May et al., 1992; Morris et al., 1992). The Addition of bromoethane sulfonic acid (BESA), which an inhibitor of methanogenesis, inhibited dechlorination of certain congeners (Williams, 1994) or even dechlorination processes (Morris et al., 1992). However, ethanol-treated, pasteurized cultures obtained from Hudson River exhibited *meta* dechlorination of Aroclor 1242 (Ye et al., 1992) and the addition of BESA did not inhibit *meta* dechlorination of 2346-CBp by a 2346-CB enrichment cultures, indicating that the methanogens may not carry out the dechlorination but influence the availability of electron donors in these cultures. The addition of sulfate (10-30 mM), an electron acceptor used by sulfate reducing bacteria, completely inhibited the dechlorination or favored one dechlorination process than others (May et al., 1992; Morris et al., 1992; Rhee, Bush, et al., 1993a). In addition, nitrate (10-16 mM) had no effect or inhibited the dechlorination (Morris et al., 1992; Rhee, Bush, et al., 1993a). Ferric oxyhydroxide (50 mM) decreased the extent of PCBs dechlorination (Morris et al., 1992). The presence of 5 mM sulfate, thiosulfate, sulfite or nitrate inhibited the

dechlorination of 2346-CBp and 246-CBp enrichment cultures. It is suggested that either these electron acceptors were preferred over PCBs or that non-halogenating bacteria using these electron acceptors were out-completed the PCB-dechlorinators for the available of electron donors.

Zwiernik et al., (1998) found $FeSO_4$ could enhance reductive dechlorination of Aroclor 1242 by microbes from Hudson River sediments. In the presence of $FeSO_4$, both Na_2SO_4 and $PbCl_2$ could stimulate *para* dechlorination. Furthermore, in the presence of Na_2SO_4 and molybdate (inhibited sulfate reducing bacteria), $Fe(OH)_3$ did not have stimulatory effect on *para* dechlorination. These researchers propose that the addition of $FeSO_4$ or $NaSO_4$ (plus $PbCl_2$) provides sulfate ions as electron acceptors which stimulated the growth of *para* dechlorinating microorganisms. The additive supplementation of Fe^{2+} or Pb^{2+} to remove sulfide ions formed during sulfate reduction and thus reduced the bioavailability and toxicity of sulfide ions and/or H_2S. These results also showed that if sulfide/H_2S concentrations are in acceptance levels, the PCB dechlorination and sulfate reduction can coexist in the sediment.

From these different results, it suggests that reductive dechlorination of PCB can occur under methanogenic, sulfidogenic, Iron(III) reducing and denitrifying conditions and which processes can be occurred or predominately depending on the physiology of the indigenous microorganisms.

2.11.9 Polychlorinated Biphenyls-Dechlorinating Populations in Contaminated Sediments

Recently, the numerous of reductive dechlorination of various halogenated aromatic and aliphatic compounds have been isolated. The potential of these halorespiring bacteria for innovative clean-up strategies of polluted anoxic environments (Smidt et al., 2000). Cutter et al., (2001) studied the identification of microorganism that links its growth to reductive dechlorination of 2356-CBps. Through denaturing gradient gel electrophoresis (DGGE) of 16S rDNA form a highly enriched *otho*-PCB dechlorinating culture. This was the first identification of a

microorganism that catalyses the reductive dechlorination of a PCBs. The organism, bacterium *0-17* has high sequence similarity with the green non-sulphur bacteria and with group that includes *Dehalococcoides ethenogenes*. Furthermore, methaogenic archaea were not involved in this dechlorination process. (Field and Sierra-Alvarez, 2008) summarized the potential of microorganisms to transform PCBs. PCBs dechlorinating microbes have not been isolated in pure culture; however, there is strongly evidence from enrich cultures that some *Dehalococcoides spp.* and other microorganisms within the *Chloroflexi* phylum could grow by linking the oxidation of H_2 to the reductive dechlorination of PCBs.

Currently, it has been isolated PCB-dechlorinating populations from contaminated sediments, PCB-free sediment spiked with Aroclor 1248 incubated with microorganisms eluted from St. Lawrence River sediments. Eubacterial and archaeal were predominated in PCB-spiked sediment cloned libraries. The result shows that sequence analysis of four eubacterial implied homology to *Escherichia coli, Lactospaera pasteurii, Clostridium thermocellum,* and *Dehalobacter restrictus*. The predominant archaeal sequence was closely related to *Methanosarcina barkeri*, this implied that a methanogenesis are involving in the PCB dechlorination processes as well (Oh et al., 2008). It could be concluded that methanogens might engage in PCB-dechlorinating microbes. However, it depended on the prevalence of microcosm existing in investigated sites.

CHAPTER III

METHODOLOGY

3.1 Characterization of Sampling Sites

Sediments and stream waters were collected from several natural water resources during 2007 to 2009. The sampling locations are shown in Figure 3.1 and Appendix A including four PCBs possibly contaminated areas and a non-PCB polluted site: 1) Hum Lum Poo Canal which receives treated and untreated effluents from the Bangpoo Industrial Estate and nearby factories and has a history of HCB contamination, ten sampling sites (HLP1 to HLP10) were specified in this canal and the sampling points are shown in Appendix A; 2) a canal receiving effluent discharge from the center wastewater treatment plant of the Bangplee Industrial Estate in Samutprakarn Province where several electronic-related factories which possibly using dielectric fluids are located (BP1 and BP2); 3) a canal receiving discharge from small material recovery facilities in Samutprakarn Province (MF1 and MF2) which possibly received PCBs leakage during waste separation and cleaning; 4) a canal nearby the South-Bangkok Power Plant (PWP) where certain amounts of used transformers and capacitors were stored; and 5) Bangplakod Canal (BPK), passing through Suksawad Road in Samutprakarn Province which has high water quality and is a habitat for many aquatic lives, was served as a control for non-PCB contaminated site. All sediments were kept in plastic bags and stored at 4^{o}C until being used.

3.2 Media Preparation

3.2.1 Sediment-Water Preparation

Sediment-water mixtures were freshly prepared by adding 5 g of sediment and 5 ml of stream water into a 20-ml serum bottle, mixed thoroughly by vortex mixer for

Figure 3.1 Sampling locations in Samuthprakarn Province of Thailand (Note: 1: HLP, 2: BP, 3: MF, 4: PWP, 5: BPK).

1 minute, sealed with butyl rubber stoppers and alumina-cap, and kept under room conditions until used.

3.2.2 Sediment Slurry Preparation

Sediment slurry was freshly prepared by mixing sediment and stream water at the ratio of 1:1 (v/v). The sediment slurry was then filtered to remove the particles larger than 0.7 mm by a 100-ml glass syringe with a 22G×2 hypodermic-needle (0.7 mm opening) and kept in a 100-ml alumina-capped serum bottle (Chen et al., 2010) until used.

3.2.3 Reductive Anaerobic Mineral Medium

A reductive anaerobic mineral medium (RAMM) was prepared and used as necessary. It consisted of 2.7 g/l of NH_4Cl, 0.1 g/l of $MgCl_2.6H_2O$, 0.1 g/l of $CaCl_2.2H_2O$, 0.02 g/l of $FeCl_2.4H_2O$, 0.27 g/l of K_2HPO_4, and 0.35 g/l of KH_2PO_4. Yeast extract (YE) was applied as a nutrient supplement at the concentration of 5 g/l.

This solution was used instead of stream water to mix with sediment to prepare nutrient-rich sediment slurry and was kept in a 100-ml alumina-capped serum bottle.

3.2.4 Sterilized Medium

In order to ensure that the disappearance of PCBs derived from microbial activity, several control experiments were performed using sterilized media. The sterilized medium was prepared by pasteurized at 75^{o}C for 20 min to kill vegetative cells and activated spore, then incubated at 23-25^{o}C for 48 hr to allow spore to germinate and finally autoclaved at 121°C for 1 hr to kill vegetative spores (Bedard and Quensen III, 1995).

3.3 Chemicals

All chemicals used in this research were reagent grade and used without further purification. Demineralized water was used to prepare all reagents.

3.3.1 PCB Congeners

For PCBs, 22 congeners (as shown in Table 3.1) which are the major components in Aroclor 1242 and Aroclor 1260, except 345-, 234- and 2345-CBps which are not presented in the Aroclor mixture, were purchased from AccuStandard, Inc. (USA) and were used to prepare the standard solutions in 99% acetone. Bedard et al. (1995) found 234- and 2345-CBps were the most susceptible to be dechlorinated because both congeners contain phenyl rings substituted with three and four chlorines and at least two of which were adjacent. As a result, the PCB congeners chosen in this research were 234- and 2345-CBps, in order to test the dechlorination abilities of anaerobic microbes. Stock solutions were separated into several 1.5-ml vials, sealed with butyl rubber stoppers and alumina-caps, and kept refrigerated until being used.

3.3.2 Organic Substrates

Acetate, lactate and pyruvate in sodium form were purchased from Sigma-Aldrich, Inc. (USA). The solutions were prepared in demineralized water and kept refrigerated until being used.

3.3.3 Halogenated Primers

Methyl 4-bromobenzene (4-BZ), 1,4-dibromobezene (14-DBZ), 4-bromobenzonitrile (4-BN), and 4-bromobezoic hydrazide (4-BH) were purchased from Sigma Aldrich, Inc. (USA). Stock and standard solutions were prepared in acetone and kept refrigerated until being used.

Table 3.1 List of PCB congeners used in this study.

PCBs	Congeners	PCBs	Congeners
Dichlorobiphenyl	22'	Tetrachlorobiphenyl	252'5'
	23'		242'5'
	24'		232'5'
	44'		253'4'
Trichlorobiphenyl	22'5		2344'
	254'		2345
	244'	Pentachlorobiphenyl	2363'4'
	234		2453'4'
	342'	Hexachlorobiphenyl	2362'4'5'
	345		2452'4'5'
			2342'3'6'
			2342'4'5'

3.4 Extraction and Analytical Methods

Quantitative comparisons of the samples were extracted by the solvent and ultrasonic extraction (EPA 3550) and the quantification comparisons were shown in Appendix B.

3.4.1 Sediment Extraction

One ml of 6 N H_2SO_4 and 1 g of copper (Cu^{2+}) were added into the serum bottle and the mixture was mixed thoroughly by a mixer. After that, 5 ml of 9:1 hexane:acetone solvent were added into the serum bottle. The bottle was then shaken by a vortex mixer followed by ultra-sonicating for 30 minutes. The mixture was centrifuged at 3,000 rpm for 20 minutes and the supernatant was transferred as much as possible to a new vial. The remaining mixture was re-extracted for another time following the similar procedures. At the second extraction, the upper layer liquid was pulled out and filled the tube up to the 5-ml mark and then 1 ml of 6 N NaOH was added. The mixture was then shaken by vortex mixer and 4 ml liquid was transferred to a new vial. A small amount of Na_2SO_4 was added to a tube to remove moisture. The PCB-containing extrants were further cleaned up by the deactivated florisil column and the column was rinsed with *n*-hexane. The elution was transferred to an analyzing vial for GC analysis. Extraction efficiencies were higher than 60% for all samples.

3.4.2 Sediment Slurry Extraction

Two ml of liquid mixture was withdrawn from the serum bottle by a needle and injected into an extraction tube. Two ml of hexane and 0.2 ml of 6 N NaOH were added. The mixture was shaken by a vortex mixer followed by ultra-sonicating for 10 minutes. The vial was centrifuged at 3000 rpm for 10 minutes. The upper layer of extracting solvent was withdrawn as much as possible afterward and injected into a new tube. The remaining mixture was re-extracted for another two times following the similar procedures. At the third extraction, the upper layer liquid was pulled out

and filled the tube up to the 5-ml mark. A small amount of Na_2SO_4 was added to remove moisture before analyzing by the GC and recovery efficiencies were higher than 80% for all samples.

3.4.3 Gas Chromatography Analysis

Application of GC analysis followed the USEPA 8082A Method using GC/μECD (quantification) and EPA 680 Method using GC/MS (qualification). The chromatograms of Aroclor 1242 and Aroclor 1260 and chromatographic data were shown in Appendices C and D. All possible intermediates from 234- and 2345-CBp could be detected under these analytical procedures. Gas chromatograph capillary column equipped with a μECD (J & W Scientific from Agilent Technologies) and 30–m×0.25-mm ID fused-silica capillary column chemically bonded with SE-54 (DB-5), 0.25-μm film thickness was used to analyze PCBs and their intermediates. All intermediate products were confirmed by the gas chromatograph mass spectrometry (GC/MS). Electron impact ionization as ion sources and quadrupole mass analyzer (J & W Scientific from Agilent Technologies) and 30-m×0.25-mm ID fused-silica capillary column chemically bonded with SE-54 (HP-5MS), 0.25-μm film thickness. Both gas chromatographs were operated under the following conditions: the oven temperature was maintained at 120°C for 2 min, raised to 225°C at 3°C/min, maintained for 3 min, and then raised again at 5°C/min to the final temperature of 270°C, which was held for 11 min. The temperature of the injector and the detector were set at 280°C and 300°C, respectively. Nitrogen and helium gases were used as the makeup (20 ml/min) and carrier (1 ml/min) gases, respectively. The split ratio was kept at 10:1.

3.4.4 Methane Analysis

Quantification of headspace methane gas in the serum bottles was done by the GC-TCD pack column with 2 analytical ports (GC-8A, Shimadzu, Japan); port 1 equipped with the UnibeadsC 80/100 mesh (4 mm OD, 3 mm ID, 3 m long), port 2 equipped with the 3% unisole 30T on flusinP 30/60 mesh (4 mm OD, 3 mm ID, 3 m

long). Temperature of the injector was maintained at 160°C. Column temperature was maintained constantly at 110°C. Nitrogen and helium (300 kPa) were employed as make-up and carrier gases, respectively.

3.5 Experimental Scenarios

3.5.1 Effect of Organic Substrates on PCBs Dechlorination

Effects of different organic substrates on microbial ability to reductively dechlorinate PCBs were studied in order to evaluate the possibility of enhancement by external supplement. Fresh sediment and stream water were tested in this task. The fresh sediment slurry (SS) and reductive anaerobic mineral medium (RAMM) were used as the media. Sterile SS or/and RAMM were used as the control sets. Sediment sample from Site 3 of the Hua Lam Poo Canal in Samutprakran Province was used.

Three organic substrates namely sodium acetate, sodium lactate, sodium pyruvate were used as the external carbon source and yeast extract was provided for nutrient supplement (Oremland, 1988). Yeast extract was found to be able to enhance the dechlorination activities of indigenous microbes (Chang, et. al., 1996). Pyruvate and lactate were chosen as the organic substrates because they are fermentation products which can promote the activities of acetogenesis and sulfate reducing bacteria. Acetate was used because it is the substrate directly used by methanogens and sulfate reducing bacteria. Each substrate was added to make the concentration of 2 g/l and yeast extract was added to make the concentration of 5 g/l before the inoculation. The 0.1 ml of 1,000 mg/l 234-CBp stock-solution was injected into the serum bottle to make an initial concentration of 2 mg/l, final acetone concentration in the medium was lower than 0.5% (v/v); hence, did not cause any interference in microbial activity. All serum bottles were kept in the dark at room temperature and were shaken from time to time on a daily basis. The mixed liquor was withdrawn and extracted for remaining CBps every 2 weeks. The producing of methane gas was also monitored.

3.5.2 Effect of Halogenated Primers on PCBs Dechlorination in Sediment Slurry

This part aimed to investigate a possibility to enhance the 234- and 2345-CBp dechlorination by using priming compounds which could stimulate the dechlorination of PCBs (Bedard et al., 1998; DeWeerd and Bedard, 1999). Microorganisms could use PCBs and other halogenated aromatic compounds as the electron acceptors; hence, presumably that the consortiums which could degrade halogenated groups, possibly could dechlorinate PCBs as well. Selected priming congeners including methyl 4-bromobenzene (4-BZ), 14-dibromobezene (14-DBZ), 4-bromobenzonitrile (4-BN) and 4-bromobenzoic hydrazide (4-BH) all of which had been found to be able to activate PCBs dechlorinating microbes during the dechlorination process (DeWeerd and Bedard, 1999). These selected primers will not persist in the natural environment and; hence, are environmental friendly chemicals (Bedard et al., 1995). Long-term storage sediment slurry under cold tempered (4^{o}C) was used in this phase.

The 0.1 ml of target PCB congener stock-solution at 1,000 mg/l was spiked into the serum bottle to make an initial concentration of 2 mg/l. Halogenated priming congeners were separately amended from the stock solution prepared at 50,000 mg/l to yield the final concentration of 75 mg/l. Added acetone in the mixture was lower than 0.5% (v/v) in order to prevent any toxicity and/or enhanced the dechlorination by acetone amended. The serum bottles were incubated in the dark at room temperature. Non-priming sets were incubated by indigenous sediment slurry (did not amended with halogenated priming congeners) under the same conditions and sterile control sets were also prepared for comparison (Figure 3.2). Samples were taken every 2 weeks and analyzed for both PCBs and halogenated primers.

3.5.3 Effectiveness of Bioaugmentation on PCB Dechlorination

According to Mongkong (2007) who also used the sediments from the similar sites as in this study to dechlorinate 234-CBp, the microbial consortiums in the sediment samples could be divided into two groups according to their 234-CBp

dechlorination abilities, i.e., active (sample from the Hua Lam Poo Canal) and inactive (samples from small material recovery facilities, South Bangkok Power Plant, Bangplee Industrial Estate, and Bangplakod Canal). It is interesting to determine the effect of bioaugmentation on PCB dechlorination by inoculating active microbes from the HLP to those non-active matrix of the small material recovery facilities, South Bangkok Power Plant, Bangplee Industrial Estate, and Bangplakod Canal. Fresh stream water and sediment were used in this part. Two PCB congeners were used, i.e., 234-CBp was used with the sediment slurry test at the concentration of 2 mg/l and 2345-CBp was used with the sediment test at the concentration of 8 mg/l.

Both sediment-water and sediment slurries were separately tested in order to compare the PCBs dechlorination ability in different environmental matrix. Working scheme for this phase is summarized in Figures 3.3 and 3.4 for sediment slurry and sediment-water, respectively. Sample was taken every month for sediment-water set and every 2 weeks for sediment slurry set.

3.5.4 Effect of Temporary Heat Treatment on PCBs Dechlorination

Temporary heat treatment might enhance the desorption of PCBs from sorbed phase to be available for microorganisms uptake; hence, promote the activity of microorganisms in order to enhance the dechlorination abilities of anaerobic microbes. In addition, high temperature in thermophilic range might also accelerate the microbial degradation rate of PCBs. Thus, this part simulated temporary heat shock to the sediments in order to promote the dechlorination ability of microorganisms as well as desorption of PCBs.

Temporary heat treatments at 50, 70 and 90°C for thermophilic conditions in sediment-water media were applied to both active and non-active microbes to investigate the effect on 234-CBp dechlorination. Treated sediment-water samples were extracted every three weeks and the working scheme for this section is summarized in Figure 3.5.

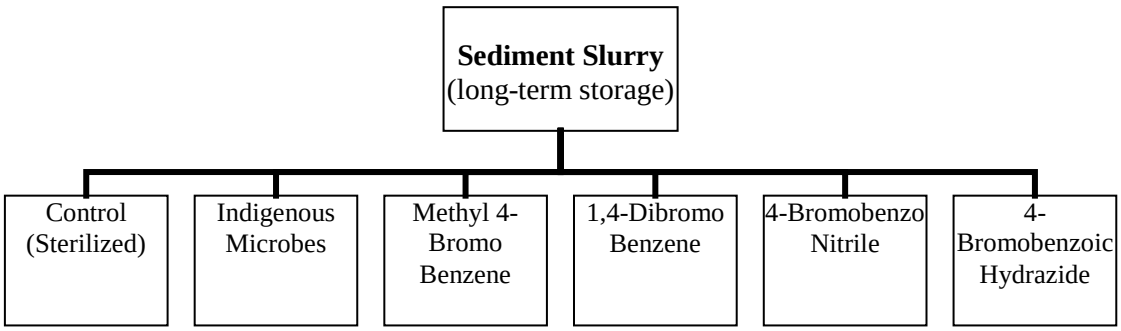


Figure 3.2 Working scheme for the effect of halogenated primers in sediment slurries on 234- and 2345-CBp dechlorination.

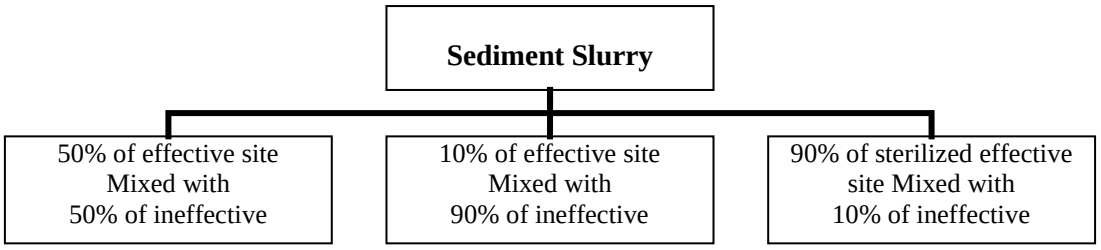


Figure 3.3 Working scheme for the bioaugmentation in the sediment slurry on 234-CBp dechlorination (Note: Effective site referred to HLP3).

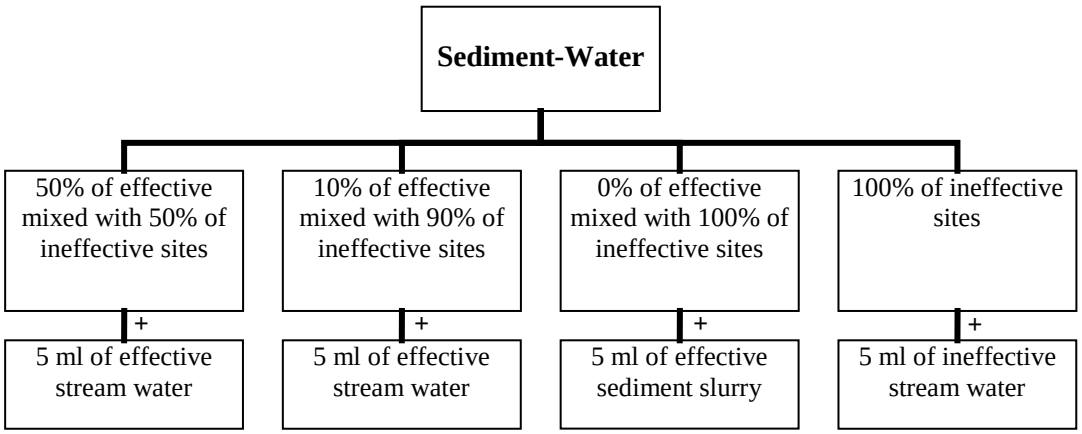


Figure 3.4 Working scheme for the bioaugmentation in the sediment-water on 2345-CBp dechlorination (Note: Effective site referred to HLP3).

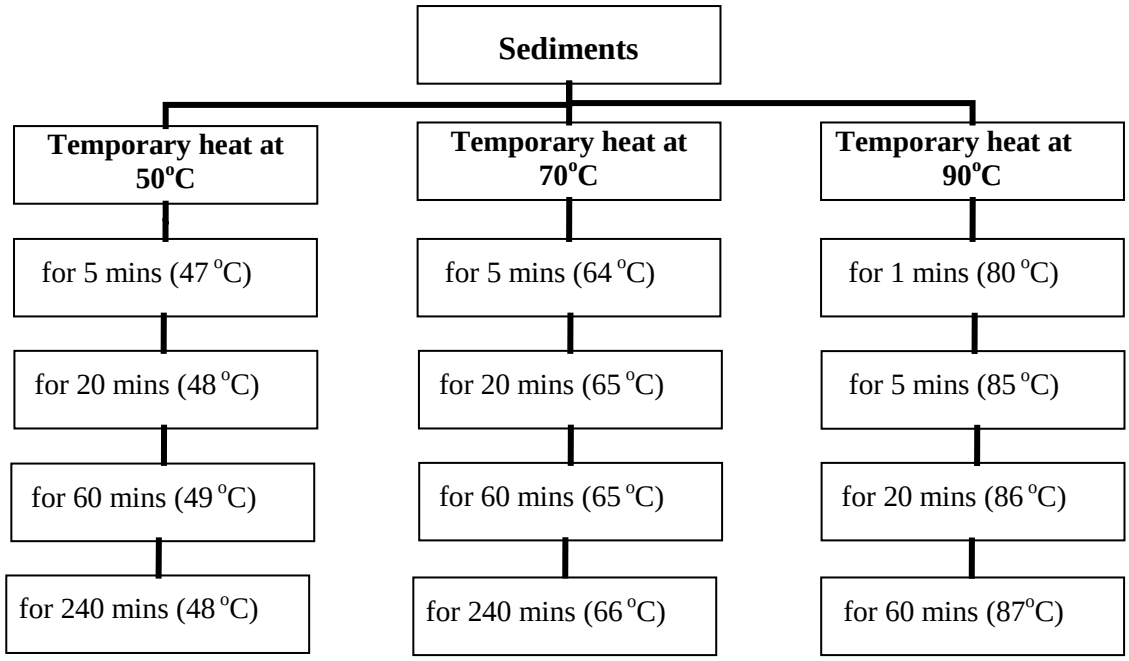


Figure 3.5 Working scheme for the heat treatment effect on 234-CBp dechlorination (Note: temperatures in core sediment were shown in parenthesis).

3.5.5 Fate of PCBs in Simulated Environmental System

In this part, the vertical movement of PCBs and dechlorination ability in the stream-simulating system were investigated. In this phase, the environmental simulating system was constructed by using 2 aquariums connected together as shown in Figure 3.6. Storage sediments under 4°C (1 year) mixed with target ingredients were added into the glass cup with 10 cm height and 5 cm in diameters (Figure 3.7) and placed at the bottom of Aquarium Box 1. These cups represented the PCBs contaminated sediments.

Fresh stream water was filled up to simulate stream condition, hence, either suspended solid particles or xenobiotic compounds might affect the microbial activities. Fresh stream water was circulated from the storage (Aquarium Box 2) to simulate the real canal flowing environment. For supplement compounds, two organic acids namely acetate and pyruvate at 2 g/kg, which served as the electron donor, and yeast extract at 5 g/kg, which served as a nutrient enrichment, were

separately provided into the sediments. No additive sets were used as the control for PCBs dechlorination and movement under natural canal environment. The oxidation reduction potential of the sediment in the cup was periodically measured in order to ensure the anaerobic conditions. The target compound in this study was 2345-CBp at 6 mg/l. One cup of each series was sampled every month and the sediment was analyzed for 2345-CBp and its intermediates.

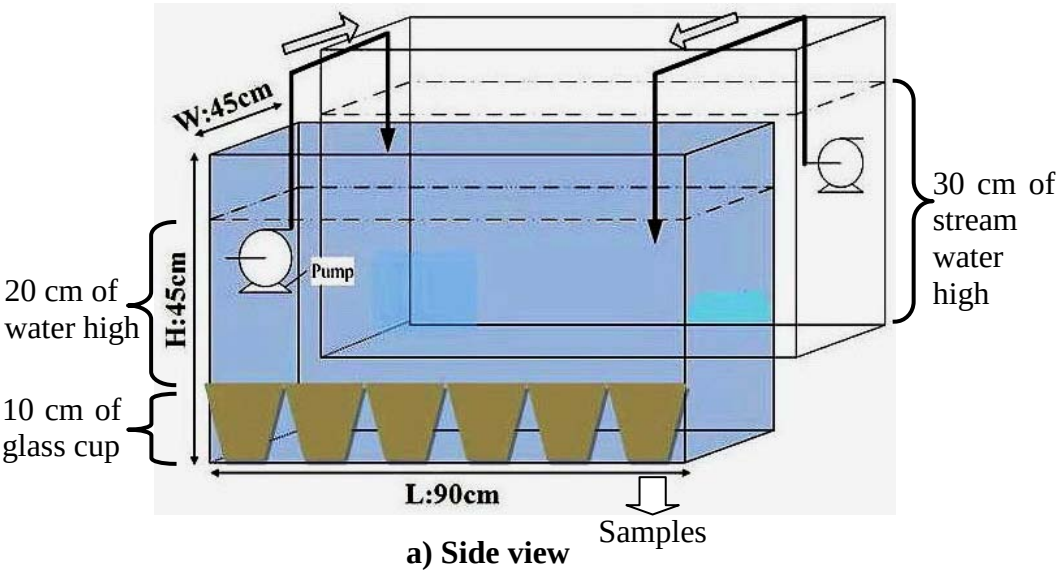


Figure 3.6 Layout and configuration of stream-simulated reactor.

