ATTENUATION OF POLYCHLORINATED BIPHENYLS UNDER ANAEROBIC CONDITIONS

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ABSTRACT

ATTENUATION OF POLYCHLORINATED BIPHENYLS (PCBs) UNDER ANAEROBIC CONDITIONS

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Polychlorinated biphenyls (PCBs) are toxic and persistent anthropogenic contaminants. Concern on their adverse health effects has led to their regulation in air, water and/or soil in addition to sludge. Hence, removal of PCBs in various matrices, including transformer oils (TO) is a priority. This study aims to investigate PCB-118 and Aroclor 1254 toxicity and dechlorination by varying certain critical experimental components including electron donor (sludge or fatty acids), inocula (unacclimated or acclimated culture) and the doses of PCB and TO under anaerobic conditions. Anaerobic toxicity assays (ATA) reactors, lab-scale anaerobic batch digesters and sediment microcosms were used for this purpose. Increase in PCB-118 and TO doses affected anaerobic digester performance by negatively influencing methanogenesis, while favoring dechlorination only with the increase in PCB-118 dose. Up to 22% PCB-118 removal was attained with unacclimated culture. Studies with acclimated cultures showed Grasse River (GR) sediment to be the most active when compared to Fox River and Baltimore Harbor sediments. In GR sediment microcosms, PCB-118 and Aroclor 1254 removal efficiencies decreased when TO was present (1%), while 10% TO inhibited PCB dechlorination. Waste activated

sludge was shown to be an effective electron donor, similar to fatty acids. Aroclor 1254 dechlorination was dechlorinated through removal of flanked *meta* and *para* chlorines, however, dechlorination pathways appeared to differ according to the presence/absence of TO. No *ortho* or unflanked chlorines were removed. Molecular tools (qPCR and DHPLC) were used to confirm the presence of active PCB dechlorinators. Dechlorination of PCBs was shown to be growth-linked.

Keywords: Anaerobic Digestion, Aroclor 1254, Molecular Tools, PCB 118, Transformer Oil

ÖZ

ANAEROBİK ŞARTLAR ALTINDA POLİKLORLU BİFENİLLERİN (PCBler) GİDERİMİ

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Poliklorlu bifeniller (PCBler) toksik ve insan kaynaklı kalıcı kirleticilerdendir. PCBlerin sağlık üzerindeki olumsuz etkilerinden kaynaklanan endişeler, onların çamurun yanı sıra havada, suda ve/veya topraktaki miktarlarının sınırlandırılmasına dair düzenlemelere yol açmıştır. Böylece, PCBlerin çeşitli matrislerden, özellikle de trafo yağlarından giderimi öncelik kazanmıştır. Bu çalışmanın amacı, PCB-118 ve Aroklor 1254'un klorsuzlaştırılması, bunlardan kaynaklı toksisitenin belirlenmesi ve değişen PCB ve trafo yağı (TO) dozlarının, elektron donör (çamur veya yağ asitleri), bakteri kültürü (aklime veya aklime olmayan kültür) gibi çeşitli kritik deneysel öğelerin etkilerinin anaerobik koşullar altında araştırılmasıdır. Bu sebeple, anaerobik toksisite testi (ATA) reaktörleri, laboratuvar ölçekli anaerobik kesikli reaktörler ve sediman mikrokozmları kurulmuştur.

PCB-118 ve TO dozundaki artışın metanojenlerin aktivitesi üzerindeki etkisi negatif iken, sadece PCB-118'in dozundaki artış klorsuzlaştırılmasında pozitif yönde katkı sağlamıştır. Aklime olmayan kültür eklenen çamur reaktörlerinde, PCB-118 giderimi en çok %22 bulunmuştur. Aklimasyon çalışmaları, Grasse River (GR) sedimanının,

Baltimore Harbor ve Fox River'a nazaran daha aktif olduğunu göstermiştir. GR reaktörlerinde, PCB-118 ve Aroklor 1254 giderim verimi %1 TO varlığında önemli olçüde düşüş gösterirken, %10 TO varlığında giderim tamamen durmuştur. Atık çamurun da yağ asitlerine benzer şekilde etkin bir eletron donör olduğu gözlenmiştir. Aroklor 1254'ün klorsuzlaştırılmasında, bakterilerin özellikle iki yanı ve/veya tek yanı klorlarla çevrili *meta* ve *para* konumlarındaki klorları hedeflediği gözlenmiş, TO varlığı ve yokluğunun klorsuzlaştırma yollarında farklılığa sebep olduğu bulunmuştur. *Ortho* veya etrafı diğer klorlarla çevrili olmayan klorlarda giderim gözlenmemiştir. Moleküler araçlar (qPCR ve DHPLC gibi) kullanılarak, PCBlerin parçalanmasında rol alan bakterilerin varlığı ispatlanmış ve klorsuzlaştırmanın bakterilerin büyüme hızıyla ilişkili olduğu tespit edilmiştir.

Anahtar Kelimeler: Anaerobik Stabilizasyon, Aroklor 1254, PCB 118, Moleküler Araçlar, Trafo Yağı

To my grandma, Derdi,
and to my brother, Zafer

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“Oysa defalarca sormuşlardı:
Büyüyünce ne olacaksın diye;
"Mutlu!". diyemedik.
Çünkü, çocuktuk; akıl edemedik....”

Nazim Hikmet Ran.

“Even though being asked many times:
What we are going to be when we grow up;
We could not say ‘Happy!’.
Because we were kids; we could not think of it…”

Nazim Hikmet Ran.

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CHAPTER 1

INTRODUCTION

1.1. General

Polychlorinated biphenyls (PCBs) are one of the most toxic and persistent man-made organic compounds formulated as biphenyl rings with various numbers of chlorine atoms substituted onto its carbon positions. Theoretically, there are 209 possible PCB compounds, called congeners (ranging from mono- to deca-chlorinated isomers), in commercial mixtures called Aroclors (Monsanto, USA and UK), Clophen (Bayer, Germany), or Kaneclor (Kanegafiichi, Japan) among the other names (Johnson *et al*., 2006). PCBs were produced by catalytic chlorination of biphenyls with anhydrous chlorine in the presence of a catalyst. The names and chemical properties of PCB mixtures depend on their degree of chlorination (UNEP, 1999).

Advantageous physico-chemical properties of PCBs, such as their heat stability, low chemical reactivity, non-flammability and high electrical resistance, enabled them to be widely used as dielectric fluids for transformers and capacitors, heat transfer fluids, sealants, as hydraulic lubricants, adhesives, cutting oils, flame retardants, paints, and in carbonless copy paper (Bedard and Quensen III, 1995). Transformer and capacitor oils commonly contain mixtures of chloro-benzenes with mostly Aroclor 1254 or 1260 (Erickson, 1997). Aroclor 1254, one of the mixtures, is a light yellow viscous liquid, consisting mostly of penta- (49%) and hexa-chlorinated PCB congeners (28%). Total chlorine content of Aroclor 1254 is 54% by weight (Hansen, 1999; Field and Sierra-Alvarez, 2007). Aroclor 1254 was primarily used in transformers as hydraulic fluids, in synthetic resins and as anti-dusting agents. According to the Monsanto (global market leader of worldwide PCB production)

sales record, Aroclor 1254 was one of the most produced and sold Aroclor mixture (Johnson *et al.*, 2006). As a result, Aroclor 1254 can be regarded as one of the most prominent PCB mixtures to be discharged into the environment.

Due to the toxicity of PCBs, they are classified as persistent organic pollutants and their production was banned by the U.S. Congress in 1977 and by the Stockholm Convention on Persistent Organic Pollutants in 2001 (Breivik *et al.*, 2007). However, due to their extensive production and continuing use in existing transformers, capacitors, heat exchangers and hydraulic equipment, PCBs are still on the earth. It was reported by Patureau and Trably (2006) that about 10 million tones, equivalent to 1/3 of the total worldwide production of PCBs, had been released into the environment.

Waste motor and transformer oils are considered the main sources of PCB emissions into the environment. Jones *et al.* (2003) reported that about 125 million transformers containing PCBs were in use as of 1999 in USA. Approximately, 61% of PCBs still in use in electrical transformers, about 12% in electrical capacitors and 27% in storage waiting for disposal constitute significant sources of potential risk. PCBs have been released into the environment through improper burning or incomplete incineration; by vaporization from paints, coatings and plastics; by direct entry or leakage into sewers and streams; landfills; and by accidental spills or by improper disposal of PCB containing materials (Tagasuka *et al.*, 2006).

PCB residues in soil, sediments and sewage sludge contribute to the overall environment pollution. There are numerous studies reporting the presence of PCBs in sewage sludge (Moreda *et al.*, 1998; Stevens *et al.*, 2001; Nakhla *et al.*, 2002; Patureau and Trably, 2006; Harrison *et al.*, 2006; Benabdallah El-Hadj *et al.*, 2007). PCB content of sewage sludge is typically around l–10 mg/kg with an elevated portion of highly chlorinated PCBs (Benabdallah El-Hadj *et al.*, 2007). The detection of PCBs in sludge is a health concern. Due to this concern, the European Union have started to regulate the PCB contents in sewage sludge before spreading it on land by

in setting limits for the EU Sewage Sludge Directive (Patureau and Trably, 2006). Concern on their adverse health effects in living organisms and other significant environmental concerns due to their suspected toxic and carcinogenic properties, as well as endocrine disruptive effects has led the European Union and many other countries to regulate the PCB contents in air, water and/or soil in addition to sludge.

Although PCBs were never produced in Turkey, transformers/capacitors containing oil with PCBs are currently known to be used in the production/transmission of electricity (UNIDO, 2002). Therefore, in Turkey, ratifying the Stockholm Convention in January 2010, together with the Regulation on Control of Equipments Containing PCB and polychlorinated terphenyls (PCTs), Turkish Ministry of Environment and Urbanization is held responsible for eliminating the use of in-place equipment containing PCBs by 2025. The Ministry is also responsible for preparing inventories, identifying polluted sites, and making efforts to accomplish environmentally friendly management of wastes with PCB amounts greater than 50 ppm and remediation of contaminated sites by 2028. It is required to prepare and submit progress reports in eliminating PCBs to the governing body of the Convention upon becoming a part of the Convention every 5 years (Stockholm, 2012).

Disposal of PCB contaminated transformer oils has been an crucial task for decades. Depending on the type and the source of contamination, there are several physicochemical and biological treatment options for PCBs. However, physico-chemical techniques are either expensive or may generate undesirable, toxic by-products, including dioxins and furans, while biological degradation alters the structures of PCB congeners to more biodegradable chemicals and may present a more environmentally friendly solution (Abramowicz, 1995).

Mainly, research on microbial PCB biodegradation has focused on contaminated soils and sediments. Despite their resistance to biodegradation, microbially mediated anaerobic reductive dechlorination of PCBs has been shown in a variety of anaerobic

sediments (Brown *et al.*, 1984; Abramowicz, 1990; Bedard *et al.*, 1986; Bedard and Quensen III, 1995; Wu *et al.*, 1998; Fava *et al.*, 2003; Kjellerup *et al.*, 2008; Payne *et al.*, 2011). It has been shown that anaerobic microbial populations are responsible for anaerobic dechlorination of PCBs thus reducing their toxicity via various reductive dechlorination mechanisms in which electron-donating substrates are present (Berkaw *et al.*, 1996; Wu *et al.*, 1998; Fagervold *et al.*, 2005, 2007 and 2011). Dechlorination under anaerobic conditions occurs due to dehalorespiration, in which halogenated compounds are served as terminal electron acceptors. As a result, an excess of a carbon source (electron donor) is needed for efficient dechlorination (Tartakovsky *et al.*, 2000).

Limited information about anaerobic reductive dechlorination of PCBs in the presence of transformer oil is available. In literature, mainly, physico/chemical dechlorination of PCBs in oil was studied (Wu *et al.*, 2005; Cafissi *et al.*, 2007). There are not many biological especially anaerobic dechlorination studies about PCBs in oil. The ones which investigated dechlorination of PCBs in oil either tested aerobic conditions and/or used commercial microorganisms or an easily degradable carbon source as an electron donor (Cedzynska and Sobiecka, 2004; Ahn *et al.*, 2006; Sobiecka *et al.*, 2009). Microbial reductive dechlorination of spiked Aroclor 1254 under anaerobic conditions has been shown in several studies (Quensen III *et al.*, 1990; Nies and Vogel, 1990; Oefjord *et al.*, 1994; Natarajan, 1998; Pakdeesusuk *et al.*, 2003, Zanaroli *et al.*, 2012) but these studies either did not investigate PCB dechlorination in the presence of transformer oil or were not extensive. Besides, either their dechlorination rate was slow and/or the lag period of dechlorination was long, as well as they generally used some easily degradable chemical as a carbon source, such as acetate, acetone, methanol or glucose.

There are few studies investigating PCB dechlorination during anaerobic digestion of activated sludge (Ye *et al.*, 1992; Phelps *et al.*, 1996; Chang *et al.*, 2002; Fava *et al.*, 2003). Anaerobic digestion is a commonly applied treatment option for waste activated sludge (WAS) stabilization because of its abilities to transform organic

wastes into biogas (60-70 vol% of methane, CH_4), and to bring out the potential of using the biogas as energy source. At the same time, digestion reduces the amount of organics and hence the ultimate sludge solids for disposal, and destroys most of the pathogens present in the sludge and controls odor problems. The European Union has put an objective to increase the amount of energy obtained from renewable sources from the 2005 level of 8.5% to 20% in 2020 (Mottet *et al.*, 2010).

1.2. Motivation and purpose of the study

A limited amount of work has been done on the anaerobic dechlorination of PCBs in the presence of transformer oil; especially using WAS as the electron donor for the dechlorination of PCBs. Hence, this study aims to investigate the degradation of PCBs in the presence of WAS and transformer oil under anaerobic conditions. To achieve this goal, several sub-goals and stepwise approaches including acclimated (exposed) or unacclimated (unexposed) cultures, among others have been developed, which are described below:

1. To investigate toxicity and toxic dose of PCBs using a model PCB congener, namely PCB 118 and transformer oil (TO), on the methane producing bacteria through anaerobic toxicity assay (ATA),

2. To investigate PCB 118 dechlorination and the effect of varying doses of PCB 118 in the presence of TO on sludge digester performance in laboratory scale anaerobic batch reactors,

3. To enrich indigenous putative dechlorinating bacteria from three historically impacted sediments, namely, Grasse River (GR), Fox River (FR) and Baltimore Harbor (BH) in USA, for PCB 118 and Aroclor 1254 dechlorination and pathway identification,

4. To determine specific dechlorination rates of single congeners for inferring clear parent-daughter congener relationships and identify the specific reductive dechlorination pathways of Aroclor 1254 by using GR sediment culture,

5. To investigate the dechlorination of PCB 118 and Aroclor 1254 in the presence of TO and an easily degradable (compared to WAS) carbon source, i.e. fatty acid mixture, by using GR sediment culture (acclimated) transferred and enriched in the previous step,

6. To investigate the dechlorination potential of PCB 118 and Aroclor 1254 in laboratory scale digesters with WAS as the electron donor (complex carbon source) using the acclimated culture enriched from the GR sediment.

1.3. Scope of the study

The scope of the thesis to fulfill the given objectives is structured as follows:

1. PCB118 is selected as a model congener since it is a toxic congener found abundantly in Aroclor 1254, a mixture that is widely used in transformer oil (TO) formulations. ATA is used to assess the toxicity of various doses of PCB 118 (0, 1, 10, 20, and 30 mg/L) and TO (0, 0.38, 0.76, and 1.52 g/L) on the anaerobic biodegradability of sludge. Digestion performance is analyzed by cumulative methane production. PCB 118 dechlorination is also investigated in ATA microcosms. The toxic dose of PCB 118 affecting the small-scale digester is determined. The inoculum used in this experiment is unacclimated mixed anaerobic culture. The study was conducted in METU Laboratories (Ankara, Turkey) and the results of this part are given under Chapter 4.

2. Larger scale lab-digester performance in the presence of PCB 118 and TO is investigated. A dose of TO determined from the previous step (ATA) is used

in this part and added as co-contaminant with PCB 118 into the anaerobic digesters. Two doses of PCB as 1 mg/L (low-level) and 20 mg/L (high-level) are used in this experiment. WAS serves as the carbon source. The inoculum used in this experiment is unacclimated mixed anaerobic culture. The study was conducted in METU Laboratories (Ankara, Turkey) and the results of this part are given under Chapter 5.

3. Three historically impacted site sediments (GR, FR, and BH) are incubated with PCB 118 or Aroclor 1254 to compare the dechlorinating activity of each sediment culture. The sediments, used as inocula, are accepted as acclimated cultures since all these sites were historically contaminated with PCBs. Fatty acid mixture serves as the carbon source. Molecular tools including enumeration by qPCR and DHPLC are used to monitor growth of dechlorinating culture and the change in the diversity of dechlorinating cultures during dechlorination. The most active culture is selected for subsequent experiments. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA) and the results of this part are given under Chapter 6.

4. The most active culture determined in the previous experiment is used as an inoculum (which is an acclimated culture) for dechlorination of Aroclor 1254 and individual PCB congers selected from Aroclor 1254. The 22 most predominant congeners in Aroclor 1254 are incubated separately with the GR sediment. Individual pathways are elucidated, and dechlorination rates are determined. By using qPCR, the relationship of dechlorination to microbial growth is determined. The relationship between predominant dechlorinating phylotypes of this study and well-defined organisms is investigated. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA) and the results of this part are given under Chapter 7.

5. The effect of doses of TO (0, 1%, and 10%, v/v) on the dechlorination of PCB 118 and Aroclor 1254 is investigated in the presence of an easily degradable carbon source, fatty acid mixture. PCB 118 and Aroclor 1254 dechlorination profile is determined. A mixture of active culture transferred from the previous set of GR and the original GR sediment is used as an acclimated culture/inoculum. Growth of dechlorinating culture is followed by using qPCR, and the relationship of dechlorination to microbial growth is assessed. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA) and the results of this part are given under Chapter 8.

6. The dechlorination of PCB 118 and Aroclor 1254 along with TO in small scale sludge digesters is investigated. WAS acts as the electron donor, giving ‘information about the behavior of a complex carbon source. A mixture of active culture transferred from previous set of GR and original GR sediment is used as an acclimated culture/inoculum. Molecular tools such as 16S rRNA based qPCR and DHPLC as well as sequencing are used to characterize, quantify and dechlorinating bacteria. The presence of dechlorinating bacteria is validated and its relations to the well-known organisms are presented by constructing a phylogenetic tree. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA) and the results of this part are given under Chapter 8.

1.4. Organization of thesis

This thesis consists of ten chapters. Chapters 1, 2 and 3 include introduction, literature review and material and methods, respectively. Mainly, Chapters 4, 5, 6, 7 and 8 discuss experiments and their findings. Chapter 9 summarizes major conclusions and Chapter 10 includes suggestions for further studies following this study. Appendices present supplementary information for experimental procedure

that includes calibration curves, supporting figures, tables for discussion of the results and some other information about PCBs.

This dissertation is organized as a collection of manuscripts that have been either submitted or will be submitted for publication. For this reason, some repetition may appear in the introduction and material and methods sections of Chapters 4, 5, 6 7, and 8.

CHAPTER 2

LITERATURE REVIEW

2.1. Polychlorinated Biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) belong to the halogenated aromatic group of environmental contaminants found in diverse environmental matrices (Safe, 1994). PCBs are composed of two benzene rings connected at the C-1 carbon (Figure 2.1). Each benzene ring can have up to 5 chlorine substituents in the *ortho*, *meta*, or *para* positions (Wiegel and Wu, 2000); positions 2, 2', 6, and 6' are called *ortho* positions, positions 3, 3', 5, and 5' are called *meta* positions, and positions 4 and 4' are called *para* positions (Figure 2.1)..

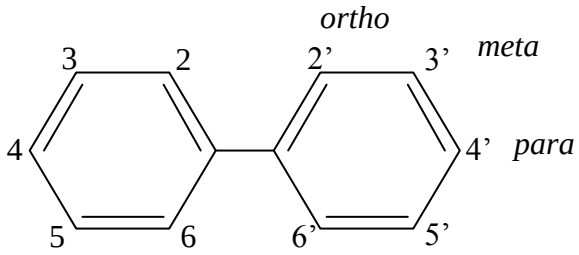


Figure 2.1. Structural formula of PCB showing chlorine attachment positions on the biphenyl structure.

PCBs are found in a complex mixture (80-110 congeners) of multiple isomers (Frame, 1997). These isomers differ in chlorine number and position yielding up to 209 possible PCBs (Bedard, 2003; Borja *et al.*, 2005). Each of them is referred to as a congener (Fish K., 1996). PCB congeners having the same number of chlorine

atoms are recognized as homologs, and the homologs with different chlorine positions are named isomers (Borja et al., 2005). The individual chlorine substitutions are further classified into singly or doubly flanked and unflanked chlorines, referring to whether the neighboring positions carry chlorine substitutions or not, respectively (Wiegel and Wu, 2000), for example when there are three chlorines on both *meta*, 3 and 5, and *para*, 4, positions on one ring of the biphenyl structure, the *para* chlorine is called as doubly flanked chlorine due to the *meta* chlorines on the both adjacent positions. The *meta* chlorines on the same ring are named as flanked chlorines. On the other hand, if the other biphenyl ring has an *ortho* chlorine, it is called an unflanked chlorine.

Ballschmiter and Zell (1980) proposed a numbering system for the PCB congeners which has been adopted by the International Union of Pure and Applied Chemists (IUPAC). In degradation studies, another notation of PCBs is commonly used, where, the positions of chlorine atoms on each ring are separated with a hyphen differentiating the rings (İmamoğlu, 2001), e.g. IUPAC no. 28 has chlorines on the positions of 2 and 4 on the first ring and another chlorine at position 4 on the other ring; hence, the designation of congener 28 is 24-4. The complete list of congeners indicating the numbering and structure of PCBs is given in Table A1 (Appendix A). In this study, the PCB congeners are referred either with a "PCB" sign or "CB" which stands for chlorinated biphenyls, in front of their numbers or following their numbers), indicating the IUPAC number of the corresponding congener.

PCBs were classified with respect to percentage of their chlorine content (by weight) and were marketed under a number of trade names. These include Aroclor and Pyroclor (USA), Clophen (Germany), Fenclor (Italy), Fenoclor (Spain), Kanechlor (Japan) and Phenoclor and Pyralene (France) (Wiegel and Wu, 2000). Aroclor mixtures are specified with a four-digit code. The first two digits indicate the type of mixture and/or number carbons in biphenyl and the last two digits indicate the approximate chlorine content by weight percent. Thus, Aroclor 1248 is a chlorinated biphenyl mixture of varying amounts of mono- through hepta-chlorinated homologs

with an average chlorine content of 48%. The exception to this code is Aroclor 1016, which contains mono- through hexa-chlorinated homologs with an average chlorine content of 41% (Quensen III *et al.*, 1990).

Although commercial production of PCBs was banned in 1978 (Fagervold *et al*., 2005), they are still present on existing transformers, capacitors, heat exchangers and hydraulic equipment. The total amount of PCBs produced in the world is estimated to be 1.7 million tons (Breivik *et al.*, 2007). Unfortunately, a considerable amount has entered to the environment due to inappropriate disposal practices, accidents and leakages from industrial facilities. There is concern about the long-term effects of these compounds (UNEP, 2002).

2.2. Physicochemical Properties of PCBs

Table 2.1 presents some physical properties of Aroclors such as solubility, vapor pressure, log K_{ow}, and Henry's law. Range of water solubilities, vapor pressures, Henry's constant and Log K_{ow} values for Aroclors are given in Table 2.2. Table 2.3 contains properties for the most toxic and the most environmentally prevalent congeners. PCBs are relatively insoluble in water, with the highest solubilities among the *ortho*-chlorinated congeners. Solubility of non-*ortho* congeners decreases especially as the *para* positions are filled, resulting in greater and more uniform perimeter electronegativity and interference with hydrogen bonding. PCBs are soluble in non-polar organic solvents and biological lipids, and the shift from water to lipid solubility is shown in Table 2.2 as an increasing K_{ow} with increased chlorination. Henry's Constant (H) used to describe environmental behavior of atmospheric pollutants and it gives the ratio between vapor pressure (saturated) and the solubility of the compound in water. PCBs, especially highly chlorinated congeners, are relatively non-volatile, with partial pressures and Henry's law constants that tend to decrease with increased chlorination, especially for *meta*- and *para*-saturated congeners (WHO, 2003). Generally, melting point and lipophilicity

increase with increasing degree of chlorination; vapor pressure and water solubility decrease.

PCB concentrations and distribution in contaminated sediments are affected by physical phenomena such as sorption, desorption, dissolution, and volatilization. The composition of the PCB mixture and the characteristics of the contaminated site control the importance of these processes (Hurme and Puhakka, 1999).

Table 2.1. Physical and chemical properties of Aroclor mixtures (Mackay et al., 2006).

Aroclor mixture	MW (g/mol)	% Cl	No.of Cl molecule	Density g/cm^3 at 25 ˚C	Distillation range ˚C	Aqueous solubility range		Vapor pressure range, PL/Pa	Henry's Law constant/H ($Pa.m^3/mol$)	Log K_{ow} range
						S (g/m^3)	C_L ($mmol/m^3$)			
Aroclor 1016	257	41	3	1.33	323-356	0.22-0.84	0.856-0.216	0.06-0.2	0.06-0.2	4.4-5.8
Aroclor 1221	192	20.5-21.5	1.15	1.15	275-320	0.59-5.0	0.307-26.0	0.89-2.0	0.89-2.0	4.1-4.7
Aroclor 1232	221	31.4-32.5	2.04	1.24	290-325	1.45	6.56-2.0	0.54	0.54	4.5-5.2
Aroclor 1242	261	42	3.1	1.35	325-366	0.1-0.75	0.383-2.87	0.05-0.13	0.05-0.13	4.5-5.8
Aroclor 1248	288	48	3.9	1.41	340-375	0.1-0.5	0.347-1.74	0.0085-0.11	0.0085-0.11	5.8-6.3
Aroclor 1254	327	54	4.96	1.5	365-390	0.01-0.30	0.306-0.92	0.008-0.02	0.008-0.02	6.1-6.8
Aroclor 1260	372	60	6.3	1.58	385-420	0.003-0.08	0.00806-0.215	0.0002-0.012	0.0002-0.012	6.3-6.8

Table 2.2. Physicochemical properties of PCB homologs at 20-25ºC (Mackay et al., 1992; Erickson 1997).

PCB homolog	MW (g/mol)	Cl (%)	PCB of isomers	Vapor pressure (Pa)	Water solubility (g/m^3)	Log K_{ow}	Bioconcentration factor, BCF in fish	Evaporation rate ($g/m^2.h$)	Henry's Law constant/H ($Pa.m^3/mol$)
Biphenyl	154.2	0	1	4.9	9.3	4.3	1000	0.92	28.6
Monochloro-	188.7	19	3	1.1	4.0	4.7	2500	0.25	42.6-75.6
Dichloro-	223.1	32	12	0.24	1.6	5.1	6300	0.065	17.0-92.2
Trichloro-	257.6	41	24	0.054	0.65	5.5	1.6×10^4	0.017	24.3-92.2
Tetrachloro-	292.0	49	42	0.012	0.26	5.9	4.0×10^4	4.2×10^{-3}	1.72-47.6
Pentachloro-	326.4	54	46	2.6×10^{-3}	0.099	6.3	1.0×10^5	1.0×10^{-3}	24.8-151
Hexachloro-	360.9	59	42	5.8×10^{-4}	0.038	6.7	2.5×10^5	2.5×10^{-4}	11.9-818
Heptachloro-	395.3	63	24	1.3×10^{-4}	0.014	7.1	6.3×10^5	6.2×10^{-5}	5.40
Octachloro-	429.8	66	12	2.8×10^{-5}	5.5×10^{-3}	7.5	1.6×10^6	1.5×10^{-5}	38.1
Nonachloro-	464.2	69	3	6.3×10^{-6}	2.0×10^{-3}	7.9	4.0×10^6	3.5×10^{-6}	-
Decachloro-	498.7	71	1	1.4×10^{-6}	7.6×10^{-4}	8.3	1.0×10^7	8.5×10^{-7}	20.8

Table 2.3. Physicochemical properties of some of the most toxic and/or environmentally prevalent PCB congeners (WHO, 2003; Johnson et al., 2005)

	PCB 1	PCB 77	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 163	PCB 169	PCB 180
Chlorine substitution (IUPAC No.)	2	34-3'4'	245-2'5'	234-3'4'	245-3'4'	234-2'4'5'	245-2'4'5'	2345-3'4'	2356-3'4'	345-3'4'5'	2345-2'4'5'
MW (g/mol)	188.7	292	326.4	326.4	326.4	360.9	360.9	390.6	390.6	360.9	395.3
Molecular formula	$C_{12}H_9Cl$	$C_{12}H_6Cl_4$	$C_{12}H_5Cl_5$	$C_{12}H_5Cl_5$	$C_{12}H_5Cl_5$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_4Cl_6$	$C_{12}H_3Cl_7$
Water solubility (mg/L at 25 °C)	4.83	0.175	1 $x10^{-2}$	0.0034	0.0134 (20 °C)	0.0159	0.00091-0.00086	0.00533	0.001195	0.000036-0.0123	0.00031-0.00656
Log K_{ow}	4.53	6.04-6.63	6.4	6.98	7.12	6.50-7.44	8.35-6.72	7.6	7.2	7.408	6.70-7.21
Vapour pressure (mmHg at 25°C)	1.38 $\times10^{-3}$	4.4 $\times10^{-7}$	8.25 $x10^{-6}$	6.531 $x10^{-6}$	8.974 $x10^{-6}$	4 $x10^{-6}$	3.8 $\times10^{-7}$	1.61 $x10^{-6}$	5.81 $\times10^{-7}$	4.02 $\times10^{-7}$	9.77 $\times10^{-7}$
Henry's law constant (atm·m3/mol at 25°C)	7.36 $\times10^{-4}$	0.43-.94 $\times10^{-4}$	-	8.25 $\times10^{-4}$	2.88 $\times10^{-4}$	0.21-.07 $\times10^{-4}$	1.3-2.78 $\times10^{-4}$	1.43× 10^{-4}	0.15 $\times10^{-4}$	0.15-0.59 $\times10^{-4}$	0.32-.07 $\times10^{-4}$

2.3. Toxic and Biochemical Effects of PCBs

The position and the number of chlorines in a particular PCB molecule determine its properties, environmental pathways and toxicity (Gdaniec-Pietryka *et al.*, 2007). PCB congeners carrying between 5 to 10 chlorine atoms mostly in the *para*- and *meta*-positions are considered as toxic congeners, however, the congeners substituted only at the 3,4, and -*ortho* positions are considered the most toxic (Borja *et al.*, 2005).

Commercial mixtures, as well as the individual PCB congeners are considered most dangerous because of their potential for "dioxin-like toxicity" (Baars *et al.*, 2004). Dioxins are organic aromatic compounds released by industrial processes, seismic emissions, or waste incineration emissions (Baars *et al.*, 2004). They can be chlorinated and are regarded as much more toxic than PCBs. The USEPA regulates dioxins as probable carcinogens, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) is considered the most toxic synthetic chemical ever produced (Halden and Dwyer, 1997). Dioxin-like PCB congeners contain two chlorines in the *para* position, at least two chlorines in the *meta* position, and at most one chlorine in the *ortho* position (Bedard, 2003). As the configuration of congeners gets closer to that of 2,3,7,8-TCDD, their potential toxicities increases. This arrangement allows the PCB molecule to rotate and assume a coplanar orientation, causing the dioxin-like behavior (Baars *et al.*, 2004). While dioxin-like PCBs are more carcinogenic, non-coplanar congeners are more disruptive of cognitive function (Faroon *et al.*, 2001). The addition of *ortho* chlorine reduces the toxicity significantly (Abramowicz, 1990). The three most toxic congeners, namely PCB 77, PCB 126, PCB 169, are found in trace quantities in Aroclor mixtures (McFarland and Clarke, 1989). However, other potentially toxic ones, such as PCB 105, PCB 118, PCB 128, PCB 138, PCB 156, and PCB 170 are present in much larger quantities in highly chlorinated Aroclors, such as Aroclor 1254 and Aroclor 1260 (Frame *et al.*, 1996; McFarland and Clarke, 1989). Tables 2.4 summarize the compositions of common Aroclor mixtures by congener prevalence and congener toxicity.