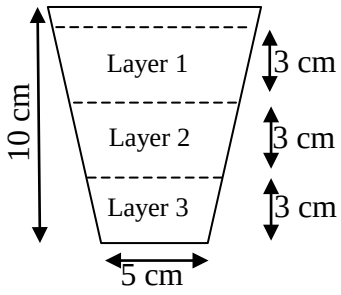


Figure 3.7 Three layers of cup test.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Characterization of Experimental Media

The sediment and water sample were collected from natural water resources during 2007 to 2009. The sediment slurries, sediments, and stream water were analyzed for their chemical properties and the results are shown in Tables 4.1 to 4.5. Low VSS:SS ratio implied low viable microbes in the sediment. Nevertheless, these VSS values are still higher than those of typical biological treatment systems. Hence, it can be expected that there were sufficient viable microbes for anaerobic digestion. The COD were quite high (all greater than 2,000 mg/l) which ensured enough carbon sources from natural and/or anthropogenic sources in order to support the activities of heterotrophic bacteria; archaea and fermentators microorganisms which play important roles in dehalogenation processes. Moreover, the COD:N:P ratios were in the range 62 to 401:3 to 11:1 implying that there were adequate nutrients for microbial requirement for anaerobic digestion. This was supported by the observation of biogas immerging from the sediments during sampling as shown in Figure 4.1. Additionally, the sediment samples after one-year storage under cold climate environment were also repeatly analyzed for SS, VSS, COD, TKN and phosphorus as shown in Table 4.5. The results suggested that all parameters were not significantly changed during the storage time line.

4.2 Determinations of PCBs Dechlorination Ability of Microbial Consortium in Natural Stream Sediments

Bioremediation can be a managed or spontaneous process derived under biological treatment in remediating contaminated environment; it is mentioned on microbiological catalysis acts on pollutants. Because of the role to estimate the

Table 4.1 Characteristics of the sediment slurries prepared from the sediments and stream waters sampled in June, 2007.

Sampling sites		COD	TKN	P	SS	VSS
Bangplee Industrial Estate	Site 1	9,350	520	50	152,630	10,800
	Site 2	2,750	780	100	125,750	11,650
Small Material Recovery Facilities	Site 1	15,130	1,020	90	100,770	8,650
	Site 2	36,160	400	90	144,320	8,560
South-Bangkok Power Plant		13,480	1,580	200	205,990	12,100
Bangplakod Canal		2,480	640	40	188,050	19,850
Hua Lum Poo Canal (Site 3)		43,040	930	320	94,950	14,700

Note: all units are mg/l by volume

Table 4.2 Characteristics of the raw sediments sampled in March, 2008.

Sampling sites		COD	TKN	P	Moisture Content (%)	VSS (%)
Bangplee Industrial Estate	Site 1	31,750	913	361	66.93	17.25
	Site 2	42,350	935	143	43.03	8.08
Small Material Recovery Facilities	Site 1	34,710	1019	296	53.69	7.14
	Site 2	42,090	930	88	61.89	15.09
South-Bangkok Power Plant		22,890	1170	252	57.04	9.20
Bangplakod Canal		18,460	930	788	53.44	7.80
Hua Lum Poo Canal (Site 3)		77,650	1,200	950	71	10.20

Note: all units are mg/g by weight

Table 4.3 Characteristics of the sediment slurries prepared from the sediments and stream waters sampled in March, 2008.

Sampling sites		COD	TKN	P	SS	VSS
Bangplee Industrial Estate	Site 1	21,030	773	280	117,040	12,240
	Site 2	28,700	952	480	140,560	12,760
Small Material Recovery Facilities	Site 1	29,560	431	200	255,180	15,520
	Site 2	26,900	515	159	218,960	15,880
South-Bangkok Power Plant		27,880	896	300	287,740	15,020
Bangplakod Canal		38,860	997	90	208,360	13,960

Note: all units are mg/l by volume

Table 4.4 Characteristics of stream waters sampled in March, 2008.

Sampling sites		COD	TKN	P	SS	VSS
Bangplee Industrial Estate	Site 1	214	13	1.5	101	25
	Site 2	236	14	1.1	20	11
Small Material Recovery Facilities	Site 1	744	28	0.6	13	10
	Site 2	565	35	0.4	15	13
South-Bangkok Power Plant		193	21	0.8	51	19
Bangplakod Canal		127	20	0.4	91	36

Note: all units are mg/l by volume

Table 4.5 Characteristics of sediment slurries prepared from the sediments and stream waters after one-year storage (prepared in May, 2009).

Sampling sites		COD	TKN	P	SS	VSS
Bangplee Industrial Estate	Site 1	11,034	933	305	740,760	17,400
	Site 2	19,862	1,053	540	751,210	18,765
Small Material Recovery Facilities	Site 1	19,310	547	241	800,680	14,760
	Site 2	24,828	1,107	179	858,910	12,985
South-Bangkok Power Plant		13,241	747	229	858,910	13,620
Bangplakod Canal		11,103	427	72	741,450	17,350
Hua Lam Poo canal (Site 3)		25,379	827	270	894,425	14,735

Note: all units are mg/l by volume

Figure 4.1 Sediment and stream water sampling site along Hua-Lam-Poo Canal.

biodegradable activity in related to the environmental impact assessment. The requirements for a potential bioremediation treatment consisted of specific microorganisms, carbon sources, electron acceptors and donors, moisture, pH, nutrients, temperature, whereas should be absent from toxicity and predators (Cookson, 1995). In order to reveal the importance of the evaluation of naturally degradation ability, it is first necessary to investigate the presence of degradation-related microorganisms at the giving sites that possessed the capacity to synthesize enzymes for the PCBs degradation. Second, it is necessary to evaluate the appropriate of carbon sources, electron donors and acceptors; and finally, it also need to test the sufficiency of moisture and the range of suitable pH values, and temperatures. Furthermore, the availability of inorganic nutrients such as nitrogen, phosphorus and trace metals are all essential to be investigated.

A preliminary test was conducted to verify microbial activity and extraction accuracy, the qualification and quantification were shown in Appendix B. Three sets of the experiment were performed by spiking the sediments with either Aroclor 1242 or 1260 and incubating at different conditions, i.e., kept in the dark under room conditions, kept in a refrigerator at 4°C, and sterilized and kept in the dark under room conditions. The results showed highly chlorinated PCB congeners could transform to lower chlorinated congeners in the non-sterilized series maintained under room conditions whereas no significantly dechlorination was observed in either refrigerated and no dechlorination in sterilized sets within the experimental period of 5 months. These results revealed two pieces of important information; first, the disappearance of PCBs under the studied conditions was surely due to microbial activity and second, extraction and analytical procedures for PCB congeners used in this study were reliable and accurate. Moreover, the PCBs were not loss during the autoclaving, which indicated that volatilization was not the major PCBs loss path in this study.

Dechlorination of PCBs in Thailand has been performed since 2006. Early study for PCB dechlorination has been conducted by Sudjarid (2006) with the PCB congeners as shown in Table 3.1. Primary determinations of 209 PCB congeners by

GC were presented in Appendix C. Certain congeners were inoculated into fresh sediments without any acclimation and nourishment. The results implied that only 234-CBp which contains three chlorine atoms located at three consecutive positions on one phenyl ring could be dechlorinated effectively by indigenous microbes from HLP canal; hence, 10 sampling sites along this canal were investigated, with lag phase and completion time for 2 and 12 weeks, respectively as shown in Table 4.6 (Sudjarid, 2006). Only 24-CBp was found as the sole intermediate from 234-CBp dechlorination. It is well documented that less chlorinated PCB congeners were less toxic but more persistent under anaerobic condition (Bedard et al., 1995). 24-CBp could not be further dechlorinated to mono-CBps and biphenyls under anaerobic condition because the energy release during the dechlorination was lower than those required by the dechlorinating anaerobes to perform the task. Nonetheless, these less chlorinated PCBs can be effectively degraded under aerobic condition (Bedard et al., 1995). Hence, sequential anaerobic/aerobic microbial processes have a potential to completely degrade PCBs.

Continuing study of the dechlorination of PCBs was studied by Mongkon (2007). This study was determined the dechlorination efficiency of indigenous microbes from other locations, but still in the industrial zone of Samuthprakarn Province in Thailand. Comparing between sediment and sediment slurry sets, under the same condition, it found that the sediment could begin to dechlorinate 234-CBp after week 5 and complete within week 14-22 (Table 4.6). It was postulated that might be due to the effect of the availability of 234-CBp to the dechlorinators. Sediment was a much thicker and heavier mixture than the sediment slurry; hence, providing less accessible condition to 234-CBp for the microbes. It is considered that soil organic matters which well absorbs to non-polar organic compounds including PCBs, then affected to decrease of their bioavailability. Besides that the particle size (sediment, without sieved and sediment slurry, sieved) were directly affected to the PCBs degradation activity.

Moreover, the investigation of 234-CBp dechlorination by using the sediment microorganisms revealed that the native microbes from HLP sites had higher potential

to dechlorinate PCBs than other sites (Table 4.6 vs Table 4.7) both in the sediment and sediment slurry. In addition, the sediments from HLP sites also had ability to dechlorinate HCB as well (Chen et al., 2010). This result is different from the study of Chen et al. (1997) who used sediment slurries from Erh-Jen River in Kaohsiung City of Taiwan and could not observe any dechlorination of 234-CBp within the incubation period of 20 weeks. This might be due to the difference in the native microbial diversity between these two countries which located in different climates, i.e., tropical climate in Thailand versus temperate climate in Taiwan as noticed by Chen et al. (2010). Furthermore, abiotic conditions also have an effect on PCB transformation due to the variation of bacterial communities, either directly (degraders) or indirectly (bacterial syntropic association with degraders) (D'Angelo and Nunez, 2010).

Besides that, Sokol et al. (1994) also found 234-CBp could be dechlorinated after 3 months but not complete within 7 months of the incubation time. This result reveals a piece of evidence to the dechlorination capability of HLP consortium which exposed to highly concentrated hazardous compounds. Moreover, the dechlorination performance of microbes has been changed at different sampling sites even incubated under the similarly condition. It seems likely that microflora proliferated at each site might be different. Not only the climate could affect the dechlorination potential but also the supplementation of substrates from the external sources and/or native consortium present at each site.

Furthermore, evaluation of the microbial ability to dechlorinate hexachlorobenzene (HCB), which is a chlorinated aromatic compound with similar property but less complication than PCBs, was also performed in the HLP canal by Chen et al., 2010. HCB dose was increased from 2 to 10, 40, 100 and 200 mg/l by volume. It found that HCB was still dechlorinated at 200 mg/l with less retardation (Chen et al., 2010). From these results, it implies that the PCBs concentrations used in this study (ranging from 2 to 8 mg/l by volume) should not be toxic to sediment microorganisms.

Additionally, all PCB congeners as shown in Table 3.1 except 2345-CBp which contained chlorines substituent in two benzene rings could not be dechlorinated within the incubation time. It implies that the potential of steric effect of bulky substitutions on the opposite ring was significant in the PCB dechlorination as suggested by VanDort et al., (1997).

Furthermore, the positions of chlorines substituted have a stereo-chemical effects on the affinity of inducible enzymes and their substrate molecule (Sylvestre and Sandossi, 1994). Therefore, the water solubility of PCBs has a vital role to its dechlorination. Highly chlorinated PCB congeners are less water soluble; thus, less accessibility to microorganisms. The results also suggested that more chlorine-substituted congeners needed much longer time to acclimate and initiate the dechlorination.

Table 4.6 Dechlorination of 234-CBp in sediments and sediment slurries from 10 sites of HLP.

Congeners	**Locations**	**Sediments[a]**		**Sediment slurries[b]**	
		Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)
234-CBp	Site 1	5	14-22	2	12
	Site 2	5	14-22	2	10
	Site 3	5-8	14-22	2	12
	Site 4	8	14-22	4	10
	Site 5	5	14-22	2	10
	Site 6	5-8	14-22	2	18
	Site 7	5	14-22	2	12
	Site 8	5	14-22	2	12
	Site 9	5	14-22	2	12
	Site 10	8	14-22	2	12

Note: 24-CBp was found only sole intermediate product.

[a]: data quoted from Mongkon, 2007, [b]: data quoted from Sudjarid, 2006.

Table 4.7 Dechlorination of 234-CBp by sediments and sediment slurries from other sites, except HLP.

Locations		Sediments[a]			Sediment slurries		
		DeCl	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)	DeCl	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)
Power Plant		O	9	21-24	O	14	20-22
Bangplee Ind. Estate	S1	N.D.	N.D.	N.D.	O	18	20
	S2	N.D.	N.D.	N.D.	O	20-22	>22
Material Recovery Facility	S1	O	18-24	>24	O	12-14	18
	S2	N.D.	N.D.	N.D.	O	14-18	24-28
Bangplakod Canal		N.D.	N.D.	N.D.	O	10-12	24-26

Note: O: dechlorination could occur, N.D.: dechlorination could not detect, DeCl: dechlorination process. 24-CBp was found only sole intermediate product. [a]: data quoted from Mongkon, 2007.

It can be noted that the PCB dechlorinators were widespread in all explored sites. Regardless of the in-effective dechlorination ability in this study might be because of inadequate of electron donors or the incubation times were not long enough. Thus, the microorganisms from HLP sites were notably the best dechlorination efficiency. This experimental part was carried out by spiking the 234- and 2345-CBps, separately. Study with these two congeners could provide several outcomes: first, the dechlorination pathways; second, the effects of chlorines substitution patterns on reactivity; third, the dechlorination capability of microbial populations and last, the terminal dechlorination products of PCBs. Furthermore, the dechlorination activity of PCBs could enhance further by using enrichment microcosm and/or pure cultures, because it was notified that the efficiency of anaerobic dechlorinating of chlorophenols was pretty much higher when tested with pure cultures (Bouchard et al., 1996; Utkin et al., 1995; Mohn and Kennedy, 1992).

Additionally, after the dechlorination ability has been verified. Enhancement of the bioavailibility was investigated in further phases of this study, thus bioavailability may often control the occurrence of the dechlorination as well as the

rate/or extent of biodegradation. In order to contribute the better understanding of the parameters which has been effected to the dechlorination, under appropriate environmental temperature.

4.3 Effect of Organic Substrates on 234-CBp Dechlorination

Effect of organic substrate on microbial ability to reductively dechlorinate PCBs was investigated in order to evaluate the possibility of dechlorination enhancement by external supplement. Sediment slurry and RAMM from the HLP site 3 was used in this part. Microorganisms can use specific organic substrates as the sources of carbon and energy for their growth; hence, supplement of certain organics can promote the activity of target microorganisms. Pyruvate and lactate were chosen because they are fermentation products which can promote the activities of acetogens and sulfate-reducing bacteria; hence, commonly support anaerobic degradation activities. Moreover, it also suggests that lactate could enhance the PCBs dechlorination (under methanogens and sulfate reducing conditions) rather than acetate or pyruvate amendment (Chang et al., 2001). Acetate was selected because it is the substrate directly used by the methanogens. It also has been found the major electron donor and/or carbon source during the degradation of PCBs (Baba and Katayama, 2007).

The results revealed that the supplement of external substrate and nutrients did not have any significant acceleration on 234-CBp dechlorination, the effect occurred at the same extension rate in all electron donor supplements; i.e., the time for the occurrence of intermediate and the completion of dechlorination were similar within 5 to 7 weeks and 17 to 21 weeks, respectively, as shown in Table 4.8. And the dechlorination profile was showed in Figure 4.2, and the another dechlorination profiles were represented in Appendix E-1. These results imply that the sediment from HLP already contained sufficient substrates and nutrients for 234-CBp dechlorination. The initial lag time was due to the preparatory period required by the 234-dechlorinators to gather enough growth substrates and produce specific enzyme(s) needed for 234-CBp metabolism. And also the insignificant variations of

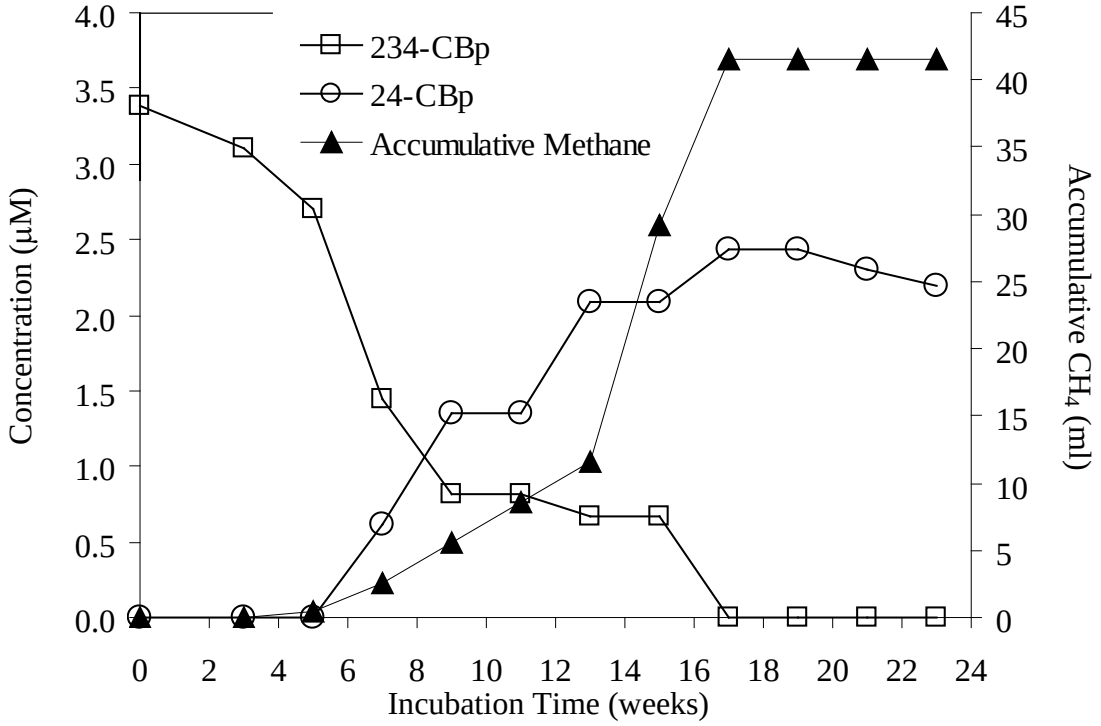
the dechlorination ability under differ of amendment of various electron sources might be postulated that the stable interaction of supporting bacteria and PCB dechlorinators were involved in the natural matrix condition. Thus, after long-term commensalism, the dechlorination consortia worked by using the medium from HLP canal always showed the best performance.

The determination of difference sources on electron donors showed some characters related to several PCB dechlorination consortia. Acetate and other organic acids could enriched the PCB dechlorinating microorganisms as classified as *Chloroflexi* (Wu et al., 2000; Pulliam Holoman et al., 1998; Cutter et al., 1998). Pyruvate also possibly consumed during dechlorination of PCBs (Cutter et al., 2001; Adrian et al., 1998; Maymó-Gatell et al., 1997). Lactate could produce acetate, pyruvate and hydrogen by fermentation of *Firmicutes*, and also production of propionate and methane. Moreover, the acetogenesis also could be metabolized lactate or pyruvate and produced either hydrogen or hydrogen carbonate; both could enhance the production of methane (Chang et al., 2001). This finding was quite different from the results of Nies et al. (1990) who found organic supplement (without yeast extract) was the most important factor controlling the dechlorination of Aroclor 1242 in soil. And the results suggested that the less-impact of stimulation indicating that the sediment had already contained sufficient level of electron donors for PCBs transforming bacteria.

Table 4.8 234-CBp dechlorination in the presence of organic substrates.

Medium	Electron Donor	Final Product	Lag Phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)
RAMM	Pyruvate	24-CBp	5	17
	Lactate	24-CBp	5	17
	Acetate	24-CBp	5	21
SS	no addition	24-CBp	7	21
	Pyruvate	24-CBp	5	21

Note: RAMM: reductive anaerobic mineral medium, SS: sediment slurry



Note: the dechlorination was not occurred under sterilized control sets.

Figure 4.2 234-CBp dechlorination profiles in reductive synthetic anaerobic mineral medium amended with lactate.

4.3.1 Dechlorination of PCBs and Methane Gas Production

To determine the principal microbes responsible for HCB dechlorination, Chen et al. (2010) used the sediment from HLP sites. The results found that, during the initiation period, the sulfate reducing bacteria (SRB) and denitrifiers (DN) were very active. Furthermore, it also suggested that neither SRB nor DN directly engaged in or serious interfered with the HCB-dechlorination. To identify the role of methanogens on HCB-dechlorination, bromoethanesulfonic acid (BES), a selective methanogenic inhibitor (Chang et al., 1997), was inoculated into the serum bottle at various concentrations. It was found that only 5 mM of BES could suppress the methanogenic activity, as could be obviously observed from less methane gas

production compared to non-amended inhibitor set. It makes an important of evidence that the dechlorinating step was directly performed by methanogenic bacteria only or maybe a variety of different species of methanogens undergoing by this role (Chen et al., 2010). Nonetheless, some studies revealed that the methanogens did not involve in the dechlorination process (Prytula and Pavlostathis, 1996) as was in contrast with some other results which suggesting that methanogens were the main HCB-dechlorinators (Change et al., 1997). This study indicated that the sediment slurry contained both methanogens and other HCB dechlorinating species (Chen et al., 2010).

Further investigation on HCB dechlorinations was conducted by using vancomycin (VAN), a strong bactericide on gram-positive bacteria, especially acetogens. The results revealed that 100 mg/l of VAN moderately retarded the dechlorination performance, and partial pressure of methane also reduced (Chen et al., 2010). From this study, it could be concluded that apart from methanogenic HCB-dechlorinators, it is possible that there were other HCB-dechlorinators naturally existing in the sediment of HLP canal (Chen et al. 2010).

From unclear results of main dechlorinators of HCB which have been tested in Thai canal, some results notified that the methanogens and SRB were main PCB dechlorinators in sediment slurry (Chang et al., 2001; Lovely et al., 1995). Moreover, It was shown that the diversity of community could be reduced in mineral medium by addition of inhibitors for methanogens (BES) and *Clostridium spp.* (VAN), without eliminating of dechlorination that was inhibited by the addition of molybdate, which inhibited SRB, by analysis of total community fro 16S rRNA (Holoman et al., 1998).

Thus, in this study the main dechlorinators of PCBs were under investigated; however, not yet focus on any specific groups of consortium. This scenario was screening simplified the relationship of methanogens and dechlorination of PCBs in HLP canal at the beginning. The relations of dechlorination of 234-CBp and $CH_{4(g)}$ production were revealed. Figure 4.3 shows the $CH_{4(g)}$ production under difference supplement of electron donors (lactate, $C_3H_6O_3$, acetate, CH_3COOH, pyruvate,

$C_3H_4O_3$), which could support the growth of various anaerobes including the PCB dechlorinators. The results were expected to determine the dominant bacteria in PCBs dechlorination consortia while the dechlorination occurred.

Among the tested electron donors, acetate showed potentially enhancing of the accumulative methane production, since it is one of the methanogenesis substrates as express in equation 4.1. However, methanogens need to compete with SRB for acetate; hence SRB would outcompleted of methanogens for acetate (Oremland and Polcin, 1982). Pyruvate shows the second potential for the activation of methane production, the quantitation of methane were not significantly different from RAMM and SS; whereas, lactate could transform to pyruvate by SRB and acetogenesis, then pyruvate transform to acetate which used by methanogens.

Moreover, the result also implies that the mineral mediums were not superior for the methanogenesis by HLP anaerobes; it might only be a medium that provided essential growth factors to HLP microorganisms that had been long-term adapted in natural sediment/water of HLP site. In lactate amended test, there was less production of methane gas, and it can be understood by the competitive interaction of SRB and methanogens for the quest of electron donors. Under sulfate reducing condition, lactate could be utilized through incomplete and complete oxidation illustrated as equation 4.2 and 4.3. Therefore, lactate can be directly use by SRB, in contrast, it needed to be degraded to acetate before the utilization by methanogens.

<u>Under methanogenic condition:</u>

$$\text{Acetate} \rightarrow CO_2 + CH_{4(g)} \qquad (4.1)$$

<u>Under SRB condition:</u>

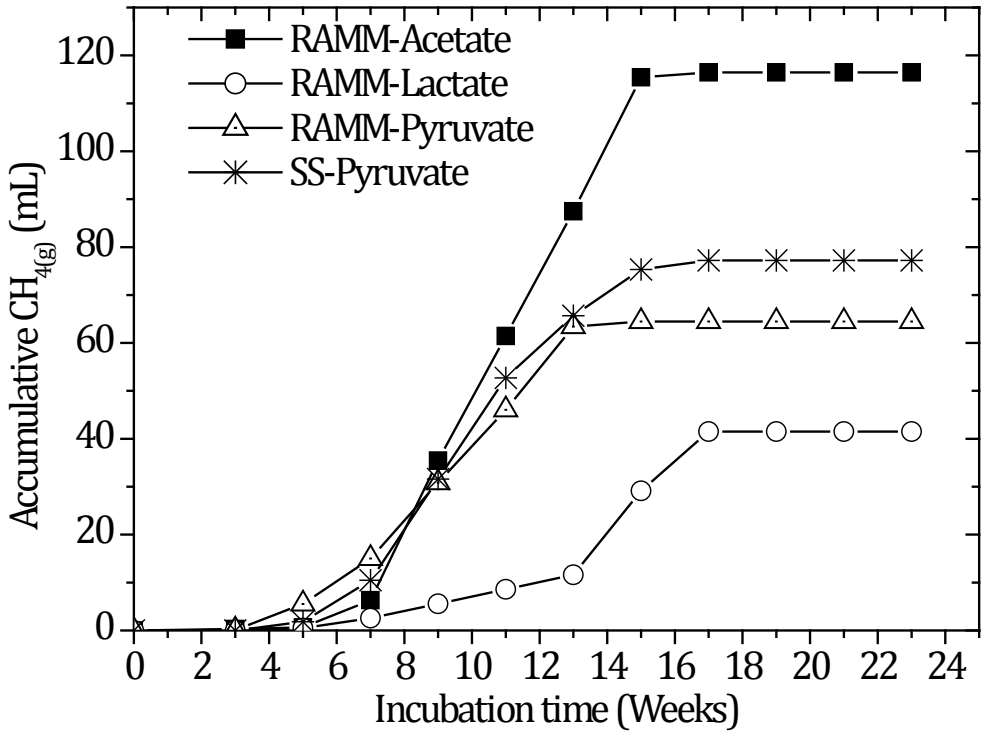
$$3\text{lactate} \rightarrow 2\text{propionate} + \text{acetate} + CO_2 \text{ (incomplete oxidation)}, \qquad (4.2)$$

$$\text{or } 2\text{lactate} + SO_4^{2-} \rightarrow 2\text{acetate} + 2CO_2 + 2H_2O + S^{2-} \text{ (complete oxidation)} \qquad (4.3)$$

To summarize the results, it was found that the tendency of producing methane gas was similarly under the acetate and pyruvate amended sets, but decrease in lactate

set. The reason might because SRB could use lactate directly and enriched as a dominant bacteria than methanogens and somewhat inhibited the methanogenesis. Although SRB need to compete with acetogens for lactate, the results also suggested that this competition did not retard SRB to be enriched. Under this sequences condition, it refers that HLP methanogens were less-enriched comparing to SRB in using external sources like lactate and pyruvate. Therefore, the role of fermentators was important in methanogenesis and possibly in dechlorination processes also.

The results in Figure 4.2 vs 4.3 showed a possibility that methanogens and sulfate reducing bacteria both could initiate the dechlorination, since methane gas was detected earlier before the occurrence of dechlorination intermediates. And this assumption will lead to a contradiction to our previous studies that methanogenesis may be not the main dechlorinators in the dechlorination of HCB and PCBs. As showed by the results from Baba et al., (2007) and Cutter et al. (2001), it was found that the methanogens were not responsible for the dechlorination process. Nonetheless, an involvement of methanogens on reductive dechlorination of PCBs could not be surely neglected. We can also assume that the highly evolving of methane been later than the occurrence of dechlorination revealed the abundance of PCB dechlorinators among the group of methanogens. Whenever the methanogenesis occurred, the dechlorination was initiated immediately. Then, before the mass production of methane gas produced to a significant amount, PCBs dechlorination was proceeding and perhaps reaching to complete. It can be roughly concluded that the methanogenesis might involve in the degradation of chlorinated aromatic compounds such as HCB and PCBs. In the other hand, SRB was seemingly has the potential to initiate the dechlorination and play a role as the dechlorinators in this study. Therefore, we cannot totally exclude the possibility that both methanogens and SRB can precede the PCBs dechlorination. Moreover, it also strongly notified that the dechlorination of PCBs or HCB might not be occurred by a sole group of anaerobes under the □tudied conditions. The dechlorination of aromatic chlorinated or polychlorinated compounds seem to derive from similar consortium, however, degradation of complicated polychlorinated compound needed longer acclimation period to initiate the dechlorination.



Note: RAMM: reductive anaerobic mineral medium, SS: sediment slurry

Figure 4.3 Accumulative methane gas productions during interval time.

4.4 Enhancement of 234- and 2345-CBps Dechlorination by Halogenated Primers

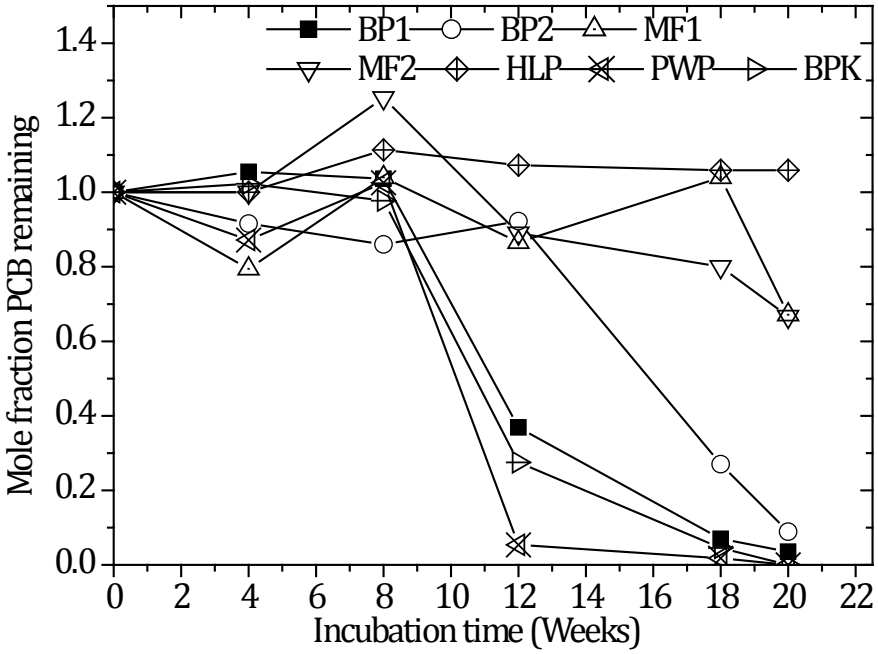
4.4.1 Dechlorination Ability by Indigenous Microbes

To investigate the dechlorination ability of the sediment slurries from all sites, the microbial mediated dechlorination of 234- and 2345-CBp was repeatedly tested by comparing between fresh and stored sediment slurries. The sustainable potential of microbial dechlorination ability after long-time in cold climate area was evaluated by using a long-term storage river sediment and water for dechlorination test. The samples were kept under cold temper in order to simulate the microbes' environment under cold climate during seasonal change, mostly in developed country, such as Europe, Russia, and Japan.

The result showed that the microbes from Site HLP, after long-term storage, totally lost their dechlorination abilities. Therefore, no HLP sediment slurry sets have a potential to dechlorinate 234-CBp. However, all sites other than HLP still could dechlorinate 2345-CBp (Fig. 4.4). This indicates that dechlorination ability in the fresh sediment was more effective than the storage sediments, although the COD, SS, VSS, TKN, and phosphorus were not significantly different (Table 4.3 vs Table 4.5). A similar findings also revealed that the dechlorination activity might be loss by stored samples in 4^{o}C for several months as well (Chen et al., 2010). There were evidences that the dechlorination activity of the PCB-dechlorinators might be lost if PCBs as well as other aromatic compounds disappear from the medium for a long period of time (Rhee et al., 1993).

Moreover, it also strongly evidenced of reasons of dechlorination of PCBs and other chlorinated aromatics was rarely occurred under the natural environment in warm or cold area; hence, it took longer period of time to initiate the dechlorination. Furthermore, under long-term storaged sample in cold climate, the microbes might die off from the sediment, whereas only spore-forming microorganisms could survive. This was notified that the dechlorination of PCBs could not initiate by only sole consortium as stated in previously scenario.

234-and 2345-CBp are classified as susceptibility single PCBs congeners to be dechlorinated, which contained chlorine atoms in one ring, in order to prevent an enantiomerics affected (Bedard and Quensen III, 1995). It found that 2345-CBp was easily to be degraded than 234-CBp. It might be due to the reaction heat (ΔH^{o}_{f}) and/or Δln RRT of 2345-CBp to 235-and 245-CBp (-14.746, -14.821/ 0.557, 0.525) were higher than 234-CBp to 24-CBp (-14.118/ 0.514); which higher values were more favorable routes for PCBs dechlorinating microbes. These values were usefulness to explain the dechlorination of persistent organic pollutants (POPs) by indigenous consortiums via energy released after used the pollutant as carbon sources (Chen, et al., 2001). These results notified the significantly difference from Chen, et. al. (2001) which 234- and 2345-CBp were not dechlorinated by indigenous microbes from Ho-Tsin River, Kaohsiung, Taiwan (20 weeks).



Note: BP1&2: Bangplee Industrial Estate Site 1&2,
MF1&2: Small Material Recovery Facilities Site 1&2,
HLP: Hua Lam Poo Canal, PWP: South-Bangkok Power Plant,
BPK: Bangplakod Canal

Figure 4.4 2345-CBp dechlorination profiles by storage indigenous microbes.

It might be due to tropical climate as well as significantly native microbes were presented in Thailand as described by Chen, et. al. (2010). Therefore, this was firmly evidence that environmental conditions and/or native microorganisms in Thailand were potentially to degrade POPs either HCB or PCBs (Anotai, et al. 2010).

4.4.2 PCBs Dechlorination by Amending Halogenated Primers

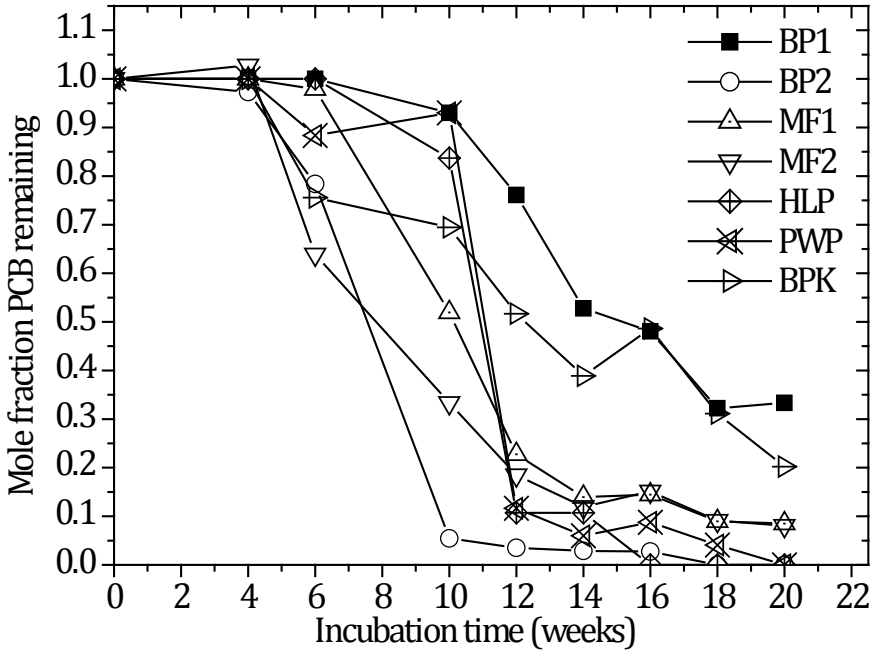
The dechlorination of PCBs could not be significantly enhanced by addition electron donors (lactate, acetate, pyruvate) in-previous experiments and the result notified that the original nutrients including electron donors in fresh river water and sediment were already adequate for the initiation and sustain of dechlorination. There still one potential strategy for stimulating PCBs dechlorination, by acceleration the production of dechlorination enzymes. It could be proceeded by introducing

alternative halogenated electron acceptors/co-substrates to the dechlorination tests, such as 26-dibromobiphenyls (26-DBB) (Bedard et al., 1998), halobenzoates (DeWeerd and Bedard, 1999), chlorobenzene and chlorophenols (Cho et al., 2002) and highly chlorinated PCB congeners such 23456-PCBs (VanDort et al., 1997). The halopriming coupled 26-BB could increase the number of PCBs dechlorinators as well as PBB dehalogenators, it suggested that halopriming could stimulating the growth of dehalorespirers (Wu et al., 1999). The other advantage is that halogenated primers were not persistent and/or toxic to microorganisms and environment (DeWeerd and Bedard, 1999).

The degradation of halogenated priming congeners can be easily measured. During the extraction of PCB congeners and its dechlorination intermediates, the halogenated priming congeners were also extracted. Qualification of halogenated primers peaks were not interfered with any PCB congeners.

In this study, the methane productions were also observed; whereas, reductive dechlorination of PCBs were well known can be occurred by directly (sulfate reducing bacteria and methanogens) and indirectly (acidogenesis, iron reducing bacteria) dechlorinators in ecosystem metabolism. The results suggest that dechlorination of PCBs were not strongly related to the production of methane gas; hence, the occurrence of methane could detect earlier before the occurrence of intermediates. It implied that methanogens may be not the sole dechlorinators in this study, the same results also showed in previously phase. However, we also noticed that halogenated primers might be degraded under methanogenesis condition; since it has been confined that methanogens always played an important role to dechlorinate HCB as describes by Chen et. al., (2010).

The dechlorination of 234-CBp by anaerobes from various sampling sites and amended priming congeners were presented in Table 4.9 and dechlorination profiles was shown in Figure 4.5, for more detail on experiment data were showed in Appendix E-2 and the dechlorinating phylums were represented in Appendix E-2-1.



Note: BP1&2: Bangplee Industrial Estate Site 1&2,
MF1&2: Small Material Recovery Facilities Site 1&2,
HLP: Hua Lam Poo Canal, PWP: South-Bangkok Power Plant,
BPK: Bangplakod Canal

Figure 4.5 234-CBp dechlorination profiles by amending with 4-bromobenzonitrile.

The result showed that halogenating primers could significantly enhance the dechlorination of 234-CBp, except the set of HLP site coupled with 4-BH. Anaerobes from all sampling sites lost their ability to degrade 234-CBp, except BPK still possessed the dechlorination activity but seemingly in a slow rate. Microorganisms from different sources had evolved the ability to dechlorinate PCBs after the addition of halogenating primers. The main point from this part was focused in HLP site. After addition of 4-BZ, 14-DBZ and 4-BN, the target PCBs could be completely dechlorinated within 4 weeks. In our previous studies, microbial consortia from HLP site were used to be the best dechlorination consortia in the tests of fresh and non-storage sediments. After the long-term storage in cold condition, their ability was collapsed.

Table 4.9 234-CBp dechlorination ability of various halogenated priming congeners amended.

Priming congeners	Locations	Occurance time of intermediates (weeks)	100% of 234-CBp dechlorination completion time (weeks)
4-BZ	BP1	14	>20
14-DBZ		14	20
4-BN		12	>20
4-BH		14	>20
Un-amended primers		N.D.	N.D.
4-BZ	BP2	10	20
14-DBZ		10	18
4-BN		10	18
4-BH		10	20
Un-amended primers		N.D.	N.D.
4-BZ	MF1	12	>20
14-DBZ		14	>20
4-BN		10	20
4-BH		10	>20
Un-amended primers		N.D.	N.D.
4-BZ	MF2	10	>20
14-DBZ		10	>20
4-BN		10	20
4-BH		10	20
Un-amended primers		N.D.	N.D.
4-BZ	PWP	10	14
14-DBZ		10	20
4-BN		12	20
4-BH		14	20
Un-amended primers		N.D.	N.D.
4-BZ	BPK	14	>20
14-DBZ		14	>20
4-BN		12	20
4-BH		10	20
Un-amended primers		12	>20
4-BZ	HLP	10	14
1,4-DBZ		10	14
4-BN		10	14
4-BH		N.D.	N.D.
Un-amended primers		N.D.	N.D.

Note: 24-CBp was detected as only sole intermediate product.

N.D. : the dechlorination was not occurred.

Surprisingly, due to the cross feeding of primers and PCBs, the PCBs dechlorination potential were seemingly recovered and completed the dechlorination. It can be recognized that the dehalorespirators might have a certain interaction with PCBs dechlorinators. Krumins, et al. (2009) found tetrachlorobenzene or pentachlorobenzene could increase native population of *Dehalococcoides spp*. under

the analysis of *Chloroflexi* 16S rRNA genes. Moreover, we could not exclude the possibility that the additive of haloprimers could increase the dehalorespirator populations and these consortia could degrade PCBs by direct metabolism or co-metabolism.

In the other hand, the consortium from BPK source was more active in PCBs dechlorination even though has been storage for a long period. And this site was chosen as a representative for the less industrial-polluted area. This result implies that the PCB dechlorinators were prevalence in all investigated sites. However, the dechlorination could not occur might be not because the lack of active microorganisms, including dechlorinators and supporting bacteria. The more critical point should be the missing of a suitable environment to initiate the dechlorination and keep supporting the dechlorinators.

Recently, it has been surveyed the PCBs degradation potential of microbes in the abandon land which did not receive pollutants after a long historical contamination (Prewchote, 2009). In these areas, dechlorination consortia might be inactive, and the activation was required for the initiation of the dechlorination. This study showed that the amending of halogenating congeners were an effective way.

Furthermore, it also showed that the supporting bacteria could significantly affect to the initiation of dechlorination. There was strongly evidence that the growth and development of dechlorinators were required the support from the other bacteria in order to co-ordinate the occurrence of dechlorination. It also referred to the comprehensive interaction of the dechlorinators and supporting bacteria which worked together as dechlorination consortia in diminishing the contamination of PCBs.

According to the findings in this study, the remediation strategy by amending only single strain of bacteria or a pure culture to clean-up the contaminated sites was usually not appropriate, because the dechlorinators of PCBs could not stand alone and

precede the degradation. PCBs dechlorination could only happen from the collaboration and interaction of the microorganisms in the dechlorination consortium.

PCB congeners, they could be degraded even by using storage samples. Prior to this study, 234- and 2345-CBp were considered as susceptible PCB congeners and their dechlorination patterns should be similarly. But our results showed the difference between two of them. The unique of intermediate products in difference sites and primers amended under various conditions, it indicated various group of consortia were prevalent in these sites and has a potential to dechlorinate a 4 chlorines of PCBs to two chlorines. Moreover, the PCB dechlorinators could remove chlorines in both *meta*- and *para*- positions, which implied that a variety of dechlorinators engaged in this study.

2345-CBp was tested in order to explore the dechlorination pathways and determined the sequence in removing chlorines. The results showed the dechlorination could occur by indigenous microbes from all sites, except HLP. The amendment of primers did not significantly stimulate the dechlorination as showed in Table 4.10 and Figure 4.6, for further details on dechlorination profiles were showed in Appendix E-2. It implies that for highly chlorinated PCB dechlorinators involving in this study for 2345-CBp was not well defined.

The dechlorination mass balances were estimated; however, it could not be well defined in dechlorination ratio. Because of during extraction sample time the PCBs might be already dechlorinated (major mechanism) and/or adsorb onto solid particles (minor mechanism); hence, it still adequate to estimate (Figure 4.7). Chlorine removal in 234-CBp was occurred only at the *meta*-position, whereas it happened at both *meta*- and *para*-positions for 2345-CBp and could be dechlorinated further to two-chlorinated congeners. The dechlorination pathways were shown in Figure 4.8.

Table 4.10 2345-CBp dechlorination ability of various halogenated priming congeners amended.

Priming Congeners	Locations	Occurance time of intermediates (weeks)	100% of 2345-CBp dechlorination completion time (weeks)	Intermediate Products
4-BZ		10	>20	235-**, 245-*
14-DBZ		10	20	235-*, 245-**, 24-/25-
4-BN	BP1	8	>20	235-**, 245-*
4-BH		8	20	235-
Un-amended		8	20	235-
4-BZ		8	20	235-**, 245-*, 24-/25-
14-DBZ		8	20	235-*, 245-**, 24-/25-
4-BN	BP2	6	20	235-, 245-
4-BH		6	18	235-**, 245-*, 24-/25-
Un-amended		8	20	235-**, 245-*, 24-/25-
4-BZ		8	20	235-, 245-, 24-/25-
14-DBZ		8	>20	235-*, 245-**, 24-/25-
4-BN	MF1	8	20	235-**, 245-*, 24-/25-
4-BH		8	20	235-, 245-, 24-/25-
Un-amended		8	>20	235-**, 245-*, 24-/25-
4-BZ		8	20	235-, 245-, 24-/25-
14-DBZ		8	>20	235-*, 245-**, 24-/25-
4-BN	MF2	6	20	235-, 245-, 24-/25-
4-BH		8	18	235-, 245-, 24-/25-
Un-amended		8	>20	235-*, 245-**, 24-/25-
4-BZ		8	18	235-, 24-/25-
14-DBZ		8	18	235-**, 245-*, 24-/25-
4-BN	PWP	8	18	235-**, 245-*, 24-/25-
4-BH		8	18	235-**, 245-*, 24-/25-
Un-amended		8	18	235-**, 245-*
4-BZ		8	18	235-**, 245-*, 24-/25-
14-DBZ		8	18	235-**, 245-*, 24-/25-
4-BN	BPK	8	18	235-**, 245-*, 24-/25-
4-BH		8	18	235-**, 245-*, 24-/25-
Un-amended		8	18	235-**, 245-*, 24-/25-
4-BZ		6	20	235-**, 245-*, 24-/25-
14-DBZ		6	14	245-, 24-/25-
4-BN	HLP	6	>20	235-**, 245-*, 24-/25-
4-BH		N.D.	N.D.	-
Un-amended		N.D.	N.D.	-

Note: **Majority occurred, *Minority occurred, N.D. : Dechlorination was not occurred during incubation period (20 weeks); 24-/25- were co-eluting peaks.

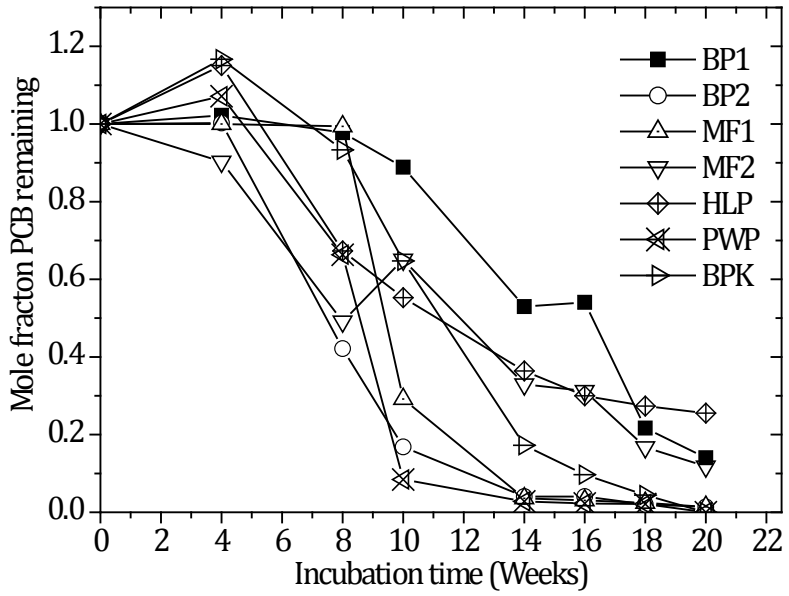


Figure 4.6 2345-CBp dechlorination profiles by amending with 4-bromobenzonitrile.

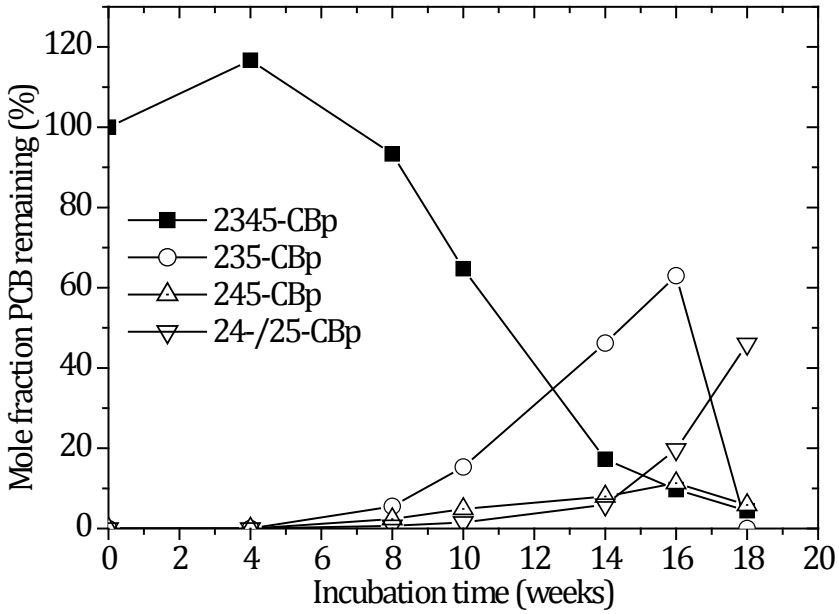


Figure 4.7 2345-CBp dechlorination profiles by amending 4-BN under sediment slurry from BPK site condition.

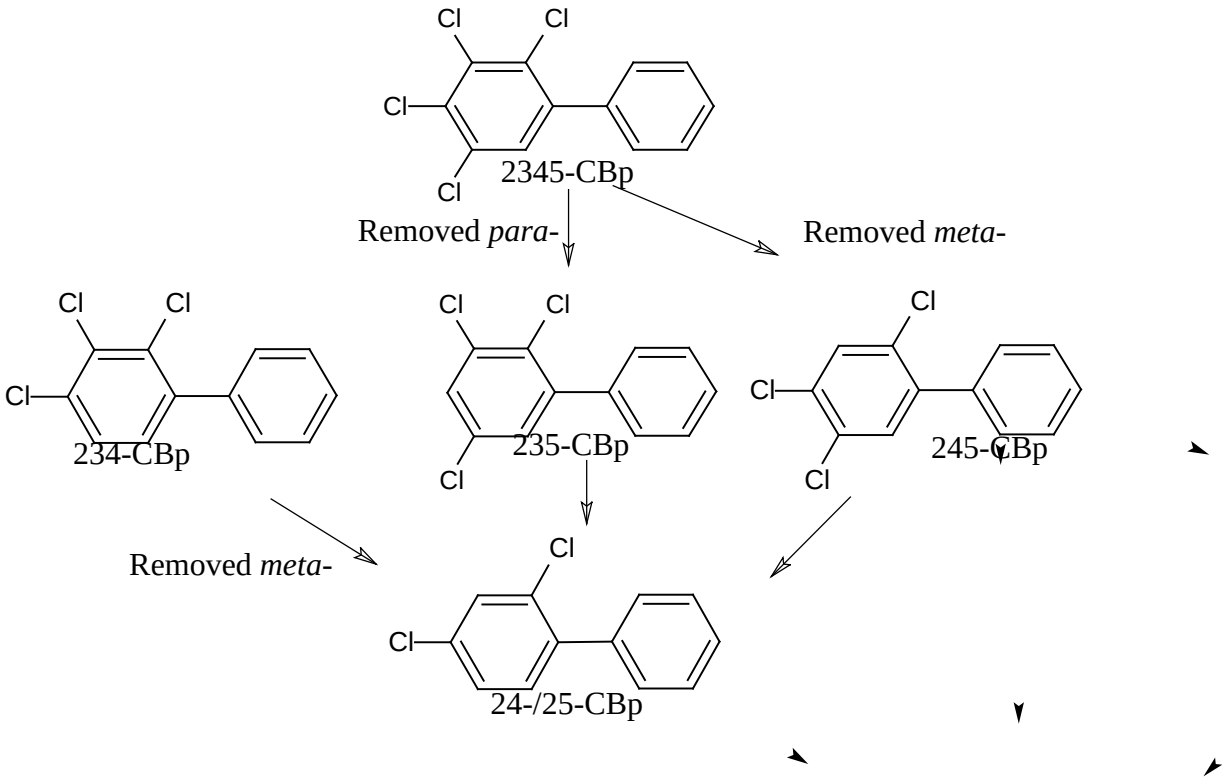


Figure 4.8 Dechlorination pathways of 234-CBp and 2345-CBp.

4.5 Effectiveness of Bioaugmentation on 234- and 2345-CBp Dechlorination

By isolation of the mixed cultures from sampling sites, several possible PCB dechlorinators; i.e., *Desulfitobacterium, Dehalobacter, Desulfuromonas, Sulfurospirillum, Anaeromyxobacter, Geobacter, and o-17/*DF-1-type *chloroflexi* microorganisms have been observed in sediments/soil samples (Fagervold et al., 2007; Yan et al. 2006; Abramoicz, 1990; Erickson and Nondello, 1993; Gibson et al., 1993). Bedard et al. (2007) reported that the *Dehalococoides* population in sediment-free mixed culture could catalyze in-situ dechlorination of Arochlor 1260. The dechlorination of tri- through penta-CBp in bioreactor was evaluated by using methanogenic granules were accomplished without any accumulated of *ortho*-chlorine substituent congeners (Natarajan et al., 1998). Furthermore, combining the results from these two studies, it suggested that bioaugmentation has the potential to enhance PCBs dechlorination rather than biostimulation techniques (Winchell and Novak, 2008). Recently, bioaugmentation with a culture containing *Dehalococcoides*

ethenogenes strain 195 coupled with pentachloronitrobenzene was proved within the contribution to dechlorination rate of PCBs (Krumins et al., 2009). In our study, there are rarely indigenous microorganisms which could dechlorinate 234-CBp effectively.

To determine the possibility of bioaugmentation of PCBs dechlorination, microbes from HLP canal site 3 were used as seeds to inoculate into the sediment slurries or sediments, which have been identified as less effective in PCBs dechlorination. And different quantity of active mixed cultures were inoculated into the less-dechlorination samples to extent the dechlorination rates of 234- and 2345-CBps Tables 4.11 and experimental data detail were showed in Appendix E-3. Figure 4.9-4.11 summarized the effect of bioaugmentation without any enrichment culture or nourishments. The result in sediment slurry sets revealed that all augmentation-ratios could dechlorinate 234-CBp, except 90% sterilized mediums. It reveals that ten times dilution of active cultures by less-effective cultures would develop a negative effect on the dechlorination. And the dechlorination was occurred after inoculated 22 weeks, it might be less of population of active microbes.

Table 4.11 234-CBp dechlorination in the sediment slurries bioaugmented with sediment slurry from Site HLP.

Locations	**90% of sterilized HLP**		**10% inoculation of HLP**		**50% inoculation of HLP**	
	Lag Phase (weeks)	100% of 234-CBp DeCl completion time (weeks)	Lag Phase (weeks)	100% of 234-CBp DeCl completion time (weeks)	Lag Phase (weeks)	100% of 234-CBp DeCl completion time (weeks)
BP1	ND	ND	8	14	8	12
BP2	ND	ND	8	12	8	14
MF1	ND	ND	2	14	8	10
MF2	ND	ND	2	12	2	12
PWP	ND	ND	8	10	2	10
BPK	ND	ND	8	14	2	10

Note: ND: Not detection within incubation time, DeCl: Dechlorination, Lag phase: Defined in term of intermediate occurrence, 24-CBp was found as the sole product.

This is a firmly evidence that promote of dechlorination activity for the less-effective sites could derive from bioaugmentation action rather than amending chemicals in the contaminated sites.