Table 2.4. Percent composition for some of the most toxic congeners in commercial Aroclors (weight %) (Frame et al., 1996).

Aroclor	**PCB 28** (24-4)	**PCB 52** (25-25)	**PCB 101** (245-25)	**PCB 105** (234-34)	**PCB 118** (245-34)	**PCB 138** (234- 245)	**PCB 153** (245-245)	**PCB 180** (2345-245)
Aroclor 1016	8.57	4.63	0.04	-	–	–	–	–
Aroclor 1221	0.62	0.22	0.07	0.05	0.08	–	-	–
Aroclor 1232	3.92	1.86	0.33	0.22	0.28	0.06	0.05	0.02
Aroclor 1242	7.31	3.64	0.78	0.52	0.66	0.16	0.09	-
Aroclor 1248	5.57	6.93	2.22	1.60	2.35	0.41	0.43	0.21
Aroclor 1254	0.19	5.38	8.02	7.37	13.59	5.95	3.77	0.67
Aroclor 1260	0.05	0.27	3.23	0.22	0.51	6.73	9.91	12.05
Aroclor 1262	0.15	0.17	1.23	0.09	0.17	3.14	7.42	14.53

Health concerns arose from PCBs suspected toxic and carcinogenic properties, as well as its endocrine disruptive effects, as well as the effects on the immune system, nervous system, reproductive system, and endocrine system (USEPA, 2008). PCBs also bioaccumulate in the food chain, with concentrations increasing by several orders of magnitude at succeeding trophic levels (Jacobson and Jacobson, 1996). Exposure to PCBs has been linked to cancer and other adverse health effects, such as reproductive deficiencies, developmental complications, immuno-suppression, and dermatological disorders in humans and animals (WHO, 2003). PCBs can be transmitted from mother to child during breast feeding (Faroon *et al.*, 2001). Since PCB pollution of the environment remains a potentially serious health threat, all congeners of PCBs must be completely removed from polluted sites available to human exposure in order to protect public health.

Since dioxin-like compounds normally exist in environmental and biological samples as complex mixtures of congeners, the concept of toxic equivalents (TEQs) has been presented for risk assessment and regulation. Applying this concept, relative

toxicities of dioxin-like compounds in relation to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (i.e. toxic equivalency factors, TEFs) are determined from *in vitro* and *in vivo* studies. For a compound to be classified as toxic; it should have structural relationship to the PCDDs and PCDFs, bind to the Ah-receptor, produce dioxin-specific biochemical and toxic responses, and be persistent and accumulate in the food chain (Safe, 1990; Barnes *et al.*, 1991; Ahlborg *et al.*, 1992). Three coplanar PCBs, i.e. 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169), show dioxin-like effects, such as Ah-receptor agonist activity (Safe, 1990; Ahlborg *et al.*, 1992). PCB 170 and PCB 180 are counted in since they are active as inducers of EROD activity and exist in significant amounts in environmental samples. Table 2.5 presents TEFs values proposed for humans, fish, and birds (the latter two for wildlife risk estimates).

2.4. Environmental Distribution of PCBs

Due to their broader use, PCBs have entered the environment through both legal and illegal use and disposal. Due to their persistence they have contaminated various environmental matrices worldwide. One-third of the total USA production of PCBs (6.35 x 108 kg) is estimated to have been released into the environment through various venues by deliberate or accidental discharges (Wiegel and Wu, 2000).

The global distribution of PCBs throughout the world, suggests that PCBs are transported in air (WHO, 1993). The ability of PCBs to co-distil, volatilize from landfills into the atmosphere (adsorption to aerosols with a particle size of < 0.05–20 μm), and their resistance to incineration at low temperatures, makes atmospheric transport the main way of global distribution. In a study in the USA, 92% of the PCBs detected were in the vapor phase (WHO, 1993).

Table 2.5. Toxic equivalency factors for coplanar and mono- and di-ortho-substituted PCBs and related values of TEFs for humans, fish, and birds.

Type	Congener Structure	PCB Congener	TEF [1]	TEF [2]		
				Humans	Fish	Birds
Non-ortho substituted	3,3',4,4'-TetraCB	PCB 77	0.0005	0.0001	0.0005	0.1
	3,3',4,4',5-PentaCB	PCB 126	0.1	0.1	0.005	0.1
	3,3',4,4',5,5'-HexaCB	PCB 169	0.01	0.01	0.00005	0.001
Mono-ortho substituted	2,3,3',4,4'-PentaCB	PCB 105	0.0001	0.0001	<0.000005	0.0001
	2,3,4,4',5-PentaCB	PCB 114	0.0005	0.0005	<0.000005	0.0001
	2,3',4,4',5-PentaCB	PCB 118	0.0001	0.0001	<0.000005	0.00001
	2',3,4,4',5-PentaCB	PCB 123	0.0001	0.0001	<0.000005	0.00001
	2,3,3',4,4',5-HexaCB	PCB 156	0.0005	0.0005	<0.000005	0.0001
	2,3,3',4,4',5'-HexaCB	PCB 157	0.0005	0.0005	<0.000005	0.0001
	2,3',4,4',5,5'-HexaCB	PCB 167	0.00001	0.00001	<0.000005	0.00001
	2,3,3',4,4',5,5'-HeptaCB	PCB 189	0.0001	0.0001	<0.000005	0.00001
Di-ortho substituted	2,2',3,3',4,4',5-HeptaCB	PCB 170	0.0001	0	0	0
	2,2',3,4,4',5,5'-HeptaCB	PCB 180	0.00001	0	0	0

[1]: Ahlborg *et al.*, 1994. [2]: WHO, 1997.

The solubility of PCBs in water decreases with increasing degree of chlorination. It changes between 6 ppm and 0.007 ppm from monochlorobiphenyl to octachlorobiphenyl, respectively. Decachlorobiphenyl, although it has higher chlorine content, has solubility twice that of octachlorobiphenyl. The solubility also varies among congeners of the same number of chlorine atom (Borja *et al.*, 2005).

Major portions of PCBs released into the aquatic environment are expected to up either adsorbed onto sediment or resting as sludges at the bottoms of rivers, lakes, and oceans and afterward they enter into the food chains via bottom living organisms. Most of the environmental data indicate that the sorption of PCBs onto suspended solids may be a significant process in the removal of these compounds from natural waters. The lipophilic nature of PCBs contributes to their tendency to accumulate in fatty deposits and thus, leads to a magnification of PCB concentrations along the food chain (Wu, 1996).

When PCBs are not bound to deeper sediment layers, they can be remobilized, and, as a consequence, could be reintroduced into the water. PCBs are characterized by average vapor-pressure values that allow their transport in the gaseous phase through the pores of the solid phase. As a result, the compounds can be transported outside the sediment layer and released into water and air (Gdaniec-Pietryka *et al.*, 2007). The transport of PCBs from sediments undergoes seasonal changes. In summer, due to the temperature increase, more PCBs are released into the water. Some PCBs are characterized by high mobility in the environment because they have high values of vapor pressure and Henry's constant. The Henry's constant for PCBs can be in the range 2–50 $Pa.m^3/mol$, which results in the tendency of analytes to volatilize, and therefore to circulate, in a gaseous form, through different environmental compartments. This process influences the global distribution of PCBs in the environment.

Organic matter, which can be a significant component of sediments, in particular its oily fractions, can undergo degradation that significantly influences the equilibrium and the kinetics of PCB desorption. The increased concentrations of analytes in the water phase were observed during degradation of the oily fractions onto which the compounds had been previously adsorbed (Gdaniec-Pietryka *et al.*, 2007).

2.5. Biological degradation of PCBs

Investigation of different alternatives in eliminating PCBs is of great importance. One crucial process that has potential is microbial degradation. Although PCBs persist in the environment, anaerobic and aerobic methods are able to transform these chemically stable compounds under a variety of conditions in the laboratory and in the environment (Furukawa, 1979; Williams, 1994; Wu *et al.*, 1998; Chang *et al.*, 1999; Tartakovsky *et al.*, 2000; Tartakovsky *et al.*, 2001; Chang *et al.*, 2001; Nakhla, 2002; Fava *et al.*, 2003; Patureau and Trably, 2006; Taharakan, 2006; Benabdallah El-Hadj *et al.*, 2007). The transformation depends on the degree of chlorination of the PCB congener, the redox conditions, and the type of microorganism involved (Correa *et al.*, 2010).

The transformation mechanisms are divided into two general classes: reactions that require external electron transfer (oxidations and reductions) and those do not (substitutions and dehydrohalogenations) (Vogel *et al.*, 1987). So, the removal mechanisms of chlorinated aliphatics can be summarized as; usage of these compounds by aerobic and anaerobic bacteria as growth substrates (electron donors), by strict anaerobes as electron acceptors (reductive dehalogenation), and co-metabolic transformation (Leisinger, 1996). During reductive dehalogenation a halogenated compound is used as an electron acceptor. To develop effective bioremediation strategies, the microbes and pathways involved in PCB degradation need to be elucidated. Several microorganisms have been isolated to degrade PCBs aerobically or anaerobically (LaMontagne *et al.*, 1998; Baba *et al.*, 2007).

Under aerobic conditions, lightly chlorinated PCB congeners can be co-metabolized as well as serve as growth supporting substrates. Under anaerobic conditions, highly PCB congeners reductively dechlorinate provided that electron-donating substrates are available (Field and Sierra-Alvarez, 2007). These processes are further described in the following sections.

2.5.1. Aerobic biodegradation of PCBs

Aerobic degradation of PCBs has been widely studied over the past ten years. The lightly chlorinated PCB congeners resulted partially from the dechlorination of highly chlorinated congeners are used as substrates by aerobic bacteria. Mainly, the PCB aerobic pathway involves a biphenyl-dioxygenase, which converts PCBs to chlorinated benzoic acids and chlorocatechols (Abramowicz, 1990). An example of the metabolic pathway, including sequential enzymatic steps, is given in Figure 2.2. Aerobic oxidative destruction involves two groups of genes. The first one is responsible for the transformation of PCB congeners to chlorobenzoic acid, and the second group is responsible for the degradation of the chlorobenzoic acid. A common growth substrate for PCB-degrading bacteria is biphenyl or monochlorobiphenyl (Borja *et al.*, 2005).

Fava *et al.* (1996) investigated dechlorination of Aroclor 1221 in an aerobic packed-bed bioreactor and Borja *et al.* (2006) demonstrated 95% removal of a mixture PCB in an aerobic fluidized bed reactor. Both studies used biphenyl to support the co-metabolism of PCB. Degradation of Aroclor 1242 was also studied in a granular biofilm reactor with limited aeration providing both anaerobic and aerobic conditions simultaneously (Tartakovsky *et al.*, 2001).

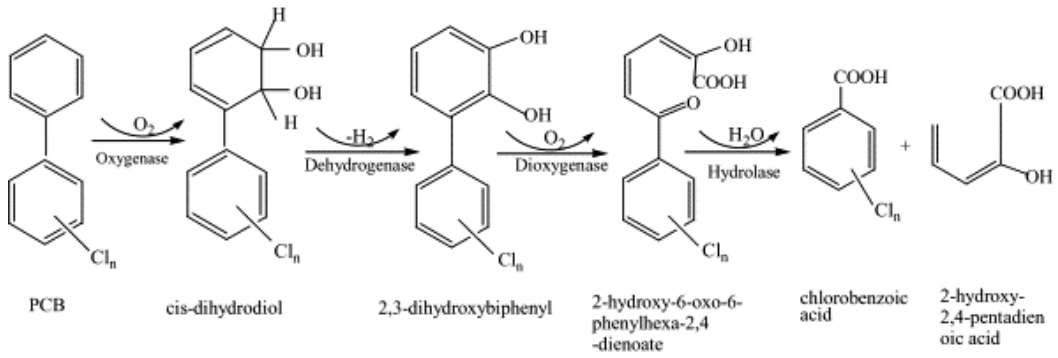


Figure 2.2. Major steps in the conversion of PCBs into chlorobenzoates (Borja *et al.*, 2005)

However, the aerobic degradation of PCBs typically attacks only lightly chlorinated congeners and aerobic PCB biodegradability decreases with increased chlorine (Furukawa, 2000). Also, the congeners having double *ortho* substituted chlorines are observed to be poorly degraded (Field and Sierra-Alvarez, 2008). Furthermore, in many instances, only the top few millimeters of sediments are aerobic. Therefore, the largest reservoirs of PCBs in rivers and lakes are the anaerobic sediments, which are not suitable for the growth of aerobic microorganisms. Effective microbial transformation of PCBs in these contaminated sediments can only occur through anaerobic processes, i.e. reductive dehalogenation (Wiegel and Wu, 2000).

2.5.2. Anaerobic dechlorination of PCBs

Anaerobic dechlorination is the process which is primarily responsible for the degradation of haloaromatic compounds under anaerobic conditions, and is the only known degradative process of many highly halogenated pollutants. The reductive dechlorination of polychlorinated biphenyls (PCBs) is important because the dechlorinated products are more susceptible to aerobic degradation, including ring opening and mineralization, thereby reducing the toxicity associated with PCBs (Bedard *et al.*, 1986; Bedard and Quensen III, 1995).

Anaerobic transformation of chlorinated organic compounds involves reductive dehalogenation where the halogenated organic compound serves as the electron acceptor; the halogen substituent is replaced with hydrogen (Equation 2.1).

$$R\text{-}Cl + 2e^- + H^+ \text{------>} R\text{-}H + Cl^- \qquad (2.1)$$

Electron acceptors generally are the limiting factors in anaerobic environments. Thus, any microorganism that could use PCBs as terminal electron acceptors would be at a selective advantage (Quensen III *et al.*, 1990).

The first examination of anaerobic dechlorination of PCBs was done by Brown and colleagues with the Hudson River sediments (Brown *et al.*, 1984). They observed the profile obtained from Hudson River sediment samples and found that it was the altered profile of the commercial PCB mixture contaminating the river, which was Aroclor 1242. The river sediment samples had a PCB profile with higher fraction of mono- or dichlorobiphenyls, higher amount of *ortho* chlorines and less proportion of tri-, tetra- and pentachlorobiphenyls, as compared to Aroclor 1242. These alterations in the original source profile was then interpreted as the reductive microbial *meta* and *para* removal in sediments since the other known physical and biological transformation processes could not account for these changes in profile (Brown *et al.*, 1984; Bedard and Quensen III, 1995). Besides this *in situ* study, a laboratory study was also conducted to confirm the environmental dechlorination. The laboratory confirmation of the anaerobic dechlorination of Aroclor 1242 in Hudson River sediments was achieved by obtaining a PCB congener profile similar to the one in the environment after a 16 week incubation of sediments in microcosms (Quensen III *et al.*, 1988).

Numerous studies have been conducted on the dechlorination of PCBs using contaminated sediments and soils (Bedard *et al.*, 1986; Quensen III *et al.*, 1988; Abramowicz, 1990; Alder *et al.*, 1993). Evidence of reductive dechlorination of PCBs in many anaerobic sediments of contaminated sites has been reported. Research investigating the ability of anaerobic sediment microcosms to dechlorinate Aroclor 1242, 1248, 1254 and 1260 demonstrated that the PCB congeners were mostly attacked from the *para* and *meta* positions. By the preferential removal of *meta* and *para* substituted chlorines, the abundance of highly chlorinated congeners decreases while that of lower chlorinated, *ortho* substituted, congeners increases. Since the *meta* and *para* chlorines are removed from the congeners, the coplanar structure, and hence, the dioxin-like toxicity of these congeners is reduced as a result of anaerobic dechlorination (Abramowicz, 1995). Moreover, the resulting lower chlorinated congeners can be degraded by aerobic bacteria, allowing for the complete biodegradation of PCBs into carbon dioxide and water via sequential anaerobic and

aerobic microbial degradation processes (Bedard and Quensen III, 1995). In addition, the depletion of highly chlorinated congeners may reduce the exposure level of PCBs, thereby, reduce the potential carcinogenicity and bioaccumulation of PCBs (Quensen III *et al.*, 1990; Abramowicz, 1995; Bedard and Quensen III, 1995; Wiegel and Wu, 2000). Due to all of these benefits of dechlorination of PCBs, which are widespread environmental pollutants, this degradation mechanism may have significant implications for risk assessment and remediation strategies (Bedard and Quensen III, 1995).

An example of an anaerobic dechlorination process is shown in Figure 2.3. As can be seen from the figure, the *meta* and *para* substituted chlorines are replaced by hydrogen atoms at each step. Via the anaerobic dechlorination of highly chlorinated congeners, the preferential removal of *meta* and *para* substituted chlorines occur, leaving the biphenyl ring intact (Abramowicz, 1995).

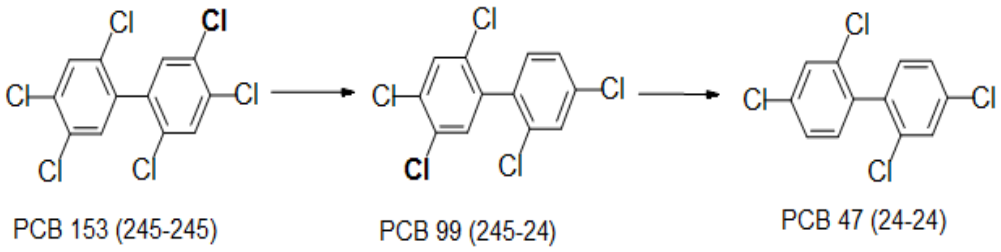


Figure 2.3. A pathway of anaerobic dechlorination of a highly chlorinated congener, PCB 153.

Laboratory studies provide the advantage of a controlled environment in which the dechlorination reactions of each congener can easily be observed and the factors affecting the dechlorination can be determined. Additionally, anaerobic microcosm studies indicate the ability of sampled sediments to reductively dechlorinate spiked PCBs (Fish and Principe, 1994; Williams, 1994; Sokol *et al.*, 1998; Wu *et al.*, 1998;

Chang *et al.*, 2001; Bedard *et al.*, 2005; Yan *et al.*, 2006a, b; Zanaroli *et al.*, 2006) in addition to aged PCBs naturally present in the sample (Zanaroli *et al.*, 2006). Natarajan *et al.* (1998) has studied dechlorination of Aroclor 1254 by the microbial granules in both sediment-free and sediment-containing medium at room temperature. They observed 70% reduction of highly chlorinated congeners without accumulation of any lightly chlorinated (mono- and di-) end products at 24 weeks, mainly the removal of chlorines from *ortho*, *meta* and *para* positions of PCBs were observed. They reported an initial accumulation of tri- and tetra-chlorobiphenyl intermediates, and their subsequent dechlorination upon further incubation which was correlated to that these congeners were dechlorinated at a slower rate in comparison to mono-, di-, penta-, hexa- and heptachlorobiphenyl congeners, and, thus, were rate limiting.

For bioremediation of contaminated sites, biostimulation is the mostly applied method which involves the modification of the environment to stimulate existing bacteria capable of bioremediation. This can be done by addition of various forms of rate limiting nutrients and electron acceptors. Biostimulation is based on the hypothesis that high concentrations of an appropriate substrate, which is susceptible to dehalogenation, will promote the growth of microorganisms. It is applied to stimulate the activity of PCB-dechlorinating microorganisms present in the sediments when the activity of these microbial populations is little or undetectable. PCBs serve as electron acceptors during this method by dechlorinating microorganisms (Bedard, 2003). The first study about biostimulation is conducted with the Housatonic River sediments, contaminated with Aroclor 1260 (Bedard *et al.*, 1996). When the sediments of the River were analyzed, the PCB congener profile showed that anaerobic dechlorination has occurred in the sediments before and the microorganisms responsible for the dechlorination were still present in the sediments but they were not active any more. In the study, 2,6-dibromobipheyl (26-BB) was added to the sediments and this amendment stimulated the activity of the indigenous PCB-dechlorinating microorganisms resulting in a 74% decrease in the PCB congeners having six or more chlorines within a year.

Another biostimulation effort was conducted on Hudson River sediments, contaminated with Aroclor 1242 by the addition of ferrous sulfate to stimulate the microorganisms for the complete *meta* and *para* dechlorination (Zwiernik *et al.*, 1998). The added ferrous sulfate stimulated microorganisms' growth, and when the sulfate was depleted, the PCB-dechlorination with processes M and Q (Table 2.6) was promoted (Zwiernik *et al.*, 1998).

Biostimulation can be enhanced by bioaugmentation. It is applied when the PCB-dechlorinating microorganisms are not present or not active in the contaminated site, by the addition of dechlorinating microorganisms enriched from the same site or another site. If the microorganisms from another site are used, the microbial ecology and biogeochemistry of the sites should be similar in terms of temperature, pH, electron acceptors and donors, organic components and mineral and sediment compositions (Bedard, 2003). A study about the bioaugmentation of River Raisin sediments, contaminated with Aroclors 1242 and 1248, was conducted with the bacterial consortium developed from another site in the form of methanogenic granules (Natarajan *et al.*, 1997). The results of this bench-scale study demonstrated enhanced dechlorination of PCBs in the sediments (Natarajan *et al.*, 1997).

The two most recent bioaugmentation studies were conducted with microcosms from Baltimore Harbor (BH) sediments. In the first study, BH sediments spiked with PCB congener PCB 151 or Aroclor 1260 to see the effects of indigenous dehalorespiring microorganisms and a mixed culture including different species enriched from BH (Fagervold *et al.*, 2011). The results of this study showed an extensive Aroclor 1260 dechlorination when the dehalorespiring microorganisms were used. Also, these dehalorespiring microorganisms effectively competed with the indigenous microbial population and changed the specific PCB dechlorination pathways (Fagervold *et al.*, 2011). In the second study, bioaugmentation with dehalorespiring bacterium, DF-1, was investigated on the mesocosms containing BH sediments contaminated with weathered Aroclor 1260 (Payne *et al.*, 2011). As a result, 56% decrease by mass was

achieved for highly chlorinated congeners, whereas the unamended controls showed little detectable activity (Payne *et al.*, 2011).

All of these bioremediation studies, biostimulation and bioaugmentation, show promising results for enhancing anaerobic dechlorination of PCBs. The identification and application of environmentally friendly and cost effective methods for the *in situ* bioremediation is of great concern.

2.5.2.1. Dechlorination patterns and processes

In anaerobic degradation of PCBs, the transformations among congeners (i.e. transformation of a mother congener into a daughter congener) are called dechlorination pathways or reactions. The specific groups of dechlorination reactions are defined under various dechlorination activity or processes. The various dechlorination patterns can be best recognized by the products that accumulate as a result of their respective dechlorination processes. There are eight distinct microbial dechlorination processes (Processes M, Q, H, P, HP, N, LP and T) which can be identified through careful comparison of the patterns of congener loss and product accumulation patterns in different sediment samples (Table 2.6) (Wiegel and Wu, 2000; Bedard, 2003).

Process P was observed in Woods Pond sediment with Aroclor 1260 and results in accumulation of PCB 52(25-25) (Bedard *et al.*, 1996). In Hudson River sediments, during Aroclor 1260 during dechlorination, Process H was observed. In this process, PCB 52 was the accumulation product (Quensen III *et al.*, 1990). Process M was mostly observed in lighter Aroclor mixtures and in Hudson River sediments (Quensen III *et al.*, 1990). Process Q was reported during the dechlorination of Aroclor 1242 in Hudson River sediments. Process C is the combined result of processes M and Q. Process LP can dechlorinate unflanked *para* chlorines and results in greater dechlorination of Aroclor 1260 when combined with process N (Bedard *et al.*, 1997). Process T observed in Woods Pond sediment microcosms with

Aroclor 1260 incubated at 50-60 degrees is defined by very restricted *meta* dechlorinating activity (Wu, 1996)

Table 2.6. Patterns of reductive dechlorination of PCBs (Bedard, 2003; Bedard *et al.*, 2005).

Dechlorination Process	**Susceptible Chlorines**	**Homolog Substrate Range**	**Reactive Chlorophenyl groups[1]**	**Primary chlorophenyl products**
P	Flanked *para*	4-6	3<u>4</u>, 23<u>4</u>, 2<u>4</u>5, 23<u>4</u>5, 23<u>4</u>56	3, 23, 25, 235, 2356
H	Flanked *para* and *meta* of 234-chlorophenyl groups	4-7	3<u>4</u>, 2<u>3</u>4, 2<u>4</u>5, 23<u>4</u>5	3, 24, 25, 235
H’	Flanked *para* and *meta* of 23- and 243-chlorophenyl groups	3-5	2<u>3</u>, 3<u>4</u>, 2<u>3</u>4, 2<u>4</u>5, 23<u>4</u>5	2, 3, 24, 25, 235
N	Flanked *meta*	5-9	2<u>3</u>4, 2<u>3</u>6, 24<u>5</u>, 2<u>3</u>4<u>5</u>, 2<u>3</u>46, 2<u>3</u>4<u>5</u>6	24, 26, 246
M	Flanked and unflanked *meta*	2-4	<u>3</u>, 2<u>3</u>, 2<u>5</u>, <u>3</u>4, 2<u>3</u>4, 2<u>3</u>6	2, 4, 24, 26
Q	Flanked & unflanked *para* and *meta* of 23- and 243-chlorophenyl groups	2-4	<u>4</u>, 2<u>3</u>, 2<u>4</u>, 3<u>4</u>, 2<u>3</u>4, 2<u>4</u>5, 2<u>4</u>6	2, 3, 25, 26
LP	Flanked and unflanked *para* and *meta* flanked by an *ortho*	2-5	2<u>4</u>, 2<u>4</u>5, 2<u>4</u>6, 3<u>4</u>, 2<u>3</u>, 2<u>3</u>4, 2<u>3</u>5	2, 25, 26, 3, 24, 25
T	Doubly flanked *meta*	7-8	2<u>3</u>45	245

[1] The targeted chlorines are underlined in each reactive chlorophenyl group.

These patterns only include *meta* and *para*-dechlorination, showing the relatively infrequent observation of *ortho*-dechlorination in many of the initial sediment studies (Quensen III *et al.*, 1988, 1990; Alder *et al.*, 1993) or later studies with enriched cultures developed from the same sediments (Bedard *et al.*, 2005). Some *ortho*-dechlorination is also observed in the field (Bedard *et al.*, 2005). However, some

studies with either enrichment cultures or sediment samples spiked with a defined congener have revealed many examples where *ortho*-dechlorination has taken place (Van Dort and Bedard, 1991; Williams, 1994; Berkaw *et al.*, 1996; Natarajan *et al.*, 1996; Wu, 1996; Wiegel and Wu, 2000). Also, *ortho* dechlorination of PCB mixture Aroclor 1260 (Wu *et al.*, 1998; Fagervold *et al.*, 2011) was achieved in laboratory studies. While mono and dichlorobiphenyl congeners are generally the products of PCB dechlorination (Williams, 1994), there are examples of PCB dechlorination to biphenyl (Natarajan *et al.*, 1996).

Dechlorination products are dependent on the initial PCBs (Aroclors) present. That is, patterns depend not just on the congener selectivity of the responsible microorganisms/enzymes, but also on the starting material (which Aroclor or mixture of Aroclors). Product formation has not been determined for all combinations of Aroclors and dechlorination processes, partly from a lack of research, and also because the dechlorination processes vary in their ability to dechlorinate highly and lesser chlorinated congeners. Processes N and P have been shown to dechlorinate Aroclor 1260 (Johnson *et al.*, 2005; Fagervold *et al.*, 2007). Processes M and Q act on relatively lower chlorinate congeners (Bedard and Quensen III, 1995). However, process H, P, and N are effective on highly chlorinated congeners, as can be seen in Figure 2.4.

In the environment, PCBs are often dechlorinated by some combination of the above processes. For example, Patten C is a combination of Processes M and Q (Johnson *et al.*, 2005). In this case, congeners substituted only at the *ortho* positions are dominant. Processes M, Q, H, H', and N have all occurred in experiments with upper Hudson River sediments (Bedard and Quensen III, 1995) and combinations of Processes P and N have been observed in experiments with Housatonic River and Woods Pond (MA) sediments (Wu *et al.*, 1997).

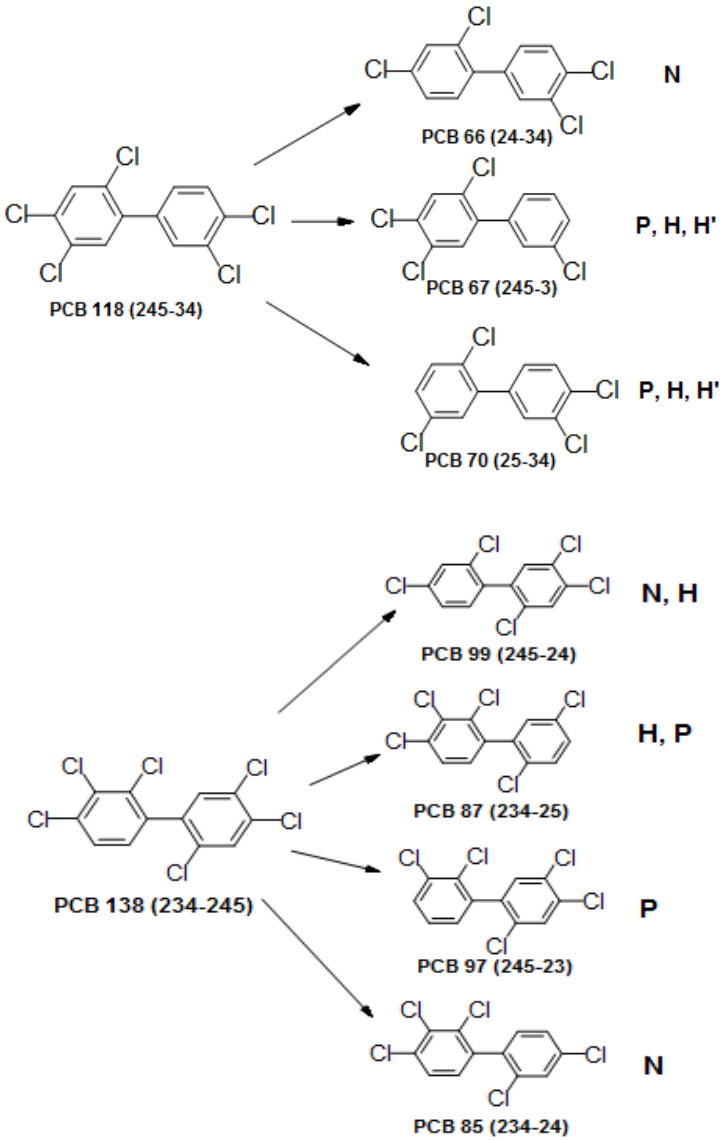


Figure 2.4. Examples of anaerobic dechlorination patways of P, N, H and H' (Imamoglu, 2002).

2.5.2.2. Factors influencing PCB dechlorination

The effectiveness of biodegradation depends on many environmental factors. Dechlorination rates vary depending on the conditions present in the environment. These factors include congener structure, solubility, concentration, degree of

chlorination, presence of toxic or inhibitory substance and competing substrates, availability of carbon sources and presence or absence of electron donors and acceptors (Wiegel and Wu, 2000; Borja *et al.*, 2005).