Comparing the results from different ratio of active cultures inoculated sets of 10% and 50% respectively, it showed that lag phase and completion time were similar. Whereas the results notified that the active consortium from HLP site could activate the dechlorination without any artificial supplements. It could act in shorten lag phase and 234-CBp dechlorination completion time. Moreover, it also suggested that only 10% of active consortia could precede the enhancement of PCBs dechlorination. All results proved the possibility to introduce active sediment slurries from active sites directly added into the less-active matrix or sites, and could promote PCBs dechlorination.

In the successive study, the sediment was used as bioaugmentation rather than sediment slurry. Under sediment-mixed sets, it showed a similarly effect as sediment slurry-mixed sets. The active sediments could promote the dechlorination of 2345-CBp within the lag phase of 6-9 weeks and the dechlorination completed time of 30 weeks. And 235- and 245-CBp were both found as the intermediates, whereas it could not be dechlorinated further to two chlorines atom. This finding revealed that low chlorinated PCB congeners were retarded under anaerobic condition as well as in sediment-water matrix. Hence, lower chlorinated PCBs were accumulated in the PCBs contamination sites. Moreover, dechlorination position could commonly happen at *meta*- and *para*- positions due to their ability of enzyme producing and enantiomeric effect of PCBs molecule. Comparing between the dechlorination mass balance of 2345-CBp under sediment slurry and sediment-water (Fig. 4.7 vs Fig. 4.12), it notices that under sediment slurry condition, the dechlorination pathway was 2345-CBp (mother congener) → 235- and/or 245-CBp (intermediate product) → 24-/25-CBp (final product). However, sediment-water has ability to transform 2345-CBp to 235- and/or 245-CBp, without any further dechlorination to di-chlorobiphenyls. It implied that the bioavailability of sediment slurry was higher than those of the sediment-water; however, sediment-water medium was more related to PCBs-

contaminated in authentic sites. Enhancing of bioavailability in sediment-water will be tested in future experiment.

Surprisingly, in the treatment of 5 ml bioaugmented cultures (equivalent as 2.5 g of sediments) in sediment slurry sets, the lag phase was only 6 weeks. It seems that the abundance of dechlorinating consortia had a positive effect to shorten the lag phase.

And the active consortia whether from sediments or sediment slurries, could also increase the dechlorination rate. This result implied that the PCBs dechlorinating microbes were abundant either in sediment slurries and sediments, and both consortia were situated in active form. A possible explanation of dechlorination could not happened in less-effective sites might be the lack of supporting bacteria (e.g. acetogenesis and acidogenic bateria), not only from the impotent main dechlorinators such as methanogenesis and SRB.

After the bioaugmentation by mixing active microbes, including supporting bacteria and dechlorinators, sooner or later, the dechlorination was initiated. Hence, nutrients supplements or carbon sources were assumed already adequate to support dechlorinating microbes in these sites. These results implied the potential of introducing the microorganisms from the remote sites could enhance PCBs dechlorination. These results were significantly different with the study of Bedard et al. (1997), which introducing of the enriched PCB-dechlorinating cultures to treat the contaminated sites, and found that the dechlorination could not be accomplished.

According to the two phases which had been constructed, in the enhancement of PCBs dechlorination by halogenated primers (using storage sediments) and in bioaugmentation treatment (using fresh sediments). The results showed a noteworthy referred that the dechlorination ability of microbes was sometimes very different even though collected from the same sources. For example, 234- and 2345-CBp could be effective dechlorinated in first phase, but showed the contrast result in another phase. HLP anaerobes always showed their powerful PCBs dechlorination ability in fresh

sediment, however, less-active in storage sediment. It suggested that the abilities of dechlorination consortia were not kept constant in fresh condition and in storage either.

This study was not perfect in-showing mass balance of PCB congeners during incubation. Because PCBs are likely to adsorb onto the sediment particles, it could lead to be less recovered from extraction procedures (Figure 4.12). Finally, as a result, it was a very promising approach that the simple bioaugmentation technique should work successfully in-field practice in Thailand and can be contribute to worldwide as well.

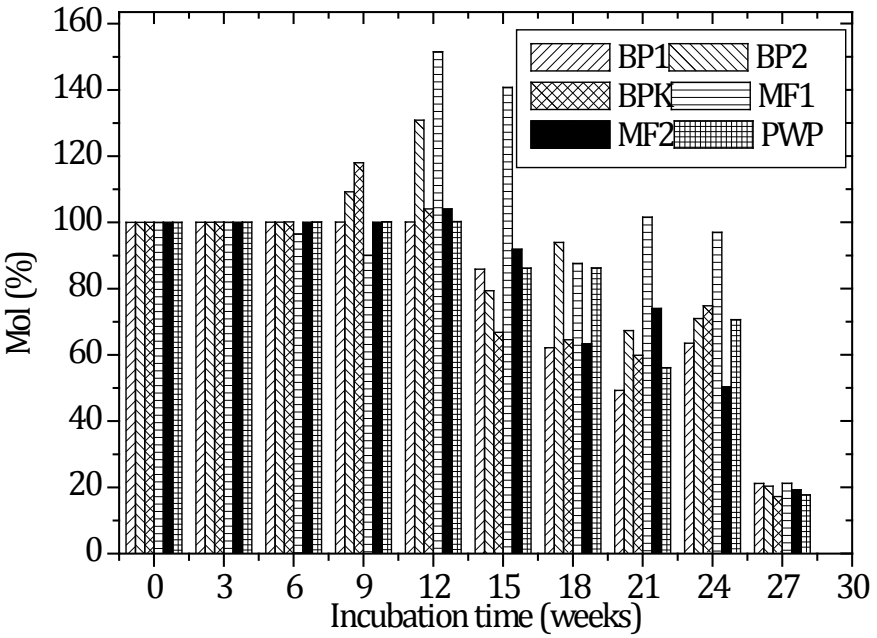


Figure 4.9 2345-CBp dechlorination in the sediments bioaugmented with 50% inoculation of less-effective sediment and 50% inoculation of effective sediment.

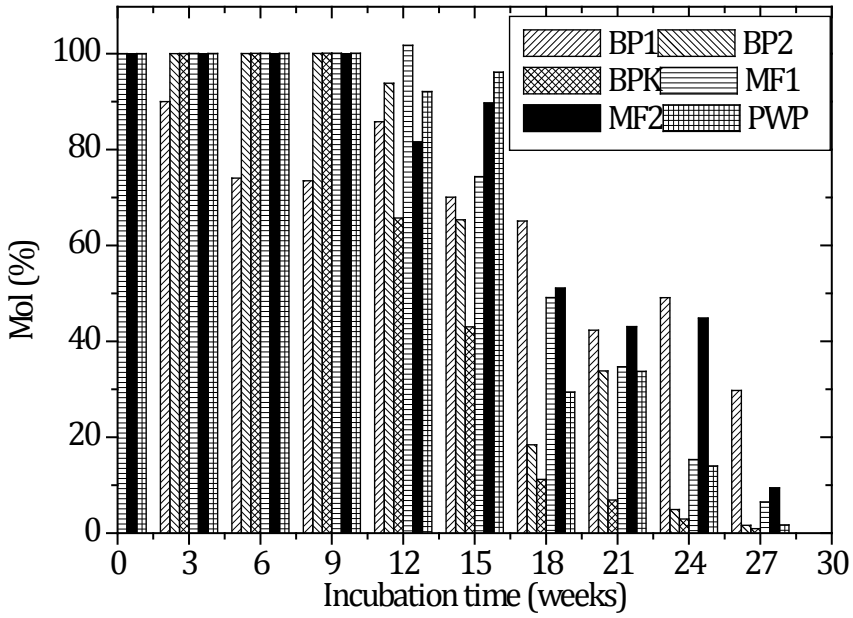


Figure 4.10 2345-CBp dechlorination in the sediments bioaugmented with 90% inoculation of less-effective sediment and 10% inoculation of effective sediment.

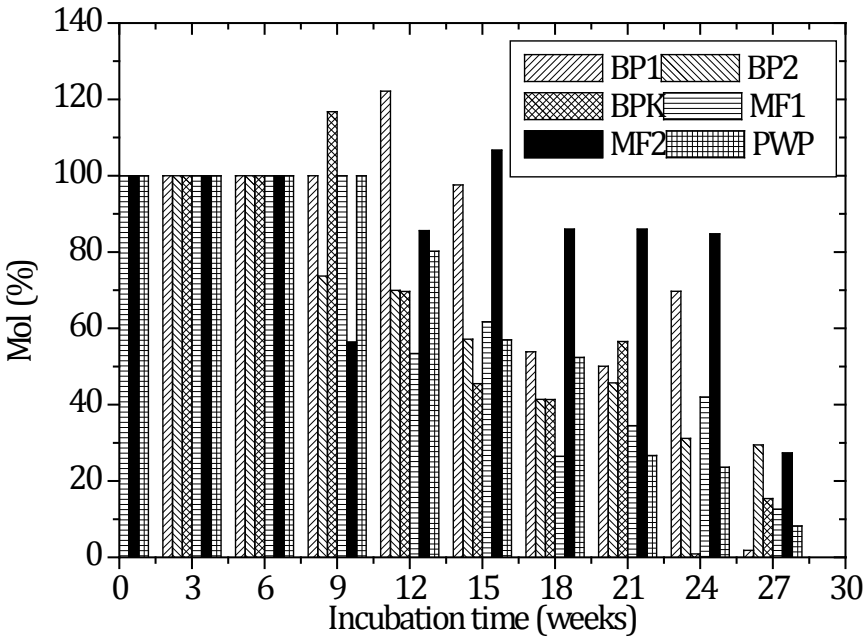


Figure 4.11 2345-CBp dechlorination in the sediments bioaugmented with 100% inoculation of less-effective sediment and 100% effective sediment slurry.

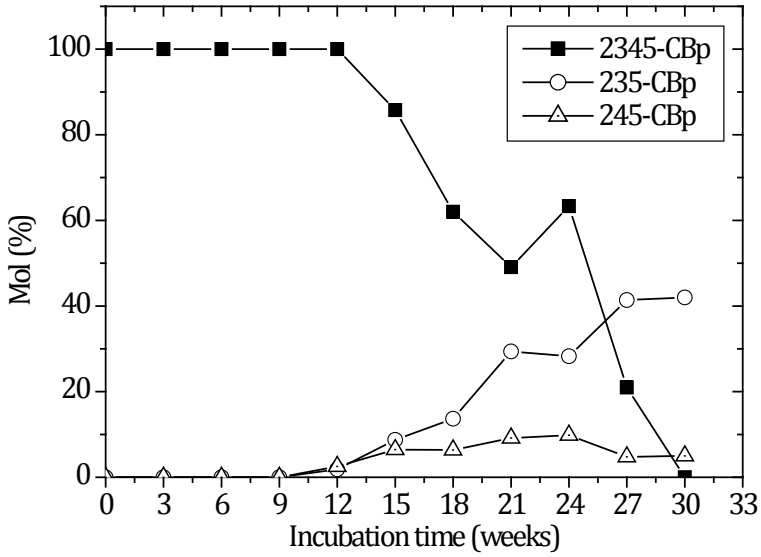


Figure 4.12 2345-CBp dechlorination profiles the sediments bioaugmented with 50% inoculation of less-effective sediment from BP1 site and 50% inoculation of effective sediment from HLP site.

4.6 Effect of Temporary Heat Treatment on PCBs Dechlorination

PCBs are hydrophobic compounds and tend to be adsorbed onto soil or sediment particles; thus, their degradation rate might be limited by their bioavailability which was strongly related to desorption from particles into the aqueous phase. The temporary heat treatment would increase the desorption rate of PCBs. In addition, a warmer environment could stimulate the production of certain enzymes that might be necessary for PCBs dechlorination. However, the heat shock could possibly give a stress effect to bacteria. Microorganisms in a natural habitat always need to adapt themselves to environmental factors; e.g. temperature, pH, salinity, which could affected on their growth, activity and enzymatic reductive dehalogenation. Different microorganisms will be predominated during various temperatures providing. Thus, temperature should be one of the main physical factors that significantly influencing on the dechlorination of PCBs.

To attempt of this phase was made for that high temperature could activate the growth of certain microbes and enhance the dechlorination. In previous researches, PCBs dechlorination have been tested under 18 conditions of temperature from 4 to 66^{o}C (Wu et al., 1996; Wu et al., 1997). The dechlorination generally occurred at 8-34^{o}C (mesophilic) and 50-60^{o}C (thermophilic). However, the optimal temperature for the chlorine removal from 2346-CBp were 18-30^{o}C. These results suggested that the dechlorination of PCBs could be effectively occurred under mesophilic and thermophilic. Furthermore, the thermophilic microorganisms should play a role in the breakdown of such a complex organic compounds. As well as, the high temperature could accelerate an enzymatic process such as PCBs dechlorination. Some bacteria were proliferated under the extremely hot conditions, including; clostridium (Oh et al., 2008) and SRB spore forming group (Fava et al., 2003), those were possible in dechlorinating PCBs. This phase could perform a roughly screening at the dechlorination consortia from the sampling sites.

Mongkong (2007) ever used the sediments from the same sites in this study to dechlorinate 234-CBp. Temporary heat treatments, of 50, 70 and 90°C respectively were applied for PCBs dechlorination test in sediment sets. And after the heat shock, the temperatures of core sediment were around 2-5^{o}C less than the given temperatures. Table 4.12 showed the results in temporary heat treatment sets, it implied that only 2 heat treatment courses within 50^{o}C could generate the dechlorination. The dechlorination occurred in week 17, but could not be completed within 25 weeks, for details of dechlorination concentration were showed in Appendix E-4. These results showed that the initiation of PCBs dechlorination could happen only under a less-severe condition such as mild heating temperature with a short period. Therefore, the heat shock might be help for some bacteria within the dechlorination consortia; it could not apply a universally favorable condition for all bacteria. For example, the microbes from HLP site show a longer lag phase from week 5-8 to 17. Furthermore, under the heat treatment courses of either 60 and 240 minutes of 50°C, and all periods of 70°C or 90°C, it showed a negative effect to the dechlorination. An extreme heat shock could damage the collaborated work of PCB dechlorination by destroy one or some bacteria in the dechlorination consortia.

Table 4.12 The dechlorination of 234-CBp in sediment by providing temporary heat treatment.

Locations	Indigenous microbes (without any supplements)[a]		Temporary heat at 50°C for 5 mins		Temporary heat at 50°C for 20 mins	
	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)	Lag phase (weeks)	100% of 234-CBp dechlorination completion time (weeks)
BP1	N.D.	N.D.	17-25	>25	17-25	>25
HLP6	5-8	14-22	17	>25	12	>25
MF2	N.D.	N.D.	17-25	>25	N.D.	N.D.
BPK	N.D.	N.D.	17-25	>25	N.D.	N.D.

Note: N.D.: dechlorination could not detect, 24-CBp was found only sole intermediate product, [a]: data quoted from Mongkon, 2007, another temperatures and heating times could not observe any dechlorination, this experimental was set up for 25 weeks only.

Hence, it could be roughly simplified that spore-forming sulfidogenic bacteria and clostridium, both groups of bacteria could survive under extremely high temperature, might not be the only members in this dechlorination consortia. On the other word, it meant that bacteria groups other than the extremely thermophilic groups might play a certain role or even a critical role in the PCBs dechlorination. There were evidences that sulfidogens could catalyze the dechlorination of PCBs and other aromatic compounds (Häggblom et al., 2000; Kuo et al., 1999; Masunaga et al., 1996). Therefore, the extreme heat shock might be killed the bacteria which is not responsible for the direct step of removing chlorination from PCB molecules. And the dechlorinators like sulfidogens could survive from this shock; however, without any supported from supporting bacteria, initiation of PCBs dechlorination could be failed.

Although, the heat shock treatment was significantly improved the dechlorination capability under sediment slurry condition (Table 4.12 vs 4.13). The dechlorination could be shorten in both lag phase and dechlorination completion time (Table 4.13). These results were strongly notified that large particles of organic matrix could retard the initiation of dechlorination. Furthermore, it could be revealed

to an alternatively enhance the capability of dechlorination by self-cleaning technique approach; hence, better understanding of this phase is require.

Table 4.13 The dechlorination of 234-CBp in sediment slurry by providing temporary heat treatment.

Conditions	Lag phase (weeks)	100% of 234-CBp DeCl Completed time (weeks)	Conditions	Lag phase (weeks)	100% of 234-CBp DeCl Completed time (weeks)
BPK-Indigenous microbes	12	15	HLP-Indigenous microbes	12	15
Heat at 50^{o}C for 3 mins*	2	4	Heat at 50^{o}C for 3 mins*	2	4
Heat at 50^{o}C for 5 mins	2	4	Heat at 50^{o}C for 5 mins	2	4
Heat at 50^{o}C for 10 mins	2	4	Heat at 50^{o}C for 10 mins	2	4
Heat at 70^{o}C for 3 mins	2	4	Heat at 70^{o}C for 3 mins	2	4
Heat at 70^{o}C for 5 mins	2	4	Heat at 70^{o}C for 5 mins	2	4
Heat at 70^{o}C for 10 mins	2	4	Heat at 70^{o}C for 10 mins	2	4
Heat at 90^{o}C for 1 mins	2	4	Heat at 90^{o}C for 1 mins	2	4
Heat at 90^{o}C for 3 mins	2	6	Heat at 90^{o}C for 3 mins	2	6
Heat at 90^{o}C for 5 mins	2	6	Heat at 90^{o}C for 5 mins	2	6

Note: DeCl: dechlorination, this table was quoted from Kanya, 2010 (in preparation), the dechlorination of indigenous microbes was served as active-control SS, *: mean the temporary heat at given temperature in difference interval time, approximately core temperature was not reached to given temperature (decreasing around 8-9^{o}C).

4.7 Fate of PCBs in a Simulated Environmental System

The simulated environmental system was constructed to observe the PCBs dechlorination under a natural stream-like condition. In addition, it could also reveal the profiles and fates of the mother compound and dechlorinated intermediates during the dechlorination. A preliminary study by using simulated aquarium boxes (SAB) had been explored by Siriruang, 2006, which work with the dechlorination of hexachlorobenzene (HCB), a similarly structural but less complicated chlorinated

aromatic compound than PCBs. The columns were made from PETE drinking water bottles as shown in Figure 4.12. In this study, a sets of sediment spiked with various concentrations of HCB were test (0.5, 2.0, 10 and 50 mg/l), separately. It was found that all the sets spiked with HCB lower than 2.0 mg/l could not initiate dechlorination within 100 days of incubation. Thus, 10 and 50 mg/l of HCB would be enough to initiate the dechlorination and the transformation was completed in all layers within an interval of 126 days.

Afterwards, Krajibthong (2007) studied HCB and 234-CBp dechlorination by using SAB with modification permeate column (MPC) (Figure 4.12). The results showed that HCB could be dechlorinated within 15 weeks by indigenous microbes in the sediment under stream-simulated system; whereas, dechlorination could not occur to 234-CBp. The results of HCB dechlorination in SAB were confirmed the suggestion of Anotai et al. (2010) that HCB may not be a concerned POPs in Thai canal.

Again, both two studies suggested that the HCB dechlorination could be found in a nature environment-simulated condition. Contrarily, the dechlorination of 234-CBp could not happen was possibly because of the lack of electron donors and some important nutritious factors, which were easily escape from the open system like SAB-MPC. If this assumption was correct, the dechlorination could occur under SAB system, after preset the deficiency of carbon and nutrients by external sources. This phase of study was dedicated as a successive work. Therefore, organic substrates and nutrients were amended to test an extra-supplement in enhancing the dechlorination in long-term storage sediment and re-circulated by fresh canal water.

The redox potential (E_h) of the sediment layers always revealed the degree of a suitability for microbes to grow and reproduce; i.e., +800mV (aerobic condition), +740mV (facultative condition), -220mV (sulfate reduction condition), -300mV (methanogenesis condition) (peper et al., 2006). Table 4.14 showed that in this SAB-MPC system, the upper layer showed a positive E_h values, which indicated an aerobic condition; whereas, at middle and bottom layers showed negative E_h values which

proposed to an anaerobic condition that suitable for acidogens, sulfate-reducing bacteria and methanogens. Additionally, SRB, methanogen were presumably the key bacteria as in PCBs dechlorination consortia. However, the dechlorination could not happen within an incubation period of 6 months and the experimental data were represented in Appendix E-5. It suggested that the extra electron donors and yeast extract supplements did not initiate the dechlorination of 2345-CBp in storage sediment under SAB system.

These results were contradicted to the results in serum bottle conditions (halogenated primers part), which the dechlorination could be succeeding; it imply an important to evidence of re-activated of over storage sediment. Moreover, the redox potential of fresh sediment slurry in serum bottle condition could be reach to lower values than under storage sediments (Chang et al., 1996). Apparently there is no relationship between lower E_h values and 234-CBp dechlorination.

Moreover, it was implied that sediments sample collected in difference occasions also showed fluctuation in their dechlorination abilities. Another explanation for the deficient dechlorination might be because the incubation period for PCBs dechlorination was not sufficient for 2345-CBp dechlorination consortia to grow and initiate the dechlorinating activity; hence under simulated condition need longer time compared with serum bottle condition. Therefore, in a natural sediment condition, it would be two basic requirements to initiate the dechlorination: one was the certain level of PCBs concentration (increasing PCB dose), and the other was the richness of applicable nutrients (adding a combination of electron donor and nutrients).

4.8 Strategy for the Application of Biostimulation and Bioaugmentation Techniques

In-situ remediation techniques is the most applicable method in recovering non-heavily PCBs contaminated site within a concentration of PCB-commercial mixtures ranged from 10 to 200 mg/kg of soil.

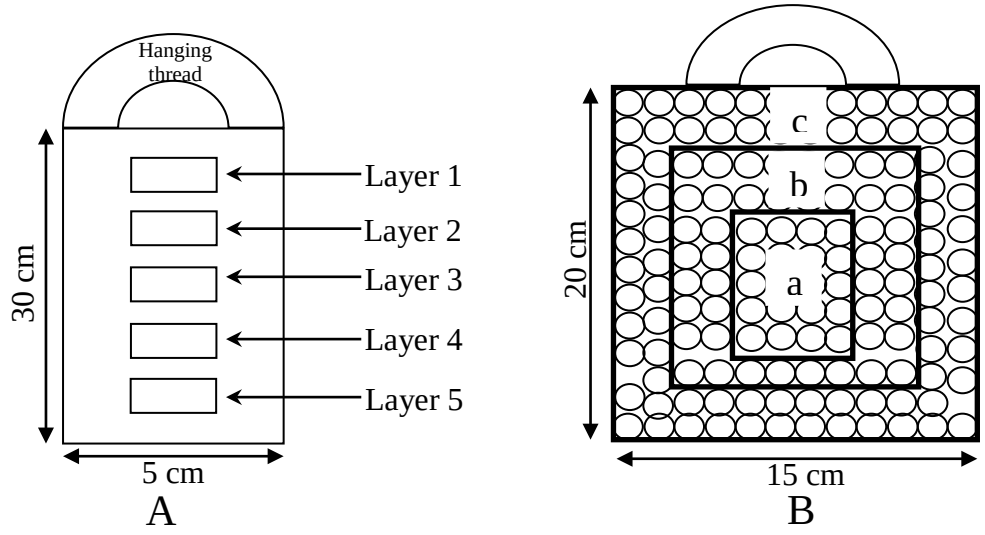


Figure 4.13 A: five layers of PETE drinking water column has been worked by Siriruang, 2006. B: MPC was making up from a 0.10 cm-perforated aluminum sheet and folded to from a half-cylinder shape. Three different sizes (a, b and c) of perforated aluminum containers of ∅ 5.0×8.0, ∅ 10.0×15.0, ∅ 15.0×20.0 cm were prepared by Kajibthong, 2007.

Table 4.14 The redox potential measurement during incubation time.

Layer	1 month	2 months	4 months	6 months
No additive				
Upper part	58	65	57	30
Middle part	-184	-175	-179	-167
Bottom part	-320	-309	-300	-338
Additive with Acetate				
Upper part	34	90	60	67
Middle part	-200	-235	-230	-316
Bottom part	-379	-400	-323	-369
Additive with Pyruvate				
Upper part	50	31	56	63
Middle part	-220	-230	-200	-274
Bottom part	-389	-390	-395	-375
Additive with Yeast Extract				
Upper part	77	83	26	37
Middle part	-167	-220	-233	-254
Bottom part	-361	-328	-285	-361

The disadvantages of dredging contaminated soil for ex-situ treatment and disposal are not only high cost of operation but also having negative impact to the indigenous ecosystem. And it leads to only serve as a reclamation option for a highly PCBs contamination sites. The dechlorination rate for lower concentration of PCBs (2 mg/kg dry weight) observed in the dechlorination test within biostimulation and bioaugmentation treatment was not difference from the test of higher concentration of PCBs (Krumins et al., 2009).

The preliminary determinations of the dechlorination ability by indigenous microbes around Samuthparakarn province, Thailand, have been investigated for 7 years. First, the evaluation of the degradation potential of chlorinated aromatic compounds; i.e. hexachlorobenzene (HCB) was tested. And a tremendous results were found as HCB could be dechlorinated very effectively by indigenous microbes using natural sediment water as sole media without any nourishing and enrichments (Anotai et al., 2010; Chen et al., 2010). Moreover, it also pointed out that HCB might not be concerned as persistent organic pollutants (POPs) in the stream of Thailand. This notified an impressive and unique observation from other countries that HCB dechlorination could only found in modified or enriched medium.

Since there was no doubt that HCB was naturally degradable, PCBs might be possibly degraded in the natural environment as well. Preliminary determination of dechlorination of PCBs was dedicated since 2006. 234- and 2345-CBp were able to be degraded by indigenous microbes, whereas other congeners were not. It suggested that the indigenous microbes from Thai Canal were able to dechlorinate some certain structural PCB congeners. The dechlorination condition was defined as terms of the lag phase, dechlorination rate, and dechlorination extent. According to our researches in Thai stream microbes, for 234- and 2345-CBps dechlorination, the dechlorination conditions were always remarkable, including a short lag phase, a steady dechlorination rate and usually more than 90% completion of PCBs dechlorination. It suggested that indigenous dechlorination consortia in Thai stream had been in an adequate environment of physical conditions, nutrients and growth factors supplement. Although, the anaerobic consortia are rich and powerful, the

bioavailability of PCB congeners was still the key to initiate dechlorination. Therefore, the enhancement of PCBs bioavailability is almost as importance as the activation of dechlorination consortia, and both of them must be carefully considered in developing bioremediation techniques.

The results from the studies of Winchell and Novak (2008) showed the effect of biostimulation (supplement of H_2 via elemental iron (Fe^0)) and bioaugmentation (amend of PCB-dechlorinating enrichment culture) in 2345-CBp dechlorination test. Insignificantly dechlorination of 2345-CBp were found in both sediment from Raisin River (historical PCBs contaminated site) and Duluth Harbor (non-contaminated site). However, in the further study by augmenting microcosms, an extensive dechlorination was observed, whereas the microcosms was enriched and grown on acetate (20 mM) under a headspace of 3% H_2 to 97% N_2. In another study, the investigation of PCBs dechlorination in Ohio River sediment under natural environment condition showed an un-accomplishment dechlorination, whereas under elevated redox status and cool conditions (D'Angelo and Nunez, 2010).

For a field remediation, it is important to investigate that the dechlorination ability of indigenous microbes from Thai Canal to chlorinated aromatic compounds. In this study, the results from stimulated system by amending volatile fatty acid reported an insignificantly dechlorination and showed that electron donors were effectively to promote the dechlorination. The occurrence of dechlorination happened after week 5 in supplemented sets, and week 7 in non-supplemented sets, and then in all sets the dechlorination completed times were similar. It revealed that these electron donors (acetate, lactate, pyruvate) were required by dechlorination consortia in this condition, whereas, the electron donors supplementation were not critical in the serum bottle conditions. In previous works showed that the organics substrate always played the important roles in reductive dechlorination processes (Nies and Vogel, 1990). Whereas the rate and extent of the dechlorination of Aroclor 1242 were greatest for methanol-, glucose- and acetone- fed batches, and least for acetate fed batches. And there was no significant dechlorination observed in non-fed batches.