The most important parameter affecting the rate of dechlorination is the presence and activity (in terms of substrate range and specificity) of the PCB-dechlorinating microorganisms (Bedard, 2003). The activity of the microorganisms is found to be related with the PCB congener profile in the sediments (Kjellerup *et al.*, 2008). Both anaerobic and aerobic metabolism methods transform PCBs. Different microorganisms show preferential attack on PCBs resulting in different patterns of degradation. The degree of chlorination of the congener is reported to be a major factor, which influences degradation potential of the compound. Kjellerup *et al.* (2008) investigated the factors affecting the rate of dechlorination by comparing the characteristics of three different contaminated sites in terms of the microorganisms present and the physicochemical properties. They reported that Grasse River which has the highest PCB concentration and total organic carbon content, showed the most extensive dechlorination with the shortest lag phase. It was concluded that the PCB congener profile of Grasse River, the high concentrations of total PCBs and presence of potential electron donors promoted the enrichment of PCB-dechlorinating microorganisms (Kjellerup *et al.*, 2008).

Water solubility of the compound, as one of the main factors, has a vital role in its degradation. Compounds with high aqueous solubility are easily accessed by microorganisms than those with low solubility. For PCBs, highly chlorinated congeners are very insoluble in water. This could explain the resistance of highly chlorinated PCB congeners to biodegradation.

Another major factor affecting biodegradation is pollutant concentration. Generally, a low pollutant concentration may not be sufficient for the induction of degradative enzymes or to sustain growth of competent organisms. On the other hand, a very high concentration may cause toxicity. At a low concentration range, degradation

increases linearly with increase in concentration until such time that the rate essentially becomes constant regardless of further increase in pollutant concentration (Borja *et al.*, 2005).

As being one of the important factors, temperature has a significant effect on the growth and the physiological activity including uptake and enzymatic dehalogenation of PCB congeners (Wiegel and Wu, 2000). Wu *et al.* (1997) investigated effect of temperature on the dechlorination of the Aroclor 1260 in Woods Pond sediment for a year at incubation temperatures from 4 to 66°C. Dechlorination occurred at 8-34°C and at thermophilic temperatures of 50-60°C.

The use of organic substrate as electron donors has also been shown to increase the rate of dechlorination. Nies and Vogel (1990) reported that the separate addition of acetate, acetone, methanol, and glucose showed similar dechlorination patterns, but, dechlorination extent and rate were different. The rate of dechlorination was greatest for methanol which was followed in a decreasing order by glucose, acetone, and acetate). The effect of co-contaminants, such as tar containing PAHs, was observed to limit the bioavailability of PCBs at a certain site, and hence hindered *in situ* dechlorination activity on Aroclor 1254 (Sokol *et al.*, 1994).

Chang *et al.* (1999) investigated the effects of modifying sludge source, temperature, pH, combining PCB congeners or keeping them separate, substrate concentration, agitation, total solid concentration, and the addition of electron donors or acceptors on the PCB-dechlorinating power of chlorophenol (CP) adapted consortium in sewage sludge. They did not find any significant difference for 2,3,4,5-CB dechlorination while changing pH from 6.0 to pH 8.0. However, they reported pH 9.o was detrimental on dechlorination. Their results showed between 1 to 10 mg/L, the higher the PCB concentration, the faster the dechlorination rate. Also, dechlorination rate was greatest in methanogenic conditions followed in a decreasing order by sulfate-reducing conditions, and denitrifying conditions. The addition of acetate, lactate, pyruvate, and ferric chloride decreased lag times and enhanced

dechlorination; however, the addition of manganese dioxide had an inhibitory effect. Dechlorination rates were also enhanced by the addition of PCB congeners, including 2,3,4-CB, 2.3,4.5-CB and 2,3,4,5,6-CB in mixture.

Environmental factors and conditions affect the growth and the variety of metabolic activities of different microorganisms differently and hence influence divergently the extent and rate of the various PCB-dechlorinating activities. As a result, a better knowledge of whether and to what extent individual environmental factors can influence PCB dechlorination is important for obtaining an understanding of the diversity of PCB dehalogenation and the conditions under which a particular PCB dehalogenation pattern can or cannot occur.

2.5.2.3. Microorganisms involved

The identification of PCB dechlorinating bacteria provides the direct evidence for biological activities in PCB dechlorination hence, assays could be developed to detect and monitor specific PCB dechlorinators at PCB contaminated sites. This information could further help to confirm whether natural attenuation is the prevailing mechanism, or if bioaugmentation or biostimulation is needed. Also, the addition of specific dechlorinating microorganisms may change the dechlorination pathways to produce products that are more vulnerable for complete degradation leading to efficient bioremediation.

Although PCBs persist in the environment, some microbial processes are able to transform these chemically stable molecules. Aerobic degradation involves biphenyl ring cleavage. Aerobic microorganisms preferentially degrade the more lightly chlorinated congeners. These organisms attack PCBs via the well-known 2,3-dioxygenase pathway, converting PCB congeners to the corresponding chlorobenzoic acids. These chlorobenzoic acids can then be degraded by indigenous bacteria, resulting in the production of carbon dioxide, water, chloride, and biomass (Abramowicz, 1995).

A large number of aerobic bacteria are capable of cometabolizing PCB with biphenyl as the primary substrate. The biphenyl metabolic enzymes encoded by the bph gene cluster are responsible for the attack of PCB (Abramowicz, 1990; Furukawa, 2000). PCB-degrading bacteria are found among the gram negative genera *Pseudomonas, Alcaligenes, Achromobacter, Burkholderia, Comamonas, Sphingomonas, Ralstonia* and *Acinetobacter* and among the Gram-positive genera *Rhodococcus, Corynebacterium* and *Bacillus* (Furukawa, 2000; Borja *et al.*, 2005).

Since PCBs are hydrophobic, they tend to adsorb to particles that settle and accumulate in the anaerobic zone of sediments, where microbial reductive dehalogenation results in the sequential removal of chlorine atoms from the biphenyl backbone (Fagervold *et al.,* 2005). Anaerobic dechlorination can attack a large array of chlorinated aliphatic and aromatic hydrocarbons. Several anaerobic dechlorinating bacteria have been isolated (Holliger *et al.,* 1998). These include *Desulfomonile tiedjei* (Mohn, and Tiedje, 1992), *Desulfitobacterium, Dehalobacter restrictus, Dehalospirillum multivorans, Desulforomonas chloroethenica, Dehalococcoides ethenogenes* and the facultative anaerobes *Enterobacter strain MS-1* and *Enterobacter agglomerans.* Some of these microorganisms reductively dechlorinate the chlorinated compound in a co-metabolism reaction; others utilize the chlorinated compounds as electron acceptors in their energy metabolism (Borja *et al.,* 2005).

The characteristics common to dehalogenators are: (a) aryl reductive dehalogenation is catalyzed by inducible enzymes, (b) these enzymes exhibit distinct substrate specificity, (c) aryl dehalogenators function in syntrophic communities and may be dependent on such communities and, (d) aryl dehalogenators derive metabolic energy from reductive dehalogenation. Microorganisms with distinct dehalogenating enzymes each exhibit a unique pattern of congener activity (Borja *et al.,* 2005). Under anaerobic condition, reductive dechlorination of PCBs occurs in soils and sediments. Different microorganisms with distinct dehalogenating enzymes are responsible for different dechlorination activities and dehalogenation routes (Borja *et al.,* 2005).

Numerous studies have described polymerase chain reaction (PCR)-based 16S rRNA gene assays for monitoring indigenous dehalogenating communities within the phylum Chloroflexi, including Dehalococcoides species (Löffler *et al.* 2003; Fagervold *et al.,* 2005; Watts *et al.,* 2005). A unique feature of the genus *Dehalococcoides* and other species within the phylum Chloroflexi is the ability to respire by reductive dehalogenation of PCBs in addition to other polychlorinated aromatic compounds. However, a limitation of these 16S rRNA gene-based assays is that Dehalococcoides species typically represent a small fraction of the total bacterial community (Major *et al.,* 2002; Lendvay *et al.,* 2003; Amos *et al.,* 2008), and their 16S rRNA genes are difficult to detect (Kube *et al.,* 2005; Sung *et al.,* 2006). The difficulty with detecting these microorganisms within the larger indigenous community based on 16S rRNA phylotypes can be overcomed by using specific primers targeting only putative dechlorinating bacteria within *Chloroflexi* since only these phylotypes have been confirmed up to date for reductive dechlorination of PCBs (Cutter *et al.,* 2001; Fennell *et al.,* 2004; Fagervold *et al.,* 2005, 2007, and 2011; Payne *et al*., 2011) and also because dehalogenators typically represent a small fraction of the total bacterial community (Major *et al.,* 2002; Lendvay *et al.,* 2003; Amos *et al.,* 2008).

DF-1, *Dehalococcoides mccartyi* strain 195 (formerly *Dehalococcoides ethenogenes*), and *Dehalococcoides sp. strains CBDB1* are well-known and characterized microorganisms which have been shown to dechlorinate polychlorinated aromatic compounds, including PCBs (Fennell *et al.,* 2004; Liu and Fennell, 2008; Adrian *et al.,* 2009). *Dehalococcoides mccartyi* strain 195 is a pure bacterial strain which uses PCE, TCE and *cis* -DCE as metabolic electron acceptors (Major *et al.,* 2002). It also dechlorinates 2,3,4,5,6-pentachlorobiphenyl and other aromatic organochlorines (Fennell *et al.*, 2004). DF-1 is also a pure strain which grow by respiratory dechlorination of several PCB congeners (Wu *et al.*, 2002; May *et al.,* 2008). Strain DF-1 is also a member of the dechlorinating *Chloroflexi,* as are *Dehalococcoides species*. Also, higher rates of dechlorination of some PCB congeners were found in enriched cultures containing strains o-17 (Cutter *et al.,*

2001) and DF-1 (Wu *et al.,* 2002). It has been shown that *Dehalococcoides sp. strains CBDB1, BAV1* and *FL2* have an active role on the dechlorination of a wide range of chlorinated organic compounds (Fennell *et al.,* 2004; Kube *et al.,* 2005; Liu and Fennell, 2008). Recently, Adrian *et al.* (2009) reported that *Dehalococcoides sp.* strain CBDB1 dechlorinated a wide range of PCB congeners with three to eight chlorine substituents in Aroclor 1260 mixture through process H.

2.6. Degradation of PCBs in transformer oil

Decontamination of transformer oil containing PCBs has been an urgent task for decades. Large amounts of PCBs had been manufactured and used as dielectric fluids in transformer and capacitors all over the world. For different social and economic reasons, significant quantities of PCBs contaminated transformer oil are still in use or in storage (UNEP, 1998, 2000). The actual and potential release of PCBs seriously threatens human beings and ecosystem. Under current regulations, equipment that contains PCBs must be specifically identified. Transformers and capacitors, the largest reservoirs of PCBs still in use today, are included in this category.

In the literature, physico-chemical (e.g., catalytic hydrodechlorination, ozonation, ultrasonic, photolytic, wet air oxidation, reaction with a sodium salt in amine solvent, etc.) removal of PCBs in transformer oil are mainly studied. Among the former, Subbanna *et al.* (1988) achieved overall PCBs destruction by passing air containing 200–1000 ppm. of Aroclor 1254 vapours through an α-alumina tube previously treated with a specific catalyst. Forni *et al.* (1997) reported a convenient method for the environmentally friendly disposal of PCBs based on catalytic dehalogenation under mild conditions. By using 0.5% Pd on carbon in a fixed bed reactor at H_2 atmospheric pressure and at 180–230°C, they obtained high conversion of PCB to HCl and, hydrocarbons mainly biphenyls. Kanbe and Shibuya (2001) described a work on recycled insulating oil in pole transformers for electric power supply contaminated with PCB traces. They investigated the relationship between

progressive stages of dismantling and cleaning needed to remove PCBs from transformer components using *n*-hexane as a solvent.

Brunelle and Singleton (1983) carried out an extensive study of Aroclor 1260 dechlorination in such non-polar media as heptane and toluene. They found that PCBs react with polyethylene glycols and potassium hydroxide under mild conditions, following a simple nucleophilic substitution by a polyethylene glycol alkoxide, probably occurring in the glycol phase. De Filippis *et al.* (1989 and 1997) applied the same chemical process to remove PCBs from contaminated dielectric and lube oils, concluding that the former are easier to dehalogenate.

Wu *et al.* (2005) treated transformer oil (q = 0.888g /mL) taken from used dielectric oil containing PCBs by nanometric sodium hydride (nano-NaH) and transition metal catalysts ($TiCl_4$, $CoCl_2$, Ni $(OAc)_2$). High destruction and removal efficiency (89.8%) were attained.

Cafissi *et al.* (2007) studied the chemical dechlorination of the polychlorinated biphenyls (PCBs) in some dielectric oils via nucleophilic substitution of the chlorine atoms by polyethylene alkoxide. Experiments were carried out with two transformer oils, henceforth named oil D (q = 0.882 g/mL) and oil E (q = 0.882 g/ml), containing different amounts of a mixture of commercial polychlorinated biphenyls (PCB content for oil D: 320 mg/kg oil, oil E: 800 mg/kg oil) known as Aroclor 1240-1250-1260. The influence of temperature, ultrasound, polyethylene glycol (PEG) and base type was investigated. Another chemical dechlorination process of PCBs along with different reaction temperatures and times in waste transformer oil (artificially loaded with 100 ppm of PCB mixtures, Aroclor 1242, 1254 and 1260) obtained from a power plant in Korea has been investigated by Ryoo *et al.* (2007).

Jones *et al.* (2003) examined the radiolytic degradation of PCBs in two electrical transformer oils containing about 95 μg/g and >800 000 μg/g PCB. Used electrical transformer oils containing low or high concentrations of polychlorinated biphenyls

(PCBs) were treated using electron, γ, and ultraviolet radiation, and the conditions for complete dechlorination were developed.

There are not many biological studies investigating dechlorination of PCBs in the presence of oil. The ones which investigated dechlorination of PCBs in oil either tested aerobic conditions and/or used commercial microorganisms or used an easily degradable carbon source as an electron donor (Cedzynska and Sobiecka, 2004; Ahn *et al.*, 2006; Sobiecka *et al.*, 2009).

As an example of PCB dechlorination in the presence of oil, but not transformer oil, was investigated in a sludge digester by Nakhla *et al.* in 2002. Poultry sludge and a commercial seed was used as an inoculum. They investigated the application of aerobic and anaerobic treatment for removal of PCBs in sludges generated from a groundwater treatment system. PCB concentration was 520 ppm, with Aroclors 1254 and 1260 accounting for 26 and 74%, respectively, as well as total organic carbon (TOC), and oil and grease (OGG) concentrations of 108,500 and 18,600 mg/L, respectively. They reported that Aroclors 1254 and 1260 concentrations decreased by about 76 to 89%, and 73 to 89%, respectively, over a period of 60 days. As opposed to numerous PCB dechlorination studies, they found that aerobic PCB biodegradation rates of Aroclors 1254 and 1260 were faster than the anaerobic biodegradation rates, which was reported to be between 13.5% and 23%, respectively. Interestingly, no dechlorination by-product was reported in that study and the initial TSS concentration was 68,800 mg/L. The dechlorination reported in the study of Nakhla *et al.* (2002) in which no dechlorination by-product was observed and extremely high solid concentration as well as TOC were used, might indicate adsorption rather than reductive dechlorination as the prevailing mechanism. In the study of Chang *et al.* (1999) higher solid concentration was shown to cause higher rate of PCB adsorption in sludge.

2.7. Degradation of PCBs in sludge

Wastewater collection systems receive organic pollutants from three main sources: household disposals, fossil fuel spillages, and urban runoff inputs that flush the organics deposited on the ground surface from vehicles or heating systems. As a result of their low aqueous solubility, organic pollutants are efficiently removed from the water during sedimentation in the wastewater treatment and resulted in the formation of sewage sludges that contain a wide range of lipids: petroleum aliphatic hydrocarbons, PCBs and polycyclic aromatic hydrocarbons. Application on land or using as fertilizer is beneficial for dealing with the huge quantities of wastewater treatment sludges. However, a significant portion of the generated sewage sludge is applied to land as an organic fertilizer or amendment contains PCBs.

In sewage sludges, which contain traces of many chemicals of natural and synthetic origin, PCBs are typically present at concentrations of l-10 mg/kg with an elevated portion of highly chlorinated PCBs (Alkock and Jones, 1993; Chang *et al.*, 1999; Benabdallah El-Hadj *et al.*, 2007). Due to their hydrophobic properties, PCBs tend to accumulate by sorption onto soil, sediments and sludges. This concern has led the European Union to regulate the PCB contents in sewage sludge before spreading on land by fixing limits for the future common EU Sewage Sludge Directive. The proposed limit values are of 0.2 mg/kg.dry weight (dw) for each PCB no. 28, 52, 101, 118, 138, 153 and 180 (Patureau and Trably, 2006). In the 3rd draft presented to EU environmental commission, the proposed PCB cut-off limit is of 0.8 mg/kg.dw for the sum of these seven PCBs (Benabdallah El-Hadj *et al.*, 2007). Turkey also regulates PCB contents in sludge with a cut-off value of 0.8 mg/kg.dw for the sum of these seven PCBs, according to Turkey's Regulation on The Use of Domestic and Urban Sludge in the Soil, which has been in force since 2010.

In this context the performance of aerobic or anaerobic digesters become important in determining whether the PCB levels can be reduced during sludge treatment. Tartakovsky *et al.* (2000) conducted a study to evaluate the performance of a

continuous upflow anaerobic sludge bed reactor inoculated with granular anaerobic sludge to treat a model mixture of Aroclor 1242, isobutanol, and Tween 80 (surfactant). To distinguish between sorption and dechlorination processes, extraction of Aroclor from the sludge was performed along with the measurements of chloride and Aroclor in the reactor effluent. A release of inorganic chloride combined with the enrichment of lightly chlorinated congeners was observed. Based on the chloride balance, the dechlorination process was found to be Aroclor concentration-limited at an Aroclor load of 0.4 mg/(gVSS day) due to the low solubility of Aroclor in water. They stated that Aroclor was toxic for anaerobic bacteria when present at a concentration significantly greater than its aqueous solubility limit (0.5 mg/l). While an addition of Tween 80 to the influent stream improved solubility of Aroclor, it led to self-inhibiting dechlorination on a load of 1.3 mg/(gVSS.day). The maximal dechlorination rate observed in that study was 0.6 mg Aroclor/(gVSS.day) and dechlorination was observed above 15 ppm and organic loading of 0.72 gCOD/L.day. They reported adsorption to be the prevailing mechanism for Aroclor disappearance.

In another study, 59.4-83.5% and 33.0-58.0% total PCB removal (PCBs 28, 52, 101, 138, 153, and 180) in anaerobic sludge digester under thermophilic and mesophilic conditions, respectively, was reported in which total initial PCB concentration was 0.5 ppm (Benabdallah El-Hadj *et al.,* 2007). Similar results were also observed by Patureau and Trably (2006). No dechlorination by-products for PCB 52, 101, 118, 138, 153 and 180 were reported in that study despite abiotic loss at about 20% and biotic PCB loss between 40-100% in anaerobic and/or aerobic reactors. The reason of the higher PCB removals in the reactors of Patureau and Trably, (2006) is possibly due to the higher VS content of the sludge (26000 mg/L) and TS content (32000 mg/L).

Chang *et al.* (1999) investigated the effects of several factors on the PCB dechlorinating power of the same CP adapted consortium in sewage sludge: sludge source, temperature, pH (6.0, 7.0, 8.0, and 9.0), mixing PCB congeners or keeping

them se*para*te, substrate concentration (1, 5, and 10 mg/L), agitation (0 and 200 rpm), total solids concentration (5 and 10 g/L), and the addition of electron donors or acceptors. Specifically, they reported that higher total solids concentrations in sewage sludge and higher concentrations of chlorine in PCB resulted in slower dechlorination rates. Also, their results showed that at concentrations of 1 to 10 mg/L, the higher the PCB concentration the faster the dechlorination rate. Chang *et al.* (1999) have shown that higher solid concentration causes higher rate of adsorption in sludge. No abiotic losses were recorded in the same study, but the experiment was conducted under batch conditions without shaking (Chang *et al.* 1999).

Patureau and Trably (2006) investigated aerobic and anaerobic biodegradation of six priority PCBs (PCBs 28, 52, 101, 138, 153, and 180) in continuous stirred tank reactors fed with naturally contaminated sewage sludge. The reactors were inoculated with long-term acclimated anaerobic and aerobic culture. In this study, the daily organic load was about 1 kgCOD/m^3.d and TS and VS concentrations in the feeding mixture were 32 and 26 g/L, respectively. They found that anaerobic and aerobic abiotic losses were higher for the lightly chlorinated PCBs but for all PCBs remained below 20%. PCB removals were reported as about 40% at most, under strict methanogenic conditions. Their finding showed PCB biodegradation of naturally contaminated sludge was limited by the strong sorption of PCBs on sludge particles, and consequently by their bioavailability.

Although not numerous, there are studies on the investigation of degradation of PCBs in treatment plant sludge. Considering the new regulations on restrictions of toxic organics in sludge and the large quantities of sludge generated in many treatment plants waiting for disposal as well as being a part in the Stockholm Convention, together with the Regulation on Control of Equipments Containing PCB and PCTs (2007), hence, responsibility of Turkish Ministry of Environment and Urbanization for eliminating the use of in-place equipment containing PCBs by 2025, this is an important sub-area of research on elimination of PCBs.

CHAPTER 3

MATERIALS AND METHODS

3.1. Chemicals

All solvents (hexane (pesticide grade), acetone (reagent grade)) were obtained from Merck Chemical Co. Germany. Individual standards of pure PCB congeners (25, 26, 33, 66, 67, 70, 118, as 10 ng/µL in iso-octane), PCB-Mix 3 (containing PCB No.s : 28, 52, 101, 118, 138, 153, 180, as 100 ng/µl each component in iso-octane), 2-4-5-6-Tetrachloro-m-xylene solution (TMX, as 200 µg/mL in methanol), and PCB-209 (2,2´,3,3´,4,4´,5,5´,6,6´-Decachlorobiphenyl, 100 ng/µL each component in iso-octane), were bought from Dr. Ehrenstorfer GmbH for preparation of calibration curves and peak identification by gas chromatographic analysis.. These were used during ATA and big batch reactor studies. All the other PCBs (99-100% purity) used in sediment experiments were purchased from AccuStandard. Tetrachloroethylene (PCE) which is used in sediment experiments was purchased from Sigma-Aldrich Co. US. Hexane (pesticide grade) used in sediment experiments was from Acros Organics.

Sulfuric acid (98%), sodium sulfate (granular), and copper powder (fine powder, GR particle size<63 µm, Cat.No: 1.02703.250) were purchased from Merck KGaA (Darmstadt, Germany), and granular copper (0.2-0.4mm) from Sigma-Aldrich GmbH (Seelze, Germany). A certified reference soil, i.e. sandy loam type soil (Cat. No: CRM 141-050) and certified reference material, i.e. sewage sludge (Cat. No: LGC6184) containing seven indicator PCBs (28, 52, 101, 118, 138, 153, and 180) were purchased from LGC standards-Germany and used for extraction purposes in order to verify the PCB extraction efficiency. Alconox detergent obtained from

Alconox Inc. (White Plains, NY, USA) was used during the cleaning of all glassware.

3.2. Basal Medium

Basal medium (BM) as described by Speece (1996) containing all the necessary micro- and macro-nutrients was used in small scale ATA experiments of Chapter 4. Composition of BM is presented in Table B1 (Appendix B). Low-sulfate (0.3 mM) estuarine medium (E-Cl), was prepared as described by Berkaw *et al.* (1996) and used in all sediment experiments of Chapter 6, 7, and 8. Composition and preparation details of E-Cl are presented in Table B2 (Appendix B).

3.3. Sludge

In this study two types of sludge, mainly waste activated sludge, were served as a carbon source in different experiments. Sludge 1 collected from the return line of secondary sedimentation tank at *Ankara Central Wastewater Treatment Plant* (Ankara, Turkey) was added into the small scale ATA reactors (details provided in Chapter 4) and larger scale batch reactors (details provided in Chapter 5). On the other hand, sludge 2 collected from the return line of secondary sedimentation tank of *Little Patuxent Water Reclamation Plant* (Baltimore, USA) was added into small scale sludge-sediment microcosms (details provided in Chapter 8). Further information is presented under the related chapters.

3.4. Seed

Two types of seed (inocula) were used in different experiments of this study. Seed 1, as an unacclimated culture, was mixed culture anaerobic digester sludge (ADS) taken from one of the mesophilic anaerobic digesters at *Ankara Central Wastewater Treatment Plant* (Ankara, Turkey). It was added into ATA reactors in Chapter 4 and laboratory scale batch reactors in Chapter 5. On the other hand, seed 2, as an

acclimated culture was mainly sediments cultures taken from historically contaminated sites such as Grasse River, Fox River and Baltimore Harbor (USA). Different sediment cultures were used in different experiments explained in Chapter 6-8. Sampling of these sediments is explained below under Section 3.5 and details of each seed and/or sediment cultures are presented under the related chapters.

3.5. Sediments

Sediments were taken from three sites such as Grasse River, Baltimore Harbor and Fox River and were stored anaerobically in glass jars sealed with Teflon-lined lids at 4°C in the dark prior to use. These sediments were obtained from Dr. Kevin Sowers' Laboratories (Institute of Marine & Environmental Technology, Baltimore, USA). All sediments were black in color and had a sulfide odor indicative of reduced anoxic conditions.

3.6. Transformer Oil

As being the main source of PCB emission into the environment, transformer oil (TO) was also considered as one of the reactor constituents. PCB-free TO (Cat. No: 40900-U) from Sigma and TK-OIL-100US from Ultra scientific) was purchased from Sigma-Aldrich Co. US and Ultra Scientific, Inc. US. Density of TO is 889.2±17.0 g/L and its VS and TS values were the same about 876.7±27.2 g/L.

3.7. Experimental Setups

3.7.1. ATA Reactors

The first experimental setup was prepared for small scale ATA reactors. In this setup, PCB118 was selected as a model congener. The toxicity of various doses of PCB 118 (0, 1, 10, 20, and 30 mg/L) and TO (0, 0.38, 0.76, and 1.52 g/L) on the anaerobic biodegradability of sludge was assessed by ATA. Waste activated sludge (sludge 1) served as the carbon source in this setup. Totally, 137 ATA reactors were prepared

and operated for 65 days. This study was conducted in METU Laboratories (Ankara, Turkey). Detailed information of this part is given under Chapter 4.

3.7.2. Laboratory Scale Batch Reactors

The second experimental setup was prepared for larger scale batch reactors. Larger scale batch reactor performance in the presence of PCB 118 and TO was investigated. A dose of TO determined from the previous step (ATA), 1.52 g/L, was used in this part and added as co-contaminant with PCB 118 into the anaerobic digesters. Two doses of PCB 118 used were 1 mg/L and 20 mg/L. Waste activated sludge (sludge 1) served as the carbon source in this setup. In total, 8 reactors were prepared and operated for 159 days. This study was conducted in METU Laboratories (Ankara, Turkey). Detailed information of this part is given under Chapter 5.

3.7.3. Enrichment of Sediment Cultures

The third experimental setup was prepared for development of culture by using three historically impacted site sediments (GR, FR, and BH). They were incubated with PCB 118 or Aroclor 1254. Fatty acid mixture served as the carbon source in this setup. Totally, 8 microcosms were prepared and operated between 60-180 days until seeing a dechlorination activity. The most active culture was selected for subsequent experiments. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA). Detailed information of this part is given under Chapter 6.

3.7.4. Dechlorination of Aroclor 1254 and 22 single PCB Congeners

The most active culture determined in the previous experiment was used as inocula for dechlorination of Aroclor 1254 and individual PCB congers selected from Aroclor 1254. Fatty acid mixture served as the carbon source in this setup. Solvent

control and sterile controls were also prepared. Individual dechlorination pathways were elucidated, and dechlorination rates were determined. Totally, 79 microcosms were prepared and operated for 180 days. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA). Detailed information of this part is given under Chapter 7.

3.7.5. Effects of Transformer Oil on Anaerobic Reductive Dechlorination of PCBs in Contaminated Sediments

The effect of doses of TO (0, 1%, and 10% of microcosms volume filled with TO) on the dechlorination of PCB 118 and Aroclor 1254 was investigated in the presence of a low molecular weight fatty acid mixture as an easily degradable carbon source. PCB 118 and Aroclor 1254 dechlorination profiles were determined. A mixture of active culture transferred from the previous set of GR and the original GR sediment was used as a culture/inocula. Solvent control, solvent plus oil and sterile control without oil were also prepared. Totally, 6 microcosms were prepared and operated between 180 and 210 days. The study was conducted in Laboratories of Institute of Marine & Environmental Technology (Baltimore, USA). Detailed information of this part is given under Chapter 8.

3.7.6. Effects of Transformer Oil on Anaerobic Reductive Dechlorination of PCBs in Sewage Sludge

Dechlorination of PCB 118 and Aroclor 1254 with and without TO were investigated in simulated small scale sludge digesters. A mixture of active culture transferred from previous set of GR and original GR sediment was used as an acclimated culture/inocula. Waste activated sludge (sludge 2) served as the carbon source in this setup. Totally, 21 microcosms were prepared and operated for 120 days. Detailed information of this part is given under Chapter 8.

3.8. Analysis of Biogas Production and Composition

3.8.1. Biogas Production

Total gas production of small scale ATA reactors was measured with a water displacement device, which simply works as an open-tube manometer, consisting of 50 mL burette connected to a 250 mL water reservoir. The hose of the system was filled with water and one end of the system was opened to the atmosphere whereas other end was connected to a needle via latex tubing for piercing the rubber stoppers of the reactors to sample biogas amount in the headspace of 250-mL reactors. Graduation intervals were 0.1 mL and gas volume was measured from the rise in the water level between two arms. A photo of the apparatus is given in Figure 3.1.

Figure 3.1. Gas volume analysis apparatus for anaerobic batch reactors

3.8.2. Biogas Composition

Gas composition (CH_4, CO_2) of small scale ATA and larger scale batch reactors was analyzed by gas chromatography (GC) with a Thermal Conductivity Detector (TCD) (Agilent Technologies 6890N). A 30.0 m x 530 µm x 40.0 µm nominal HP-Plot Q capillary column was used for the gas composition analysis. The column temperature was maintained at 45°C for 1 minute, then programmed to reach 65°C at a rate of 10°C/min with a carrier gas (He) flow rate of 3 mL/min (average velocity of 29 cm/sec). A certified calibration gas consisting of nitrogen (10%), methane (65%), and carbon dioxide (25%) was used for the development of calibration curves. Four-point linear calibration curves were prepared for CH_4, CO_2 and N_2 by injecting three replicas for four different volumes (100, 75, 50, and 25 µl) of the certified gas (Figure 3.2). A 100 µL gas tight Hamilton Syringe was used for the injection.