In another study about microbial dechlorination under various conditions, the results showed the maximum dechlorination rate happened under methanogenesis and sulfate reducing condition rather than denitrifying condition (Chang et al., 2001). Additionally, under methanogenesis and sulfate reducing condition coupled with additional organic substrate (lactate, pyruvate, acetate) amended could increase the dechlorination rate. It suggested that the dechlorination was more likely to be initiated and worked well in an environment could transit to methogenesis and SRB conditions. These conditions could be developed by the addition of organic substrates

Furthermore, halogenated organochemicals as primers used to activate the PCBs dechlorination needed to be friendly to the natural environment, such as non-persistent or less-biohazardous. The stimulation by adding high concentration of single PCB congeners like 23456-CBps to anaerobic consortium for activating the dechlorination of PCBs was tested and showed its applicability (VanDort et al., 1997). However, most important is that we have to concern about the chemicals' environmental characters, especially the half-life and toxicity in natural sites. If the chemicals could not readily degrade, they might make more problems rather than the original contaminants.

In the sites been lack of appropriate PCB dechlorination consortium, bioaugmentation techniques could be applied. Augment of PCBs dechlorination consortium had been evaluated by introducing enriched or pure cultures (Bedard et al., 2007; Hartkamp-Commandeur et al., 1996). The enriched cultures were obtained by Aroclor or individual PCB congeners pre-enrichment. The results showed that both cultures isolated from Aroclor-adapted or single PCB congeners-adapted mixed cultures, the dechlorination conditions were similarly (Cutter et al., 2001; Pulliam Holoman et al., 1998; Williams, 1997; Zanaroli et al., 2010).

In this study, we introduced sediments from active sites directly to less-active sediment samples without any pretreatment. A significant improvement of PCBs dechlorination ability was found to the mixed cultures. For overall dechlorination processes, it is a collaborating works by a dechlorination consortium including

members such as the dechloirnators and the supporting bacteria. Therefore, the improvements of the impotent sediments might be from the strengthening of either one. The answer was not yet evaluated, and it needs different methods to finalize this matter such as enrichment PCBs-dechlorinating consortium and used it as active seeds in order to improve the degradation capability of less-active dechlorination site, or isolate pure culture from HLP canal and re-inoculation to treat sediment from other sites.

Herein the dechlorination test by sediment and river water, it was dedicated to simulate a real soil/sediment condition rather than those by sediment slurries. And its results could be used to define the possibility for the relevance of site remediation. Within our findings, both introducing of active sediments or sediment slurries could improve the dechlorination capability of the impotent sources. It notified the value of active inoculums from natural source to improve the clean-up ability of a contaminated but impotent site. This achievement would be lead to a novel alternative in evaluating the degradation capability of microbes from natural sites even some of the sites had never been contaminated.

CHAPTER V

CONCLUSIONS

5.1 Conclusions

234- and 2345-CBp were separately tested in difference objectives. Results from this research were listed as following:

-The reductive dechlorination of PCBs could be mediated by stream anaerobes in sediment-water and sediment slurry study conditions. And the dechlorination capability was observed in either contaminated or non-contaminated sites. It revealed that a PCBs-contamination history was not necessary to develop the PCBs dechlorination consortia, and the dechlorination-active consortia were widespread in Thai streams. The introduction of biostimulation and bioaugmentation techniques were effective in enhancing PCBs degradation.

-Natural environmental matrix in Hua-Lam-Poo Canal, Samuthprakarn, Province, Thailand could initiate the dechlorination of PCBs, not only in sediment slurry but also in sediment-water condition. However, other sites were tested in sediment-water condition, not included HLP site. It found that only the Power Plant and Material Recovery Facility 1 sites could proceed the dechlorination; whereas, all investigated sites were potential to degrade 234-CBp, nevertheless, HLP sites showed the best performance.

-The amendment of the organic substrates and mineral ingredients could not promote 234-CBp dechlorination. It revealed that the electron donors (acetate, lactate, pyruvate) were already adequate to support the growth of dechlorination consortia in Hua-Lam-Poo canal.

-The observation of methane gas production was not coupled with the degradation of 234-CBp. It implied that the methanogens might not be the only PCBs dechlorinators, and/or merely few enriched methanogens could perform the dechlorination well enough. However, the contribution of methanogens still could not be neglected.

-Long-term storage of the sediment in cold condition without any further nutrient supplement would eternally deactivate the 234-CBp dechlorination consortia from seven investigation sites. Nevertheless, the dechlorination still occurred to 2345-CBp by some sediments, except HLP, MF site1 and MF site2. It revealed that the dechlorination consortia responsible for specific PCB congeners were very different and possessed variant characters.

-Halogenated primers (4-BH, 4BZ, 14-DBZ and 4-BN) could promote the activity of 234-CBp dechlorinators which were thought dechlorination-impotent in original condition; however, all primers did not have any significant stimulation on the 2345-CBp dechlorinators which were proved dechlorination-active already.

-Directly mixing the microbes from effective site (HLP site3) to the less-effective matrix could improve the dechlorination activity. Additionally, in sediment slurry condition the mixed cultures were showed more effective in dechlorination than the sediment-water condition.

-Temporary heat treatment showed no positive effect under sediment-water condition. Moreover, it could retard the dechlorination within the treatment of extremely high temperature and a long period of providing heat.

-Supplement of fresh canal water to the cold-storage sediment could not recover its dechlorination activity, while the indigenous microbes were kept in a $4^{o}C$ within 6 months.

-This research could revealed that the bioremediation techniques, whether bioaugmentation by directly adding the effective mixed culture microbes to improve the dechlorination capability in less-effective sites or biostimulation by adding halogenated compounds; could be applied directly to PCBs-contaminated sites in Thailand.

5.2 Recommendations for Further Studies

This research was working on a bench scale, and not yet upgrade to a pilot scale. And the mechanisms, criteria, and parameters which involved initiation, rate and extend of the dechlorination was not completely explored. Therefore, the PCB dechlorinators cultivation and dechlorination influence factors are still important issues waiting to be investigated. Further studies need be contributed to fulfill these issues and make a better understanding by researches estimated as followed:

- Testing the dechlorination of commercially PCB mixtures, i.e., Aroclor 1242, Aroclor 1260; since we have found that even simple PCB congeners showed different potential of dechlorination. The dechlorination of Aroclors maybe contribute more attempts to evaluate the remediation potential.

- Increasing the bioavailability of PCBs could be carried out by using commercial and bio-surfactant to enhance desorption of PCB molecules from sediment particles into the liquid phase.

- Restoring the dechlorinating activity of cold-storage samples could be done by various treatments of enrichment, warm up process and bioaugmentation.

- Investigating PCBs dechlorination in natural contaminated sites (Hua-Lam-Poo Canal will be the first priority) by introducing modified permeated column (MPC) system as the dechlorination vessels, could be gathered more data under simulated real canal flowing for preparation of remediation of PCBs contaminated sites.

- Contributing these experimental data to the environmental evaluation modeling system, in order to construct a best strategy could be managed the contaminated sites as well as regulatory to prevent the risk of human exposure.

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APPENDICES

APPENDIX A

Geographic Mapping Sampling Sites

Table A.1 Global positioning system (GPS) of the sampling sites in Samuthprakran Province.

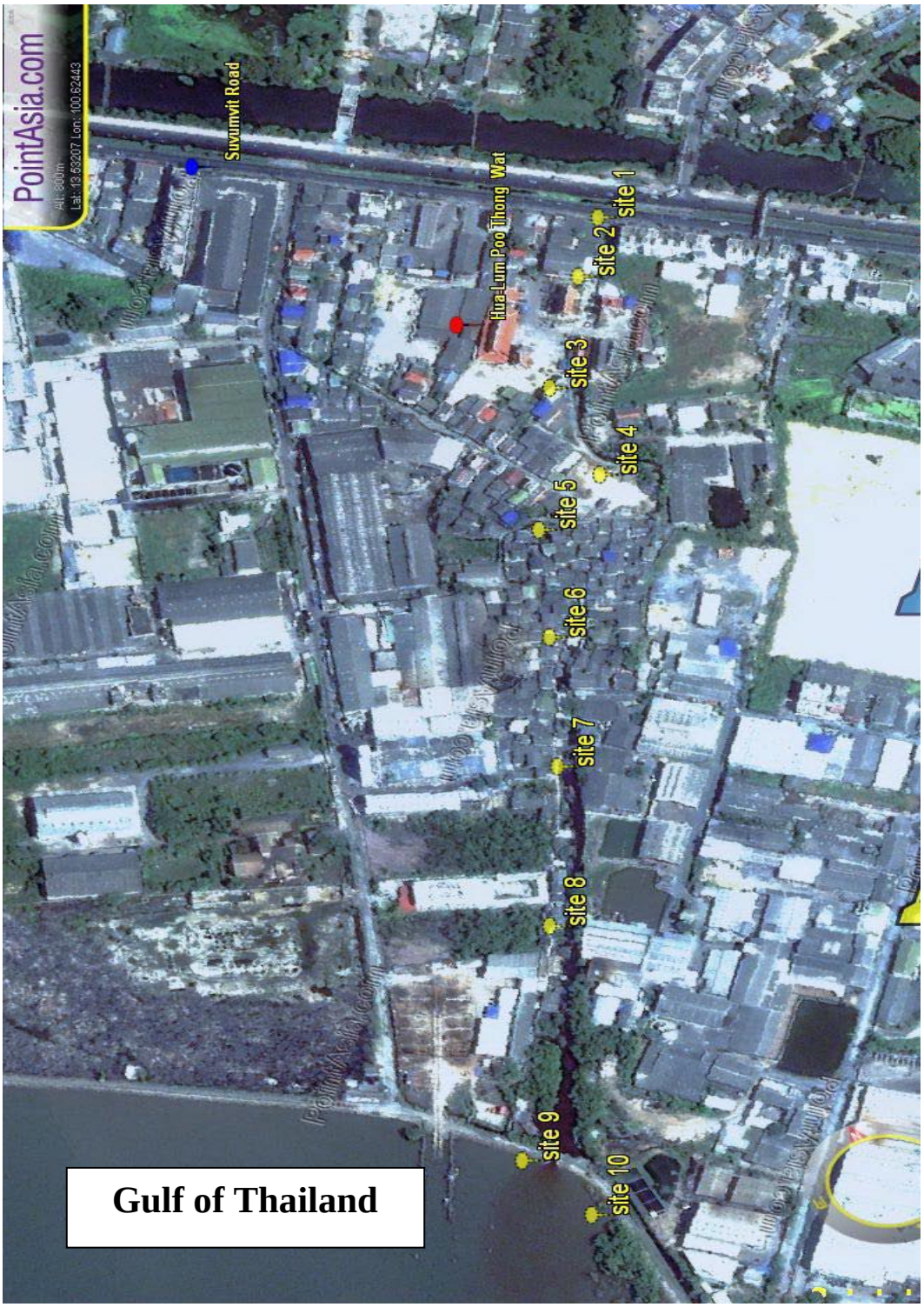
Sampling sites		North (N)	East (E)
South-Bangkok Power Plant		13^{o} 37’ 27 N	100^{o} 33’ 31 E
A canal receiving discharge from wastewater treatment plant of Bangplee Industrial	Site 1	13^{o} 32’ 56 N	100^{o} 47’ 26 E
	Site 2	13^{o} 33’ 47 N	100^{o} 47’ 43 E
A canal receiving discharge from small material recovery facilities	Site 1	13^{o} 31’ 44 N	100^{o} 47’ 43 E
	Site 2	13^{o} 45’ 39 N	100^{o} 46’ 52 E
Bang Pla Kod Canal		13^{o} 35’ 39 N	100^{o} 33’ 39 E

Table A.2 Global positioning system (GPS) of the sampling site in Hua Lam Poo Canal, Samuthprakran Province.

Sampling Sites	North (N)	East (E)
Site1	13' 32.105N	100' 37.595E
Site2	13' 32.073N	100' 37.562E
Site3	13' 32.049N	100' 37.547E
Site4	13' 32.018N	100' 37.535E
Site5	13' 31.991N	100' 37.518E
Site6	13' 31.946N	100' 37.482E
Site7	13' 31.897N	100' 37.454E
Site8	13' 31.848N	100' 37.430E
Site9	13' 31.740N	100' 37.365E
Site10	13' 31.713N	100' 37.375E

(www.pointasia.com)

Figure A.1 Locations of the sampling sites of this study, location 1: HLP sites, 2: BP sites, 3: MF sites, 4: PWP site and 5: BPK site.



(www.pointasia.com)

Figure A.2 Geographic information system of the sampling sites along HuaLam Poo Canal, Samuthprakarn Province (10 sampling sites).

(www.pointasia.com)

Figure A.3 Geographic information system of the sampling site at the South-Bangkok Power Plant, Samuthprakarn Province (1 sampling site).

(www.pointasia.com)

Figure A.4 Geographic information system of the sampling site along a canal receiving effluent discharge from the center wastewater treatment plant (WWTP) of Bangplee Industrial Estate in Samut Prakarn Province (2 sampling sites).

(www.pointasia.com)

Figure A.5 Geographic information system of the sampling site along a canal receiving discharge from small material recovery facilities in Samut Prakarn Province (2 sampling sites).

(www.pointasia.com)

Figure A.6 Geographic information system of the sampling site at Bang Pla Kod Canal, Suksawad Road (1 sampling site).

APPENDIX B

PCBs extraction Efficiency and Concentration Calculation

B-1 Extraction Efficiency

A preliminary test was conducted to verify microbial activity and extraction accuracy. Three sets of the experiment were performed by spiking the sediments with either Aroclor 1242 or 1260 and incubating at different conditions, i.e., kept in the dark under room conditions, kept in a refrigerator at 4°C, and sterilized and kept in the dark under room conditions. The results showed highly chlorinated PCB congeners could transform to lower chlorinated congeners in the non-sterilized series maintained under room conditions whereas no dechlorination was observed in either refrigerated or sterilized sets within the experimental period of 5 months. These results revealed two pieces of important information; first, the disappearance of PCBs under the studied conditions was surely due to microbial activity and second, extraction and analytical procedures for PCB congeners used in this study were reliable and accurate.

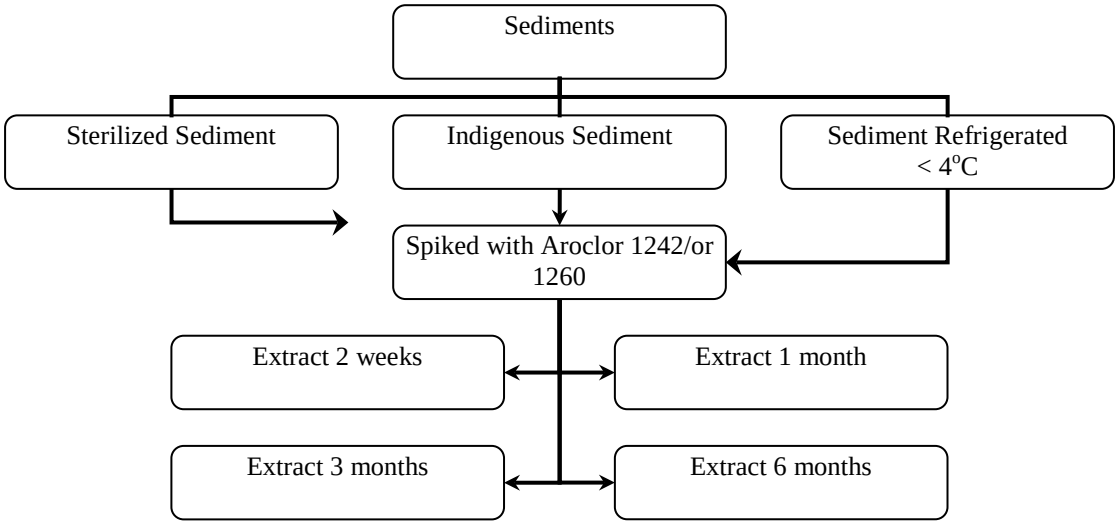


Figure B-1 Experimental set ups to differentiate between biotic, abiotic and adsorption mechanisms.

B-2 Quantification and Qualification of Samples and Standard Concentration

B-2-1 Qualification of PCB Congeners

Injection standard PCB mix congeners, 10 μg/mL Aroclor 1260 and 20 μg/mL Aroclor 1242 were analyzed before being test the extractant samples. Comparisons each peak and retention time were required, in order to confirm this analytical procedures could be recovered all PCB congeners being analysis.

B-2-2 Quantification of PCB congeners

Calculation methods of PCBs congeners could be divided onto 4 calculative methods as following:

B-2-2-1 ESTD method: External standard calculation

Absolute amount of x = $\mathbf{Response_x \times RF_x \times M \times D}$

Where, $Response_x$ is the response of peak x

RF_x is the response factor for component x, (= $Amount_x$ / $Response_x$)

M is the multiplier

D is the dilution factor

B-2-2-2 ESTD% method

Relative amount of x = {(Absolute amount of x) × 100} / Sample amount

Where, $Response_x$ is the response of peak X

RF_x is the response factor for component X, (= $Amount_x$ / $Response_x$)

M is the multiplier

D is the dilution factor

Note: this method have to calculate the amount of sample injection

B-2-2-3 Norm% method

Norm% of x = {(Response$_x$ × RF$_x$ × 100 × M × D) / Σ(Response × RF)}

Where, *Response$_x$* is the area / or height of peak X

RF$_x$ is the response factor

M is the multiplier

D is the dilution factor

Σ(Response × RF) is the total of all the Response multiply by Response factor products for all peaks including peak x

Note: this method have to calculate the amount of sample injection

B-2-2-4 ISTD method: Internal Standard calculation

Actual amount of x = (Response Ratio × RF$_x$) × (Actual amount of ISTD) × M × D

Where, *Response Ratio* is Response$_x$/Response$_{ISTD}$

RF$_x$ is the response factor of component X

M is the multiplier

D is the dilution factor

B-2-2-5 ISTD%

Relative amount of x = {(Actual amount of x) × 100} / Sample amount

Note: this method have to calculate the amount of sample injection

This study was quantified by using ISTD method to measure PCBs concentration, the calculation was followed;

%Recovery = (Area of extractant) / (Area of internal standard) × D × concentration of internal standard × 100

For example,	Area of 2 µg/mL of 234-CBp, (dimensionless)	= 81,475
	Dilution factor	= 2.5
	Area of extractant	= 25,916
	%Recovery = (25,916/81,475) × 2.5 × 2 µg/mL × 100%	= 80%

Table B-1 Extraction efficiency in sediment slurry under this analytical method.

Sampling sites	%Recovery
BP1	80
BP2	84
MF1	106
MF2	97
HLP	147
PWP	116
BPK	114

Remarks: acceptable extraction efficiencies were in raged of 70-120%.

APPENDIX C

Calibration curve

C-1 PCBs calibration curves

Calibration curve was analyzed following EPA 680A method. PCB isomer calibration mix solutions were purchased from AccuStandard Inc, USA. Table C-1 showed the PCB isomers were applied and concentrations were measured in rage 0.39 to 6.25 mg/l, under analytical observation. Each level of chlorination substituent was represented as each PCB congeners in that group, presumably that they have same sensitivity and precision. The graphs showed linear relationships, it implied to the accuracy of concentration of PCBs were analysis under these ranges.

Table C-1 PCB isomer calibration mix.

Level of Chlorination	**Isomer Selected**	**BZ#**	**RF value vs. Chrysene-d_{12}**	**Mean RF value vs. Chrysene-d_{12}**
1	2-mono	1	0.899	0.925
2	23-di	5	0.651	0.642
3	245-tri-	29	0.411	0.411
4	22′46-tetra	50	0.305	0.431

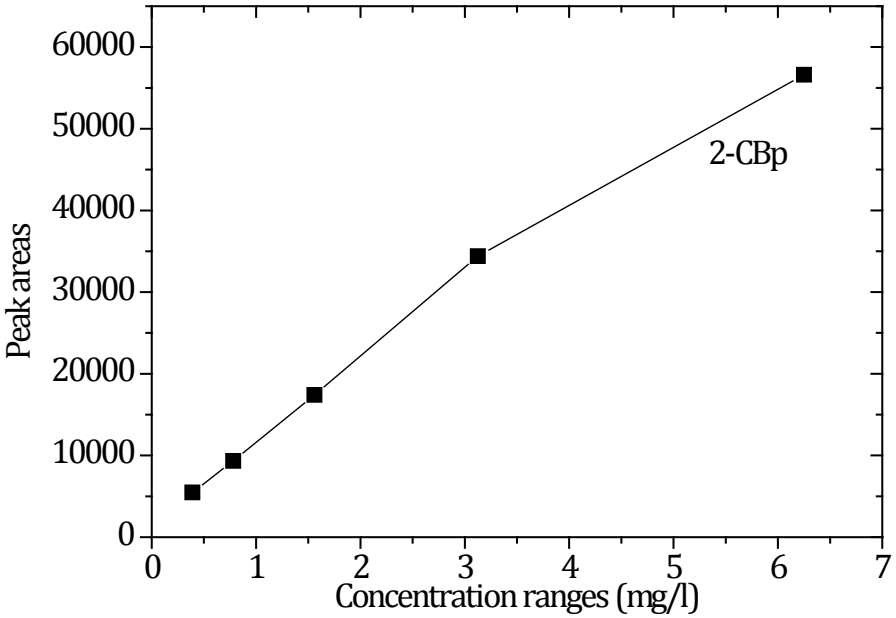


Figure C-1 The calibration curve of mono-chlorine isomers.

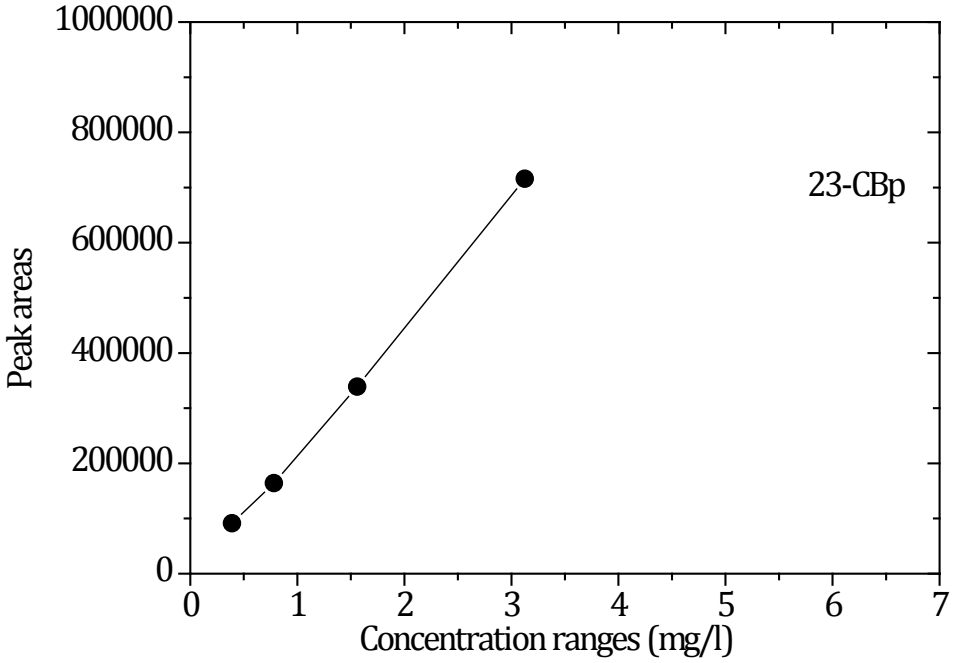


Figure C-2 The calibration curve of di-chlorine isomers.

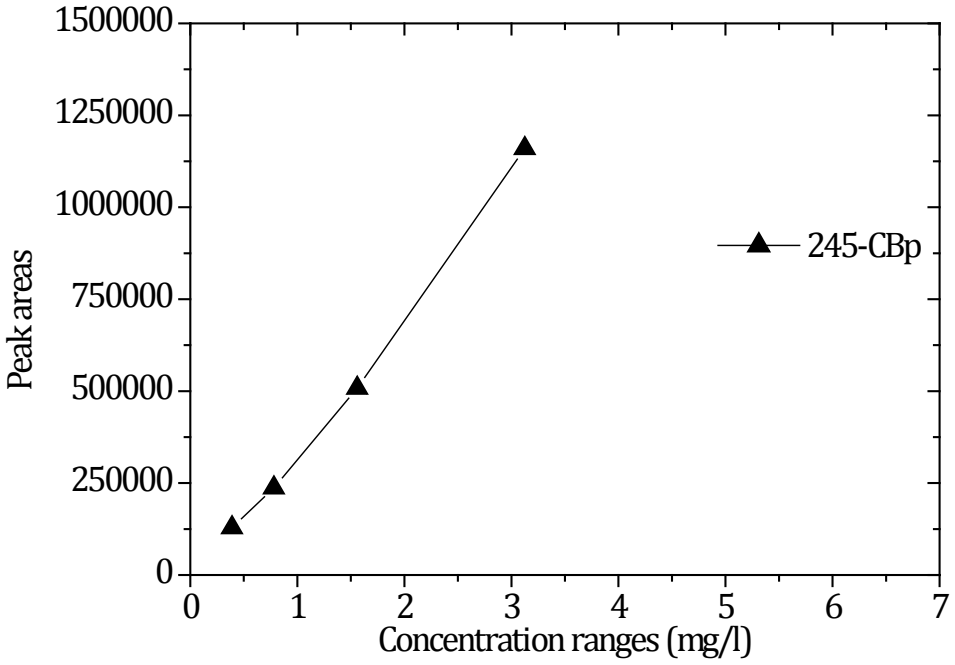


Figure C-3 The calibration curve of tri-chlorine isomers.

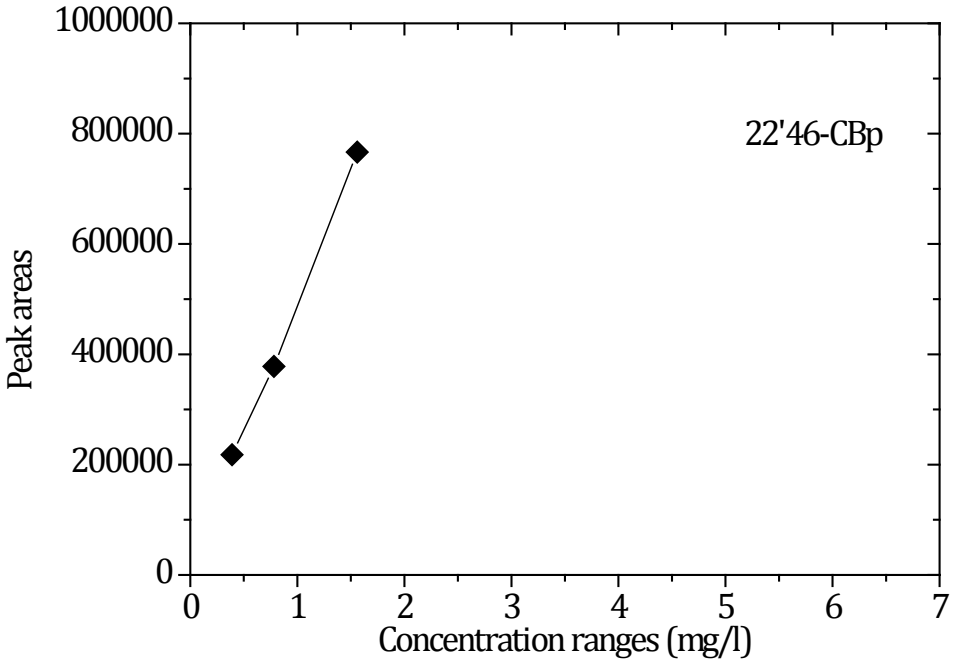


Figure C-4 The calibration curve of tetra-chlorine isomers.

APPENDIX D

Chromatographic Data of PCB Congeners

D. 1 Qualification and Quantification of PCB Congeners

To identify all 209 PCB congeners through gas chromatography retention time (RT). This methodology was used in case of available of Aroclors in ours laboratory, since they already sold as a commercially standard for all 209 PCB congeners by AccuStandard, Inc. (USA). However, this procedure could examine the possibly intermediate products before confirmed by GC-MS. Plot of relative retention times and response factor vs isomer number for all 209 congeners have been observed and showed in Figure D-1 and D-2.

Single PCB congeners were showed in Table 3.1 and commercially PCB congeners; including, 20 mg/l of Aroclor 1242 and 10 mg/l of Aroclor 1260 were individually analyzed by GC, the distribution of chromatography peaks were expressed in Figure D-3 and D-4. Injection of all standards were evaluated under analytical criteria, i.e., sequences and condition, in order to prevent the analytical error from the instrument e.g. sensitivity, precision etc. From this step, the retention time (RT) for each PCB congeners and commercial PCB congeners were attained, then namely each PCB congeners in D-1 column 1. The response factors for all 209 congeners were already synthesized (Mullin et al., 1984; Chen et al., 2001; Vetter and Bester, 2006; Kaliszan, 2007). Moreover, the relative retention times were used quantitation methods which had been describe by Chen et al., 2001. Due to their correlations of response factors, concentration of area/or height of chromatographic peaks could express the equation as followed:

Area of compound$_x$ = Response factor$_x$ × Concentration of compound$_x$

Hence: it is based on the analytical program instruments, Agilent 6890 GC and ChemStation operation.

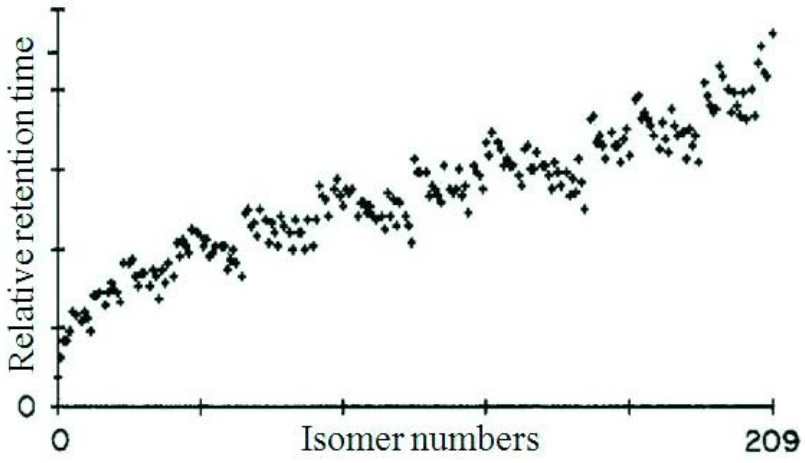


Figure D-1 Plot relative retention times of PCBs vs isomers numbers (Mullins et al., 1984).

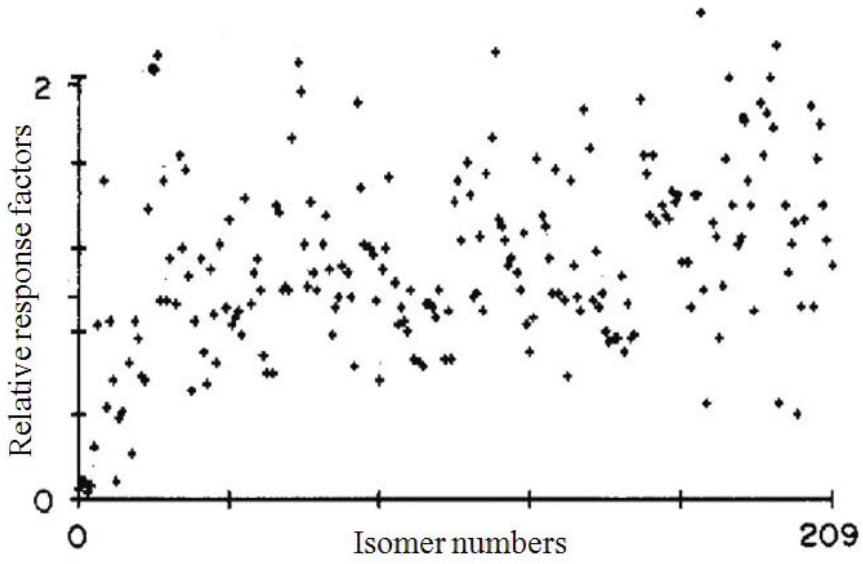


Figure D-2 Plot relative response factors of PCBs vs isomers numbers (Mullins et al., 1984).

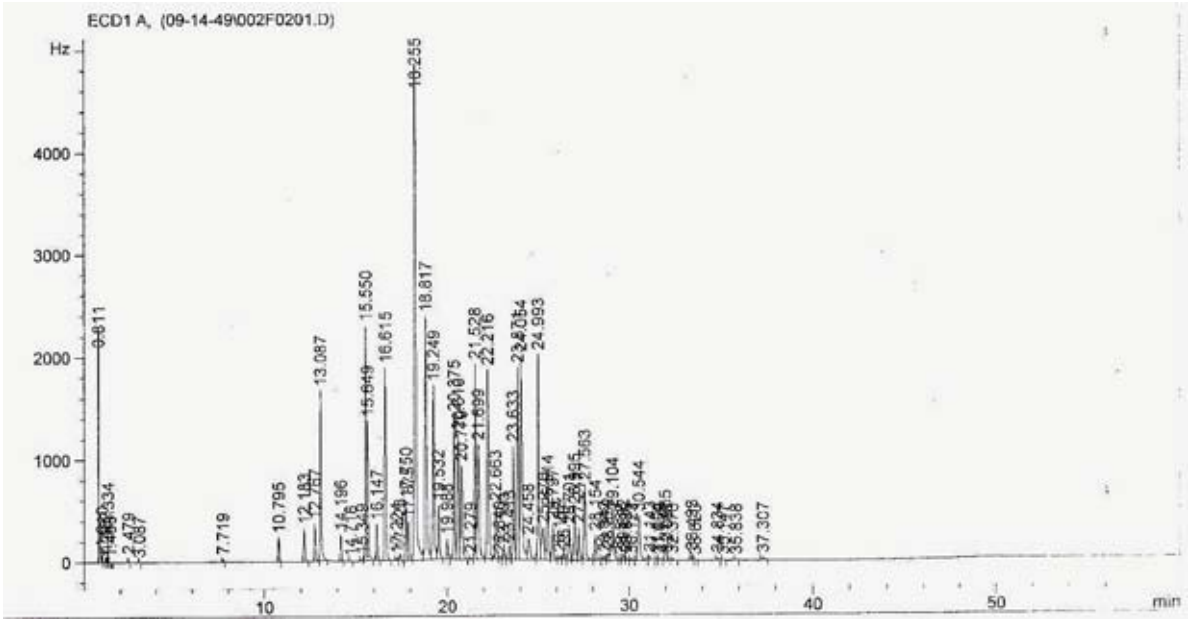


Figure D-3 Chromatograms of 20 mg/l of Aroclor 1242 under analytical criteria.

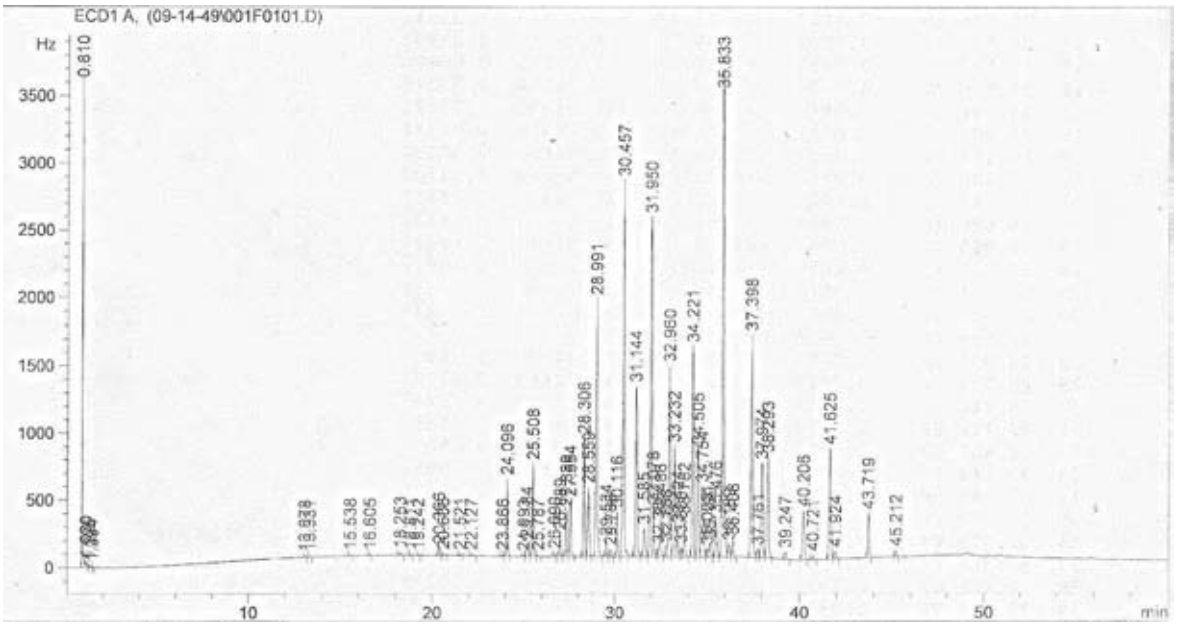


Figure D-4 Chromatograms of 10 mg/l of Aroclor 1260 under analytical criteria.

Table D-1 The chromatographic data of PCBs by gas chromatography.

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (μM)[e]
1[d]	**1**	**2**	**6.87**	**0.0393**				**1.9152**
2	2	3	8.44	0.0400				1.8817
3	3	4	8.71	0.0193				3.8999
4	4	22'	10.42	0.0374	3.01			1.6947
4	**10**	**26**	**10.42**	**0.2620**	**0.20**			**0.2688**
5	7	24	11.82	**0.6900**	0.60			0.1120
5	9	25	11.82	0.3880	0.54			0.1900
6	6	23'	12.38	0.3800	1.38			0.1940
7	5	23	12.68	0.1190	0.06			0.5326
7	8	24'	12.68	0.2060	7.65			0.3419
8	**14**	**35**	**13.37**	**0.3047**				**0.2419**
9	19	262'	13.77	0.3037	0.53			0.2102
10	11	33'	14.27	0.0449				1.4116
10	**30**	**246**	**14.27**	**0.8202**				**0.0873**
11	**12**	**34**	**15.00**	**0.1790**				**0.3934**
11	13	34'	15.00	0.2000				0.3521
12	15	44'	15.21	0.1070	1.51			0.5924
12	18	252'	15.21	0.3130	6.28	0.41		0.2040
13	17	242'	15.72	0.4120	2.88	0.19		0.1774
14	24	236	16.17	**0.7930**	0.22			0.0931
14	27	263'	16.17	0.4950	0.28			0.1477
15	16	232'	16.85	0.4470	2.01			0.1635
15	32	264'	16.85	0.2780	0.88			0.2194
16	**23**	**235**	**16.95**	**0.5000**				**0.1476**
16	34	352'	16.95	0.6092				0.1099
17	**29**	**245**	**17.04**	**0.6339**	**0.10**			**0.1056**
17	54	262'6'	17.04	0.3643				0.1545
18	26	253'	17.32	0.6030	1.33			0.1224
19	25	243'	17.46	0.5000	0.79			0.1476
20	31	254'	17.79	0.5620	4.59	0.22	0.05	0.1313

Note: a: quoted from Mullin et al., 1984, b: qouted from Vetter and Bester, 2006 and Erickson, 1986, d: numerical peaks from surrogate standard, e: peak areas concentration in μM

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (μM)[e]
21	28	244'	17.86	0.8540	6.52	0.25	0.05	0.0741
21	50	2462'	17.86	0.6817				0.0865
22	20	233'	18.27	0.7238	0.29			0.0925
22	**21**	**234**	**18.27**	**1.0598**				**0.0578**
22	33	342'	18.27	0.4470	4.79	0.14		0.1635
22	53	252'6'	18.27	0.3606	0.64	0.09		0.1561
23	22	234'	18.27	1.0935	3.41			0.0560
23	51	242'6'	18.36	0.6000	0.23			0.1084
24	36	353'	18.70	0.2948				0.2166
24	45	2362'	18.70	0.5400	1.16			0.1205
25	39	354'	19.31	0.3470				0.1840
25	46	232'6'	19.31	0.4680				0.1377
26	52	252'5'	19.92	0.4180	4.04	5.18	0.56	0.1541
26	69	2463'	19.92	0.8024	0.11			0.0811
26	73	263'5'	19.92	0.5805				0.1121
27	49	242'5'	20.16	0.6480	3.60	1.64		0.0910
28	**38**	**345**	**20.18**	**0.4698**				**0.1556**
28	43	2352'	20.18	0.5030				0.1293
28	47	242'4'	20.18	0.8480	0.94	0.17	0.11	0.0658
28	48	2452'	20.18	0.5560	0.84	0.14	0.09	0.1170
28	75	2464'	20.18	0.6461	0.11			0.0913
29	35	343'	20.32	0.3746				0.1704
29	62	2346	20.32	1.1478		0.23		0.0471
29	**65**	**2356**	**20.32**	**0.8408**				**0.0663**
30	**44**	**232'5'**	**21.06**	**0.5240**	**3.20**			**0.1241**
30	104	2462'6'	21.06	0.4561		2.03		0.1264
31	37	344'	21.23	0.5800	0.27			0.1272
31	42	232'4'	21.23	0.7920	0.83			0.0822
31	59	2363'	21.23	0.6000	0.34			0.0274
32	41	2342'	21.75	0.5469	1.86	0.64	0.14	0.1189
32	64	2364'	21.75	0.6070	1.64	0.45		0.1072

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (µM)[e]
32	71	263'4'	21.75	0.4680				0.1377
32	72	252'5'	21.75	0.5515				0.1180
33	68	243'5'	21.99	0.7260				0.0813
34	40	232'3'	22.19	0.7220	0.89	0.20		0.0817
34	96	2362'6'	22.19	0.4308		0.08		0.1338
35	57	2353'	22.75	0.6000				0.1084
35	103	2462'5'	22.75	0.6068	0.01	0.29	0.09	0.0959
36	67	2453'	22.82	0.6000	0.06	0.02	0.02	0.1084
36	100	2462'4'	22.82	0.5871		0.10		0.0991
37	58	233'5'	22.98	0.6090				0.0969
37	63	2354'	22.98	0.7280	0.23	0.05		0.0810
38	**61**	**2345**	**23.16**	**1.2227**				**0.0442**
38	74	2454'	23.16	0.6710	2.17	0.78		0.0879
38	94	2352'6'	23.16	0.4514				0.1277
39	70	253'4'	23.42	0.6580	3.89	3.21	0.09	0.0897
39	76	3452'	23.42	0.5795				0.1123
39	98	2462'3'	23.42	0.6246				0.0845
40	66	243'4'	23.58	0.6460	1.66	0.59		0.0913
40	80	353'5'	23.58	0.7278				0.0811
40	93	23562'	23.58	0.6676				0.0791
40	95	2362'5'	23.58	0.4430	2.87	6.02	3.04	0.1301
40	102	2452'6'	23.58	0.4561				0.1264
41	55	2343'	23.98	0.8290				0.0761
41	88	23462'	23.98	0.6892				0.0766
41	91	2362'4'	23.98	0.5710	0.17	0.83		0.1019
41	121	2463'5'	23.98	0.7659				0.0760
42	56	233'4'	24.52	0.8290	1.60	0.58		0.0761
42	60	2344'	24.52	1.0164	1.33	0.54		0.0531
42	84	2362'3'	24.52	0.3860	0.72	1.95	0.25	0.1304
42	92	2352'5'	24.52	0.5375	0.25	1.58	0.59	0.1083
42	155	2462'4'6'	24.52	0.5860				0.0899

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (µM)[e]
43	89	2342'6'	25.04	0.5610				0.1038
43	90	2352'4'	25.04	0.6110	0.32	0.93	0.56	0.0864
43	101	2452'5'	25.04	0.6680	1.33	7.94	5.02	0.0790
44	79	343'5'	25.32	0.8810				0.0633
44	99	2452'4'	25.32	0.6130	0.86	3.60	0.11	0.0861
44	113	2363'5'	25.32	0.6040				0.0238
44	123	3452'4'	25.32	0.6645		0.81		0.0794
45	112	23563'	25.71	0.8286				0.0682
45	119	2463'4'	25.71	0.8239	0.05	0.14		0.0685
45	150	2362'4'6'	25.71	0.5676				0.0928
46	78	3453'	25.93	1.1151				0.0484
46	83	2352'3'	25.93	0.6339	0.12	0.45		0.0833
46	109	23463'	25.93	0.9625				0.0502
46	152	23562'6'	25.93	0.5235				0.1006
47	86	23452'	26.22	0.7968				0.0731
47	97	2452'3'	26.22	0.6310	0.65	2.55	0.23	0.0837
48	81	3454'	26.52	0.7159				0.0824
48	87	2342'5'	26.52	1.0210	0.77	3.78	0.77	0.0473
48	111	2353'5'	26.52	0.6601				0.0800
48	115	23464'	26.52	1.1328		0.30	0.05	0.0427
48	117	23564'	26.52	0.8895				0.0561
48	125	3452'6'	26.52	0.5560				0.1047
48	145	23462'6'	26.52	0.6789				0.0704
49	**116**	**23456**	**26.62**	**1.3987**				**0.0345**
50	85	2342'4'	26.74	0.7396	0.53	1.66	0.05	0.0714
50	120	2453'5'	26.74	0.7444				0.0782
50	136	23623'6'	26.74	0.4440	0.07	1.12	2.23	0.1175
50	148	2352'4'6'	28.33	0.5540				0.0951
51	77	343'4'	27.08	0.3812	0.45			0.1476
51	110	2363'4'	27.08	0.6500	1.53	5.85	1.90	0.0812
52	82	2342'3'	27.67	0.7730	0.44	0.95		0.0753

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (µM)[e]
52	151	23562'5'	27.67	0.7850		1.17	3.67	0.0672
52	154	2452'4'6'	27.67	0.5700				0.0924
53	124	3452'5'	28.18	0.8480				0.0589
53	135	2352'3'6'	28.18	0.7031	0.08	1.62	2.56	0.0680
53	144	23462'5'	28.18	0.8764				0.0516
54	107	2353'4'	28.34	0.8183	0.07	0.72		0.0690
54	108	2343'5'	28.34	1.0654				0.0454
54	147	23562'4'	28.34	0.6000				0.0878
55	106	23453'	28.63	1.0046				0.0481
55	118	2453'4'	28.63	0.8700	1.62	6.39	0.57	0.0574
55	139	23462'4'	28.63	0.7219				0.0662
55	140	2342'4'6'	28.63	0.6732				0.0710
55	149	2362'4'5'	28.63	0.5720	0.63	2.21	7.83	0.0921
56	134	23562'3'	28.63	0.7331		0.42	0.38	0.0652
56	143	23452'6'	28.63	0.7088				0.0674
57	114	23454'	29.04	1.0261	0.01	0.25	0.03	0.0471
57	122	3452'3'	29.04	0.7247				0.0728
57	131	23462'3'	29.04	0.8492	0.03	0.17	0.09	0.0532
57	133	2352'3'5'	29.04	1.1480				0.0381
57	142	234562'	29.04	1.2180				0.0359
58	146	2352'4'5'	29.25	0.7280		2.43	2.71	0.0656
58	161	23463'5'	29.25	0.9672				0.0452
58	165	23563'5'	29.25	1.0777				0.0406
58	188	23562'4'6'	29.25	0.7337				0.0595
59	132	2342'3'6'	29.99	0.7303	0.30	1.98	3.69	0.0654
59	153	2452'4'5'	29.99	0.6880	0.68	4.26	10.80	0.0695
59	184	23462'4'6'	29.99	1.0046				0.0398
60	105	2343'4'	30.08	0.9400	0.86	3.83	0.07	0.0531
60	127	3453'5'	30.08	0.5834				0.0998
60	168	2463'4'5'	30.08	0.8375				0.0539
61	141	23452'5'	30.66	1.3520		1.04	2.56	0.0324

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (μM)[e]
61	179	23562'3'6'	30.66	0.8237		0.21	1.79	0.0567
62	130	2342'3'5'	30.79	0.9520		0.63	0.08	0.0459
63	137	23452'4'	31.09	1.1120		0.25	0.06	0.0393
63	176	23462'3'6'	31.09	1.0589		0.32	0.95	0.0377
64	138	2342'4'5'	31.47	0.8270	0.54	3.20	6.13	0.0618
64	158	23463'4'	31.47	1.1320		0.77	1.55	0.0386
64	160	234563'	31.47	1.1914			0.05	0.0367
64	163	23563'4'	31.47	0.9976				0.0438
64	164	23463'5'	31.47	0.9848				0.0444
64	186	234562'6'	31.47	1.2236				0.0327
65	126	3453'4'	31.60	0.4757				0.1212
65	129	23452'3'	31.60	0.9970		0.23	1.11	0.0439
65	178	23562'3'5'	31.60	0.6210		1.35	1.62	0.0703
66	159	23453'5'	31.99	0.9934				0.0440
66	166	234564'	31.99	1.0421				0.0420
66	175	23462'3'5'	31.99	0.3810		0.05	0.23	0.1093
66	182	23452'4'6'	31.99	1.1272				0.0354
66	187	23562'4'5'	31.99	1.1220		0.32	3.97	0.0356
67	162	2353'4'5'	32.30	1.0322				0.0424
67	183	23462'4'5'	32.30	0.9760		0.17	1.76	0.0409
68	128	2342'3'4'	32.47	1.1880		2.07	1.06	0.0368
68	167	2453'4'5'	32.47	1.0658		0.21	0.26	0.0410
69	185	234562'5'	32.74	1.4370			1.34	0.0278
70	174	23452'3'6'	32.94	0.8060		0.34	3.85	0.0597
70	181	234562'4'	32.94	1.6046				0.0249
71	177	23562'3'4'	33.29	1.0090		0.21	2.21	0.0396
72	156	23453'4'	33.72	1.3890	0.09	1.62	0.88	0.0315
72	171	23462'3'4'	33.72	1.1712	0.05	0.50	2.16	0.0341
72	202	23562'3'5'6'	33.72	1.1650			0.50	0.0316
73	157	2343'4'5'	34.01	1.1965			0.14	0.0366
73	173	234562'3'	34.01	2.0440		0.09	0.36	0.0195

Table D-1 The chromatographic data of PCBs by gas chromatography (Cont').

Peak No.	IUPAC No.	Congeners	Retention Time	Response factor[a]	% in Aroclor 1242[b]	% in Aroclor 1254[b]	% in Aroclor 1260[b]	A=10,000 (µM)[e]
77	200	234562'3'6'	35.48	0.3690			0.45	0.1039
78	169	3453'4'5'	35.71	0.8355			0.05	0.0541
79	170	23452'3'4'	35.90	0.7500	0.11	0.31	3.91	0.0642
79	190	234563'4'	35.90	1.3100		0.08	0.79	0.0305
80	198	234562'3'5'	36.89	1.0700			0.09	0.0344
80	199	23452'3'5'6'	36.89	1.1508			1.31	0.0320
81	196	23462'3'4'5'	37.43	1.2321			0.69	0.0299
77	200	234562'3'6'	35.48	0.3690			0.45	0.1039
78	169	3453'4'5'	35.71	0.8355			0.05	0.0541
79	170	23452'3'4'	35.90	0.7500	0.11	0.31	3.91	0.0642
79	190	234563'4'	35.90	1.3100		0.08	0.79	0.0305
80	198	234562'3'5'	36.89	1.0700			0.09	0.0344
80	199	23452'3'5'6'	36.89	1.1508			1.31	0.0320
81	196	23462'3'4'5'	37.43	1.2321			0.69	0.0299
81	201	23462'3'5'6'	37.43	0.8030		0.68	0.99	0.0552
81	203	234562'4'5'	37.43	1.6290			0.99	0.0226
82	189	23453'4'5'	37.73	1.5091			0.11	0.0265
83	195	234562'3'4'	38.67	0.4150			0.68	0.1058
83	208	234562'3'5'6'	38.67	1.1756			0.17	0.0289
84	207	234562'3'4'6'	39.55	1.3257			0.05	0.0256
85	194	23452'3'4'5'	41.04	1.8680			1.30	0.0197
86	205	234563'4'5'	41.37	1.4060			0.15	0.0262
87	206	234562'3'4'5'	43.21	1.6730			0.45	0.0203
88	209	234562'3'4'5'6'	44.74	1.1390			0.05	0.0279

APPENDIX E

Experimental Data (tabular or graphical)

E-1 The relations of Dechlorination and Methane Production from Study of Effect of Organic Substrates on 234-CBp Dechlorination

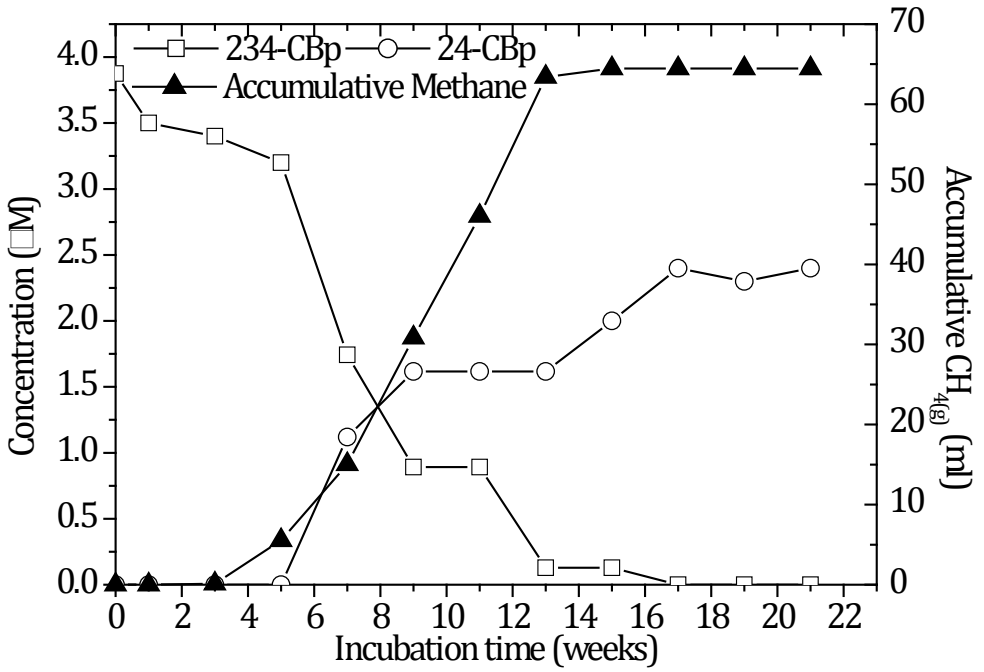


Figure E-1-1 234-CBp dechlorination profiles in reductive synthetic anaerobic mineral medium amended with pyruvate.

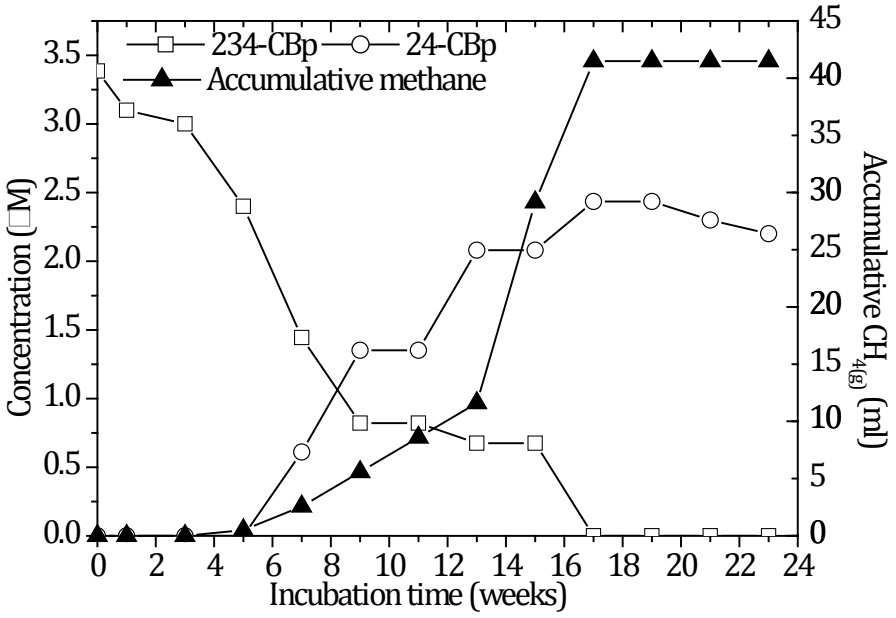


Figure E-1-2 234-CBp dechlorination profiles in reductive synthetic anaerobic mineral medium amended with lactate.