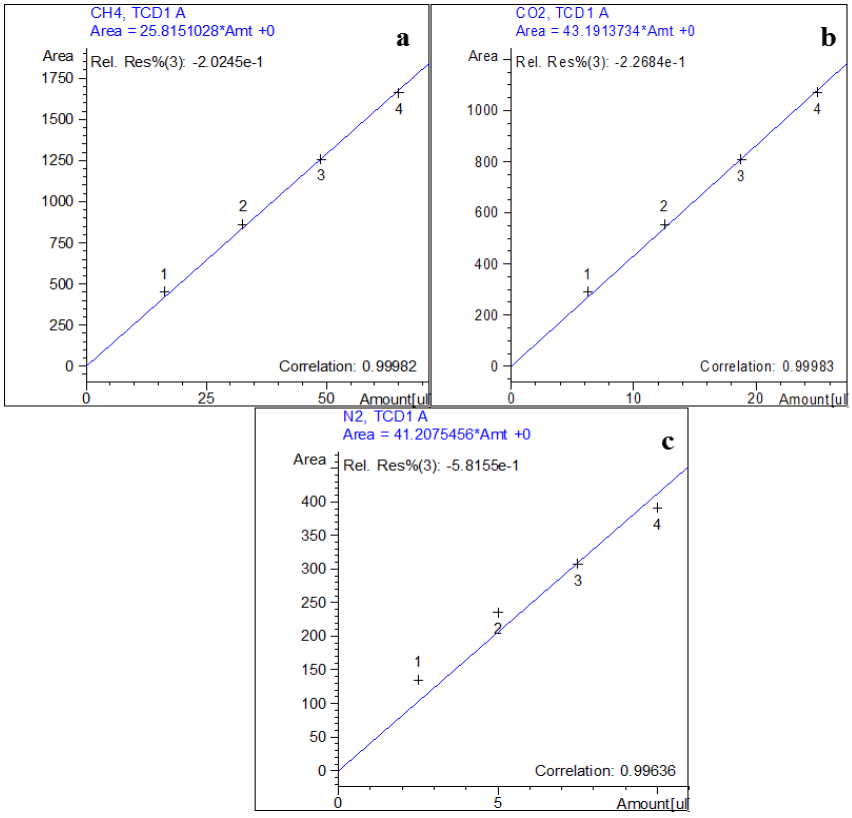


Figure 3.2. Calibration curves of a) CH_4, b) CO_2 and c) N_2 gases

Methane analysis of small scale sludge-sediment microcosms was done by taking 100 µl gas with a gas tight syringe (SGE) and injecting into a GC (Hewlett Packard 5890A) equipped with flame ionization detector (FID). The column was 0.32- by 182.88-cm stainless steel packed with silica gel (80/100 mesh; Supelco). The column oven was operated at 110°C, and He was the carrier gas. Purified methane (Matheson) was used as a standard (Sowers and Ferry, 1983). A calibration curve was prepared before every analysis by injecting a standard methane gas. As seen from Figure 3.2., r^2 values of all curves were higher than 0.995.

3.9. Analysis of Soluble Chemical Oxygen Demand and Total Chemical Oxygen Demand

COD measurements were only carried out for sludge containing reactor samples such as for experiments of Chapter 4, 5 and 8. Soluble chemical oxygen demand (sCOD) and total chemical oxygen demand (tCOD, can also be seen as COD in the text, indicating tCOD) contents of reactors were measured according to an USEPA approved reactor digestion method (Jirca and Carter, 1975) (for a COD range of 0-1500 mg/L) by using Hach DR2000 spectrophotometer at 620 nm as given in Hach Water Analysis Handbook (1989). For COD analysis, Hach Spectrophotometer (Model 45600-02, Cole Parmer Instrument Co., USA) vials and laboratory-made COD solutions were used. Prior to sCOD analysis, samples were filtered through 0.45 µm pore sized filters (Millipore).

A COD calibration curve (Figure 3.3) was prepared in order to check the accuracy of the laboratory-made COD solutions by using a standard substance, i.e. potassium acid phthalate (KHP), which was prepared according to the Hach Water Analyses Handbook (1998). As placed in Figure 3.3, the r^2 (0.999) of the calibration curve indicates the accuracy of the laboratory-made COD solutions.

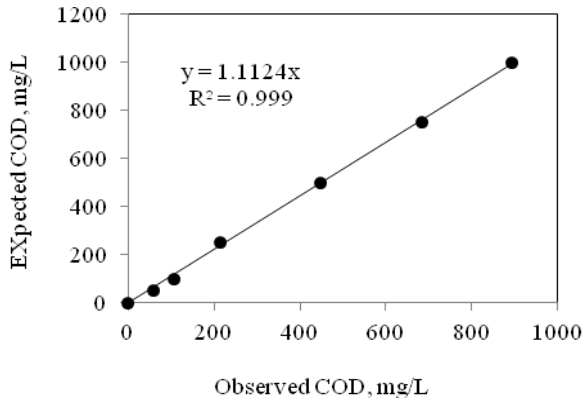


Figure 3.3. The calibration curve of the laboratory-made COD solution.

3.10. PCB Analysis

Two PCB analysis methods were used for the experiments of this study. The first PCB analysis method was based on absolute concentration of PCB congeners and used for small scale ATA (Chapter 4) and larger scale batch reactors (Chapter 5). In those reactors, analysis of PCB 118 and its expected daughter products (PCBs 25, 26, 33, 66, 67, and 70) in slurry samples of small scale ATA (Chapter 4) and larger scale batch reactors (Chapter 5) were done according to first PCB analysis method by using TMX as a surrogate and PCB 209 as an internal standard. The second PCB analysis method was more comprehensive and used for the measurement of 148 PCB congeners. PCB analysis of sediment studies (Chapter 6-8) were carried out according to second PCB analysis method in which PCB 30 and PCB 204 were internal standards while PCB 166 served as surrogate. Details of each method are given below.

3.10.1. First PCB analysis Method

3.10.1.1. Calibration Standards, Tables and Curves

Identification and quantification of PCBs were made using internal standard calibration according to USEPA Method 8082A (USEPA, 2000). Internal standard

(IS) is a pure analyte added to a sample extract, or standard solution in known amount(s) and used to measure the relative responses of other method analytes and surrogates that are components of the same solution. The response of the target compound is normalized to the response of the internal standard which is contained within the aliquot of the sample or sample extract that is actually injected into the instrumentation. A constant amount of the internal standard is added to all samples or extracts. That same amount of the internal standard is also included in each of the calibration standards. The ratio of the peak area (or height) of the target compound in the sample or sample extract to the peak area (or height) of the internal standard in the sample or sample extract is compared to a similar ratio derived for each calibration standard. This ratio is termed the response factor (RF) or relative response factor (RRF), indicating that the target compound response is calculated relative to that of the internal standard.

Since the study aims to investigate the degradation products of PCB 118, encountering a wide range of concentration; three ranges of calibration were chosen and the tables were performed accordingly. Low, medium and high calibration ranges were chosen as [0.1-2] ppb, [1-50] ppb, and [50-500] ppb, respectively. Calibration curves were prepared with a minimum of five points. These calibration curves include PCB 118 and the possible daughter products (PCB 25, 26, 33, 66, 67, and 70) and surrogate compound (TMX) by USEPA Method 8082A (USEPA, 2000). The accuracy and the precision of the PCB measurements are controlled by calculating surrogate recovery as:

$$\text{Surrogate recovery (\%)} = \frac{C_s}{C_a} \text{x} 100 \qquad (3.1)$$

Where; Cs is the amount of surrogate measured in the sample and Ca is the actual amount of surrogate, TMX, spiked into a sample. Surrogate recovery is evaluated for each sample, spiking TMX) into the sample before extraction procedure to evaluate overall laboratory performance and efficiency of the extraction technique. For each

of the initial calibration standards, the RF values should be calculated for each target compound relative to the internal standard as shown in Equation 3.2.

$$RF=\frac{A_s.\,C_{is}}{A_{is}.C_s} \qquad (3.2)$$

Where: A_s is the peak area (or height) of the analyte or surrogate. A_{is} is peak area (or height) of the internal standard. C_s is Mass of the analyte or surrogate in the sample aliquot introduced into the instrument, in µg/L. C_{is} is the mass of the internal standard in the sample aliquot introduced into the instrument, in µg/L.

To evaluate the linearity of the initial calibration, the mean RF (internal standard calibration), the standard deviation (SD), and the relative standard deviation (RSD) should be calculated as shown in Equation 3.2.

$$SD=\sqrt{\frac{\sum_{i=1}^{n}(RF_i-\overline{RF})^2}{n-1}}\;; \qquad \text{mean RF}=\overline{RF}=\frac{\sum_{i=1}^{n}RF_i}{n}\;; \qquad RSD=\frac{SD}{\overline{RF}}x100 \qquad (3.3)$$

Where n is the number of calibration standards and RSD is expressed as a percentage (%). The RSD of the calibration or response factors needs to be less than or equal to 20% over the calibration range, which shows the slopes of the lines for each standard are sufficiently close to one another and the use of the linear model is generally appropriate over the range of standards that are analyzed. Given the potentially large numbers of analytes that may be analyzed in some methods, it is likely that some analytes may exceed the 20% acceptance limit for the RSD for a given calibration. In order for the linear regression model to be used for quantitative purposes, r or r^2 must be greater than or equal to 0.99 according to USEPA Method 8000C and 8082A (USEPA, 2000).

Accordingly, calibration standards were prepared by diluting the stock standards in hexane. Target concentration values for the calibration curves, retention times (RT),

the response factors which were calculated according to internal standard calibration (RF) and relative standard deviations (RSD %) for each concentration range were given in the following tables (Table 3.1, 3.2, and 3.3). For all of the calibration ranges, RSD % of the all congeners that is used to show the reliability of internal calibration was lower than 20%, meeting the quality criteria given in the EPA Method 8082. As it is given on the PCB calibration curves (Figure B1-B3, Appendix B), all correlations coefficients, r^2, were above 0.998.

Table 3.1. Low range calibration table of PCBs and TMX showing RTs, RFs, and RSDs

RT (min.)	Compound	Lvl	Amt (ppb)	Area	RF	mean RF	SD	RSD (%)
8.493	TMX	1	0.1	62.17	1.90	1.66	0.18	11.11
		2	0.25	138.59	1.70			
		3	0.5	307.41	1.73			
		4	1	529.30	1.50			
		5	2	1013.30	1.45			
13.662	PCB 26	1	0.1	32.59	1.00	0.91	0.08	8.30
		2	0.25	76.99	0.94			
		3	0.5	171.15	0.96			
		4	1	296.71	0.84			
		5	2	578.87	0.83			
13.786	PCB 25	1	0.1	36.27	1.11	1.01	0.09	8.42
		2	0.25	84.76	1.04			
		3	0.5	190.78	1.08			
		4	1	325.41	0.92			
		5	2	649.29	0.93			
14.606	PCB 33	1	0.1	30.91	0.95	0.85	0.07	8.79
		2	0.25	66.07	0.81			
		3	0.5	161.05	0.91			
		4	1	285.35	0.81			
		5	2	538.77	0.77			
18.337	PCB 67	1	0.1	43.41	1.33	1.14	0.13	11.57
		2	0.25	92.66	1.13			
		3	0.5	214.20	1.21			
		4	1	370.53	1.05			
		5	2	693.74	0.99			
19.011	PCB 70	1	0.1	48.81	1.49	1.25	0.16	12.99
		2	0.25	101.77	1.25			
		3	0.5	232.29	1.31			
		4	1	402.58	1.14			
		5	2	750.57	1.07			
19.179	PCB 6	1	0.1	50.89	1.56	1.30	0.17	13.05
		2	0.25	105.02	1.29			
		3	0.5	241.36	1.36			
		4	1	418.67	1.19			
		5	2	782.81	1.12			
22.59	PCB 118	1	0.1	51.09	1.56	1.27	0.19	14.83
		2	0.25	103.10	1.26			
		3	0.5	231.32	1.30			
		4	1	401.62	1.14			
		5	2	753.14	1.08			
31.673	PCB 209	1	5	1634.20	1.00	1.00	0.00	0.00
		2	5	1633.70	1.00			
		3	5	1773.80	1.00			
		4	5	1763.90	1.00			
		5	5	1748.50	1.00			

Table 3.2. Medium range calibration table of PCBs and TMX showing RTs, RFs, and RSDs

RT (min.)	Comp.	Lvl	Amt [ppb]	Area	RF	mean RF	SD	RSD %
8.489	TMX	1	1	551.35	1.28	1.14	0.10	9.14
		2	2	1022.9	1.23			
		3	5	2557.4	1.14			
		4	10	5131.1	1.15			
		5	30	14093	1.02			
		6	50	23914	1.03			
13.66	PCB 25	1	1	318.86	0.74	0.59	0.11	18.93
		2	2	577.67	0.70			
		3	5	1363.7	0.61			
		4	10	2550.6	0.57			
		5	30	6542.5	0.47			
		6	50	10831	0.47			
13.783	PCB 26	1	1	353.41	0.82	0.67	0.12	17.60
		2	2	643.63	0.78			
		3	5	1525.6	0.68			
		4	10	2885.5	0.65			
		5	30	7447.2	0.54			
		6	50	12471	0.54			
14.603	PCB 33	1	1	302.91	0.70	0.56	0.11	19.98
		2	2	545.98	0.66			
		3	5	1276.6	0.57			
		4	10	2377.4	0.53			
		5	30	6070.8	0.44			
		6	50	10011	0.43			
18.334	PCB 67	1	1	395.6	0.92	0.73	0.14	19.83
		2	2	708.26	0.85			
		3	5	1664.8	0.74			
		4	10	3086.5	0.69			
		5	30	7943.2	0.58			
		6	50	13103	0.57			
19.005	PCB 70	1	1	434.04	1.01	0.79	0.16	19.76
		2	2	769.82	0.93			
		3	5	1814.3	0.81			
		4	10	3363.3	0.76			
		5	30	8703.7	0.63			
		6	50	14369	0.62			
19.173	PCB 66	1	1	450.97	1.05	0.83	0.16	18.59
		2	2	803.81	0.97			
		3	5	1913	0.85			
		4	10	3556.7	0.80			
		5	30	9281.4	0.67			
		6	50	15397	0.66			
22.585	PCB 118	1	1	453.67	1.05	0.85	0.15	18.01
		2	2	811.04	0.98			
		3	5	1942.9	0.87			
		4	10	3576.8	0.80			
		5	30	9526	0.69			
		6	50	15676	0.68			
31.672	PCB 209	1	5	2152.6	1.00	1.00	0.00	0.00
		2	5	2071.6	1.00			
		3	5	2243.5	1.00			
		4	5	2222.2	1.00			
		5	5	2301.3	1.00			
		6	5	2315.8	1.00			

Table 3.3. High range calibration table of PCBs and TMX showing RTs, RFs, and RSDs.

RT (min.)	Comp.	Lvl	Amt [ppb]	Area	RF	mean RF	SD	RSD %
8.493	TMX	1	50	22506	0.95	0.98	0.06	5.89
		2	100	44817	1.07			
		3	200	86947	1.03			
		4	300	124500	0.98			
		5	400	157980	0.95			
		6	500	188520	0.92			
13.662	PCB 25	1	50	10291	0.44	0.42	0.03	7.25
		2	100	19340	0.46			
		3	200	35574	0.42			
		4	300	50630	0.40			
		5	400	64521	0.39			
		6	500	80148	0.39			
13.786	PCB 26	1	50	11862	0.50	0.48	0.03	6.14
		2	100	21437	0.51			
		3	200	42135	0.50			
		4	300	58636	0.46			
		5	400	74492	0.45			
		6	500	92619	0.45			
14.606	PCB 33	1	50	9529	0.40	0.39	0.03	6.75
		2	100	17729	0.42			
		3	200	33407	0.39			
		4	300	47354	0.37			
		5	400	60141	0.36			
		6	500	74078	0.36			
18.337	PCB 67	1	50	12540	0.53	0.51	0.03	6.48
		2	100	23362	0.56			
		3	200	44525	0.53			
		4	300	62663	0.49			
		5	400	80129	0.48			
		6	500	98022	0.48			
19.011	PCB 70	1	50	13795	0.58	0.55	0.04	6.37
		2	100	24993	0.60			
		3	200	48707	0.58			
		4	300	67417	0.53			
		5	400	86188	0.52			
		6	500	107900	0.53			
19.179	PCB 66	1	50	14778	0.63	0.60	0.04	6.34
		2	100	27328	0.66			
		3	200	52210	0.62			
		4	300	73090	0.57			
		5	400	93786	0.56			
		6	500	116400	0.57			
22.59	PCB 118	1	50	14865	0.63	0.61	0.04	5.90
		2	100	27687	0.66			
		3	200	53036	0.63			
		4	300	74982	0.59			
		5	400	95027	0.57			
		6	500	119430	0.58			
31.673	PCB 209	1	5	2363.7	1.00	1.00	0.00	0.00
		2	5	2086.1	1.00			
		3	5	2114.9	1.00			
		4	5	2126.5	1.00			
		5	5	2088.6	1.00			
		6	5	2050.1	1.00			

3.10.1.2. PCB Measurement

After a detailed literature survey (Table B3, Appendix B), various temperature programs were tried to identify PCB congeners and to separate peaks from each other. Agilent ChemStation software installed on PC was used for instrument control and data analysis (integration, quantification and reporting). Pure congener standards were used to identify retention times of all PCB congeners of interest. Details of PCB analysis on GC/μ-ECD is given in Table 3.4.

Table 3.4.GC conditions for PCB analysis

GC/ECD Model	: Agilent Model 6890 N GC equipped with μ-ECD.
Column	:HP-5MS (Agilent) 5% Phenyl Methyl Siloxane capillary GC column with dimension of 30mx0.25mmx0.25 mm.
Carrier gas	: He at constant flow rate of 1.5 ml/min (37 cm/sec)
Makeup gas	: N_2 at a flow rate of 20 ml/min.
Inlet temperature	: 250ºC
Detector temperature	: 350ºC
Initial Temperature	: 100ºC
Temperature program	: from 100ºC to 160 at 20ºC/min, hold 2 min and then from 160ºC to 200ºC at 3ºC/min, 200ºC to 240ºC, at 8ºC/min, hold 5 min, 240ºC to 290ºC, at 30ºC/min.
Final Temperature	: 290ºC, hold 3 min.
Total run time	: 33 min
Injection volume	: 1 μL
Injection mode	: splitless

3.10.1.3. Development of PCB extraction method

For the extraction of PCBs, EPA has suggested various methods such as method 3550C-ultrasonic extraction, method 3540C-soxhlet extraction, and method 3546-microwave extraction. Among those methods, soxhlet and ultrasonic extraction

methods were first tried for PCB extractions in this study. For soxhlet extraction, EPA Method 3540C was followed. Briefly, two samples were prepared to test extraction efficiency of this method. For this purpose, duplicate soil samples, each 20.0 g, which were previously cleaned with the same procedure and dried at 105ºC, was taken and put into cellulose thimble. Then, they were placed into Soxhlet apparatus, a known amount of 2,4,5,6-Tetrachloro-m-xylene was added as a surrogate standard and 2 grams of granular copper (reduced) was added to remove sulfur during the extraction process. Samples were extracted for 17 hours at 4-6 cycles/hour with 1:1 hexane-acetone (300 mL) mixture. Following extraction, residual moisture in extract was dried via passing the extract through a column packed with anhydrous Na_2SO_4. Soxhlet apparatus (25 mL) and drying column (100 mL) were washed with hexane to get any residual PCBs on them into extract. The extract was reduced to about 10 mL using a Kuderna-Danish (KD) evaporator with a 3-ball Snyder column. If the extract obtained from the KD process was colored, then, final portion was transferred to a vial and 2 mL of concentrated H_2SO_4 was added and vial was shaken for about 30 sec. to remove interfering substances and then, sample was centrifuged at 2500 rpm for 5 minute to separate phases consisting of clear extract (top layer) and sulfuric acid (bottom layer). Then, top clear extract was purified with Na_2SO_4. Lastly, extract was concentrated to 2 mL using a gentle stream of nitrogen. The average recovery of 2 replicas was around 96% (93% and 98%). Despite the high recoveries, total extraction time was around 18-16 h and only 2 extractions were possible at a time due to limited number of materials (soxhlet apparatus and related heating devices, and even place under the fume hood). Last but not least, the required solvent (hexane, suprasolve grade, most expensive one) volume (300-500 ml/sample) and sample amount (3-20 g) is very high. The large amout of sample required was not feasible for this study. Therefore, the soxhlet method was abandoned.

Ultrasonication method is mostly used for soil and sediment samples (Kimbrough *et al.*, 1994; Kodba and Marsel, 1999; Abrha and Raghavan, 2000; Hadnagy *et al.*, 2007). For ultrasonic extraction, EPA method 3550C was followed. Briefly, high-

performance ultrasonic homogenizer (Labsonic P, Sartorious) was used with output control knob set at 11 and the mode was on pulse. A soil sample (10.0 g) was mixed with 100 mL of acetone-hexane (1:1, v/v) and sonicated for 3 min. the procedure was repeated 3 times and a total volume of 300 mL was centrifuged at 2000 rpm for 5 min and combined. The extracts were passed through anhydrous Na_2SO_4 and were concentrated down to 10 mL with Kuderna-Danish evaporator with a 3-ball Snyder column. If the extract obtained from the KD process was colored, then, 2 mL of concentrated H_2SO_4 were added to the final portion to remove interfering substances. After shaking for 30 sec., sample was centrifuged at 2500 rpm for 5 min. to separate phases consisting of clear extract (top layer) and sulfuric acid (bottom layer). Then, top clear extract was purified with Na_2SO_4. Lastly, extract was concentrated to 2 mL using a gentle stream of nitrogen.

During the extraction, soil samples sticked on to the probe, which was hard to get them back into the solution and also during the sonication some solvent inevitably evaporated due to heat production on the probe. After several trials, it was understood that ultrasonic extraction method was also not a feasible method for this study. Even though extraction time of this method was short compared to that of soxhlet, the required extraction solvent volume is almost the same for both extraction methods. Other important drawbacks of this method are solvent and sample loss during the extraction as well as the requirement of high amount of sample which is not applicable for sludge experiments; hence, sonication method was also abandoned.

After a detailed literature survey (Table B4, Appendix B), a method, which is called as *vial extraction method* in this study, was developed and used for the extraction of PCBs. This method requires very low amount of sample and extraction solvent. Also, the extraction procedure is simple. Bedard *et al.* (2005) has also used a similar method and has proven that quantitative comparisons of samples extracted by this simple procedure and by laborious Soxhlet procedure (EPA 3540) showed no difference. Mcdonough and Dzombak (2005) who also used a similar method

showed that this technique yielded better extraction efficiencies for synthetic sediment than ultrasonication method.

In the literature, for this type of extraction method, various types of shakers, extraction solvents, extraction times and sample amounts/volumes were used when extracting PCBs (Table B4, Appendix B). For this method, first, anhydrous diethyl ether was considered as an extraction solvent since it is known as a very efficient extraction solvent. However, anhydrous diethyl ether was not preferred in this study due to its carcinogenicity and volatility. Instead, a procedure was developed using hexane, which was used extensively for PCB extractions in the previous studies (Table B4, Appendix B). For the cleanup of samples, USEPA procedures were performed such as 3660B (Sulfur cleanup) and 3665A (Sulfuric acid cleanup). Details of the extraction method are given below.

Vial extraction procedure

A 2-mL sample was taken into a 22-mL glass vial sealed with PTFE screw cap. After the addition of 50 mg reduced copper (cleaning procedure is given below) to remove sulphur compounds based on EPA method 3660B (Sulfur cleanup) and known-amount of TMX to calculate extraction efficiency, PCBs in the sample were extracted by vigorous overnight-shaking on a horizontal platform shaker at 350 rpm with 10 mL of n-hexane. To separate sample and hexane, centrifugation was applied at 2500 rpm for 5 min. If the hexane extract has a yellow, brown or a dark color then sulphuric acid clean-up procedure (based on EPA method 3665A) was applied to remove non-PCB organics. Briefly, at this step, by using a 10-mL syringe, about 9-10 ml of the hexane extract was transferred into a clean 22-mL vial with PTFE cap, and 2 mL of sulphuric acid was added carefully. The vial was capped tightly and vortexed for 30 sec. Then, it was centrifuged at 2500 rpm for 5 minute to separate phases consisting of clear extract (top layer) and sulfuric acid (bottom layer). The hexane from the top layer (the exact volume extract regained was recorded for volume loss correction), with a 10 mL syringe (gas tight) was passed through a column packed with glass wool covered with a layer of anhydrous Na_2SO_4, then

Cu/fluorisil (1:4, w/w) and finally a layer of anhydrous Na_2SO_4 (dried by heating at 400ºC for 4 hours before use and kept closed in a clean and dry bottle) for removal of moisture and non-PCB compounds. The eluate was collected into a clean vial. Afterwards, to get the adsorbed PCB rinsed off from the column, an extra 2-mL of hexane was run through the column. Lastly, the extract was concentrated down to 2-mL by using a gentle stream of nitrogen and 1-mL of this eluate was taken into 2-mL borosilicate glass GC vials and ISTD was added before GC analysis. The rest was put into a GC vial capped with PTFE stopper and stored in the refrigerator.

Cleaning of Cu-powder used for PCB extractions

After purchasing, copper powder (fine powder, GR particle size<63 µm, Merck, Cat. no: 1.02703.250), is first rinsed with 1 N H_2SO_4 until the blue color has disappeared from the copper and the blue color is only in the acid phase. This required thorough washing a minimum of 6 times, where the total volume is replaced. Then, the copper was rinsed with deionized water at least 12 times. This was followed by rinsing 4 times with acetone and 2 times with low-grade hexane and drying with N_2 on low heat in the fume hood until dry (alternatively dry without heat with N_2 overnight, apply both). If the copper had a dark color (such as brownish or very dark red) to it at any step in the process it means that was oxidized and it was necessary to start all over again. Copper was treated as many times as necessary to obtain the red color. Then, reduced and dried copper was placed in a 1-L bottle with a stopper to reduce its contact with air (oxygen).

3.10.1.4. Recovery experiments for slurry samples by using vial extraction method

For this purpose, two experiments were carried with different PCB concentrations.

Experiment 1: a medium level (10 ppb) concentration was chosen for testing the efficiency of the extraction method. For this purpose, as explained above, 0.5 g of clean soil samples were taken into clean extraction tubes and distilled water was

added to bring sample volume to 2-mL. Then, PCB Mix-3 and TMX were spiked onto these samples. Then, the vial extraction procedure was followed and results are presented in Table 3.5.

Experiment 2: a medium (40 ppb) and a high level (80 ppb) concentration were chosen to test the extraction efficiency of the method. Samples were prepared in the same way but with different concentration. From the main PCB-Mix-3 stock and TMX stock, the intended concentrations were prepared in slurry samples. For each level triplicate samples were prepared and extracted by applying vial extraction procedure and average results are presented in Table 3.5.

3.10.1.5. Recovery experiments for liquid samples by using vial extraction method

Experiment 3: Laboratory control samples were prepared by introducing PCB stock solution in acetone to water. For this purpose, two low levels (1 and 2 ppb) and one medium level (20 ppb) concentration was chosen to test the extraction efficiency of the method. From the main PCB stock, the intended concentrations were prepared in water for extraction experiment. Then, the water sample was vortexed in order to homogenize the sample. From this sample 2 ml was taken into test tube and 10 ml of extraction solvent (hexane) was added. Then, the vial extraction procedure was followed. For each concentration, 6 replicas were prepared and the results are presented in Table 3.6.

Experiment 4: After the good recovery results of PCB Mix-3 congeners and surrogate, experiment 4 was prepared for PCB 118 and its possible daughter products (PCB congeners: 25, 26, 33, 66, 67, and 70). For this purpose, a PCB stock containing all of these congeners was prepared and two different concentrations (1 ppb as low level, and 20 ppb as medium level) were chosen to be tested and for each concentration nine samples were prepared for extraction, in total of 18 extractions were done. The recovery results of each congener are presented in Table 3.7.

Table 3.5. Results of slurry extraction experiment

Compound	Experiment 1*						Experiment 2					
	Medium level (10 ppb)			Medium level (10 ppb)			Medium level (40 ppb)			High level (80 ppb)		
	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)
TMX	11.28	11.25	99.7	10.91	11.241	103	4.3±0.18	4.7±0.21	108.6±3.5	8.83±0.05	7.6±1.2	86.1±4.9
PCB-28	11.28	11.51	102	10.91	10.19	93.4	42.8±1.85	39.4±2.1	92.2±4.8	88.3±0.54	74.7±3.4	84.6±3.1
PCB-52	11.28	11.35	100.6	10.91	11.76	107.8	42.8±1.85	39.8±1.8	93±2.6	88.3±0.54	75.5±5.2	85.4±1.9
PCB-101	11.28	10.22	90.6	10.91	10.39	95.2	42.8±1.85	39.3±1.6	91.9±2.3	88.3±0.54	74±3.6	83.8±3.7
PCB 118	11.28	11.22	99.5	10.91	10.01	91.8	42.8±1.85	38.5±2.2	90±4.5	88.3±0.54	73.5±2.7	83.2±2.5
PCB- 138	11.28	10.67	94.6	10.91	9.49	87	42.8±1.85	38.4±1.4	89.7±2.6	88.3±0.54	74.2±2.2	84.1±3.4
PCB-153	11.28	12.56	111.3	10.91	12.26	112.4	42.8±1.85	37.7±0.9	88.1±1.3	88.3±0.54	73.1±5.1	82.8±3.8
PCB-180	11.28	11.47	101.7	10.91	9.92	90.9	42.8±1.85	35.4±1.4	82.8±3.0	88.3±0.54	75±3.9	84.9±3.2

*: due to duplicate sample analysis standard deviations could not be calculated for this experiment.

Table 3.6. Results of liquid extraction experiment spiked with PCB-Mix-3

Compound	Experiment 3											
	Low level 1(1 ppb)			Low level 2 (2 ppb)			Medium level 1 (20 ppb)			Medium level 2 (20 ppb)		
	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)
TMX	0.96±0.01	1.06±0.08	110.4±4.8	0.98±0.01	0.89±0.15	90.8±6.8	18.6±0.5	18.9±0.6	101.7±5.5	19.6±0.1	18.9±0.4	96.6±1.78
PCB-28	2.89±0.03	2.66±0.08	92.0±1.1	0.98±0.01	1.02±0.04	105.2±5.0	27.9±0.8	26.2±0.9	93.8±5.0	19.6±0.1	19.7±0.7	100.4±3.3
PCB-52	2.89±0.03	2.59±0.08	89.6±0.8	0.98±0.01	0.9±0.04	92.8±4.9	27.9±0.8	26.3±0.9	94.4±4.9	19.6±0.1	19.5±0.7	99.5±3.0
PCB-101	2.89±0.03	2.56±0.3	88.6±8.8	0.98±0.01	0.88±0.03	90.7±3.1	27.9±0.8	25.9±0.9	92.8±5.0	19.6±0.1	19.2±0.6	98.1±2.5
PCB 118	2.89±0.03	2.69±0.46	93.8±3.5	0.98±0.01	1.09±0.01	112.4±0.5	27.9±0.8	25.8±1.0	92.3±5.3	19.6±0.1	19.7±0.5	100.5±2.8
PCB-138	2.89±0.03	2.38±0.06	82.4±0.8	0.98±0.01	0.91±0.01	93.8±0.1	27.9±0.8	24.9±1.35	89.4±6.4	19.6±0.1	19.2±0.6	97.9±2.4
PCB-153	2.89±0.03	2.32±0.06	80.3±0.3	0.98±0.01	0.9±0.01	92.8±0.2	27.9±0.8	25.3±1.17	90.8±5.8	19.6±0.1	19.2±0.6	97.9±2.4
PCB-180	2.89±0.03	2.47±0.08	85.5±0.8	0.98±0.01	1.03±0.01	106.2±0.5	27.9±0.8	24.9±1.39	89.4±6.3	19.6±0.1	17.1±0.6	87.3±2.6

Table 3.7. Results of liquid extraction experiment spiked with PCB 118 and its possible daughter products

Compound	Experiment 4					
	Low level (1 ppb)			Medium level (20 ppb)		
	Expected (ppb)	GC Result (ppb)	Recovery (%)	Expected (ppb)	GC Result (ppb)	Recovery (%)
TMX	0.97±0.01	0.86±0.1	88.7±1.4	19.6±0.1	16.9±0.2	86.1±1.4
PCB-25	0.97±0.01	0.79±0.01	81.6±2.5	19.6±0.1	17.3±0.1	88.3±1.4
PCB-26	0.97±0.01	0.79±0.0	81.7±1.9	19.6±0.1	17.2±0.1	87.5±1.3
PCB-33	0.97±0.01	0.8±0.0	84.7±2.5	19.6±0.1	17.8±0.2	90.6±0.9
PCB-67	0.97±0.01	0.79±0.1	82.1±2.3	19.6±0.1	17.7±0.0	90.2±1.0
PCB-70	0.97±0.01	0.84±0.1	86.8±2.5	19.6±0.1	17.5±0.2	89.0±1.4
PCB-66	0.97±0.01	0.79±0.01	82.3±2.3	19.6±0.1	17.4±0.1	88.5±1.6
PCB 118	0.97±0.01	0.99±0.0	102.3±1.1	19.6±0.1	18.2±0.1	92.8±0.7

3.10.1.6. Recovery experiments for certified reference materials by using vial extraction method

Evaluation of extraction efficiency and analytical accuracy was also done by using two types of certified reference material (CRM) parallel with reactor samples. CRMs of PCBs on sandy loam (CRM141-050) and PCBs in sewage sludge (LGC61844) were extracted by using vial extraction method (n=6) and the results are given in Table 3.8 and Table 3.9, respectively. Extraction results for reference soil and sewage sludge were in the range of 85-99% and 82-103%, respectively.