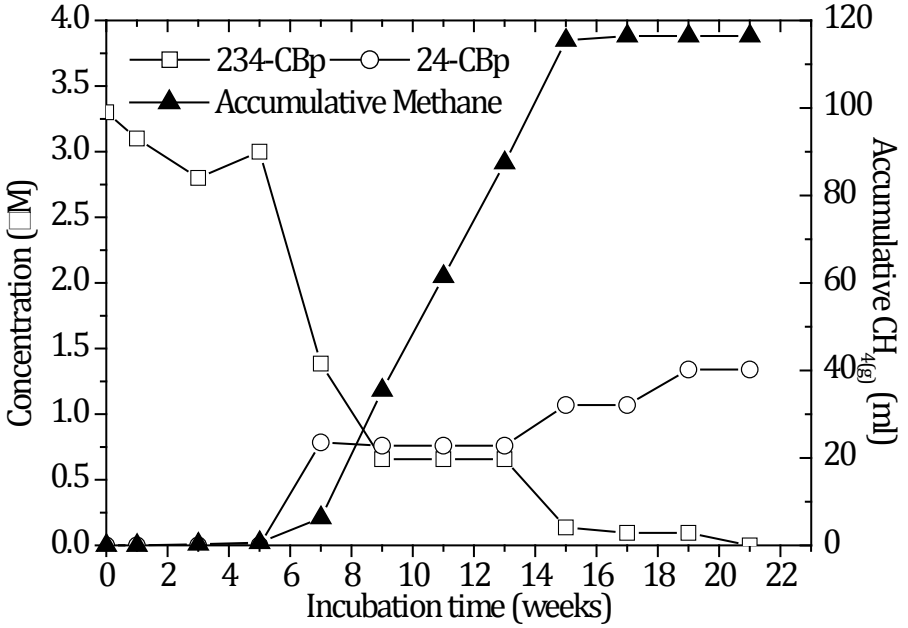


Figure E-1-3 234-CBp dechlorination profiles in reductive synthetic anaerobic mineral medium amended with acetate.

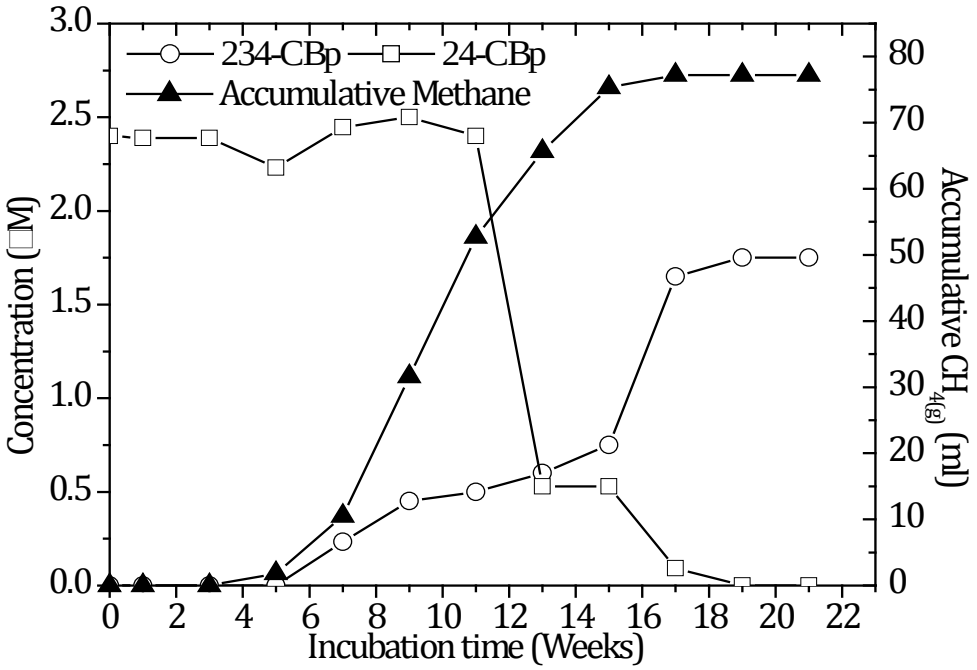


Figure E-1-4 234-CBp dechlorination profiles in sediment slurry amended with pyruvate.

E-2 Dechlorination profiles of enhancement of 234- and 2345-CBps dechlorination by halogenated primers.

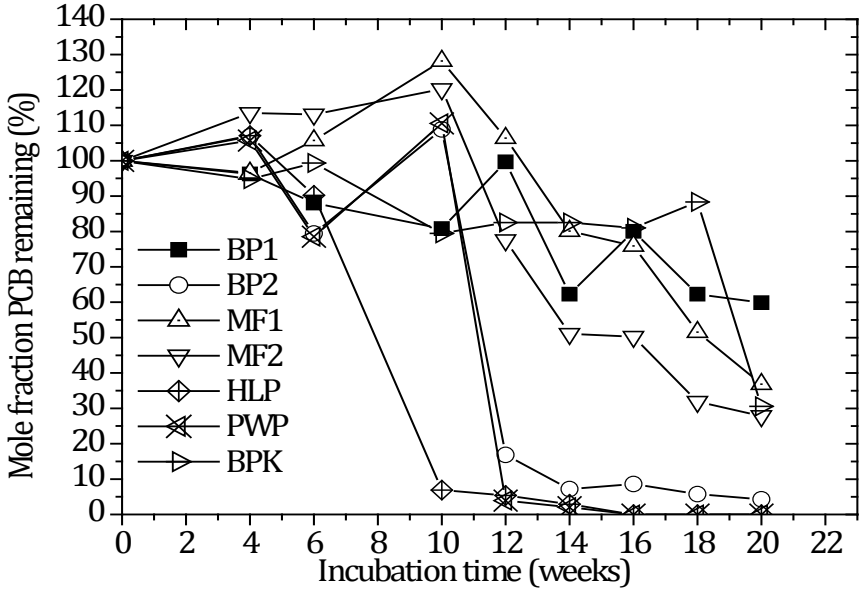


Figure E-2-1 234-CBp dechlorination profiles by amending methyl 4-bromobenzene.

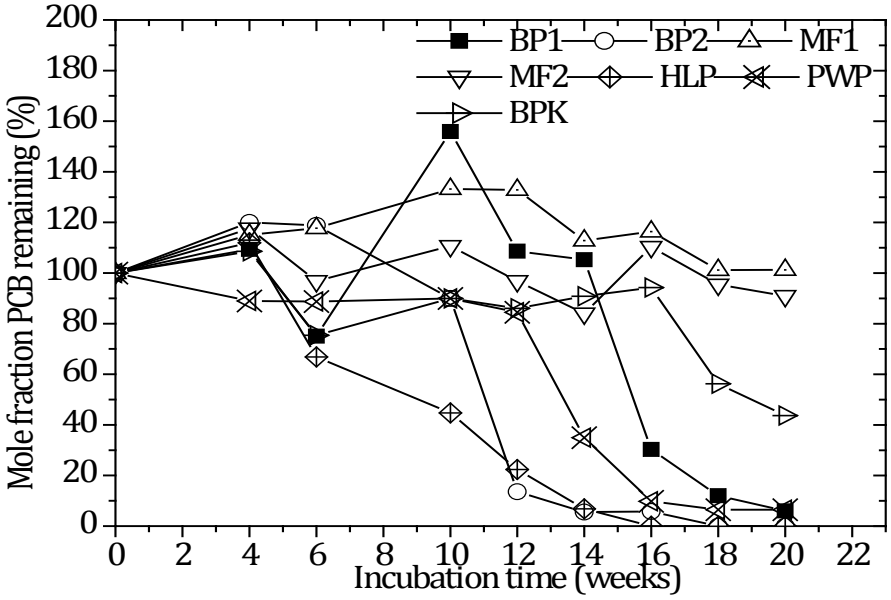


Figure E-2-2 234-CBp dechlorination profiles by amending 14-dibromobenzene.

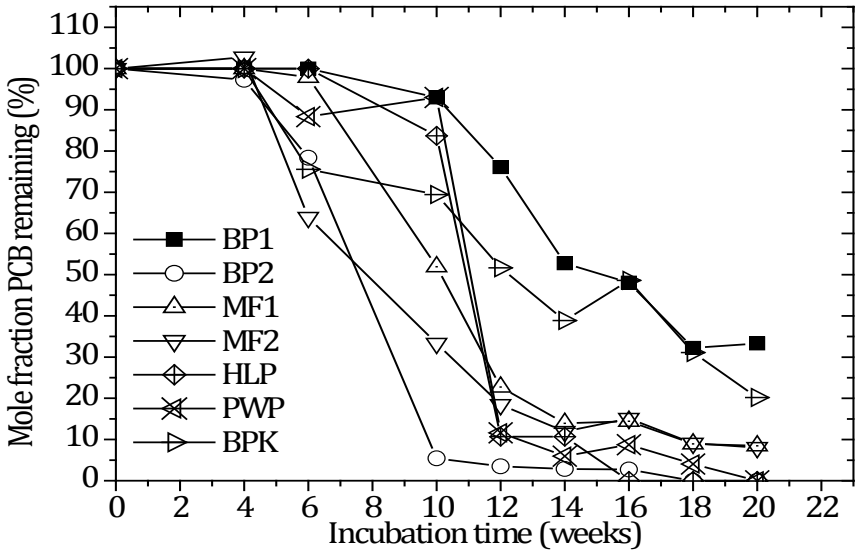


Figure E-2-3 234-CBp dechlorination profiles by amending 4-bromobenzonitrile.

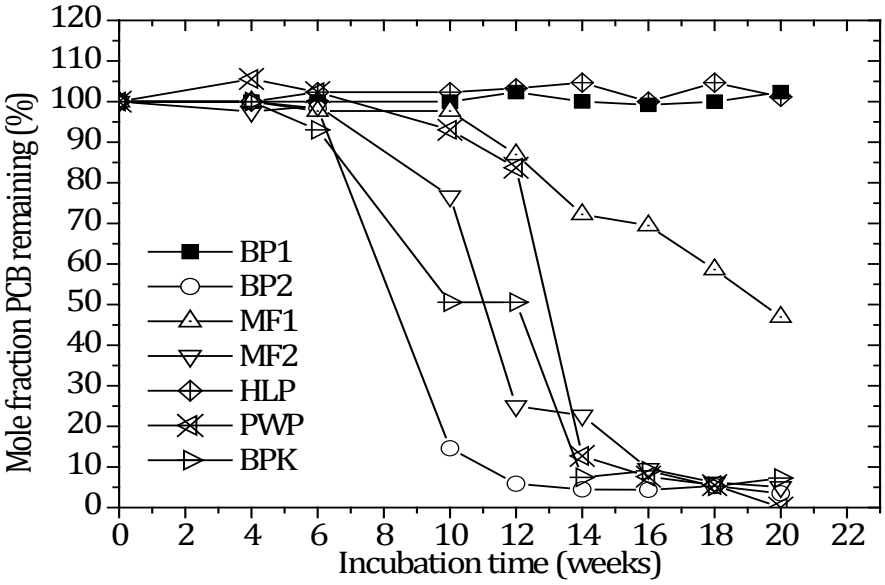


Figure E-2-4 234-CBp dechlorination profiles by amending 4-bromobenzoic hydrazide.

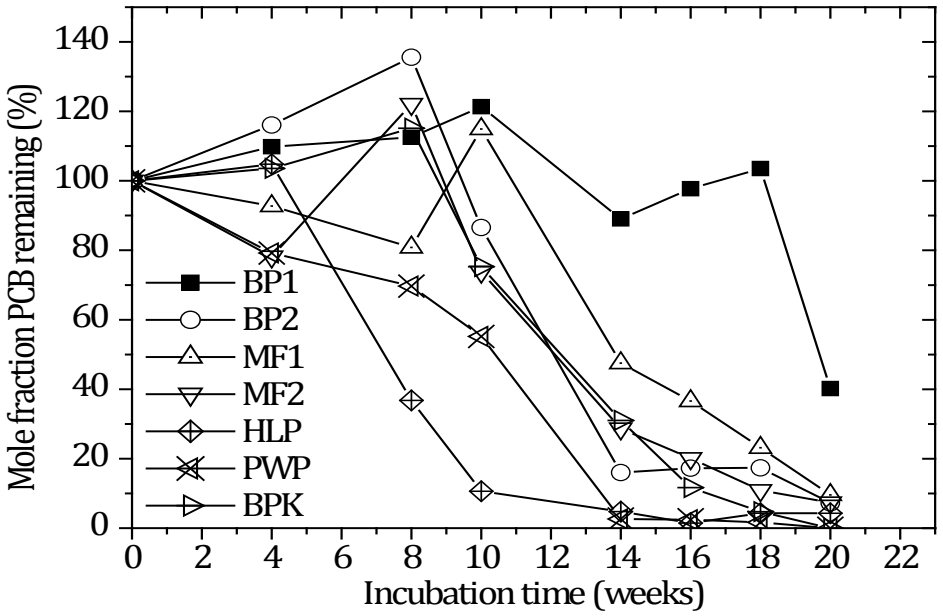


Figure E-2-5 2345-CBp dechlorination profiles by amending methyl 4-bromobenzene.

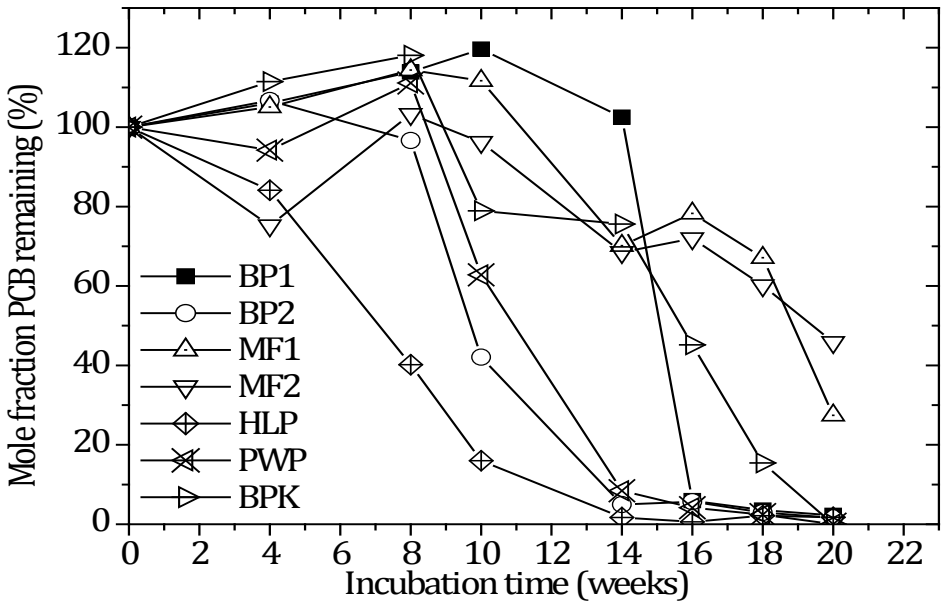


Figure E-2-6 2345-CBp dechlorination profiles by amending 14-dibromobenzene.

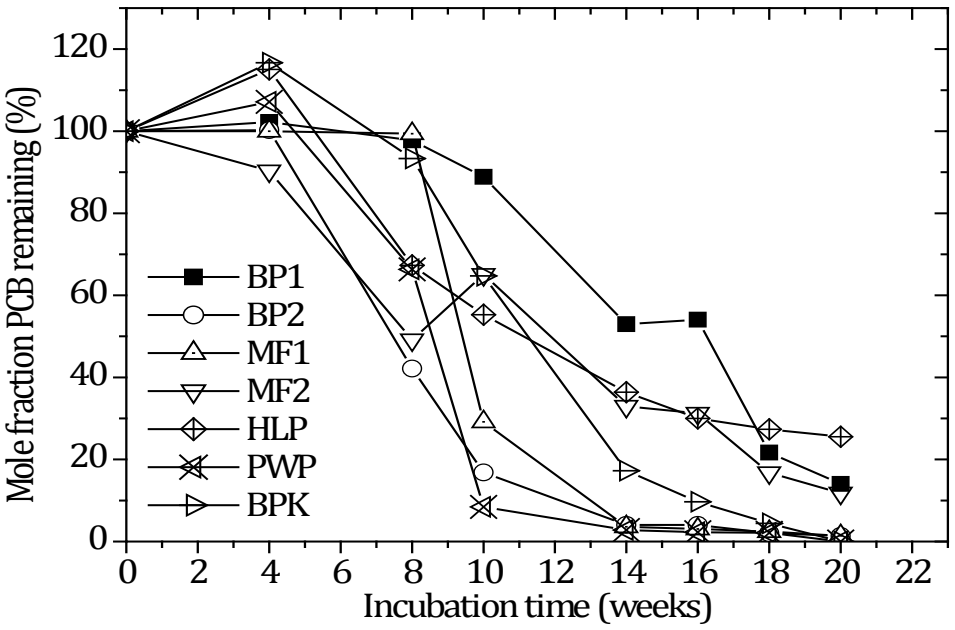


Figure E-2-7 2345-CBp dechlorination profiles by amending 4-bromobenzonitrile.

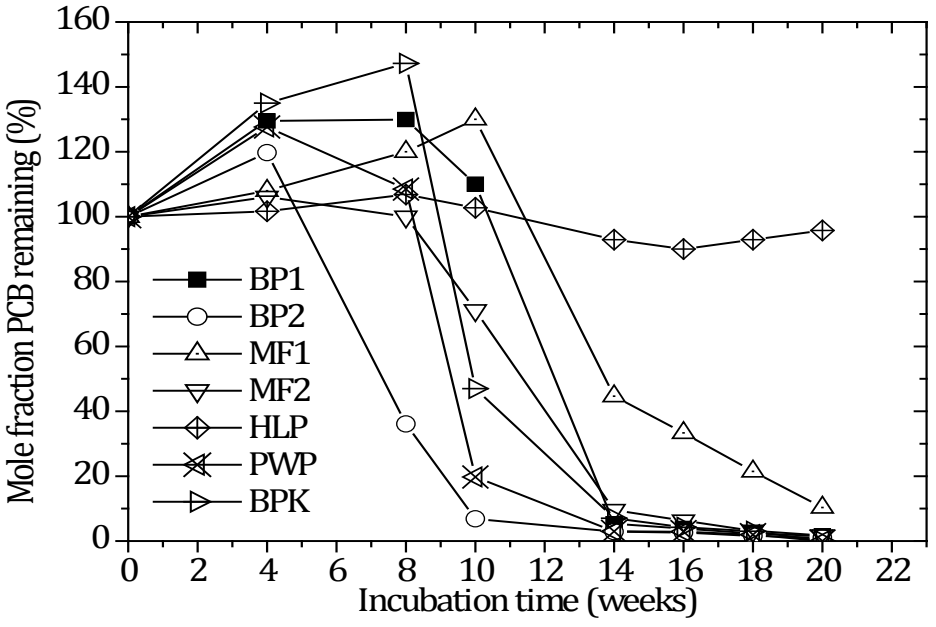


Figure E-2-8 2345-CBp dechlorination profiles by amending 4-bromobenzoic hydrazide.

E-2-1 Identification of Reductive Microorganisms to Dechlorinate 234-Trichlorobiphenyls and 2345-Tetrachlorobiphenyls by Using Halogenated primers

This study part was successive work which continuing from the experimental scenario 2. Effective sites amended with effective halogenated primer have been chosen in order to primary screen the phylum might be gathered in PCB dechlorinating consortium. As recently, researchers have been isolated the PCB dechlorinators to pure cultures, for more references see scenario 3. Furthermore, the gene analytical methods were approach into the future.

E-2-1-1 Inoculating Soil Cultures for DNA Extraction

After the dechlorination was completed in each experimental set, selected cultures were chosen from effective dechlorination inoculated cultures. Effective cultures were included BP2, PWP and BPK sites primed with 4BZ and 4BN and then after one time of serial transfer with sediments into the sterilized medium with 0.5% yeast extract (20%, v/v), all inoculating cultures were being used.

E-2-1-2 DNA Extraction and PCR Amplification

One gram of sediment slurry was used to DNA extracted procedures; full speeds centrifuged and discard the supernatant. DNA extraction were used UltraClean®Soil DNA kit (MOBIO Laboratories, Inc, USA), the extraction were followed the manufacture's protocols. The genomics DNA were further to increase the sensitivity whereas nested PCR technique was required. The DNA was diluted 10 times with sterile PCR-quality water, and 2 ng of the templates were used for 50μL reaction mixture for PCR amplification with Universal bacteria primers EUB_{8f} (5′-AGAGTTTGATCCTGGCTCAG-3′) and U_{1492r} (5′-GGTTACCTTGTTACGA-3′) (Orphan V.J. et al., 2001). Within 50 μL were contained 0.1μM of each primer and 1 U of *Taq* DNA polymerase (Qiagen, Germany). The PCR amplification program was 5 min at 95°C and then 25 cycles of 0.5 min at 95°C, 0.3 min at 55°C, 2 min at 72°C,

followed by a final extension at 72 °C for 7 min and a final hold at 4°C. Reamplified PCR products for DGGE analysis were used primers $338_{GC\text{-}f}$ (5'-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGACTCCTACG GGAGGCAGCAG-3') and 518_r (5'-ATTACCGCGGCTGCTGG-3') (Orphan V.J. et al., 2001). The amplification program was 5 min at 95°C and then 30 cycles of 0.5 min at 95°C, 0.3 min at 60°C, 0.5 min at 72°C, followed by a final extension at 72 °C for 7 min and a final hold at 4°C. PCR products were analyzed on 1.2% Agarose gel to collect molecular size.

E-2-1-3 Denaturing Gradient Gel Electrophoresis Analysis

Eighteen microlitters of reamplified PCR products with 338GC-f were loaded onto 7.5% acrylamide gels in 1xTris-acetate-EDTA (TAE). The percents denaturant were ranged from 40 to 55, which 100% denaturant contains 5.6 M urea and 40% (v/v) formamide in 1xTAE. The DGGE was performed and modified form Muyer et al.'s protocol by using a DGGE-2000 system instrument (CBS Scientific Company, Del Mar, CA). The electrotrophoresis was carried out at 60°C for 5 hr in 0.5xTAE buffer at a constant voltage of 200 V. The gel was stained with STBR Green nucleic acid stain (Molecular Probes, Eugene, OR, USA) for 30 min and DNA fragment were visualized on an UV transilluminator (Biovision CN 1,000/26M, Vilber Lourmat, France).

E-2-1-4 Nucleotide Sequencing and Phylogenic Analysis

The individual intensity DGGE bands were collected; each band was resuspended into 20 μL MilliQ water and stored at 4°C overnight. The DNA were eluted form acrylamide gel, elution was used as a template to reamplify by using the primer set without GC-clamp. The PCR products were purified by using Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Ltd., USA).

E-2-1-5 Results and Discussion

E-2-1-5-1 Abundance of PCBs Dechlorinating Population and Its Characterization

Fifteen effective inoculating cultures were selected and identified the PCBs dechlorinating microbes. The sampling sites and priming congeners were selected including BP2, PWP, BPK and 4-BZ, 4-BN, respectively. The microbial community was analyzed the DNA fragment by PCR-DGGE analysis of partial 16S rRNA genes of bacteria. Figure F-1 shows the fragment DGGE bands pattern of the PCB-dechlorinating microbes after 2345-CBp dechlorination completed (4 months) and one time serially transferred with sediments cultures. Table F-1 shown the phylogenic analysis of DGGE bands, the intensity bands were indicated the PCB-dechlorinating microbes. The same levels of the band were indicated the same dechlorinating groups. The results suggested that *Firmicutes* and *Proteobacteria* were mainly anaerobic dechlorinating microbes, whereas, Katayama and Fujie, (2000) also founds *Firmicutes* was the mainly anaerobes consortiums containing in menaquinone-6 and -7 degraded in sediments. BP2 and PWP were collected from PCBs possibly contaminated area, but could not be detected the commercially PCB congeners contaminated at the background; whereas, BPK was served as non and/or less-polluted site. DNA bands of dechlorinating microbes were not detected in the original soil. These results revealed that dechlorinating microbes DNA bands were shown after enriched cultures with contaminated chemicals and/or primers amended.

Under the treatment of 2345-CBps included bands 1, 2, 3, 4, 5, 6 and 7. The results revealed that mainly PCBs dechlorinating microbes were included bands 2 and 3, which is *uncultured gamma proteobacterium* and PCB dechlorinating bacterial communities in river sediment. Moreover, the results suggested that the group of microbes were depended on PCB congeners contaminated rather than the primer amended, which is reason for 234-CBp could not dechlorinate in this study, see scenario 2. Moreover, PWP site treated with 2345-CBps could be found the *Pseudomonas putida* which diverse metabolism exploited for bioremediation of

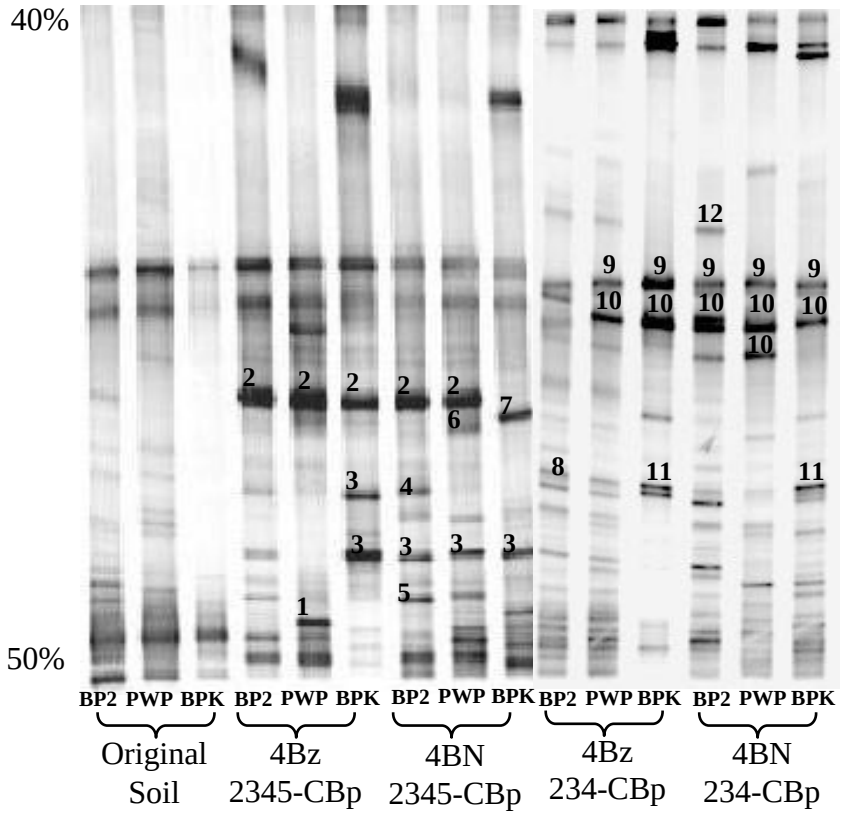


Figure E-2-1 The PCR-DGGE profiling of PCB dechlorinating cultures.

aromatic compounds in contaminated soil. The dechlorination of 234-CBp, DNA bands were composed of 8, 9, 10, 11 and 12, whereas, bands 9 and 10 were presented the majority of DNA bands and could be detected in all samples investigated. These bands were namely *uncultured gamma proteobacterium* and *uncultured bacterium clone Z552*, respectively.

The notice point was found PCBs dechlorinating microbes in BPK, which represented as less-polluted area but still observed PCBs dechlorinaing microbes and effectively dechlorination. These results strongly evidence that PCB dechlorinating microbes were prevalence in every sites investigated; hence, the dechlorination could not be occurred might be because of unsuitable condition and/or de-active PCBs dechlorinators. However, the *Chloroflexi phylum*, which well known to contain PCB-

dechlorinating strain, were not detected in this study (Fennell et al., 2004). It is imply that the specified primers to increase the sensitivity of PCR were required.