Table 3.8. Recovery results for certified reference material for PCB in soil (CRM141-50)

PCB Congener	Certificate value (µg/kg)	95% confidence interval (µg/kg)	95% prediction interval (µg/kg)	Extraction result (µg/kg)	Extraction recovery (%)
PCB-28	44.9	40.3-49.5	31.1-58.7	38.2±2.8	85.1±6.3
PCB-52	64.6	58.7-70.5	47-82.2	58.3±4.0	90.3±6.1
PCB-101	45.7	41.4-50	32.7-58.7	45.1±3.1	98.7±6.8
PCB 118	24	22.2-25.8	18.6-29.5	21.8±0.6	90.9±2.5
PCB-138	41.3	38.2-44.3	32.1-50.5	39.1±1.9	94.8±4.5
PCB-153	63	58-68	48.1-78	55.5±4.1	88.1±6.6
PCB-180	54.7	50.5-58.9	42.2-67.2	54.4±6.3	99.4±11.5

Table 3.9. Recovery results for certified reference material for PCB in Sewage Sludge (LGC6184)

PCB Congener	Certificate value (µg/kg)	Extraction result (µg/kg)	Extraction Recovery (%)
PCB-28	28±8	24.8±2.7	88.5±9.8
PCB-52	14±4	12.8±0.3	91.5±2.2
PCB-101	37±3	31.4±0.9	84.8±2.5
PCB 118	17±2	17.5±2.2	103.2±12.2
PCB-138	77±7	68.6±5.7	89.1±1.3
PCB-153	112±8	92.6±1.4	82.3±1.3
PCB-180	78±10	80.2±0.4	102.8±0.6

As a result, all recovery results were sufficiently good and within the acceptable range, 80-120%, which is given in USEPA Method 8082 (USEPA, 2000). After all these recovery experiments, it was concluded that the vial extraction procedure is sufficient and accurate and can be used for both slurry and liquid samples of small scale ATA and larger scale batch reactors (Chapter 4 and 5).

3.10.2. Second PCB analysis Method

PCB extractions and analytical procedures of sediment containing sets

One mL samples from each triplicate bottle were extracted with 5 mL of hexane on a wrist shaker overnight for measurement of PCBs. The organic phase was passed through a copper/Florisil (1:4) column and PCB congeners were analyzed using a Hewlett Packard 5890 series II GC equipped with ^{63}Ni electron capture detector as described previously (Fagervold *et al*., 2005) and a DB-1 capillary column (30mx0.25mmx0.25μm). PCB congeners in a mixture containing 250 μgL^{-1} Aroclor 1232, 180 μgL^{-1} Aroclor 1248 and 180 $\mu g\ L^{-1}$ Aroclor 1262 (also known as ‘‘Mullin’s mix’’ (Swackhamer *et al*., 1996) were used to make with a 10-point calibration curve with PCB 30 and PCB 204 used as internal standards.

Fifty-five additional congeners not present in the Aroclor mixture that were potential dechlorination products were also added to the PCB calibration table. A calibration mixture containing the internal and surrogate standards and Mullin’s mix was run with every batch of samples to determine the response factor of each peak relative to the internal standards and to correct/check shift in the retention times of peaks. PCB 166 was used as a surrogate compound and its recovery was about 85%. Congener concentrations were not corrected for surrogate recoveries, because of the possible bias in calculated mole fractions of lesser or more chlorinated congeners in the case of differential recoveries of the different surrogates. Peaks for the surrogate and internal standards and those that co-eluted with sulfur (which was sometimes not completely removed by the copper cleanup) were disregarded.

During analytical determination of PCBs, in the chromatograms, a peak may represent an individual congener or a group of congeners. The congeners that appear together in the same peak during chromatographic analysis are named as coeluting congeners. These coeluting congeners are designated by slashes separating their congener numbers. For data calculations, co-eluting congeners and homologues were

assumed to be present in equal proportions. Dechlorination curves were made for all PCB congeners in 30-day intervals over the course of incubation. The PCB concentrations were measured as µg PCB/mL of microcosm slurry by GC/ECD and converted to mol% which was calculated by using the formula below (Equation 3.4):

$$\text{mol\%} = \frac{\frac{C_i}{MW_i}}{\sum \frac{C_i}{MW_i}} .100 \qquad (3.4)$$

where MW_i is the molecular weight of each detected PCB congener (g/mol) and C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry). The extent of dechlorination was calculated from the weighted average number of chlorines ($Cl_{average}$) in samples before and after incubation. $Cl_{average}$ was reported as average number of Cl per biphenyl, and was calculated according to the formula below (Equation 3.5):

$$Cl_{average} = \frac{\sum C_i . n_i}{\sum C_i} \qquad (3.5)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents. The dechlorination rate was calculated within the linear slope of the dechlorination curve by dividing total chlorine removed per biphenyl with the time elapsed in days and calculated by using the formula below (Equation 3.6):

$$\text{Rate} = \frac{\left(\frac{\sum C_i . n_i}{\sum C_i}\right)_{initial} - \left(\frac{\sum C_i . n_i}{\sum C_i}\right)_{final}}{t} \qquad (3.6)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents and t is the incubation time elapsed (days).The average rate and the standard deviation were calculated from triplicate cultures.

3.11. Cleaning Of Glass Ware

Cleaning steps are given in a numbered order below;

1. All extraction tubes and other glass ware were rinsed with alcohol.
2. They were then soaked into alconox (detergent) added hot water for several hours.
3. All were brushed.
4. Rinsed with tab water to remove foam due to alconox.
5. Rinsed with acetone, hexane, methanol, acetone, hexane and methanol (2 times with all) in this order.
6. Then they were transferred to the 2 L beaker containing acid solution (10% v/v H_2SO_4) stayed there overnight.
7. Removed from acid solution and soaked in water for at least 3 hours before they are brushed.
8. They were finally rinsed with distilled water and dried at 105ºC.

If desired one more time rinsing with hexane might be done prior to use.

3.12. Molecular Analysis

All molecular analysis was carried out only in the sediment microcosms (Chapter 6-8).

3.12.1. DNA extraction

DNA was extracted (in triplicate) from microcosms samples for three time points (time points are shown in text and/or on the graphs with letter "D" followed with time in days) and was used in downstream processes such as enumeration and community analysis of PCB dechlorinating bacteria. DNA was extracted by adding 0.25 mL of slurry samples from each microcosm to an individual Power Bead microfuge tube of a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc.) or to a 96-well bead beating plate for rapid and thorough homogenization. In the former, the Power Bead tubes were mixed by brief vortex prior to 30 s of bead beating at speed "4.5" using a FastPrep120 (Q-Biogene, CA). In the latter, instead of FastPrep120 (Qiagen, Resch), a TissueLyser is used. The TissueLyser allows the

usage of 96-well plate and like FastPrep120, it provides disruption of biological samples through high-speed shaking. In the DNA extraction process, cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the spin column. Total DNA was then isolated from the Power Bead tubes or 96-well plates according to the manufacturer's directions. DNA was eluted in 100 µL of Tris buffer (provided with kit) and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). All DNA samples were diluted to 2ng/µL in Tris buffer.

3.12.2. Enumeration of PCB dehalorespiring bacteria by quantitative PCR

The quantification of putative dechlorinating Chloroflexi in the microcosm samples was performed (in triplicate) by quantitative polymerase chain reaction (qPCR) using iQ SYBR green supermix (Bio-Rad Laboratories). DNA was amplified with primer Chl348F (5'-CGCCCGCCGCGCGCGGGAGGCAGCAGCAAGGAA-3') and Dehal884R (5'-GGCGGGACACTTAAAGCG-3') targeting the16S rRNA genes of the members within Chloroflexi including *Dehalococcoides* groups (Fagervold *et al.*, 2005).

Each qPCR reaction volume (25-µL) contained 1x iQ SYBR green supermix, 400 nM forward and reverse primers and 1 µL of sample DNA. PCR amplification and detection were conducted in an iCycler (Bio-Rad Laboratories). QPCR conditions were as follows: initial denaturation for 15 min at 95°C followed by 35 PCR cycles of 30 s at 95°C, then 30 s at 61°C, then 30 s at 72°C. One copy of the gene per genome was assumed based on the genomes of *Dehalococcoides mccartyi* strain 195 and *Dehalococcoides* sp, strain CBDB1 (Seshadri *et al.*, 2005: Kube *et al.*, 2005). QPCR data were analyzed with MJ Opticon Monitor Analysis Software v3.1 and compared to a standard curve of purified DF-1 348F/884R 16S rRNA gene product. The standard curve consisted of duplicate dilutions over 7 orders of magnitude. The specificity of qPCR amplification was verified by melting curve analysis followed by gel electrophoresis 1.2% (w/v) high-melt agarose gel.

3.12.3. Community analysis of PCB dechlorinating bacteria by denaturing High Pressure Liquid Chromatography

Denaturing high pressure liquid chromatography (DHPLC) analyses were performed using a WAVE 3500 HT system (Transgenomic, Omaha, NE) equipped with a florescence detector (excitation 490 nm, emission 520 nm). Primers 348F/884) were used both for DHPLC following PCR or qPCR (sometimes qPCR products were used) as described below.

PCR reactions were performed in 50 μL reaction volumes using the following GeneAmp reagents (PE Applied Biosystems, Foster City, CA): 10 mM Tris-HCl, a mixture of dNTPs (200 nM each), 1.5 mM $MgCl_2$, 100 nM of each primer, 0.8% dimethyl sulfoxide (DMSO), 2 units of Ampli *Taq* DNA polymerase, 200 nM of each primer, 1 μL of DNA template and 34.5 μL of nuclease-free water. Amplification was performed in a PTC200 thermal cycler (MJ Research, Watertown, MA.) with the following cycle parameters: Initial denaturing (1 min at 95°C), 40 cycles of denaturation (45 s at 95°C), annealing (45 s at 61°C), and elongation (45 s at 72°C), followed by a final extension (30 min at 72°C). PCR products of the correct length (about 500 bp) were confirmed by electrophoresis using a 1.2% high melting agarose gel prior to analysis by DHPLC. The 16S rRNA gene fragments were analyzed in a 30 μL injection volume by DHPLC with a DNASep® cartridge packed with alkylated non-porous polystyrene-divinyl benzene copolymer microspheres for high-performance nucleic acid separation (Transgenomic, Omaha, NE). The oven temperature was 63.0°C and the flow rate was 0.5 mL min^{-1} with a gradient of 55–35% Buffer A and 45–65% Buffer B from 0 to 13 min. The analytical solutions used for the analyses were such that Buffer A constituted 0.1 M triethyl ammonium acetate (TEAA) at pH 7, Buffer B was made of 0.1 M TEAA and 25% acetonitrile at pH 7, Solution D included 25% water and 75% acetonitrile. Syringe Wash Solution was supplied from Transgenomic, Omaha, NE. Analysis was performed using the Wavemaker version 4.1.44 software. An initial run was used to identify individual PCR fragments and determine their retention times. Individual

peaks were eluted for sequencing from a subsequent run and collected with a fraction collector based on their retention times. The fractions were collected in 96-well plates (Bio-Rad, Hercules, CA) and dried using a Savant SpeedVac system (Thermo Electron Corporation, Waltham, MA) followed by dissolution in 30 μL of nuclease-free water. Re-amplification was performed following the protocol described above. The PCR amplicons were electrophoresed in a 1.2% high melting agarose and the excised fragment was purified for sequencing using Wizard® PCR Preps DNA Purification Resin (Promega, Madison, WI) and then sequenced (BASLab, University of Maryland, IMET, Baltimore ,USA).

3.12.4. Sequencing and analysis

Each DHPLC fraction was sequenced in the 5′ and 3′ direction with 100pM of primer 348F or 884R using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA). Sequences were examined for errors and assembled using the software Finch TV software package (FinchTV, 2011). Alignments were then refined manually. Chimera formation was examined using Chimera Check (Cole *et al.*, 2003). The sequence alignments were done by using Ribosomal Database Project (RDP) produced by Cole *et al.* (2009). Phylogenetic tree was generated based on published Chloroflexi sequences over 1200 base pairs by the neighbour joining approach using default settings in RDP.

After comparative sequence analyses of DNA obtained from the DHPLC, 10 phylotypes (Figure 8.12) were sequenced and checked to find regions of local similarity between sequences by using Basic Local Alignment Search Tool (BLAST), which is available on the web on the National Center for Biotechnology Information (NCBI, USA) website. This program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Also, BLAST can be used to infer functional and evolutionary relationships

between sequences as well as help identify members of gene families (Altschul *et al.*, 1990).

DHPLC fractions was sequenced in the 5′ and 3′ direction with 100 pM of primer 348F or 884R using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA). Sequences were examined for errors and assembled using the software Finch TV software package (FinchTV, 2011). Alignments were then refined manually. Chimera formation was examined using Chimera Check (Cole *et al.*, 2003). The sequence alignments were done by using Ribosomal Database Project (RDP) produced by Cole *et al.* (2009). Phylogenetic tree was generated based on published Chloroflexi sequences over 1200 base pairs by the neighbor joining approach using default settings in RDP.

3.13. Other Analyses

TS and VS determinations were carried out as described in Standards Methods were done according to Method 2540B and Method 2540E, respectively, (APHA, 2005). Determination of TSS and VSS were done according to Standards Methods 2540D and 2540E, respectively (APHA, 2005).

pH measurements were performed with a pH meter (Model 510) and a pH probe (EC-PH510/21S, Eutech Instruments Pte Ltd., Spain). Calibration of probes was done before each reading, by using standard pH solutions of 4, 7, and 10.

ORP measurements were performed for small scale ATA and larger scale batch reactors with a pH meter (Model 510) equipped with an ORP electrode (Recorder S-500C- Sensorex, Eutech Instruments Pte Ltd., Spain).

CHAPTER 4

IMPACT OF PCB 118 AND TRANSFORMER OIL TOXICITY ON ANAEROBIC DIGESTION OF SLUDGE: ATA RESULTS

4.1. Introduction

Polychlorinated biphenyls (PCBs) are one of the most toxic and persistent man-made contaminants which were commercially produced as complex mixtures starting from the late 1920s. Due to their physical and chemical stability, they were used widely in many industrial applications (Erickson, 1997). Waste motor and transformer oils are considered as main sources of PCB emissions into the environment. It is estimated that about 125 million transformers containing PCBs were in use as of 1999 in USA (Jones *et al.*, 2003). Although both the production and the discharge of PCBs have been banned in USA since 1978 (Borja *et al.*, 2005), contamination with PCBs still occurs and is of great public concern. This concern has led the European Union and many other countries to regulate the PCB contents in air, water and sludge. Accordingly, the Stockholm Convention has listed PCBs as priority chemicals for eventual elimination by 2025 and the parties of the Convention must apply environmentally sound management of PCB wastes by 2028 (Stockholm Convention, 2012).

Depending on the type and the source of contamination, there are several physicochemical and biological treatment options for PCBs. In literature, mainly, physico/chemical dechlorination of PCBs in oil was studied (Wu *et al.*, 2005; Cafissi *et al.*, 2007). However, physicochemical techniques are either expensive or may generate undesirable, even toxic by-products such as dioxins and furans, while

biological degradation alters the structures of PCB congeners to more biodegradable chemicals and seems to be a more environmentally friendly solution (Abramowicz, 1995). It is known that, dechlorination under anaerobic conditions occurs due to dehalorespiration, where halogenated compounds are used as terminal electron acceptors (Berkaw *et al.*, 1996; Fagervold *et al.*, 2011). Consequently, an excess of a carbon source (electron donor) is required for efficient dechlorination (Tartakovsky *et al.*, 2000). There are not many biological-especially anaerobic dechlorination-studies of PCBs in oil. The ones which investigated dechlorination of PCBs in oil either tested aerobic conditions and/or used commercial microorganisms or used an easily degradable carbon source as an electron donor (Cedzynska and Sobiecka, 2004; Ahn *et al.*, 2006; Sobiecka *et al.*, 2009). This study aims, on the other hand, to treat PCB 118 under anaerobic conditions of sludge digesters by the use of a complex carbon source/electron donor (waste activated sludge) in the presence of transformer oil (TO).

For this purpose PCB 118 (245-3'4'-pentachlorobiphenyl) is selected as an indicator since it is a toxic congener found abundantly in Aroclor 1254, a TO formulation (Frame *et al.*, 1996). Anaerobic toxicity assays (ATAs) which was developed by Owen *et al.* (1979) was used to evaluate the toxicity of various doses of PCB 118 and TO on the anaerobic biodegradability of sludge and digester performance by analyzing cumulative methane production in the test bottles.

4.2. Material and Methods

4.2.1. Chemicals

All solvents (n-hexane and acetone) were purchased from Merck Chemical Co. Germany. All individual standards of pure PCB congeners (118, 70, 67, 66, 33, 25, and 26), surrogate standard, TMX (2456-Tetrachloro-m-xylene solution), and internal standard (IS), PCB-209 were purchased from Dr. Ehrenstorfer GmbH. PCB-

free TO is purchased from Sigma-Aldrich Co. USA. A certified reference soil, i.e. sandy loam type soil containing seven indicator PCBs (28, 52, 101, 118, 138, 153, and 180) was purchased from LGC standards-Germany (Cat. No: CRM 141-050) and used for quality assurance of extraction and analyses.

4.2.2. Experimental Setup

Waste activated sludge (WAS) was taken from the return line of secondary sedimentation tanks of Ankara Municipal Wastewater Treatment Plant in Turkey and used to constitute the digestion microcosms in this work. The conventional biological treatment plant is in operation since 1997 with a current average wastewater flow rate of 746,000 m^3/day. Mixed culture anaerobic digester sludge (ADS) taken from one of the mesophilic anaerobic digesters at the same plant was used as seed. The digesters are operated at 35°C with a sludge retention time value of 14 days. Prior to use, ADS and WAS were homogenized and filtered through a screen with a mesh size of 1 mm, and stored in refrigerators at 4°C. TSS and VSS values of WAS were 14,020±85 mg/L and 10,080±113 mg/L, respectively, and for ADS, 45,100±820 mg/L and 16,680±123 mg/L, respectively. F/M ratio was set to about 1.1 gVS WAS/gVSS ADS for all sets of reactors. A basal medium (BM) adopted from Speece (1996) was used in all microcosms to supply the necessary macro and micro nutrients. TO density was 889.2±17.0 g/L with the same VS and TS values of 876.7±27.2 g/L.

A total of 5 different sets of reactors (microcosms) were prepared for varying doses of TO (Table 4.1). First set (Set-1) was operated as TO-free set, while the other four sets, Set-2, Set-3, Set-4, and Set-5 were prepared with varying TO doses, such as 0.38g/L (low-TO), 0.76g/L (medium-TO), 0.76g/L (medium-TO), and 1.52g/L (high-TO), respectively. In each set, four different PCB 118 doses (1, 10, 20, and 30 mg/L) were applied (Table 4.1). C1 reactors were operated as one of PCB-free control reactors and were prepared for each set-except for Set-4. C1 of Set-1 did not receive

any TO, while C1 of other sets received varying amounts of TO according to the applied TO dose. C2 reactors were also operated as PCB-free control reactors, but differing from C1 reactors, they did not receive WAS, hence, allowing to assess the effect of WAS on the anaerobic digester performance in the absence and presence of varying TO doses. Set-4 did not receive any WAS and was prepared similar to Set-3 (Table 4.1) in order to evaluate the effect of WAS absence on the PCB 118 dechlorination and digester performance.

Table 4.1. Detailed composition of ATA reactors.

Sets	**Reactors**	**TO Concentration (g/L)**	**PCB 118 Concentration (mg/L)**
Set-1: No-TO	S1-1	0	1
	S1-10	0	10
	S1-20	0	20
	S1-30	0	30
	S1-C1	0	0
	S1-C2	0	0
Set-2: Low-TO	S2-1	0.38	1
	S2-10	0.38	10
	S2-20	0.38	20
	S2-30	0.38	30
	S2-C1	0.38	0
	S2-C2	0.38	0
Set-3: Medium-TO	S3-1	0.76	1
	S3-10	0.76	10
	S3-20	0.76	20
	S3-30	0.76	30
	S3-C1	0.76	0
	S3-C2	0.76	0
Set-4: Medium-TO, No WAS	S4-1	0.76	1
	S4-10	0.76	10
	S4-20	0.76	20
	S4-30	0.76	30
Set-5: High-TO	S5-1	1.52	1
	S5-10	1.52	10
	S5-20	1.52	20
	S5-30	1.52	30
	S5-C1	1.52	0
	S5-C2	1.52	0

Serum bottles with 160-mL volume were used as ATA reactors into which an aliquot of 20 mL of ADS which was not previously acclimated to neither PCB 118 nor TO,

10 mL of BM and 20-mL of WAS were added (WAS was not added in Set-4 and C2 reactors). A working liquid volume of 100 mL was provided by the addition of distilled water. The bottles were flushed with N_2 gas for 3-4 min. and they were immediately sealed with rubber stoppers and spiked with PCB 118 as required. Abiotic controls autoclaved at 121^oC for 20 min. before PCB 118 spike were prepared for all sets, but, only for one dose of PCB 118 (10 mg/L). These reactors are referred to as A-10 ppm in the text/graphs. PCB 118 stock solution was prepared in acetone. ATA reactors were operated as triplicate and incubated in dark at 35±1^oC throughout 65 days. The reactors were shaken manually once a day.

A number of parameters were measured such that: total gas volume and gas composition at predetermined time intervals and COD, TS, VS, TSS, VSS, pH, oxidation-reduction potential (ORP), and PCBs at the beginning and at the end of the reactor operation. Reactors were operated until the decelerated methane production phase was reached.

4.2.3. PCB extraction procedure

PCB extraction was done by taking 2-mL reactor slurry sample into a 22-mL glass vial sealed with PTFE screw cap. Then, 50 mg reduced copper (pre-washed with hexane, acetone and dried with N_2) to remove sulphur compounds and known-amount of TMX to calculate the extraction efficiency and 10 mL n-hexane were added. By vigorous overnight-shaking on a horizontal platform shaker at 350 rpm, PCBs were transferred into hexane phase. To separate sample and hexane, centrifugation was applied at 2500 rpm for 5 min. After passing the extract through a column packed with anhydrous sodium sulfate (Na_2SO_4), it was concentrated down to 2 mL with nitrogen gas and 1-mL of this eluate was taken into a 2-mL borosilicate glass GC vial and IS was added before GC analysis. Due to a small volume change during the extraction, final results were volume corrected. PCB analysis was done using a GC/ECD (Agilent/6890N) equipped with HP-5 MS capillary column (30mx0.25mmx 0.25μm). IS calibration procedure was followed in accordance with

EPA Method 8082 (USEPA, 2000). A 5-point calibration curve was used for each congener with relative standard deviations lower than 20% and r^2 >0.999.

4.2.4. Analytical Methods

GC experimental conditions for PCB analyses were as follows: Helium was the carrier gas at 1.5 mL/min.; nitrogen was the make-up gas at 20 mL/min.; injection mode/temperature was splitless/250°C; detector temperature was 350°C; temperature program was: initial temperature 100°C; 100-160°C at 20°C/min.; 160°C for 2 min.; 160-200°C at 3°C/min.; 200-240°C at 8°C/min.; 240°C for 5 min.; 240-290°C at 30°C/min.; 290°C for 3 min. 1 µL sample was injected for each analysis.

Gas production was measured with a water replacement device and gas composition (CH_4, CO_2) was analyzed with GC/TCD (Agilent/6890N) equipped with HP-PlotQ capillary column (30.0mx530µmx 40.0µm). GC experimental conditions for gas analyses were as follows: He was the carrier gas at 3 mL/min.; injection mode/temperature was split with ratio of 20:1 at 60 mL/min/100°C; detector temperature was 250°C; Temperature program was: initial temperature 45°C for 1 minute, 45-65°C at 10°C/min. 100 µL sample was injected for each analysis. A four-point linear calibration curve was used for the quantification of CH_4 and CO_2 gases with r^2 >0.99.

COD analysis was conducted according to EPA approved method (Jirca and Carter, 1975). Solid analysis was done according to Standards Methods (APHA, 2005). pH and ORP values were measured with a pH/ORP meter (EC-PH510/21S, Eutech Inst.).

4.2.5. Statistical Evaluation

All statistical evaluations were performed using *paired t test* with a statistical significance of $p<0.005$ (n=14).

4.2.6. Quality Assurance/Quality Control (QA/QC)

The accuracy and the precision of the PCB measurements were controlled by calculating the surrogate recoveries, which were between 88-110% and in the acceptable range of USEPA Method 8082, 80-120% (USEPA, 2000). Accordingly, for each batch of 10 sludge samples, a method blank, laboratory control sample and a duplicate sample were analysed. Method detection limits (n=7) for PCB 118 and TMX were 57 ppt and 32 ppt, respectively. Limit of quantitation (LOQ) (n=7) were 180 ppt and 100 ppt for PCB 118 and TMX, respectively. All targets were lower than the LOQ in method blanks. Surrogate recovery correction or blank subtraction was not performed. All the standard deviations of replicates (<10%) were sufficiently low, showing that the quality of measurements was good and acceptable. Also, strict USEPA glass ware cleaning procedures was followed (USEPA, 2007).

4.3. Results and Discussion

4.3.1. Methane production

Figure 4.1 summarizes the results of cumulative methane production with respect to time under different operating conditions of reactors. This figure can be evaluated from several different points. For all the microcosms investigated, the highest methane production was observed for 1 ppm PCB dosed microcosms. Gas production rates of these microcosms were initially lower than those of control reactors which received no PCBs (C1s) however, after the third week of incubation, their methane productions started to increase and exceeded those of C1s; indicating no adverse effect of PCB 118 at 1 ppm on methanogens (Figure 4.1). In fact, this effect was more like a “stimulate” effect since higher gas production obtained compared to control reactors. A similar situation was observed in the study of She *et al.* (2005) for 2,4-dinitrophenol (2,4-DNP) and 3-nitrophenol (3-NP) where similar or higher concentrations compared to this study was tested with no adverse effect on

methanogenesis. In a study of Tartakovsky *et al.* (2000), no toxicity of Aroclor 1242 was observed in ATA test even at a very high concentration (1 g/L). This finding was attributed to low solubility of the mixture. In this work, at PCB 118 doses other than 1 ppm, it was observed that the cumulative methane productions decreased with increasing PCB doses. A statistically significant decrease ($p<0.005$) in methane production was observed proportional to an increase in PCB 118 concentration. These results indicate that PCB 118 may inhibit the methane producing microorganisms at high concentrations.

In addition to volume of methane produced, fraction in total gas produced might also be used as an indication of inhibition. In PCB microcosms, CH_4 content decreased with the increase in PCB 118 dose, indicating PCB 118 inhibition. To determine a magnitude of the inhibition, we expressed the gas produced in each reactor as % of control reactor values which is similar to maximum rate ratio (MRR) explained previously by Owen *et al.* (1979). MRR is calculated by dividing the gas production of sample to the gas production of control and it is stated that a MRR of less than 95% suggests possible inhibition and one less than 90% suggests significant inhibition. The MRR values of 10 ppm PCB 118 microcosms were about 93%, suggesting some inhibition, while at 20 ppm and 30 ppm the inhibition observed in microcosms were significant (Table 4.2), with the highest inhibition in S4-30 ppm reactors (MRR value was 75.2%). Overall, notable inhibition was observed for the 20 and 30 ppm PCB reactors.

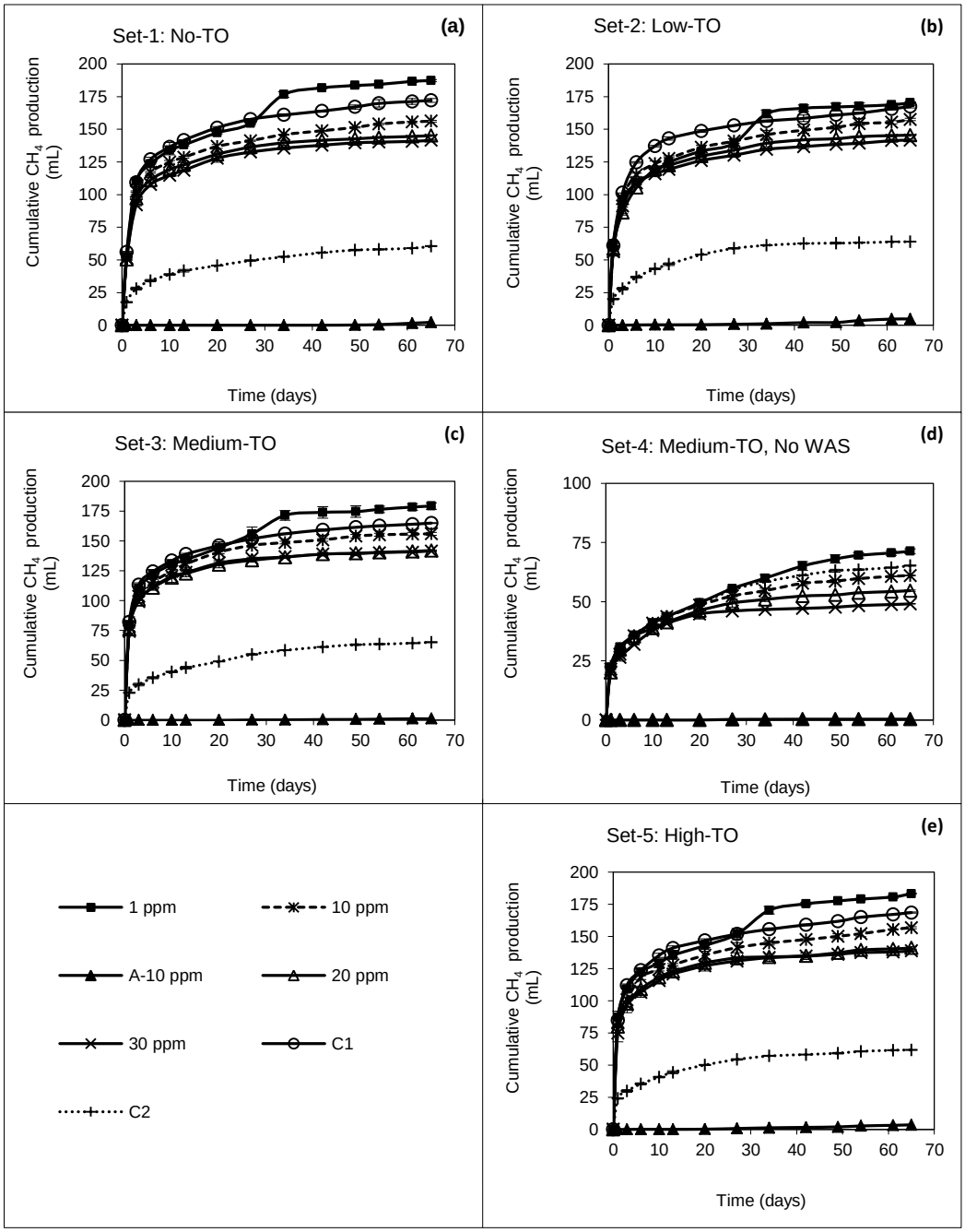


Figure 4.1. Cumulative methane productions of all microcosms with respect to varying doses of PCB 118 throughout the incubation (TO: transformer oil, A: autoclaved reactor).