Table E-2-1 Phylogenic analysis of DGGE bands quoted from BP2, PWP and BPK primed with 4-MBZ and 4-BN

Band[a]	Closest relative	Accession number	% Identity	Phylum/ Class
1	Uncultured Anaerobranca sp. clone SRB2	DQ069229	94	*Firmicutes*
2	Uncultured gamma proteobacterium clone Aerocomp_NB39 16S ribosomal	FJ754851	90	*Proteobacteria*
3	Uncultured bacterium clone AR018 16S ribosomal RNA gene, PCB dechlorinating bacterial communities in river sediment	GQ860186	98	*Proteobacteria*
4	Uncultured bacterium partial 16S rRNA gene	AJ621948	94	*Fimicutes*
5	Uncultured Firmicutes bacterium clone Z273MB13 16S ribosomal RNA gene	FJ484645	100	*Firmicutes*
6	Pseudomonas putida strain c204 16S ribosomal RNA gene	FJ950581	83	*Proteobacteria*
7	Uncultured bacterium clone 3-2 16S ribosomal RNA gene	GQ324229	94	*Proteobacteria*
8	Uncultured bacterium clone Er-MS-11 16S ribosomal RNA gene, Effects of chemical structure and concentration on the pathways and microbial communities during dechlorination of coplanar PCBs in sediment slurries	EU542432	84	*Fimicutes*
9	Uncultured gamma proteobacterium clone Aerocomp_NB39 16S ribosomal RNA gene	FJ754851	94	*Proteobacteria*
10	Uncultured bacterium clone Z552 16S ribosomal RNA gene	AY979304	92	*Fimicutes*
11	Uncultured bacterium clone AR062 16S ribosomal RNA gene, PCB dechlorinating bacterial communities in river sediment	GQ860277	96	*Proteobacteria*
12	uncultured Clostridium sp.	FJ609997	88	*Firmicutes*

Note: [a]: The DNA bands were separated in Fig.E-2-1

E-2-1-6 CONCLUSIONS

In conclusion, it was found that in long-term storage sediments could de-active the degradation of PCBs. Moreover, the results suggested the congeners of PCBs had been affected on the dechlorinating microbes rather than primers amended. The majority groups of PCBs dechlorinating microorganisms were included; *Proteobacteria* and *Firmicutes* classes.

E-3 Effectiveness of Bioaugmentation on 234- and 2345-CBp Dechlorination.

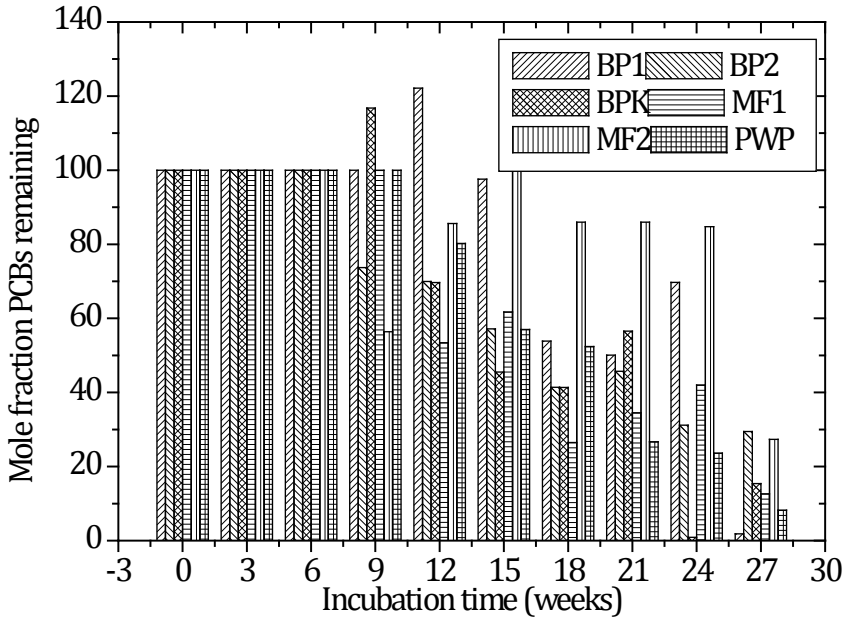


Figure E-3-1 2345-CBp mole fraction dechlorination patterns under sediment bioaugmentation condition by mixing 5g (100%) of ineffective sites with 5 ml of effective sediment slurry (HLP).

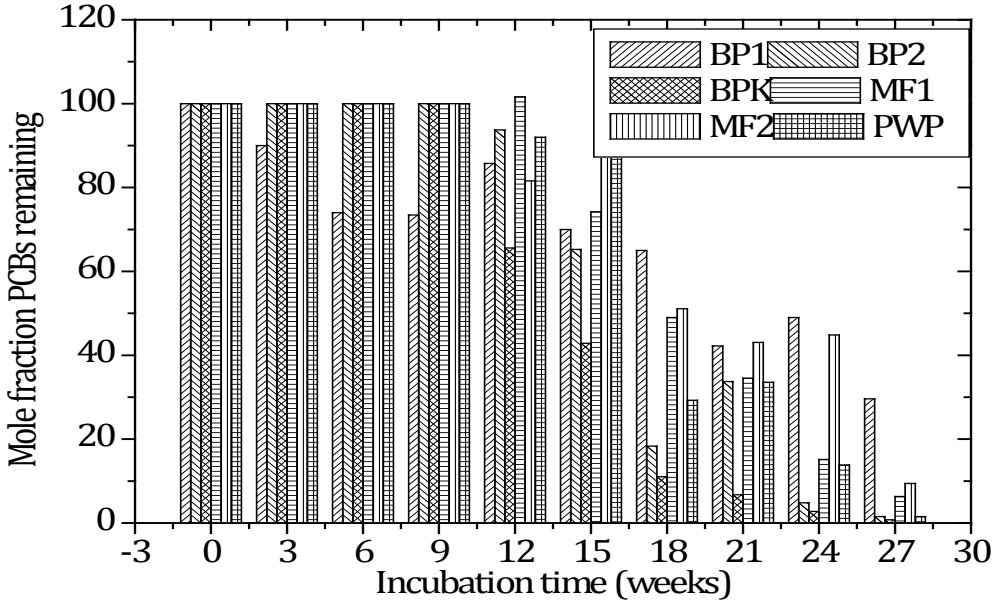


Figure E-3-2 2345-CBp mole fraction dechlorination patterns under sediment bioaugmentation condition by mixing 4.5g (90%) of ineffective sites and 0.5g (10%) of effective sites (HLP) with 5 ml of effective stream water (HLP).

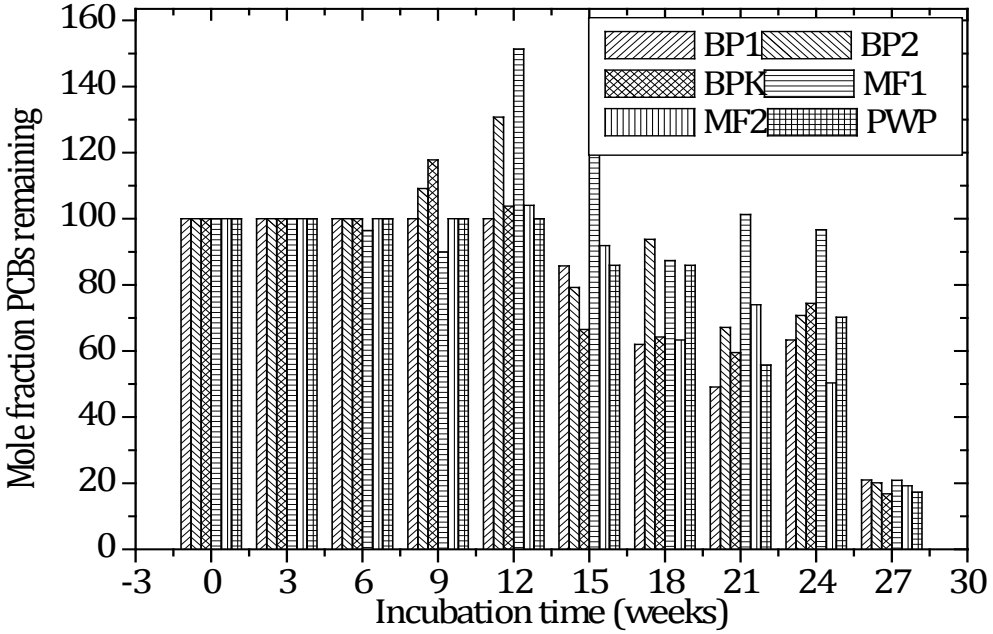


Figure E-3-3 2345-CBp mole fraction dechlorination patterns under sediment bioaugmentation condition by mixing 2.5g (50%) of ineffective sites and 2.5g (50%) of effective sites (HLP) with 5 ml of effective stream water (HLP).

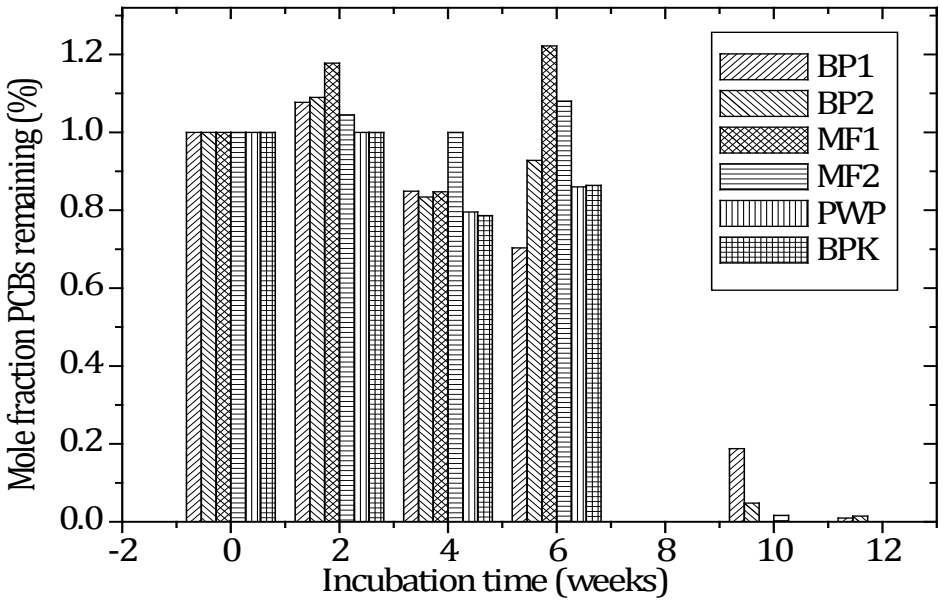


Figure E-3-4 234-CBp mole fraction dechlorination patterns under sediment slurry bioaugmentation condition by mixing 25 ml (50%) of ineffective sites and 25 ml (50%) of effective sites (HLP).

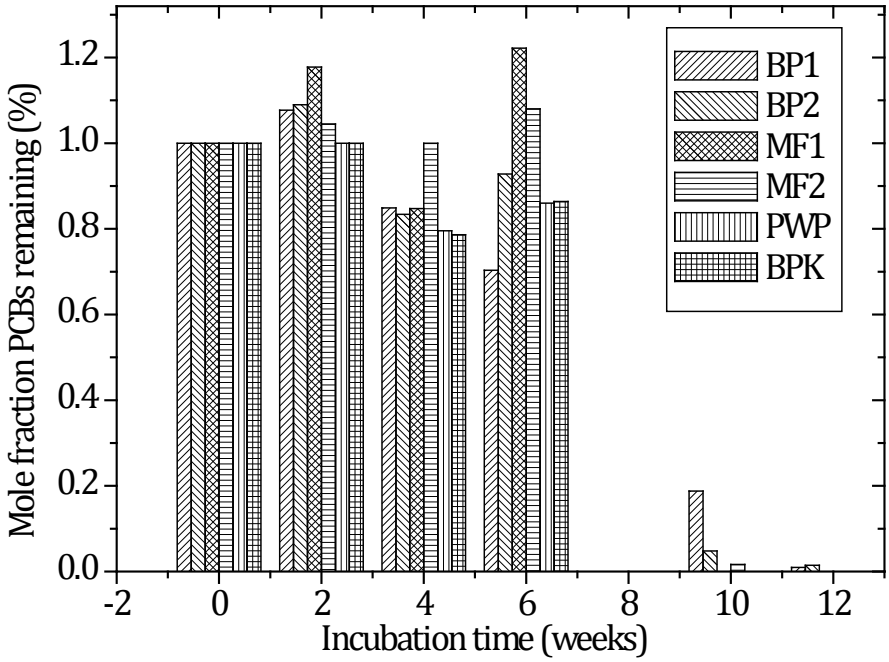


Figure E-3-5 234-CBp mole fraction dechlorination patterns under sediment slurry bioaugmentation condition by mixing 5 ml (10%) of ineffective sites and 45 ml (90%) of effective sites (HLP).

E-4 The Dechlorination Tables of Investigation of Effect of Temporary Heat Treatment on PCBs Dechlorination.

Table E-1 Concentration of dechlorination of 234-CBp in BP1 by providing 50^{o}C in various temporary heating times, (mg/kg).

Weeks	For 5 minutes		For 20 minutes		For 1 hour		For 4 hours	
	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp
0	3.87	0	3.08	0	2.97	ND	3.84	ND
3	1.38	0	2.18	0	1.31	ND	2.22	ND
6	1.82	0	3.02	0	1.94	ND	3.29	ND
9	1.81	0	2.64	0	2.84	ND	3.16	ND
13	1.50	0	2.31	0	3.09	ND	2.85	ND
17	1.96	0	1.33	0.55	1.77	ND	1.91	ND
25	0.82	1.533	0.60	0.96	1.71	ND	1.63	ND

Note: ND: the dechlorination was not detected during incubation period 25 weeks.

Table E-2 Concentration of dechlorination of 234-CBp in HLP site6 by providing 50^{o}C in various temporary heating times, (mg/kg).

Weeks	For 5 minutes		For 20 minutes		For 1 hour		For 4 hours	
	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp
0	2.90	0.00	2.73	0.00	2.18	ND	2.39	ND
3	0.98	0.00	1.82	0.00	1.49	ND	1.42	ND
6	1.88	0.00	2.71	0.00	1.94	ND	1.94	ND
9	1.58	0.00	1.64	0.00	1.94	ND	2.36	ND
13	4.36	0.00	1.95	2.16	2.62	ND	2.15	ND
17	0.29	2.81	2.02	2.05	1.19	ND	2.23	ND
25	0.20	1.97	0.09	2.76	1.45	ND	1.41	ND

Note: ND: the dechlorination was not detected during incubation period 25 weeks.

Table E-3 Concentration of dechlorination of 234-CBp in MF site 2 by providing 50^{o}C in various temporary heating times, (mg/kg).

Weeks	For 5 minutes		For 20 minutes		For 1 hour		For 4 hours	
	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp
0	2.74	0.00	4.00	ND	4.64	ND	4.60	ND
3	2.07	0.00	2.45	ND	2.96	ND	1.42	ND
6	3.72	0.00	3.39	ND	2.85	ND	2.90	ND
9	3.34	0.00	3.20	ND	1.98	ND	3.52	ND
13	1.53	0.00	3.49	ND	3.64	ND	3.84	ND
17	1.72	0.00	1.57	ND	0.94	ND	1.49	ND
25	1.35	0.18	1.29	ND	2.10	ND	1.88	ND

Note: ND: the dechlorination was not detected during incubation period 25 weeks.

Table E-4 Concentration of dechlorination of 234-CBp in BPK by providing 50^{o}C in various temporary heating times, (mg/kg).

Weeks	For 5 minutes		For 20 minutes		For 1 hour		For 4 hours	
	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp	234CBp	24CBp
0	1.02	0	1.59	ND	2.50	ND	2.00	ND
3	0.57	0	0.77	ND	2.27	ND	1.23	ND
6	0.89	0	1.16	ND	2.32	ND	1.47	ND
9	1.37	0	1.67	ND	1.67	ND	1.42	ND
13	2.25	0	0.18	ND	1.45	ND	2.55	ND
17	1.29	0	1.09	ND	1.19	ND	2.64	ND
25	0.69	1.6425	1.54	ND	1.07	ND	2.63	ND

Note: ND: the dechlorination was not detected during incubation period 25 weeks.

Table E-5 Concentration of 234-CBp by providing 70^{o}C for 5 minutes.

Weeks	Concentration (mg/kg), temporary heat for 5 minutes*			
	BP1	HLP6	MF2	BPK
0	2.70	3.60	5.06	1.74
3	1.82	1.93	2.09	2.36
6	2.32	1.31	3.14	1.90
9	2.30	1.46	2.80	1.33
13	2.09	1.33	2.55	1.21
17	1.92	1.57	1.68	1.55
25	2.85	3.75	1.93	2.63

Note: *: the dechlorination could not happen.

Table E-6 the concentration of 234-CBp by providing 70^{o}C for 20 minutes.

Weeks	Concentration (mg/kg), temporary heat for 20 minutes*			
	BP1	HLP6	MF2	BPK
0	3.62	4.00	5.12	1.29
3	1.48	1.30	1.95	1.17
6	2.26	2.13	3.19	1.05
9	2.64	1.83	3.16	1.62
13	3.09	2.31	1.30	1.25
17	1.83	1.13	1.06	1.30
25	2.23	1.13	3.94	1.21

Note: *: the dechlorination could not happen.

Table E-7 the concentration of 234-CBp by providing 70^{o}C for 1 hour.

Weeks	Concentration (mg/kg), temporary heat for 1 hour*			
	BP1	HLP6	MF2	BPK
0	3.28	2.62	4.48	1.58
3	2.33	1.74	3.11	1.91
6	2.73	2.48	2.42	1.33
9	1.39	1.50	3.12	1.75
13	2.44	1.80	2.91	1.58
17	1.04	1.06	1.68	1.64
25	2.57	1.43	1.81	1.27

Note: *: the dechlorination could not happen.

Table E-8 the concentration of 234-CBp by providing 70^{o}C for 4 hours.

Weeks	Concentration (mg/kg), temporary heat for 4 hours*			
	BP1	HLP6	MF2	BPK
0	4.06	3.06	2.74	2.95
3	2.18	1.66	1.46	2.45
6	1.80	1.81	3.79	1.59
9	2.22	1.60	2.74	2.20
13	3.09	1.84	4.82	2.00
17	1.09	1.38	1.09	2.08
25	1.59	1.26	2.25	2.25

Note: *: the dechlorination could not happen.

Table E-9 the concentration of 234-CBp by providing 90^{o}C for 1 minute.

Weeks	Concentration (mg/kg), temporary heat for 1 minutes*			
	BP1	HLP6	MF2	BPK
0	2.64	1.23	4.88	1.94
3	2.24	2.60	3.29	1.20
6	1.07	2.13	1.25	1.60
9	2.34	2.10	3.68	1.58
13	2.60	1.67	3.09	1.87
17	1.96	1.05	2.12	1.96
25	1.88	1.16	1.54	1.81

Note: *: the dechlorination could not happen.

Table E-10 the concentration of 234-CBp by providing 90^{o}C for 5 minutes.

Weeks	Concentration (mg/kg), temporary heat for 5 minutes*			
	BP1	HLP6	MF2	BPK
0	2.42	1.62	4.68	2.18
3	4.36	2.65	3.82	2.18
6	1.57	1.47	1.51	1.64
9	2.00	2.66	1.82	1.19
13	2.44	1.41	3.51	1.73
17	2.26	1.07	1.53	1.14
25	1.88	0.79	2.63	1.83

Note: *: the dechlorination could not happen.

Table E-11 the concentration of 234-CBp by providing 90^{o}C for 20 minutes.

Weeks	Concentration (mg/kg), temporary heat for 20 minutes*			
	BP1	HLP6	MF2	BPK
0	2.14	4.06	4.98	1.67
3	3.55	1.54	3.44	1.21
6	1.45	1.43	2.50	1.01
9	2.58	2.74	3.96	1.23
13	2.00	1.29	3.16	1.43
17	2.36	1.34	1.89	1.45
25	2.31	1.33	1.43	0.81

Note: *: the dechlorination could not happen.

Table E-12 the concentration of 234-CBp by providing 90^{o}C for 1 hour.

Weeks	Concentration (mg/kg), temporary heat for 1 hour*			
	BP1	HLP6	MF2	BPK
0	3.00	2.68	6.70	2.60
3	3.20	3.24	4.09	1.82
6	1.39	1.44	1.33	1.23
9	2.94	2.74	5.56	1.14
13	3.04	2.04	2.91	1.89
17	1.13	1.64	1.89	0.62
25	1.50	4.88	2.53	1.05

Note: *: the dechlorination could not happen.

E-5 The Dechlorination Profiles in Study of Fate of PCBs in Simulated Environmental System.

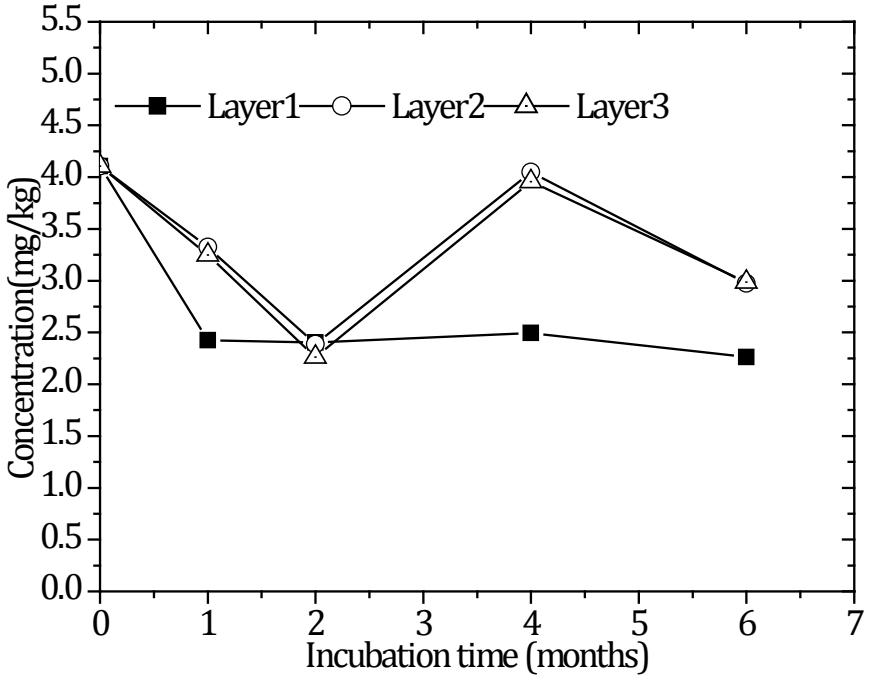


Figure E-5-1 2345-CBp dechlorination profiles under simulated aquarium boxes by indigenous microbes.

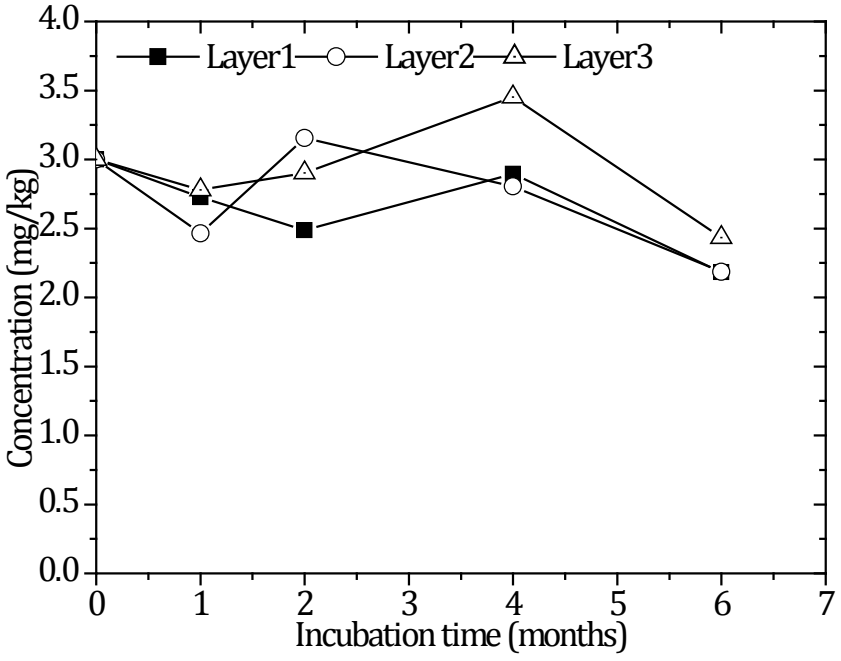


Figure E-5-2 2345-CBp dechlorination profiles under simulated aquarium boxes by amendment with yeast extract.

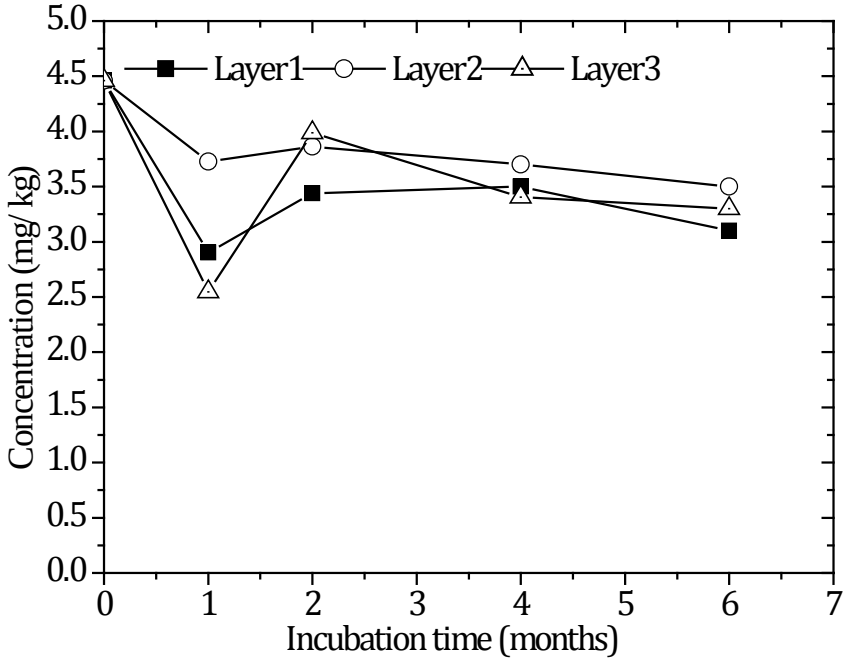


Figure E-5-3 2345-CBp dechlorination profiles under simulated aquarium boxes by amendment with sodium pyruvate.

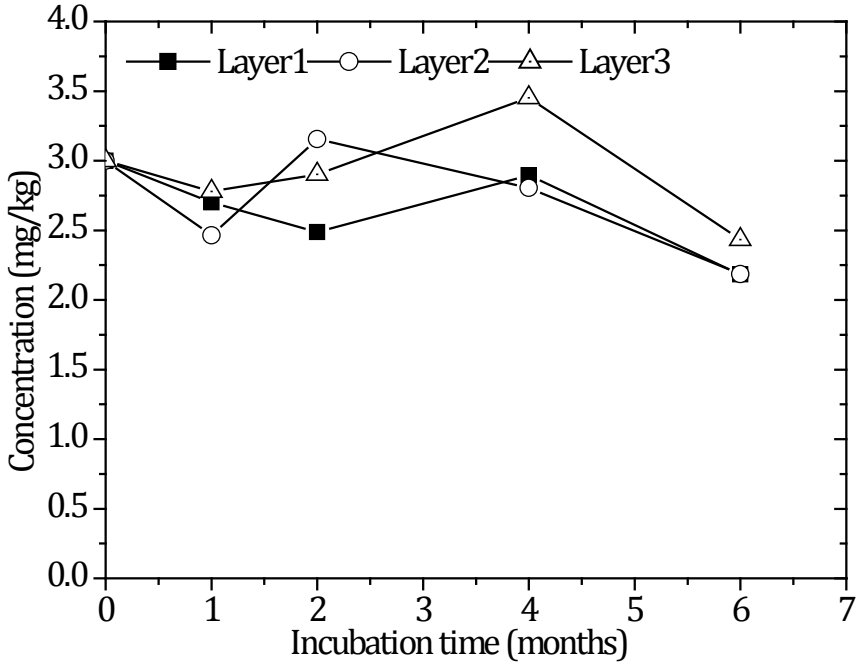


Figure E-5-3 2345-CBp dechlorination profiles under simulated aquarium boxes by amendment with sodium acetate.

BIOGRAPHY

Miss Wichidtra Sudjarid was born on January 30st, 1981 in Sakhonnakorn, Thailand. She received her Bachelor's and Master's degree in Environmental Engineering from Department of Environmental Engineering, King Mongkut's University of Technology Thonburi, Thailand in 2005 and 2007, respectively. She pursues her Philosophy of Doctoral Degree studies in the International Program in Environmental Management (Hazardous Waste Management), Interdisciplinary Program, Graduate School, Chulalongkorn University, Bangkok, Thailand on May 2007. She finished her Degree of Doctor of Philosophy Program in Environmental Management in April, 2011.