Table 4.2. CH_4 content (%), ultimate total gas and ultimate CH_4 productions as well as MRR values of all microcosms within five sets observed at the end of 65 days of incubation.

Sets	Reactor name	CH_4 %	Total gas production (mL)	CH_4 gas production (mL)	MRR[1] for Total gas	MRR[1] for CH_4
Set 1: No-TO	S1-C1	63	227.6 ± 1.0	172.1 ± 1.4	100.0	100.0
	S1-C2	33	79.9 ± 1.8	60.4 ± 1.6	35.1	35.1
	S1-1	63	253.4 ± 1.7	187.2 ± 0.9	111.3	108.8
	S1-10	59	216.3 ± 0.7	156.2 ± 0.6	95.0	90.7
	S1-A10	9	3.0 ± 4.7	2.2 ± 3.8	1.3	1.3
	S1-20	56	207.0 ± 0.0	145.2 ± 0.0	90.9	84.4
	S1-30	55	204.1 ± 2.3	141.6 ± 0.5	89.7	82.2
Set 2: Low-TO	S2-C1	63	222.7 ± 1.8	167.3 ± 0.9	100.0	100.0
	S2-C2	35	84.6 ± 0.7	63.9 ± 0.3	38.0	38.2
	S2-1	65	229.9 ± 0.9	172.0 ± 0.6	103.2	102.8
	S2-10	60	216.5 ± 1.3	155.9 ± 1.0	97.2	93.1
	S2-A10	3	14.4 ± 4.2	4.8 ± 3.1	6.5	2.8
	S2-20	58	206.2 ± 0.0	144.8 ± 0.0	92.6	86.5
	S2-30	55	200.4 ± 0.8	141.6 ± 0.7	90.0	84.6
Set 3: Medium -TO	S3-C1	60	224.7 ± 0.4	165.1 ± 0.3	100.0	100.0
	S3-C2	35	95.1 ± 1.0	65.2 ± 1.2	42.3	39.5
	S3-1	61	244.1 ± 2.2	179.3 ± 2.7	108.6	108.6
	S3-10	58	219.4 ± 0.6	156.0 ± 1.1	97.6	94.5
	S3-A10	1	5.2 ± 1.9	1.2 ± 0.8	2.3	0.7
	S3-20	55	203.0 ± 0.0	142.1 ± 0.0	90.3	86.1
	S3-30	54	198.2 ± 0.6	141.8 ± 0.4	88.2	85.9
Set 4[2]: Medium-TO, No WAS	S4-1	38	102.0 ± 1.0	71.3 ± 1.0	107.3	109.4
	S4-10	35	83.1 ± 1.0	61.1 ± 0.1	87.4	93.6
	S4-A10	0.2	1.3 ± 0.3	0.4 ± 0.3	1.3	0.5
	S4-20	31	74.6 ± 0.0	54.7 ± 0.0	78.4	83.8
	S4-30	28	63.8 ± 0.9	49.1 ± 0.1	67.1	75.2
Set 5: High-TO	S5-C1	62	225.1 ± 1.6	168.5 ± 0.4	100.0	100.0
	S5-C2	34	82.4 ± 0.6	61.8 ± 1.0	36.6	36.7
	S5-1	65	244.1 ± 1.0	183.2 ± 0.5	108.4	108.7
	S5-10	60	215.3 ± 1.1	156.7 ± 0.8	95.6	93.0
	S5-A10	1	7.9 ± 2.1	3.6 ± 2.0	3.5	2.1
	S5-20	55	197.6 ± 0.0	141.0 ± 0.0	87.8	83.7
	S5-30	55	194.9 ± 2.1	138.8 ± 0.2	86.6	82.4

[1]: MRR: maximum rate ratio: calculated as dividing sample gas production (spiked) to control gas production.
[2]: MRR of Set 4 was calculated by using the results S3-C2 reactor which was also the control reactor of this set.

For the effect of WAS presence and varying doses of PCB 118 on the biogas production, methane production results of Set-3 microcosms were compared with their no-WAS counterparts in Set-4. Total methane productions of Set-3 microcosms

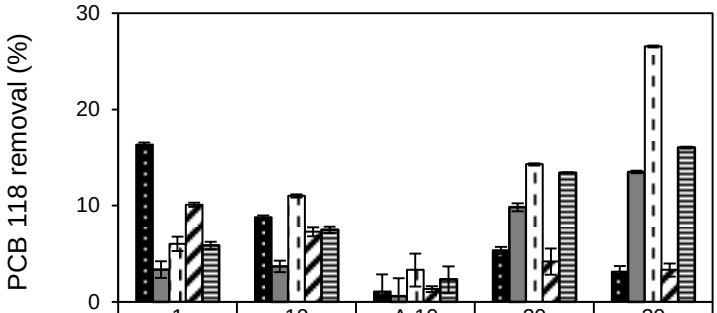
were found much higher than those of Set-4. Total cumulative methane volume of S3-C2 was about 65 mL at day 65 (Figure 4.1(d)), which also served as a control for Set-4. This result is higher than all those produced by PCB microcosms of Set-4- except for 1 ppm PCB reactor. The decrease in cumulative methane production in Set-4 was about 31%, while it was around 21% in Set-3 (Table 4.2) when PCB 118 concentration increased from 1 ppm to 30 ppm. As expected, the absence of WAS caused lower methane productions for the studied range of PCB 118. A negligible amount of methane production (if not none) was observed in autoclaved reactor (Figure 4.1).

When the effect of TO on methane production is examined, not a clear single trend can be stated. The highest methane productions occur at zero TO doses at some PCB concentrations (e.g. 1 and 20 ppm); whereas zero TO dose does not yield the maximum methane amounts in other PCB doses (10 and 30 ppm). Therefore, it is not easy to make a clear-cut judgment for TO effect on gas production (Table 4.2). When the cumulative CH_4 productions of C1 reactors were compared with respect to varying TO concentrations, S1-C1 was found as the most productive reactor with an ultimate CH_4 production of 172 mL (Figure 4.1). The ultimate cumulative CH_4 productions of WAS- and PCB-free control reactors, C2, changed between 11.1 ±0.2 mL and 11.7 ±0.0 mL with respect to varying TO concentrations (0-1.52 g/L). These sets produced the smallest amount of methane since “food” (WAS) was not provided. These results show the methane production capacity of seed (ADS) only.

4.3.2. PCB 118 removal

The total changes in PCB 118 contents of the reactors at the end of the incubation period are depicted in Figure 4.2. Additionally, Table 4.3 presents initial and final PCB 118 concentrations of all microcosms. Two distinct trends in PCB 118 removal were observed from the results. Sets containing no TO and no WAS showed a decreasing PCB removal with increasing PCB concentration. On the other hand, the

rest of the sets (low, medium and high-TO containing sets of 2, 3, and 5) showed an increasing PCB removal trend with increasing PCB concentration with the highest removal in S3-30 reactors by about 26.5±0.1% (Figure 4.2). It is known that a low pollutant concentration is generally insufficient for the stimulation of degradative enzymes or to sustain growth of competent organisms (Borja et al., 2005). In that sense, higher PCB 118 concentrations in our microcosms possibly triggered the higher production of degradative enzymes which eventually led to the higher degradation percentages. Chang *et al.* (1999) found that in the range of 1-10 ppm, the higher the PCB concentration was, the faster the dechlorination rate was. Higher dechlorination rates was observed in a previous study investigating anaerobic treatability of Aroclor 1260 in oil (Ahn *et al.*, 2006), however, in that study, methanol was used as a carbon source, which is readily degradable compared to WAS to obtain the energy required by microorganisms for reductive dechlorination.



	1 ppm	10 ppm	A-10 ppm	20 ppm	30 ppm
Set-1: No-TO	16.3	8.8	1.1	5.3	3.1
Set-2: Low-TO	3.4	3.7	0.6	9.8	13.5
Set-3: Medium-TO	6.0	11.0	3.3	14.3	26.5
Set-4: Medium-TO, No WAS	10.1	7.3	1.3	4.2	3.3
Set-5: High-TO	5.9	7.5	2.3	13.4	16.0

Figure 4.2. Comparison of percent removal of PCB 118 with regard to spiked PCB 118 doses. (TO: transformer oil, A: autoclaved reactor).

Table 4.3. PCB 118 values of all microcosms within five sets together with calculated PCB 118 removals (%).

Sets	Reactors	initial PCB 118 (ppb)	final PCB 118 (ppb)	PCB 118 Removal (%)
Set 1: No-TO	S1-1	1068.3 ± 39.2	893.8 ± 10.9	16.3 ± 0.2
	S1-10	11500.0 ± 61.7	10491.4 ± 212.2	8.8 ± 0.2
	S1-A10	11274.5 ± 74.1	11154.3 ± 201.5	1.1 ± 1.8
	S1-20	21063.8 ± 399.3	19937.6 ± 88.4	5.3 ± 0.4
	S1-30	30824.0 ± 516.9	29862.4 ± 254.3	3.1 ± 0.6
Set 2: Low-TO	S2-1	933.1 ± 5.5	901.7 ± 26.6	3.4 ± 0.9
	S2-10	11427.5 ± 240.0	11005.1 ± 68.2	3.7 ± 0.6
	S2-A10	11940.8 ± 105.3	11871.3 ± 78.7	0.6 ± 1.9
	S2-20	21633.7 ± 850.3	19506.3 ± 166.8	9.8 ± 0.4
	S2-30	31293.2 ± 67.0	27067.0 ± 559.7	13.5 ± 0.1
Set 3: Medium-TO	S3-1	1016.6 ± 45.6	955.3 ± 2.8	6.0 ± 0.7
	S3-10	11168.1 ± 185.5	9938.0 ± 58.1	11.0 ± 0.2
	S3-A10	11774.3 ± 173.3	11384.7 ± 646.7	3.3 ± 1.7
	S3-20	21719.5 ± 127.7	18614.6 ± 319.0	14.3 ± 0.1
	S3-30	31521.5 ± 512.9	23156.1 ± 683.7	26.5 ± 0.1
Set 4: Medium-TO, No WAS	S4-1	1016.1 ± 0.3	913.5 ± 23.7	10.1 ± 0.2
	S4-10	11320.6 ± 3.7	10497.3 ± 382.4	7.3 ± 0.5
	S4-A10	11033.3 ± 39.7	10888.2 ± 18.3	1.3 ± 0.3
	S4-20	21511.5 ± 1145.9	20609.6 ± 440.5	4.2 ± 1.4
	S4-30	31428.7 ± 226.6	30386.7 ± 673.4	3.3 ± 0.7
Set 5: High-TO	S5-1	925.9 ± 3.3	871.7 ± 20.9	5.9 ± 0.4
	S5-10	12166.5 ± 224.2	11255.8 ± 214.5	7.5 ± 0.3
	S5-A10	11155.1 ± 310.3	10896.4 ± 179.8	2.3 ± 1.4
	S5-20	21942.1 ± 124.1	18998.6 ± 19.1	13.4 ± 0.0
	S5-30	31497.6 ± 326.8	26448.5 ± 341.6	16.0 ± 0.1

Possible dechlorination by-products of PCB 118 (PCB 25, 26, 33, 66, 67, and 70) were checked. An increase in PCB 70 (25-3’4’-tetrachlorobiphenyl) and PCB-67 (245-3’-tetrachlorobiphenyls) concentrations as well as in PCB 26 (25-3’-trichlorobiphenyl) concentration which might be attributed to sequential para-chlorine removal of PCB 118 was observed mostly in 20 or 30 ppm PCB 118 reactors. However, the amount of these congeners, compared to the reduction in the mother congener (PCB 118) was negligible. The observed concentrations of daughter

products were less than 10 ppb in general (data not shown). Since the sampling and measurement for daughter products was done only at reactor termination, some transformations products might be lost. Additionally, it can be hypothesized that dehalogenation was limited either by low concentration of mother congener or low organic load. Indeed, in the study of Nies and Vogel (1990), insignificant dehalogenation in batches receiving no organic substrate was observed. Also, in another study, at low organic and Aroclor 1242 loading rates, it was seen that adsorption was the prevailing mechanisms of Aroclor disappearance (Tartakovsky *et al.*, 2000). In that study, dechlorination was observed for the Aroclor 1242 loading above 15 ppm and organic loading of 0.72 gCOD/L.day, while in our study organic loading was about 0.3 gCOD/L.day. Also, Aroclor 1242 comprises mostly congeners with chlorine numbers around 4 or lower (Frame et al., 1996), while PCB 118 has 5 chlorines, which makes it harder to dechlorinate.

When compared with Set-3 PCB 118 removals, lower PCB 118 removals in Set-4 indicate the necessity of WAS, a carbon and energy source, for degradation of PCB. As it is known that the use of organic substrate as electron donors is essential for dehalorespiring bacteria to degrade PCBs (Nies and Vogel, 1990). In our results, it seems presence of WAS together with TO had a synergistic effect on the degradation of PCB 118, as it was observed in Set-2, 3 and 5 at higher PCB doses such as 20 and 30 ppm. Indeed, this finding was supported with the previous finding of Cedzynka and Sobiecka (2004) with that the presence of hydrocarbons has a positive influence on the degradation of PCBs. It has been reported in the literature that anaerobic reductive dechlorination occurs under methanogenic conditions (Borja *et al.*, 2005), but, it has also been observed that methanogenesis decreased when dechlorination increased (Boyle *et al.*, 1993). This finding supports our findings since we also observed as the PCB concentration increased, methane production decreased (Figure 4.1), where dechlorination was highly pronounced (Figure 4.2).

4.3.3. Anaerobic digester performance

Typical indicators of anaerobic digester performance are COD and VS reductions. Therefore, all reactors are evaluated in terms of these parameters. As can be seen from Figure 4.3(a), within all sets COD reductions decreased with the increase in PCB 118 dose, with the highest COD removals being attained in 1 ppm reactors among the PCB-receiving microcosms. A summary of the initial and final COD analyses are given in Table 4.4. The reductions for control reactors (S1-C1, S2-C1, S3-C1, and S5-C1) which can be taken as references were as about 65%, 64%, 57%, and 47%, respectively, decreasing with the increase in TO concentration (Table 4.4). These removals are in line with those found in the literature for COD removal during anaerobic digestion of WAS as 40-60% (Benefield and Randall, 1980; Tapana and Pagilla, 2000). The highest removal was observed in S1-1 as 63% among the PCB containing reactors and as the PCB dose was increased to 30 ppm (S1-30) COD removal decreased to 22.2%. The same trend was observed in all sets. Apart from the negative influence of increasing PCB 118 doses, increasing TO doses also negatively influenced the COD reduction in reactors (Table 4.4).

Another important performance parameter of anaerobic digesters is the organic/solids reduction which is typically measured by VS reduction rates. TS and VS removals are plotted in Figure 4.3(b) and 4.3(c), respectively. The reductions in VS and TS of C1 reactors were between 49-59% and 36-41%, respectively (Table 4.5 and 4.6). From the trends observed in Figure 4.3(b) and 4.3(c), it can be concluded that as PCB 118 concentration increased, VS and TS removals decreased considerably. The reductions of Set-4 were found to be significantly lower than the others (Figure 4.3(b) and 4.3(c)) for all of the studied PCB 118 doses. No major change in TS or VS removal was observed with varying TO doses. Depending on the operating conditions, anaerobic digestion reduces volatile solids by 35 percent to 60 percent (USEPA, 1992). Even though the reductions were observed in VS and TS removal with PCB addition, the performance of anaerobic digesters were satisfactory and around the typical levels expected (Rulkens, 2008; USEPA, 1992). VSS and TSS

removals of most of the reactors were negatively affected by the increase of TO (Table 4.7).

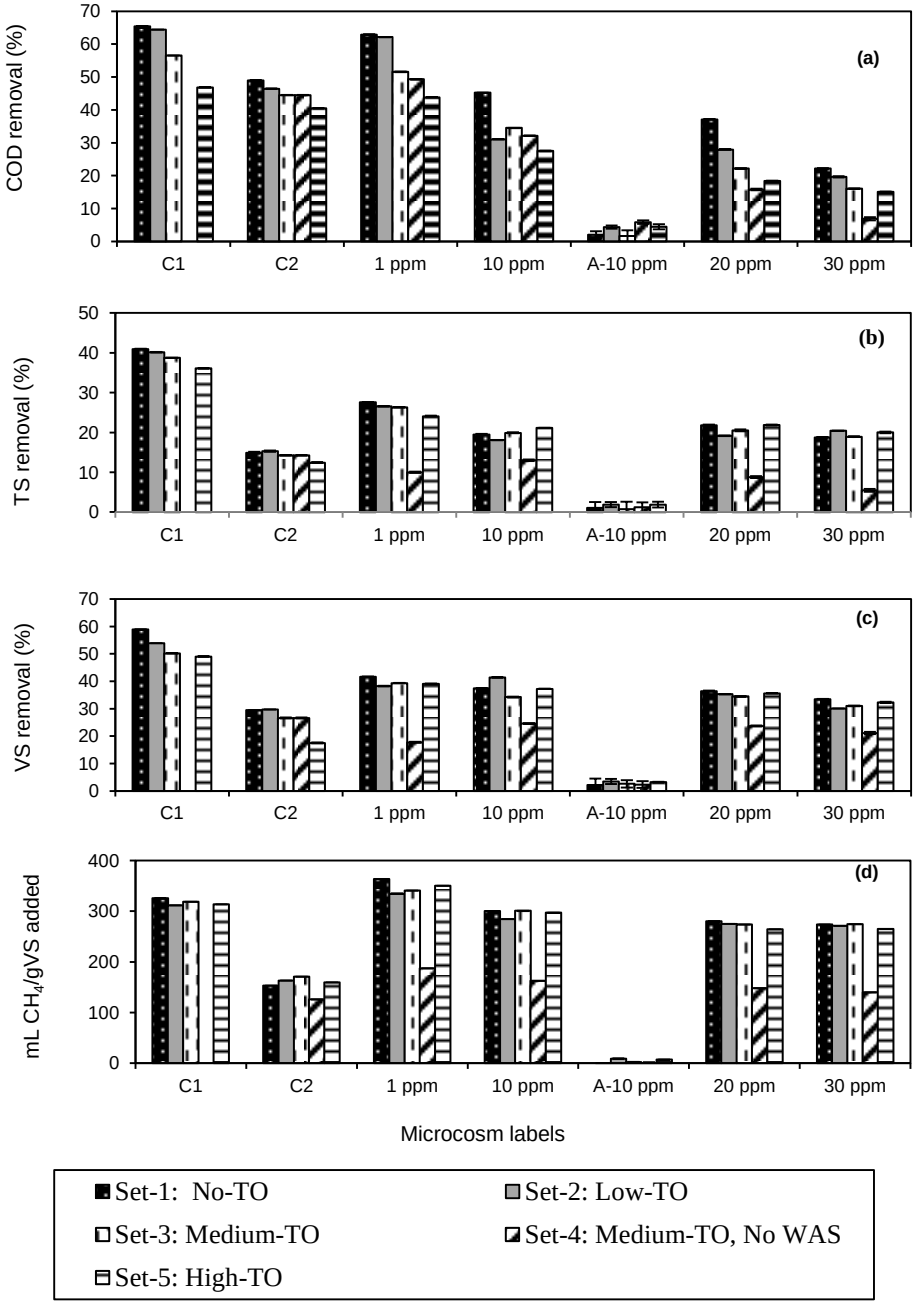


Figure 4.3. Comparisons of (a) COD, (b) TS, (c) VS reduction percentages of microcosms, and (d) mL CH_4/gVS added values of microcosms under different conditions (TO: transformer oil, A: autoclaved reactor).

Table 4.4. COD values of all microcosms within five sets together with calculated COD reductions (%).

Sets	Reactors	initial	final	COD reduction %
		COD (mg/L)	COD (mg/L)	
Set 1: No-TO	S1-C1	19739 ± 527	6827 ± 350	65.4 ± 0.1
	S1-C2	17491 ± 362	8929 ± 126	49.0 ± 0
	S1-1	21207 ± 400	7867 ± 290	62.9 ± 0
	S1-10	21509 ± 350	11786 ± 381	45.2 ± 0.1
	S1-A10	22404 ± 374	21958 ± 307	2.0 ± 1.1
	S1-20	24339 ± 129	15310 ± 253	37.1 ± 0
	S1-30	28303 ± 664	22025 ± 106	22.2 ± 0.1
Set 2: Low-TO	S2-C1	19271 ± 211	6856 ± 324	64.4 ± 0
	S2-C2	17782 ± 47	9522 ± 180	46.5 ± 0
	S2-1	20360 ± 195	7707 ± 61	62.1 ± 0
	S2-10	22642 ± 231	15613 ± 49	31.0 ± 0
	S2-A10	22276 ± 460	21304 ± 146	4.4 ± 0.5
	S2-20	26599 ± 168	19167 ± 305	27.9 ± 0
	S2-30	28862 ± 795	23189 ± 752	19.7 ± 0.2
Set 3: Medium - TO	S3-C1	20558 ± 122	8939 ± 134	56.5 ± 0
	S3-C2	17128 ± 139	9508 ± 210	44.5 ± 0
	S3-1	21545 ± 1033	10433 ± 106	51.6 ± 0.1
	S3-10	23147 ± 274	15163 ± 257	34.5 ± 0
	S3-A10	22708 ± 295	22341 ± 585	1.6 ± 1.8
	S3-20	26436 ± 165	20577 ± 318	22.2 ± 0.1
	S3-30	28522 ± 62	23960 ± 446	16.0 ± 0.1
Set 4: Medium-TO, No WAS	S4-1	18232 ± 307	9243 ± 230	49.3 ± 0
	S4-10	22258 ± 202	15106 ± 413	32.1 ± 0.1
	S4-A10	23344 ± 62	21982 ± 716	5.8 ± 0.5
	S4-20	24123 ± 16	20314 ± 936	15.8 ± 0.2
	S4-30	27140 ± 621	25263 ± 706	6.9 ± 0.5
Set 5: High-TO	S5-C1	21128 ± 38	11238 ± 379	46.8 ± 0
	S5-C2	19015 ± 724	11325 ± 235	40.4 ± 0.1
	S5-1	21245 ± 408	11934 ± 341	43.8 ± 0.1
	S5-10	23893 ± 187	17321 ± 412	27.5 ± 0.1
	S5-A10	22445 ± 597	21438 ± 500	4.5 ± 0.8
	S5-20	26820 ± 379	21903 ± 643	18.3 ± 0.2
	S5-30	28372 ± 545	24109 ± 396	15.0 ± 0.2

The methane yield for an anaerobic digestion process is commonly expressed as a function of VS load or the reduction of either VS or COD. Here, it is evaluated as a function of VS load and the yield range lied between 265-365 mL CH_4/gVS added as a function of PCB dose. When the methane yields of PCB microcosms were compared, it is seen that yield significantly decreased with the increase in PCB 118 dose, indicating inhibition to varying extents (Figure 4.3(d)). On the other hand,

methane yield/g VS added was slightly decreased as TO concentration increased (Table 4.5). Generally, the range of yield found in this study was close to those reported in literature, 370-506 mL/gVS added (Benefield and Randall, 1980).

Initial pH values were between 7.0-7.2 for all reactors except for the autoclaved ones which pH values were around 8.7. The reason of such a high pH in the autoclaved reactors could be the solubilisation of the cellular material at 121^{o}C and the release of CO_2 due to high temperature. At the end of the incubation period, pH values of all biological reactors were around 7.2 indicating proper conditions for anaerobic digestion (Speece, 1996) and dechlorination, for which optimal pH was reported as 7.0-7.5 (Borja *et al.*, 2005). ORP values of the microcosms of this study were between -214 and -330 mV showing appropriate conditions of the reactors for anaerobic degradation (Gerardi, 2003).

Table 4.5. VS values together with calculated VS reductions (%) and ml CH_4 produced per gVS removed data of all microcosms within five sets.

Sets	Reactors	initial	Final	VS reduction (%)	mL CH4 produced / gVS added
		VS (mg/L)	VS (mg/L)		
Set 1: No-TO	S1-C1	5292 ± 109	2178 ± 170	58.8 ± 0.1	325 ± 0.0
	S1-C2	3944 ± 24	2780 ± 20	29.5 ± 0.0	153 ± 0.0
	S1-1	5150 ± 127	3007 ± 140	41.6 ± 0.1	364 ± 0.0
	S1-10	5200 ± 85	3253 ± 216	37.4 ± 0.1	300 ± 0.0
	S1-A10	5183 ± 94	5070 ± 242	2.2 ± 2.3	0 ± 0.1
	S1-20	5190 ± 71	3300 ± 368	36.4 ± 0.2	280 ± 0.1
	S1-30	5170 ± 127	3440 ± 164	33.5 ± 0.1	274 ± 0.0
Set 2: Low-TO	S2-C1	5262 ± 99	2427 ± 58	53.9 ± 0.0	312 ± 0.0
	S2-C2	3862 ± 22	2713 ± 30	29.8 ± 0.0	163 ± 0.0
	S2-1	5140 ± 85	3173 ± 200	38.3 ± 0.1	335 ± 0.0
	S2-10	5480 ± 57	3213 ± 214	41.4 ± 0.1	284 ± 0.0
	S2-A10	5283 ± 47	5100 ± 159	3.5 ± 0.9	9 ± 0.7
	S2-20	5270 ± 42	3410 ± 71	35.3 ± 0.0	275 ± 0.0
	S2-30	5220 ± 28	3650 ± 0	30.1 ± 0.0	271 ± 0.0
Set 3: Medium -TO	S3-C1	5177 ± 85	2578 ± 107	50.2 ± 0.0	319 ± 0.0
	S3-C2	3815 ± 131	2800 ± 87	26.6 ± 0.2	171 ± 0.0
	S3-1	5230 ± 71	3173 ± 42	39.3 ± 0.0	340 ± 0.0
	S3-10	5190 ± 42	3413 ± 221	34.2 ± 0.1	301 ± 0.0
	S3-A10	5225 ± 130	5087 ± 130	2.6 ± 1.3	2 ± 0.7
	S3-20	5190 ± 240	3400 ± 174	34.5 ± 0.1	274 ± 0.1
	S3-30	5170 ± 127	3567 ± 31	31.0 ± 0.1	274 ± 0.0
Set 4: Medium-TO, No WAS	S4-1	3940 ± 85	3240 ± 60	17.8 ± 0.2	187 ± 0.0
	S4-10	3950 ± 99	2980 ± 40	24.6 ± 0.1	163 ± 0.0
	S4-A10	3920 ± 85	3827 ± 76	2.4 ± 1.2	1 ± 0.9
	S4-20	3870 ± 127	2953 ± 83	23.7 ± 0.2	148 ± 0.0
	S4-30	3830 ± 212	3020 ± 212	21.1 ± 0.3	140 ± 0.1
Set 5: High-TO	S5-C1	5254 ± 141	2678 ± 63	49.0 ± 0.1	313 ± 0.0
	S5-C2	3880 ± 28	3200 ± 87	17.5 ± 0.1	159 ± 0.0
	S5-1	5230 ± 71	3187 ± 189	39.1 ± 0.1	350 ± 0.0
	S5-10	5280 ± 85	3313 ± 162	37.2 ± 0.1	297 ± 0.0
	S5-A10	5200 ± 0	5033 ± 31	3.2 ± 0.5	7 ± 0.5
	S5-20	5290 ± 42	3407 ± 122	35.6 ± 0.1	264 ± 0.0
	S5-30	5240 ± 226	3547 ± 242	32.3 ± 0.1	265 ± 0.1

Table 4.6. TS values of all microcosms within five sets together with calculated TS reductions (%).

Sets	Reactors	initial	Final	TS reduction (%)
		TS (mg/L)	TS (mg/L)	
Set 1: No-TO	S1-C1	16738 ± 207	9883 ± 423	41.0 ± 0.1
	S1-C2	13359 ± 180	11367 ± 392	14.9 ± 0.2
	S1-1	16900 ± 57	12240 ± 139	27.6 ± 0.1
	S1-10	16900 ± 198	13600 ± 227	19.5 ± 0.1
	S1-A10	15975 ± 82	15807 ± 240	1.1 ± 1.5
	S1-20	17270 ± 99	13500 ± 537	21.8 ± 0.2
	S1-30	16920 ± 198	13740 ± 191	18.8 ± 0.1
Set 2: Low-TO	S2-C1	16862 ± 326	10094 ± 356	40.1 ± 0.1
	S2-C2	13508 ± 196	11440 ± 280	15.3 ± 0.2
	S2-1	17190 ± 240	12627 ± 153	26.5 ± 0.1
	S2-10	16490 ± 99	13507 ± 101	18.1 ± 0.0
	S2-A10	15900 ± 71	15600 ± 178	1.9 ± 0.6
	S2-20	16620 ± 255	13430 ± 71	19.2 ± 0.1
	S2-30	16650 ± 127	13247 ± 70	20.4 ± 0.0
Set 3: Medium -TO	S3-C1	17000 ± 218	10411 ± 220	38.8 ± 0.1
	S3-C2	13869 ± 11	11893 ± 147	14.2 ± 0.1
	S3-1	16830 ± 297	12400 ± 246	26.3 ± 0.1
	S3-10	16950 ± 71	13573 ± 352	19.9 ± 0.1
	S3-A10	15342 ± 106	15220 ± 196	0.8 ± 1.8
	S3-20	17200 ± 735	13667 ± 378	20.5 ± 0.2
	S3-30	17110 ± 71	13867 ± 81	19.0 ± 0.0
Set 4: Medium-TO, No WAS	S4-1	13240 ± 141	11913 ± 181	10.0 ± 0.2
	S4-10	13410 ± 325	11660 ± 106	13.0 ± 0.2
	S4-A10	13120 ± 14	12953 ± 200	1.3 ± 1.2
	S4-20	13030 ± 240	11887 ± 101	8.8 ± 0.3
	S4-30	13720 ± 255	12967 ± 95	5.5 ± 0.3
Set 5: High-TO	S5-C1	17277 ± 22	11039 ± 98	36.1 ± 0.0
	S5-C2	13310 ± 42	11653 ± 180	12.4 ± 0.1
	S5-1	17360 ± 481	13180 ± 140	24.1 ± 0.1
	S5-10	16940 ± 28	13353 ± 81	21.2 ± 0.0
	S5-A10	16800 ± 226	16480 ± 53	1.9 ± 1.1
	S5-20	17390 ± 212	13587 ± 374	21.9 ± 0.1
	S5-30	17420 ± 622	13933 ± 530	20.0 ± 0.2

Table 4.7. VSS and TSS values of all microcosms within five sets together with calculated VSS and TSS reductions (%).

Sets	Reactors	initial		Final		VSS reduction (%)	TSS reduction (%)
		VSS (mg/L)	TSS (mg/L)	VSS (mg/L)	TSS (mg/L)		
Set 1: No-TO	S1-C1	5067 ± 424	12716.7 ± 589	2317 ± 47	7283 ± 29	54.3 ± 0.2	42.7 ± 0.1
	S1-C2	3740 ± 170	10420.0 ± 481	2370 ± 99	6973 ± 42	36.6 ± 0.2	33.1 ± 0.1
	S1-1	5373 ± 129	12973.3 ± 323	2687 ± 50	8787 ± 12	50.0 ± 0.1	32.3 ± 0.1
	S1-10	5160 ± 124	12440.0 ± 170	2913 ± 95	9060 ± 106	43.5 ± 0.1	27.2 ± 0.1
	S1-A10	5007 ± 129	12080.0 ± 70	4233 ± 118	11800 ± 57	15.4 ± 0.2	2.3 ± 0.3
	S1-20	5100 ± 57	12320.0 ± 174	3140 ± 0	9220 ± 28	38.4 ± 0.0	25.2 ± 0.1
	S1-30	5013 ± 83	11613.3 ± 122	3740 ± 57	9510 ± 184	25.4 ± 0.1	18.1 ± 0.1
Set 2: Low-TO	S2-C1	5222 ± 84	12655.6 ± 184	2633 ± 24	7567 ± 24	49.6 ± 0.0	40.2 ± 0.0
	S2-C2	3600 ± 69	10626.7 ± 442	2307 ± 23	7247 ± 90	35.9 ± 0.1	31.8 ± 0.1
	S2-1	5080 ± 113	12140.0 ± 368	3070 ± 14	8910 ± 42	39.6 ± 0.1	26.6 ± 0.2
	S2-10	5260 ± 141	12240.0 ± 0	3300 ± 174	9293 ± 318	37.3 ± 0.1	24.1 ± 0.1
	S2-A10	4360 ± 170	11320.0 ± 396	4150 ± 132	11125 ± 88	4.8 ± 1.0	1.7 ± 2.1
	S2-20	5240 ± 69	12190.0 ± 208	3407 ± 61	9307 ± 167	35.0 ± 0.1	23.7 ± 0.1
	S2-30	5193 ± 23	12260.0 ± 288	3680 ± 57	9440 ± 220	29.1 ± 0.0	23.0 ± 0.1
Set 3: Medium -TO	S3-C1	5178 ± 505	12544.6 ± 102	2725 ± 83	7933 ± 47	47.4 ± 0.2	36.8 ± 0.0
	S3-C2	3580 ± 141	12000.0 ± 339	2540 ± 57	7490 ± 71	29.1 ± 0.2	37.6 ± 0.1
	S3-1	4940 ± 141	12600.0 ± 0	3440 ± 28	9120 ± 141	30.4 ± 0.1	27.6 ± 0.1
	S3-10	4987 ± 151	11586.7 ± 260	3427 ± 58	9547 ± 325	31.3 ± 0.1	17.6 ± 0.2
	S3-A10	4120 ± 113	11880.0 ± 113	3933 ± 57	11600 ± 53	4.5 ± 0.7	2.4 ± 0.4
	S3-20	4960 ± 57	12080.0 ± 113	3500 ± 113	9580 ± 225	29.4 ± 0.1	20.7 ± 0.1
	S3-30	4933 ± 23	11960.0 ± 144	3453 ± 50	9647 ± 162	30.0 ± 0.0	19.3 ± 0.1
Set 4: Medium-TO, No WAS	S4-1	3900 ± 28	9580.0 ± 28	2507 ± 61	7580 ± 100	35.7 ± 0.0	20.9 ± 0.1
	S4-10	4000 ± 113	9880.0 ± 113	2250 ± 14	7150 ± 71	43.8 ± 0.1	27.6 ± 0.1
	S4-A10	3347 ± 46	8620.0 ± 85	3200 ± 20	8510 ± 42	4.4 ± 0.3	1.3 ± 0.9
	S4-20	4060 ± 85	10060.0 ± 255	2360 ± 85	7340 ± 141	41.9 ± 0.1	27.0 ± 0.1
	S4-30	4000 ± 113	9880.0 ± 113	2407 ± 23	7460 ± 40	39.8 ± 0.1	24.5 ± 0.1
Set 5: High-TO	S5-C1	4960 ± 226	12083.3 ± 212	2976 ± 64	7591 ± 122	40.0 ± 0.1	37.2 ± 0.1
	S5-C2	3660 ± 28	9420.0 ± 85	2800 ± 113	7960 ± 453	23.5 ± 0.1	15.5 ± 0.3
	S5-1	4933 ± 101	11893.3 ± 23	3227 ± 115	9360 ± 271	34.6 ± 0.1	21.3 ± 0.1
	S5-10	4980 ± 28	11840.0 ± 0	3350 ± 141	9430 ± 99	32.7 ± 0.1	20.4 ± 0.0
	S5-A10	4880 ± 23	11188.0 ± 336	4753 ± 103	10980 ± 85	2.6 ± 0.5	1.9 ± 1.7
	S5-20	5040 ± 0	11560.0 ± 396	3470 ± 99	9700 ± 141	31.2 ± 0.1	16.1 ± 0.2
	S5-30	4947 ± 101	11720.0 ± 106	3430 ± 184	9920 ± 85	30.7 ± 0.1	15.4 ± 0.1

4.4. Conclusions

In this study, the effect of different doses of PCB 118 and transformer oil on the anaerobic digestion was evaluated. Also, PCB concentrations that can be tolerated by the anaerobic culture were assessed. These findings can be summarized as follows. In the range of TO doses between 0.38 and 1.52 g/L, TO effect on CH_4 production was insignificant. Although the methane percentage of the reactors was affected slightly by the presence of TO, its range lied between 54-63%, which was acceptable based on the typical performance of anaerobic digester parameters (De la Rubia *et al.*, 2002). Methanogenesis was negatively affected by the increase in PCB 118 dose, while dechlorination was favored. No toxicity for PCB 118 at 1 mg/L was observed. The presence of WAS increased the performance of the methanogens and their tolerance to higher doses of PCB 118. This could be an indication of the requirement of a carbon source during dechlorination. Also, the presence of WAS together with TO had a synergistic effect on the degradation of PCB 118, while COD degradation was negatively affected to varying extents with the increase in PCB 118 and TO doses. Increase in PCB 118 concentration also showed negative effect on VS and TS removals. Methane yield/gVS added decreased as the TO and PCB 118 concentration was increased. Presence of dechlorination by-products can be considered as an indication of reductive dechlorination of PCB 118. Some of the daughter products (PCB 70, 67, and 26) were detected in small quantities at the end of reactor operation providing evidence for dechlorination. This study showed that currently existing anaerobic digesters can possibly serve as a treatment option for PCB contaminated transformer oil. To improve the dechlorination performance, a culture which is previously acclimated to PCBs or frequent loading in the continuous mode of operation may help, since methanogenesis was not completely inhibited even at 30 ppm PCB 118.

CHAPTER 5

ANAEROBIC MESOPHILIC DIGESTION OF WASTE ACTIVATED SLUDGE IN THE PRESENCE OF 2,3',4,4',5-PENTACHLOROBIPHENYL PCB 118

5.1. Introduction

Activated sludge process is the most preferred treatment option for the conventional municipal wastewater treatment, which results in excess waste activated sludge (WAS) consisting complex organics, mainly protein (approximate 30%), carbohydrate (approximate 40%) and lipids (approximate 30%) in particulate forms. Proper disposal of WAS is an important problem in municipal wastewater treatment. It must be stabilized sufficiently to reduce its organic content so that it can be safely disposed of without causing odor problems and/or spread of pathogens. Anaerobic digestion is a commonly applied treatment option for WAS stabilization because of its ability to transform organic wastes into biogas (60-70 vol% of methane, CH_4), bringing the potential of using the biogas as energy source. At the same time digestion reduces the amount of organics and hence the final sludge solids for disposal, and destroys most of the pathogens present in the sludge and controls odor problems. The European Union has put an objective to increase the amount of energy obtained from renewable sources from the 2005 level of 8.5% to 20% in 2020 (Mottet *et al.*, 2010). In this context, anaerobic digestion of WAS may contribute to reaching this objective.

Several aliphatic and aromatic also halogenated hydrocarbons, heavy metals, detergents and long-chain fatty acids widely found in municipal wastewaters either non-biodegradable or are slowly biodegradable. Since most of these chemicals are

highly hydrophobic they accumulate on sludge to various extents (Rinzema, 1988; Hwu *et al.*, 1996; Leitao *et al.*, 2006). PCBs are among these chemicals that are also considered persistent organic pollutants. They are persistent in the environment; able to accumulate in fatty tissues in the body; and they have toxic properties (USEPA, 2008). Although both the production for industrial use and the discharge of PCBs have been banned in many countries since 80s, contamination with PCBs still occurs and is of great public concern. This concern has led the European Union and many other countries to regulate the PCB concentrations in air, water and sludge. Accordingly, the Stockholm Convention has listed PCBs as priority chemicals for eventual elimination by 2025 and the parties of the Convention must apply environmentally sound management of PCB wastes by 2028 (Stockholm Convention, 2012). Although PCBs were never produced in Turkey, transformers containing oil with PCBs are currently known to be used in the production/transmission of electricity and PCB containing transformers of thousands of tons in weight are still in use in Turkey (Unido, 2002). Therefore, in Turkey, ratifying the Stockholm Convention on January 2010, with the Control of Equipments Containing PCB and PCT Regulation (2007), Ministry of Environment and Urbanization is held responsible for the preparation and implementation of waste management plans regarding the elimination of equipments containing PCBs. Disposal of these and especially oil containing PCBs constitutes a major problem. The biological degradation of PCBs under anaerobic conditions has been observed in a variety of contaminated matrices, such as sediments (Berkaw *et al.*, 1996; Fagervold *et al.*, 2011; Payne *et al.*, 2011) and some anaerobically digested activated sludge (Ye *et al.*, 1992; Phelps *et al.*, 1996; Chang *et al.*, 2002; Fava *et al.*, 2003). Hence, such an elimination strategy for PCBs may prove to be useful.

One of the aims of this study is to investigate the potential for treatment of PCB-containing transformer oils in anaerobic mesophilic digesters by analyzing PCB biotransformation. The additional purpose is to determine digester performance in the presence of PCBs. Since PCB concentrations in naturally contaminated sludge are generally reported between 1-10 ppm (Benabdallah El-Hadj *et al.*, 2007; Bertin *et al.*, 2007; Patureau and Trably, 2006), 1 mg/L was chosen as low-level, while 20

mg/L was chosen as high-level dose of PCB 118 for this study. WAS was also considered to serve as the carbon source.

5.2. Material and Methods

5.2.1. Chemicals

Sulfuric acid (98%), sodium sulfate (granular), and fine copper powder (Cat. no: 1.02703.250), n-hexane and acetone were purchased from Merck KGaA (Darmstadt, Germany). All individual standards of pure PCB congeners (nos.118, 70, 67, 66, 33, 25, and 26), surrogate standard, TMX (2456-Tetrachloro-m-xylene solution), and internal standard (IS), PCB-209, and PCB-Mix-3 ((nos.28, 52, 101, 118, 138, 153, and 180) were purchased from Dr. Ehrenstorfer GmbH. PCB-free transformer oil (TO) was purchased from Sigma-Aldrich Co. USA. A certified reference material (Cat. no: LGC6184), i.e. sewage sludge containing seven indicator PCBs, PCB-Mix-3 congeners, was purchased from LGC standards-Germany and used for quality assurance of extraction and analyses. Alconox detergent (White Plains, NY, USA) was used for cleaning of all glassware.

5.2.2. Experimental Setup

Mixed culture anaerobic digester sludge (ADS) used as seed was taken from one of the anaerobic digesters of Ankara Central Municipal Wastewater Treatment Plant in Turkey. Digesters were operated in mesophilic range at 35°C with a sludge retention time (SRT) value of 14 days. Waste activated sludge (WAS) was taken from the return line of secondary sedimentation tanks from the same plant. The plant is in operation since 1997 with a current average wastewater flow rate of 746,000 m^3/day. ADS was filtered through a screen with a mesh size of 1 mm to eliminate coarse suspended particles present in. Both WAS and ADS were settled for 12 h to increase the solids concentration decanting overhead water and stored at 4°C prior to use. Sludge samples were analyzed for their TS, VS, TSS, and VSS concentrations before adding into the reactors and the results as mean and standard deviation values are

given in Table 5.1. Initial F/M ratio (gVS/gVSS) was set to about 1.1, which was also checked for all reactors at day 0 prior to PCB spike to PCB receiving reactors.

Table 5.1. TS, VS, TSS, and VSS values of WAS and ADS

Sludge	TS (mg/L)	VS (mg/L)	TSS (mg/L)	VSS (mg/L)
WAS	11460±85	8647±19	11197±231	8550±14
ADS	27017±259	12333±94	26460±453	12130±325

Anaerobic batch reactor system consisted of 8 reactor sets, and each consisting of a 3.2 L anaerobic reactor with a 4-L graduated cylinder gas collector connected to it from the top by PTFE pipes. Before filling up the reactors, all the connections and parts were examined for leak-proof condition. The gas collectors were filled with brine solution (10% NaCl, w/v and 2% H_2SO_4, v/v) at the beginning of reactor set-up as. Gas collectors were graduated 10 mL intervals and filled with brine solution in order to prevent CO_2 solubility (Parajulii, 2011). As the gas is produced, the solution level is pushed down and the measured volume is recorded as gas produced. Schematic representation of anaerobic batch reactor with the gas collector is given in Figure 5.1.

Reactors designated as R-1 and R-20 were biotic reactors spiked with PCB 118 at doses of 1 mg/L and 20 mg/L PCB 118, respectively. R-A was operated as abiotic control reactor which was autoclaved at 121^{o}C for 1 hour prior to PCB 118 spike as final concentration of 1 mg/L. As being main source of PCB emission into environment, TO was also considered as one of the reactor constituent and it was added to the reactors at 1.52 g/L. PCB-free TO density used in this study was 889.2±17.0 g/L with VS and TS values about the same: 876.7±27.2 g/L. Also, another pair of reactors, R-C, that received neither TO nor PCB 118 was operated as biotic control. Table 5.2 shows reactor set-up conditions for PCB 118 and TO.

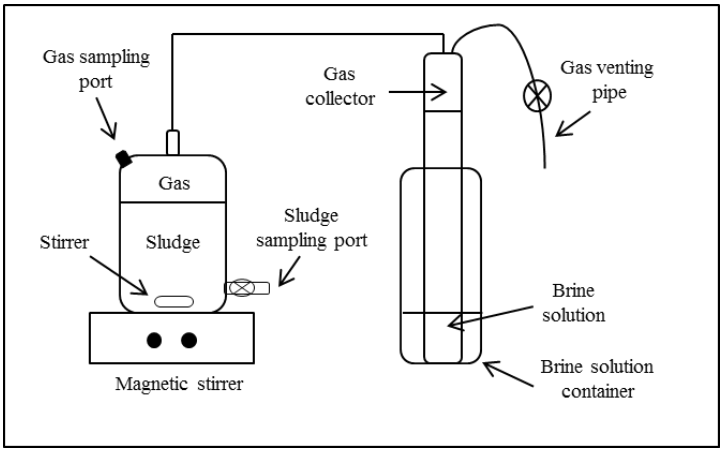


Figure 5.1. Anaerobic batch reactor together with the gas collector

Reactors were filled with 1200 mL of WAS and 1400 mL of ADS. Any basal medium was not added to the reactors assuming that sludge contains enough nutrients and buffer. Indeed, it was lately shown that there was not any significant difference in biogas productions of BM receiving and no-receiving reactors (Bozkurt, 2011). Thus, 2.6 L of 3.2 L reactor volume was filled with WAS, ADS, and TO, hence, 0.6 L was left empty as headspace. After filling all the reactors, each reactor was purged with N_2 gas for 10 minutes to remove oxygen from the system. Then, the reactors were sealed with natural rubber stoppers and incubated at 35±1^{o}C in the dark. Continuous mixing was supplied by magnetic stirrers at 250 rpm throughout 159 days. Prior to PCB spike, the reactors were operated for two days to provide seed adaptation to WAS. PCB 118 stock solutions were prepared in acetone, and spiked to the reactors to supply the desired concentrations (Table 5.2). About 2 h after PCB 118 spike, all reactors were sampled to reveal the initial conditions and also to check whether the desired PCB 118 concentration was reached. A number of parameters: total gas volume and gas composition were analysed initially and then at predetermined intervals. Total COD (tCOD), soluble COD (sCOD), TS, VS, TSS, VSS, pH, oxidation-reduction potential (ORP), and PCBs were also analysed initially and then at following days: as 11, 23, 29, 35, 41, 47, 54, 61, 74 and 159.

PCB 118 and its possible daughter products (PCB 25, 26, 33, 66, 67, and 70) were analyzed in each sample. PCBs were analyzed both in slurry samples (containing solids particles and liquid of reactor content) and the filtrate of slurry samples. Duplicate reactors were operated for 159 days. At each sampling all analyses were done on duplicate samples. Therefore the results presented here are the averages of these four measurements and represent the data after the PCB spike.

Table 5.2. Reactor compositions

Reactor *	TO	PCB 118 dose (mg/L)	WAS	ADS	Autoclave
R-1	+	1	+	+	-
R-A	+	1	+	+	+
R-20	+	20	+	+	-
R-C	-	-	+	+	-

*Each set operated in duplicate reactors.

5.2.3. PCB extraction procedure

For PCB extraction, a 2-mL slurry sample was taken into a 22-mL glass vial sealed with PTFE screw cap. After the addition of 50 mg reduced copper (pre-washed with hexane, acetone and dried with N_2) to remove sulphur compounds and surrogate (TMX), PCBs in the sample were extracted by vigorous overnight-shaking on a horizontal platform shaker at 350 rpm with 10 mL of n-hexane. Afterwards, sample and hexane were separated by centrifugation at 2500 rpm for 5 min. After passing the extract through a glass column packed with anhydrous sodium sulfate (Na_2SO_4), it was concentrated down to 2 mL with nitrogen gas and 1-mL of this eluate was taken into a 2-mL borosilicate glass GC vial and IS was added before GC analysis. The same PCB extraction procedure was applied to the filtrate of sludge samples taken from the reactors and filtered through 0.45 µm pore sized filters. Due to the small volume loss during the extraction, final results were volume corrected.

5.2.4. Analytical Methods

PCB analysis was done using a GC/ECD (Agilent/6890N) equipped with HP-5MS capillary column (30mx0.25mmx0.25μm). GC experimental conditions for PCB analyses were as follows: He as a carrier gas at 1.5 mL/min.; Nitrogen as a make-up gas at 20 mL/min.; injection mode/temperature, splitless/250°C; detector temperature, 350°C; Temperature program: initial temperature, 100°C; 100-160°C at 20°C/min.; 160°C for 2 min.; 160-200°C at 3°C/min.; 200-240°C at 8°C/min.; 240°C for 5 min.; 240-290°C at 30°C/min.; 290°C for 3 min. IS calibration procedure was followed in accordance with USEPA Method 8082 (USEPA, 2000). A 5-point calibration curve was used for each congener with relative standard deviations <20% and r^2 >0.999.

Total gas production was directly read from the gas collectors. Gas composition (CH_4, CO_2) was analyzed with GC/TCD (Agilent/6890N) equipped with HP-PlotQ capillary column (30.0mx530μmx 40.0μm). GC experimental conditions for gas analyses were as follows: He was as a carrier gas at 3 mL/min; initial temperature, 45°C for 1 minute; 45-65°C at a rate of 10°C/min. A four-point linear calibration curve was used for the quantification of CH_4 and CO_2 gases with r^2 >0.99.

COD analysis was conducted according to USEPA approved method (Jirca and Carter, 1975). Prior to sCOD analysis, samples were filtered through 0.45 μm pore sized filters (Millipore). Solid analyses were done according to Standards Methods (APHA, 2005). pH and ORP values were measured with a pH/ORP meter (EC-PH510/21S, Eutech Inst.).

5.2.5. Statistical Evaluation

All statistical evaluations were performed using Student's t test with a statistical significance of p<0.05 and n as 14 for evaluation of methane productions, and 5 for anaerobic digester performances.

5.2.6. Quality Assurance/Quality Control (QA/QC)

The accuracy and precision of the PCB measurements were controlled by calculating the surrogate recoveries as well as recoveries from certified reference sludge, which were between 82-108% and 82-103%, respectively, in the acceptable. range of USEPA Method 8082 (80-120%). Accordingly, for each batch of 10 sludge samples, a method blank, laboratory control sample and a duplicate sample were analysed. Method detection limits (MDL) (n=7) for PCB 118 and TMX were 57 ppt and 32 ppt, respectively. Limit of quantitation (LOQ) (n=7) were 180 ppt and 100 ppt for PCB 118 and TMX, respectively. All targets were lower than the LOQ in method blanks. Surrogate recovery correction or blank subtraction was not performed. USEPA Method SW-846 Chapter 4 (USEPA, 2007) was followed for all glassware cleaning. Coefficient of variance was typically low than 10% for all digester performance parameters, showing that the quality of measurements was good and acceptable.

5.3. Results and Discussion

5.3.1. Anaerobic Digester Performance

Figure 5.2 shows cumulative methane productions of all reactors with respect incubation time. Initially, in all of the reactors relatively high biogas production rates were observed. Control reactors produced the highest amount of methane among all the reactors operated. As biodegradation proceeded with time, depletion of available substrates resulted in gradual decrease in biogas productions. As soon as the fifth day of operation reached, the methane productions in PCB containing reactors fell behind the control reactor.

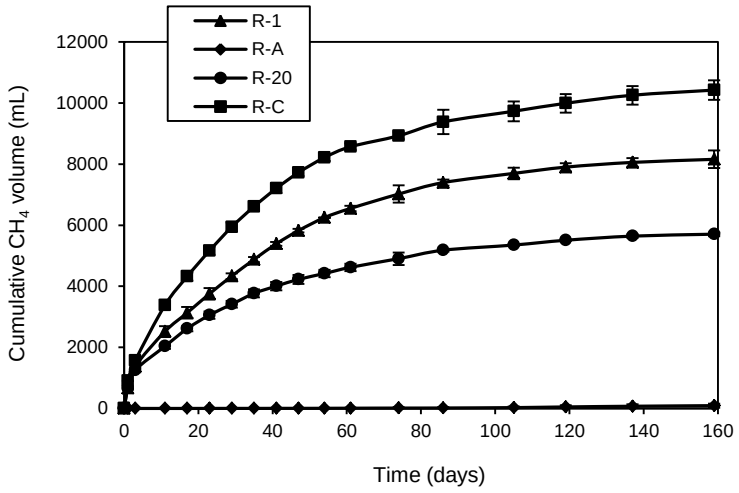


Figure 5.2. Cumulative methane productions of reactors with respect incubation time.

As can be seen from Figure 5.2, the cumulative CH_4 production showed consistent decrease as PCB 118 receiving dose was increased and hence, the highest methane production was observed in PCB-free control reactor (R-C) with the total production of 10427.4±55.4 mL in 159 days. This was followed by that of R-1 and R-20 as 8159.2±45.0 mL and 5706±33.2 mL, respectively. There was 22% decrease in R-1 and 45% in R-20 compared to control reactor. Also, as the PCB concentration increased from 1 mg/L to 20 mg/L, the methane production at 159 days decreased by about 30%, as a clear indication of the negative effect of PCB 118 presence and dose. So, as the higher the PCB dose was the lower the methane production obtained.

The variations of TS and VS with operation time are plotted for each reactor in Figure 5.3a and 3b, respectively. As can be seen from these figures, TS and VS reduction in PCB containing reactors are lower than that observed in the control reactor. The reduction observed in R-C, R-1, and R-20 were about 40%, 31%, and 24% in terms of TS removal, respectively, and about 57%, 45%, and 35%, for VS, respectively (Table 5.3) showing the negative effect of PCB 118 over the digester performances.

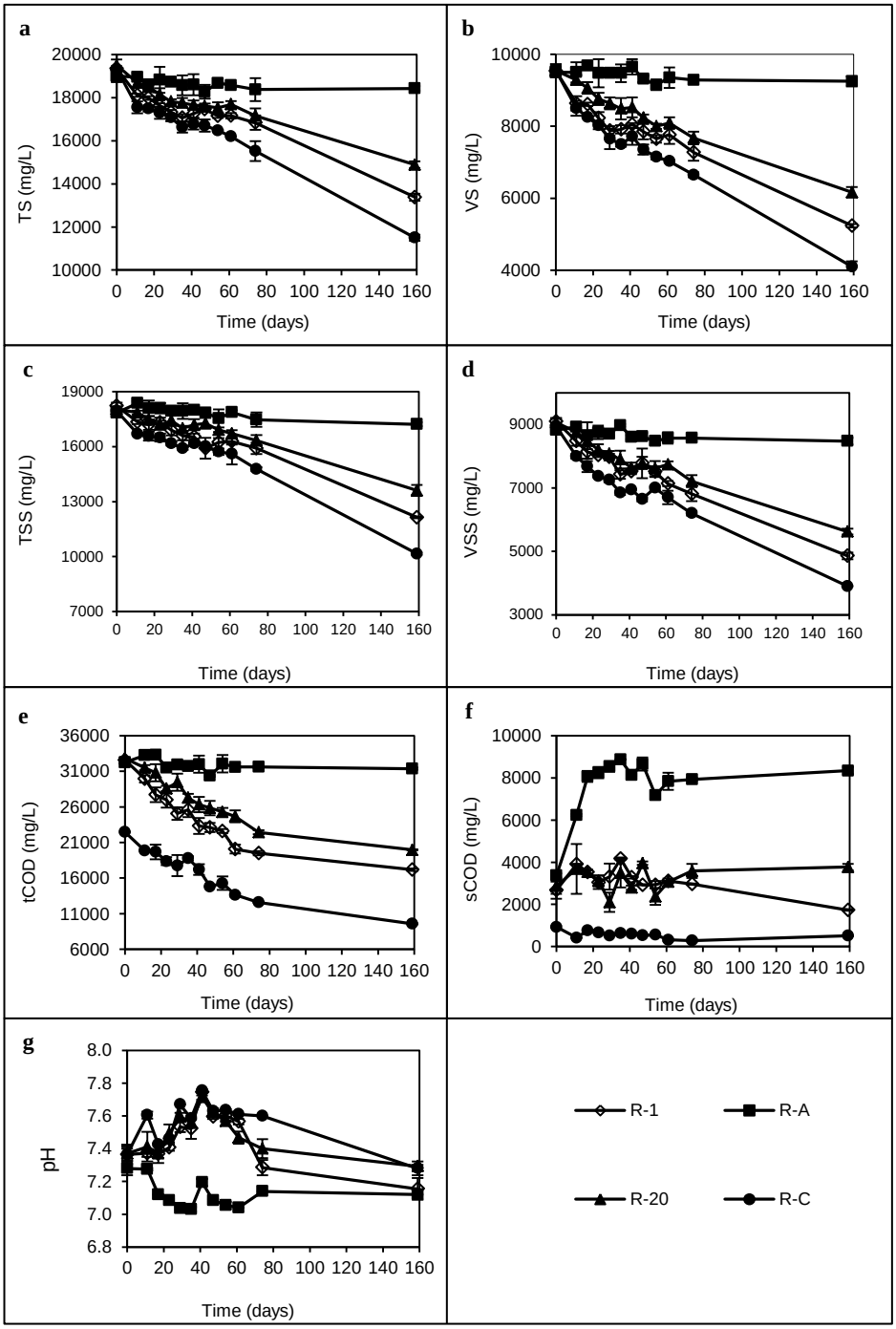


Figure 5.3. Variation of a) TS, b) VS, c) TSS, d) VSS, e) tCOD, f) sCOD, and g) pH values of the anaerobic reactors throughout 159 days of incubation.

The TSS and VSS variations for each reactor are given in Figure 5.3c and 3d. The same trends observed in TS and VS removals were also observed for TSS and VSS removals of the reactors. TSS and VSS removals were in the range of 43-25% and 56-38%, respectively, with the highest removals observed in PCB-free, reactor (R-C) and then decreasing with increase in PCB 118 dose.

The results for tCOD and sCOD for each reactor are plotted Figure 5.3e and 3f, respectively. There was a fluctuation in sCOD values of the reactors (Figure 5.2b) throughout the operation time as expected because wastes are hydrolyzed/solubilized and then converted to methane during anaerobic digestion. The high temperature during autoclave operation resulted in an increase in the soluble waste (solubilization of COD) explaining the relatively high initial sCOD of R-A (Figure 5.3f). sCOD continued to increase in this reactor, indicating that hydrolysis was active, but not methanogenesis since negligible amount of methane production was observed in this reactor (Figure 5.2b). The observed pH decrease during the operation of R-A (Figure 5.3g) also confirms this hypothesis. When the data in Table 5.3 is examined, it can be seen that there is considerable tCOD reduction in all biotic reactors, with the highest tCOD removal achieved in R-C (biotic control) at about 57%. This was followed in a decreasing order by 1 mg/L and then by 20 mg/L PCB 118 receiving reactor with about 47% and 39% reductions, respectively. tCOD reduction of abiotic reactor were insignificant, it was concluded that R-A was well sterilized and was representative of the abiotic losses. The tCOD reduction results obtained in biotic reactors are in the range of typical digester performance values reported in the literature for COD between 40-60% under mesophilic conditions (depending on SRT) (Appels *et al.*, 2008).

pH values of the live reactors are presented in Figure 5.3g and they were within optimum pH range for methane-forming bacteria (6.8-8, Speece (1996)) and also mostly in the range for optimum PCB-dechlorination (7.0-7.5, Borja *et al.* (2005)). Furthermore, all the ORP values determined were between -214 and -330 mV (data not shown), indicating proper anaerobic environment (Gerardi, 2003).

The methane yield for an anaerobic digestion process is commonly expressed as a function of either the reduction of volatile solids or COD or volatile solids added to the reactor. When the methane yields of PCB microcosms were compared as a function of VS addition, it is seen that the yield decreased with the increase in PCB 118 dose (Table 5.3). The specific gas production of R-C, 443.1 mL/gVS_{added} were consistent with the literature data reported as 370-506 mL/gVS_{added} (Benefield and Randall, 1980) showing proper anaerobic digestion of WAS under mesophilic conditions.

Table 5.3. Comparison of the reactors in terms of treatment performances.

Reactor	VS removal, %	TS removal, %	VSS removal, %	TSS removal, %	tCOD removal, %	mL CH_4/ gVS_{added}	Final pH
R-1	45±1.2	31±1.4	46.6±0.7	33.3±0.4	47.2±0.7	347.2	7.16±0.07
R-A	2.6±0.1	3.1±0.8	3.9±0	3.5±0.2	2.7±1	0.9	7.12±0.0
R-20	35.4±0.6	23.6±0.7	37.9±0.5	24.5±1.1	39.1±0.3	242.5	7.29±0.01
R-C	57±1.1	40.4±0.6	56.4±0.3	43.4±0.5	57.3±0.5	443.1	7.28±0.04

5.3.2. PCB 118 removal

The results of PCB 118 measured in slurry samples of the reactors throughout operation time are presented in Figure 5.4. PCB concentrations show a decreasing trend, more so for 20 ppm when compared to 1ppm. The highest PCB 118 removal was attained in R-20 at about 22% (Figure 5.4b), while it was 12% in R-1 (Figure 5.4a). The observation of a greater reduction in 20 ppm reactors may suggest the presence of anaerobic dechlorination in these reactors. This result is consistent with the suggestion that PCB concentration is one of the main limiting factors during the dechlorination and the higher the PCB concentration was, the higher the removal observed (Borja *et al.*, 2005). Also, Chang *et al.* (1999) reported that in the range of 1-10 ppm, the higher the PCB concentration was, the faster the dechlorination rate observed.

Although not major, there was 5% PCB 118 loss in abiotic reactor, R-A (Figure 5.4c). When PCB 118 results of biotic and abiotic reactors throughout the 159 days of incubation were statistically evaluated, it is found that PCB 118 removals of these two reactors were significantly different ($p<0.05$), which would mean that PCB 118 removal in biotic reactor (R-1) was significant.

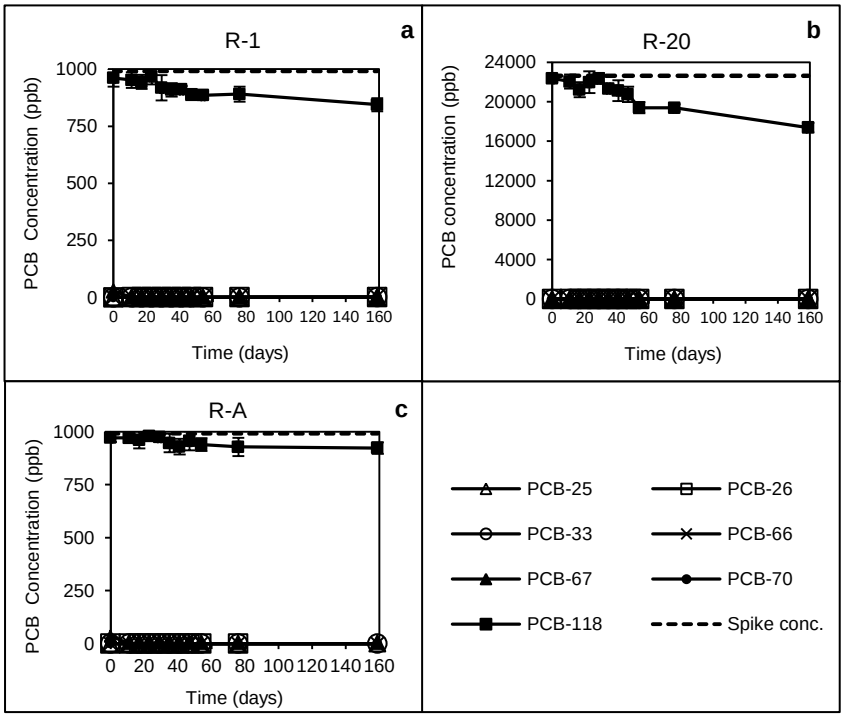


Figure 5.4. Variation of PCB 118 and its possible daughter product concentrations with respect to incubation time, which were analysed in slurry samples taken from the reactors: a) R-1, b) R-20, and c) R-A.

Possible daughter products (PCB 25, 26, 33, 66, 67, and 70) of PCB 118 dechlorination were also checked in both slurry and filtrate of slurry samples. There was a fluctuating increase in PCB 66 (23'44'-tetrachlorobiphenyl) and PCB-70 (23'4'5-tetrachlorobiphenyls) as well as in PCB-26 (23'5-trichlorobiphenyl) concentration in mostly R-20 which received higher dose of PCB 118. PCB 66 production results from meta-chlorine removal from PCB 118, while presence of PCB-26 was considered because of sequential para-chlorine removal from PCB 118

and PCB-70. However, the amounts of these congeners were less than 10 ppb in general and when compared to the reduction in the mother congener, PCB 118, they were negligible.

The amount of PCB 118 in the filtered samples and it was generally below 50 ppb (data not shown). None of the possible daughter products were observed in the filtered samples. It is probable that all the PCBs were adsorbed to the solids when considering the low solubility and high adsorption properties of them.

Similar results were also observed by Patureau and Trably (2006). No dechlorination by-products for none of the PCBs (52, 101, 118, 138, 153 and 180) were reported in that study despite abiotic loss at about 20% and biotic PCB loss between 40-100% in anaerobic and/or aerobic reactors. The reason of the higher PCB removals in the reactors of Patureau and Trably, (2006) is possibly due to the higher solids content of the sludge (26000 mg/L) and TS content of which (32000 mg/L) was about 3 and 1.5 times higher than that of our study. Indeed, by comparing the dechlorination rates of samples having a total solid concentration of 10 g/L and 5 g/L, Chang *et al.* (1999) have shown that higher solid concentration causes higher rate of adsorption in sludge. No abiotic losses were recorded in the same study, but the experiment was conducted under batch conditions without shaking (Chang *et al.* 1999). Also, Chang *et al.* (1999) reported PCB degradation only in the reactors containing acclimated consortia. They also reported (only) one of the daughter products accumulation at about 0.4-0.5ppm when 1ppm PCBs were added initially.

There may be a number of reasons/factors for no detection or detection of some daughter products in very small quantities (typically in the ppb range) in this study such as no adapted consortium was added into the reactors of this study because the main aim here was to observe the change in performance of typical anaerobic digesters in the presence of PCBs and transformer oil. However, this in turn resulted in a longer lag phase for microorganisms for the degradation of PCBs. During this time, abiotic loss of PCBs through volatilization or other mechanisms may have taken place-as indicated by the loss of PCBs in the abiotic control reactors. It is known that abiotic loss of PCBs occurs as result of volatilization, photo degradation

or chemical combination with organic matter because of the semi-volatile and hydrophobic nature of PCBs (Patureau and Trably, 2006; Wu, 1996). Photo degradation is not a concern here since experiments were conducted in the dark. Moreover, average vapor-pressure values of PCBs allow their transport in the gaseous phase (Gdaniec-Pietryka *et al.*, 2007). However, the abiotic losses of PCBs in the present study were limited to about 5%, which could be considered as a considerable but not as a main effect for PCB 118 removal. Behaviors of daughter congeners are expected to be somewhat different when compared to the behavior of PCB 118 in reactors. They are expected to be more volatile, more soluble and hence more mobile. Therefore, there is a chance that these congeners may have been produced, but lost during operation.

Also, Beyer and Biziuk (2009) stated that PCBs, especially those that have experienced significant microbial dechlorination, are very susceptible to volatilization. When the results of the present study is assessed under the light of these findings and statements, it can be concluded that about 5% PCB 118 removal might be due to abiotic losses, while the remainder removals might have been due to biological means which results in the formation of daughter products. Also, QA/QC repeatability results of this study shows that these degradation was not because of analytical measurement errors since coefficient of variance between the replicate results was lower than 5%.

5.4. Conclusion

The main objective of this study was to investigate the mesophilic anaerobic digestion of WAS. Study aimed to evaluate the methane productions of reactors under different concentrations of PCB 118 in the presence of TO. As a result, digester performances were negatively affected by the presence of PCBs. This was clearly evident from the consistently less methane formation biotic control, 1 ppm, 20 ppm and abiotic control reactors. Monitoring of operational parameters such as pH and ORP indicated the establishment of favorable conditions (in terms of optimum pH for anaerobic digestion and PCB dechlorination) in biotic control, 1

ppm and 20 ppm reactors, and even in abiotic control to some degree (though exhibiting lower pH than others). Monitoring of TS, VS, TSS, VSS suggest, although to a lesser extent, the same trend exhibited in methane formation: consistently less removal in biotic control, 1 ppm, 20 ppm and abiotic control reactors. All the results of anaerobic digester performance parameters were in accordance and to provide evidence that the presence of PCB 118 and increase in its dose affected anaerobic digester performances negatively to a certain extent. PCB 118 removal was attained at most in R-20 reactor which contained 20 mg/L of PCB 118 by about 22%. We may conclude that our results seem to be somewhere in between actual anaerobic dechlorination and abiotic loss. Use of higher solids concentrations, together with a lack of consortia might have led to suppressed PCB degradation, preventing its differentiation between abiotic versus biotic degradation mechanisms. If the operation time of reactors was doubled or an acclimated consortium was added, it is believed that the PCB concentrations would decrease further and daughter congeners in notable concentrations would have been observed.

CHAPTER 6

REDUCTIVE DECHLORINATION OF PCBS USING DECHLORINATING CULTURE ENRICHED FROM THREE HISTORICALLY IMPACTED SITES: GRASSE RIVER, FOX RIVER AND BALTIMORE HARBOR

6.1. Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants found in the atmosphere, water, sediments, fish and wildlife. PCBs were released into the environment through the production and use of commercial mixtures called Aroclors (Monsanto, USA and UK) and Clophen (Bayer, Germany), among other names. PCBs have been widely used over the last 50 years in industrial applications as hydraulic fluids, heat transfer fluids, plasticizers, flame retardants organic diluents and dielectric fluids (Chang *et al.*, 1999). It is estimated that about 10 million tones, equivalent to 1/3 of the total worldwide production of PCBs, has been released into the environment (Patureau and Trably, 2006). PCBs are persistent, lipophilic and strongly hydrophobic. Therefore, they have a high potential for bioaccumulation in living organisms. Environmental PCB exposure is a major health concern due to the toxic, carcinogenic, and endocrine disruptive effects of PCBs (USEPA, 2008).

Therefore, evaluating different ways of PCB degradation is of great environmental importance. In this context, one potential process for PCB degradation is through microbial degradation (bioremediation). Anaerobic and aerobic microorganisms can transform PCBs under a variety of conditions both in the laboratory and in environment (Williams, 1994; Wu *et al.*, 1998; Tartakovsky *et al.*, 2001; Nakhla,

2002; Fava *et al.*, 2003; Taharakan, 2006; Benabdallah El-Hadj *et al.*, 2007). Several microorganisms have previously been isolated that degrade PCBs aerobically or anaerobically (LaMontagne *et al.*, 1998; Fagervold *et al.*, 2005; Baba *et al.*, 2007). To develop effective bioremediation strategies, the microbes and pathways involved in PCB degradation need to be elucidated. Generally, highly chlorinated congeners are more susceptible to anaerobic dechlorination, whereas lightly chlorinated congeners are more susceptible to aerobic degradation; unfortunately however, no one microorganism has been isolated that can degrade all 209 PCB congeners.

Since the aerobic degradation of PCBs usually attacks lightly chlorinated congeners and aerobic PCB biodegradability decreases with increased chlorine (Furukawa, 2000). Also, in many instances, only the top few millimeters of sediments are aerobic. Therefore, the largest reservoirs of PCBs in rivers and lakes are the anaerobic sediments, which are not suitable for the growth of aerobic microorganisms. Effective microbial transformation of PCBs in these contaminated sediments can only occur through anaerobic processes, i.e. reductive dehalogenation (Wiegel and Wu, 2000).

It has been shown that anaerobic microbial populations are responsible for the anaerobic dechlorination of PCBs via various reductive dechlorination mechanisms. The observed pathways of anaerobic degradation are very dependent on particular enrichment cultures. Dechlorination under anaerobic conditions occurs due to halorespiration, where halogenated compounds are used as terminal electron acceptors. Consequently, an excess of an electron donor (such as a carbon source, hydrogen, or sometimes a reduced metal) is required for dechlorination (Tartakovsky *et al.*, 2000). In general, microbial dechlorination preferentially removes *meta* and *para* chlorines, resulting in a depletion of highly chlorinated PCB congeners with corresponding increases in lower chlorinated, *ortho*-substituted PCB congeners (Abramowicz, 1995).

PCB removal from impacted sites has been a regulatory priority for several decades. In some cases, PCB contaminated sites have been shown to contain native

populations of bacteria that can degrade or dechlorinate PCBs. Three such sites chosen in this study, Grasse River, Fox River and Baltimore Harbor, have been shown previously to have active indigenous native populations of anaerobic dechlorinating bacteria, although the sediments were not assayed for their ability to stimulate degradation of Aroclor 1254 or PCB 118 (Wu, 1998; Imamoglu, 2004; Kjellerrup *et al.*, 2008; Payne *et al.*, 2011). This study aims to develop enrichment cultures capable of anaerobically dechlorinating PCB 118, the most toxic and abundant congener found in Aroclor 1254 which is the major constituent of transformer oil. Microcosm assays were conducted to investigate the dechlorination potential of the indigenous microorganisms from these sediments. In addition, molecular tools including quantitative polymerase chain reaction (qPCR) and denaturing high pressure liquid chromatography (DHPLC) were used to characterize the dechlorinating community.

6.2. Materials and Methods

6.2.1. Chemicals

All PCBs (99-100 % purity) were purchased from AccuStandard. Transformer oil was purchased from Ultra Scientific. PCE was purchased from Sigma-Aldrich. Hexane (pesticide analyzed grade) was from Acros Organics. All other chemicals were reagent grade.

6.2.2. Sediment Locations and Collections

Sediments collected previously from three different sites, Grasse River, Fox River and Baltimore Harbor, were stored anaerobically in glass jars sealed with Teflon lined tops at 4°C in the dark prior to use. Sediments from all three sites were black in color and had a sulfide odor indicative of reduced anoxic conditions and information related to these sites is given in the following subsections.

6.2.2.1. Grasse River

The Grasse River (GR) site is located in the lower GR in the Village of Massena, NY in the United States where ALCOA (Aluminum Company of America) has been producing aluminum since 1903. It has been reported that GR was contaminated with A1248 that was used for aluminum production at this industrial site since the 1930s (USEPA, 2005). The GR is a U.S. Environmental Protection Agency (USEPA) Superfund site that contains high levels of PCBs. Historic disposal of process wastes into onsite landfills and lagoons, a practice that was common and widely accepted at that time, resulted in the release of PCBs into the lower GR from 30s to 70s. The PCBs were discharged to GR with the wastewater during routine operations through four permitted outfalls and resulted in contamination of a seven mile stretch of the river (Kjellerup *et al.*, 2008). Total PCB concentration in GR sediments was found as 6829 ng/g and the average chlorine numbers per biphenyl was reported to be 3.8. Also 87% of PCBs contained less than 6 chlorine atoms and 73% of PCBs with less than 5 chlorine atoms (Kjellerup *et al.*, 2008). In 2008, Kjellerup *et al.* showed that the GR PCB profile was most similar to Aroclor 1248, confirming the historical information about the main sources of contamination for GR. GR sediments were collected during Spring 2008 with a ponar sampler from the top 30 cm of sediment layer, NY (44°57.06 N 74°51.06 W), and was used as inoculum in GR microcosms in this study.

6.2.2.2. Fox River

Fox River (FR) is located in central and northeastern Wisconsin, United States. It is the largest tributary in the state reaching Lake Michigan. PCBs were used in industries for manufacturing of carbonless copy paper between 1954 and 1971. Due to severe contamination, the area was included in the National Priority List of the United States Environmental Protection Agency (USEPA, 1998). The total PCB mass in FR is estimated to be 30000 kg, with more than 22000 kg stored downstream of De Pere dam (Velleux and Endicott, 1994). Therefore, the PCB pollution particularly in this section of Fox River is likely a major long-term source of PCBs to

Green Bay and Lake Michigan (Imamoglu *et al.*, 2004). The total PCB concentration in FR was previously reported to be 0.2-6.8 ppm for the upstream and 0.3-17.6 ppm for the downstream cores of the study area (Imamoglu *et al.*, 2004). The major PCB source of the Fox River sediments was identified as Aroclor 1242, for both the upstream and the downstream sediments. Total PCB concentration in FR sediments was found as 2021 ±174 ng/g. The average chlorine numbers per biphenyl was 3.8. In addition, analysis of FR sediment before inoculation showed that 92% of PCBs with lower than 6 chlorine atoms and 83% PCBs had lower than 5 chlorine atoms.

6.2.2.3. Baltimore Harbor

Baltimore Harbor (BH) is a coastal embayment located in a highly urbanized watershed of the Chesapeake Bay, USA. PCB impacted BH sediments were sampled in late Spring 2009 from the Northwest Branch of BH, with a petite ponar grab sampler at 39°16.8 N, 76°36.2 W (Payne *et al.*, 2011). Relatively high amounts of PAHs, trans-nonachlor and heavy metals have been reported near this site (Baker *et al.*, 1997). It has been reported that Aroclor 1260 was predominant with smaller amounts of Aroclor 1254 in BH sediments. Also, less chlorinated congeners were detected as the potential weathered products of Aroclor 1260. Total PCB concentration in BH sediment samples was found as 1334 ng/g. An average of chlorines per biphenyl was found as 5.57 (Payne *et al.*, 2011).

6.2.3. Microcosm Setup

Sediments (GR, FR, and BH) obtained from Prof. Dr. Kevin R. Sowers' Laboratory (Institute of Marine and Environmental Biotechnology, University of Maryland, Baltimore, MD) and used in this study were sampled and stored at 4°C in the dark.

In all microcosms, low-sulfate (0.3 mM) estuarine medium (E-Cl) was prepared and used as described by Berkaw *et al.* (1996) to supply essential metals for growth of bacteria. E-Cl medium was anaerobically dispensed into 160-mL serum bottles.

Bottles were sealed under N_2/CO_2 (80:20) with 20-mm Teflon-coated butyl stoppers (West Co., Lionville, PA) secured with aluminum crimp seals. The medium was autoclaved at 121°C for 20 minutes. The final pH was 6.8. All subsequent additions into E-Cl medium containing microcosms were performed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Michigan, USA) containing N_2:CO_2:H_2 (75:20:5). Preparation details of sediment microcosms are described in Table 6.1.

Table 6.1. Experimental setup of GR, BH, and FR microcosms.

Microcosm Label	**Sediment type**	**PCB/Aroclor type and spike concentration**
GR -118	GR	PCB 118:50 mg/L
GR- 1254	GR	Aroclor 1254: 100 mg/L
FR- 118	FR	PCB 118:50 mg/L
FR- 1254	FR	Aroclor 1254: 100 mg/L
BH -118	BH	PCB 118:50 mg/L
BH- 1254	BH	Aroclor 1254: 100 mg/L

In the anaerobic chamber, sediment slurries were prepared by mixing 1 volume of wet sediments (GR, FR, and BH), (about 10 mL), with 5 volumes of E-Cl medium, (50 mL), and a fatty acid (FA) mixture (acetate, propionate, and butyrate, at a final concentration of 2.5 mM each). FA was supplied as electron donor and carbon source (Berkaw *et al.*, 1996). Also, tetrachloroethylene (PCE) at a final concentration of 200 µM was spiked into the microcosms to stimulate growth of dehalorespiring bacteria. Finally, Aroclor 1254 and PCB 118 solubilized in acetone (about 0.2% v/v) were separately added to microcosms at a final concentration of 100 mg/L and 50 mg/L, respectively. After addition of all ingredients (E-Cl, sediment, PCE, FA, and Aroclor 1254 or PCB 118), all microcosms were resealed in the anaerobic glove with the same butyl stoppers secured with aluminum crimp seals and incubated statically at 30ºC in the dark until the next sampling. Microcosms (Table 6.1) were sampled initially after addition of all materials and sampling continued at every 30 days. GR, FR and BH microcosms with PCB 118 were monitored for a total of 60, 180 and 180 days, respectively. The operation times of GR, FR and BH microcosms with Aroclor

1254 were 120, 180 and 180 days, respectively. In text and/or on the graph, results referring to a sampling time are shown as “Dtime” such as D120 means day 120.

6.2.4. Analytical techniques

6.2.4.1. PCB extractions and analytical procedures

One mL samples from each triplicate bottle were extracted with 5 mL of hexane on a wrist shaker overnight for measurement of PCBs. The organic phase was passed through a copper/Florisil (1:4) column and PCB congeners were analyzed using a Hewlett Packard 5890 series II GC equipped with ^{63}Ni electron capture detector as described previously (Fagervold *et al.*, 2005) and a DB-1 capillary column (30mx0.25mmx0.25μm). PCB congeners in a mixture containing 250 μgL^{-1} Aroclor 1232, 180 μgL^{-1} Aroclor 1248 and 180 $\mu g\ L^{-1}$ Aroclor 1262 (also known as ‘‘Mullin’s mix’’) (Swackhamer *et al.*, 1996) were used to make with a 10-point calibration curve with PCB 30 and PCB 204 used as internal standards.

Fifty-five additional congeners not present in the Aroclor mixture that were potential dechlorination products were also added to the PCB calibration table. A calibration mixture containing the internal and surrogate standards and Mullin’s mix was run with every batch of samples to determine the response factor of each peak relative to the internal standards and to correct/check shift in the retention times of peaks. PCB 166 was used as a surrogate compound and its recovery was about 85%. Congener concentrations were not corrected for surrogate recoveries, because of the possible bias in calculated mole fractions of lesser or more chlorinated congeners in the case of differential recoveries of the different surrogates. Peaks for the surrogate and internal standards and those that co-eluted with sulfur (which was sometimes not completely removed by the copper cleanup) were disregarded.

During analytical determination of PCBs, in the chromatograms, a peak may represent an individual congener or a group of congeners. The congeners that appear

together in the same peak during chromatographic analysis are named as coeluting congeners. These coeluting congeners are designated by slashes separating their congener numbers. For data calculations, co-eluting congeners and homologues were assumed to be present in equal proportions. Dechlorination curves were made for all PCB congeners in 30-day intervals over the course of incubation. The PCB concentrations were measured as µg PCB/mL of microcosm slurry by GC/ECD and converted to mol% which was calculated as to the Equation 6.1:

$$\text{mol \%} = \frac{\frac{C_i}{MW_i}}{\sum \frac{C_i}{MW_i}} .100 \qquad (6.1)$$

where MW_i is the molecular weight of each detected PCB congener (g/mol) and C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry). The extent of dechlorination was calculated from the weighted average number of chlorines ($Cl_{average}$) in samples before and after incubation. $Cl_{average}$ was reported as average number of Cl per biphenyl, and was calculated according to the Equation 6.2:

$$Cl_{average} = \frac{\sum C_i.n_i}{\sum C_i} \qquad (6.2)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents.

The dechlorination rate was calculated within the linear slope of the dechlorination curve by dividing total chlorine removed per biphenyl with the time elapsed in days and calculated by using the formula below (Equation 6.3):

$$\text{Rate} = \frac{\left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{initial} - \left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{final}}{t} \qquad (6.3)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents and t is the incubation time elapsed (days).The average rate and the standard deviation were calculated from triplicate cultures.

6.2.4.2. DNA extraction

DNA was extracted (in triplicate) from microcosms samples for three time points (time points are shown in text and/or on the graphs with letter "D" followed with time in days) and was used in downstream processes such as enumeration and community analysis of PCB dechlorinating bacteria. DNA was extracted by adding 0.25 mL of slurry samples from each microcosm to an individual Power Bead microfuge tube of a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc.) or to a 96-well bead beating plate for rapid and thorough homogenization. In the former, the Power Bead tubes were mixed by brief vortex prior to 30s of bead beating at speed "4.5" using a FastPrep120 (Q-Biogene, CA). In the latter, instead of FastPrep120 (Qiagen, Resch), a TissueLyser is used. The TissueLyser allows the usage of 96-well plate and like FastPrep120, it provides disruption of biological samples through high-speed shaking. In the DNA extraction process, cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the spin column. Total DNA was then isolated from the Power Bead tubes or 96-well plates according to the manufacturer's directions. DNA was eluted in 100 µL of Tris buffer (provided with kit) and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). All DNA samples were diluted to 2ng/µL in Tris buffer.

6.2.4.3. Enumeration of PCB dehalorespiring bacteria by quantitative PCR

The quantification of putative dechlorinating Chloroflexi in the microcosm samples was performed (in triplicate) by quantitative polymerase chain reaction (qPCR) using iQ SYBR green supermix (Bio-Rad Laboratories). DNA was amplified with primer Chl348F (5'-CGC CCG CCG CGC GCG GGA GGC AGC AGC AAG GAA-3') and

Dehal884R (5'-GGCGGGACACTTAAAGCG-3') targeting the16S rRNA genes of the members within Chloroflexi including *Dehalococcoides* groups (Fagervold *et al.*, 2005).

Each qPCR reaction volume (25-µL) contained 1x iQ SYBR green supermix, 400 nM forward and reverse primers and 1 µL of sample DNA. PCR amplification and detection were conducted in an iCycler (Bio-Rad Laboratories). QPCR conditions were as follows: initial denaturation for 15 min at 95°C followed by 35 PCR cycles of 30 s at 95°C, then 30 s at 61°C, then 30 s at 72°C. One copy of the gene per genome was assumed based on the genomes of *Dehalococcoides mccartyi* strain 195 and *Dehalococcoides* spp., strain CBDB1 (Seshadri *et al.*, 2005: Kube *et al.*, 2005). QPCR data were analyzed with MJ Opticon Monitor Analysis Software v3.1 and compared to a standard curve of purified DF-1 348F/884R 16S rRNA gene product. The standard curve consisted of duplicate dilutions over 7 orders of magnitude. The specificity of qPCR amplification was verified by melting curve analysis followed by gel electrophoresis 1.2% (w/v) high-melt agarose gel.

6.2.4.4. Community analysis of PCB dechlorinating bacteria by denaturing High Pressure Liquid Chromatography

Denaturing high pressure liquid chromatography (DHPLC) analyses were performed using a WAVE 3500 HT system (Transgenomic, Omaha, NE) equipped with a florescence detector (excitation 490 nm, emission 520 nm). Primers 348F/884) were used both for DHPLC following PCR or qPCR (sometimes qPCR products were used) as described below.

PCR reactions were performed in 50 µL reaction volumes using the following GeneAmp reagents (PE Applied Biosystems, Foster City, CA): 10 mM Tris-HCl, a mixture of dNTPs (200 nM each), 1.5 mM $MgCl_2$, 100 nM of each primer, 0.8% dimethyl sulfoxide (DMSO), 2 units of Ampli *Taq* DNA polymerase, 200 nM of each primer, 1 µL of DNA template and 34.5 µL of nuclease-free water. Amplification was performed in a PTC200 thermal cycler (MJ Research, Watertown,

MA.) with the following cycle parameters: Initial denaturing (1 min at 95°C), 40 cycles of denaturation (45 s at 95°C), annealing (45 s at 61°C), and elongation (45 s at 72°C), followed by a final extension (30 min at 72°C). PCR products of the correct length (about 500 bp) were confirmed by electrophoresis using a 1.2% high melting agarose gel prior to analysis by DHPLC. The 16S rRNA gene fragments were analyzed in a 30 μL injection volume by DHPLC with a DNASep® cartridge packed with alkylated non-porous polystyrene-divinyl benzene copolymer microspheres for high-performance nucleic acid separation (Transgenomic, Omaha, NE). The oven temperature was 63.0°C and the flow rate was 0.5 mL min^{-1} with a gradient of 55–35% Buffer A and 45–65% Buffer B from 0 to 13 min. The analytical solutions used for the analyses were such that Buffer A constituted 0.1 M triethyl ammonium acetate (TEAA) at pH 7, Buffer B was made of 0.1 M TEAA and 25% acetonitrile at pH 7, Solution D included 25% water and 75% acetonitrile. Syringe Wash Solution was supplied from Transgenomic, Omaha, NE.

Analysis was performed using the Wavemaker version 4.1.44 software. An initial run was used to identify individual PCR fragments and determine their retention times. Individual peaks were eluted for sequencing from a subsequent run and collected with a fraction collector based on their retention times. The fractions were collected in 96-well plates (Bio-Rad, Hercules, CA) and dried using a Savant SpeedVac system (Thermo Electron Corporation, Waltham, MA) followed by dissolution in 30 μL of nuclease-free water. Re-amplification was performed following the protocol described above. The PCR amplicons were electrophoresed in a 1.2% high melting agarose and the excised fragment was purified for sequencing using Wizard® PCR Preps DNA Purification Resin (Promega, Madison, WI) and then sequenced (BASLab, IMET, University of Maryland, Baltimore ,USA).

6.3. Results and Discussion

6.3.1. PCB 118 dechlorination

Addition of sediments from all three sites to microcosms spiked with 50 mg/L PCB 118 resulted in the rapid dechlorination of PCB 118 (Fig. 6.1) after a variable initial lag period. For example, there was a 30-day lag period in the GR sediment microcosm, while this lag was extended up to 120 days in BH sediment microcosm (Fig. 6.1). After this initial lag, PCB 118 was rapidly dechlorinated with a rate of $30.8x10^{-03}$ total Cl^- removed per day per biphenyl (Cl^-/day) in the GR sediment microcosm, while the rate of PCB 118 dechlorination in the BH sediment microcosm was $15x10^{-03}$ Cl^-/day. However, in the FR sediment microcosm, PCB 118 dechlorination was very low and the rate of dechlorination was $0.6x10^{-03}$ Cl^-/day. According to these results, the most active sediment microcosm was GR, followed by BH, and finally FR.

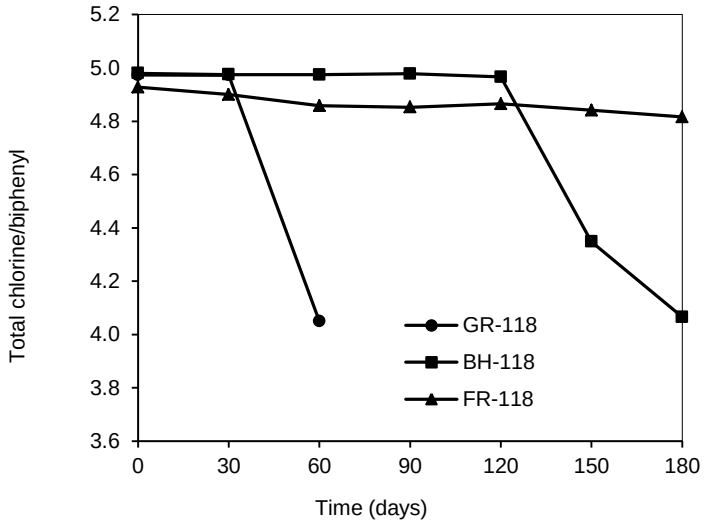


Figure 6.1. Changes in total chlorine per biphenyl values of three sediment microcosms with PCB 118.

Figure 6.2 presents the concentration of PCB 118 and all observed daughter products at each sampling time. As seen from Figure. 6.2a, in the GR sediment microcosm, about 74 mol% PCB 118 (245-34) was dechlorinated in 30 days, which resulted in the production of PCB 66 (24-34) to a final of about 50 mol% (Figure 6.2b). There was also an increase in PCB 28 (24-4) with a final mol % of 24% (Figure 6.2a). Also, there was less than 1 mol% increase in PCB 25 and PCB 74, indicating flanked *para* dechlorination of PCB 118. These results indicate that the GR sediment culture preferentially dechlorinated PCB 118 through sequential *para* flanked *meta* chlorine removal (Figure 6.3). In contrast, FR sediment microcosms dechlorinated about 11 mol% of PCB 118 (Figure 6.2c) to mostly PCB 70 (11.3 mol%) and to lesser extent to PCB 66 (2.87 mol%) after 180 days (Figure 6.2d). Other congeners were detected at lower than 1 mol%, such as PCB 26 (a product of PCB 70 dechlorination), PCB 28 (a product of all three PCB 118 intermediate dechlorination products PCB 66, PCB 70 and PCB 74). FR dechlorinating culture targeted mainly flanked *para* chlorines removal as well as flanked *meta* removal to a lower extent (Figure 6.3).

After 120 days lag time, BH sediment microcosms rapidly dechlorinated about 90 mol% of the added PCB 118 (Figure 6.2e). An increase of 83 mol% was observed in PCB 66 suggesting the same PCB 118 dechlorination pathway as observed in GR sediment microcosms. Final dechlorination products were PCB 28 and PCB 25 at about 3 and 0.4 mol% (Figure 6.2f). GR sediment was the most active culture for PCB 118 dechlorination which was followed with BH sediments with a longer lag period, and the least active sediment was the FR sediment. The dechlorination pathways of PCB 118 was inferred as based on the PCB 118 dechlorination results observed in three sediment microcosms, and are given in Figure 6.3.

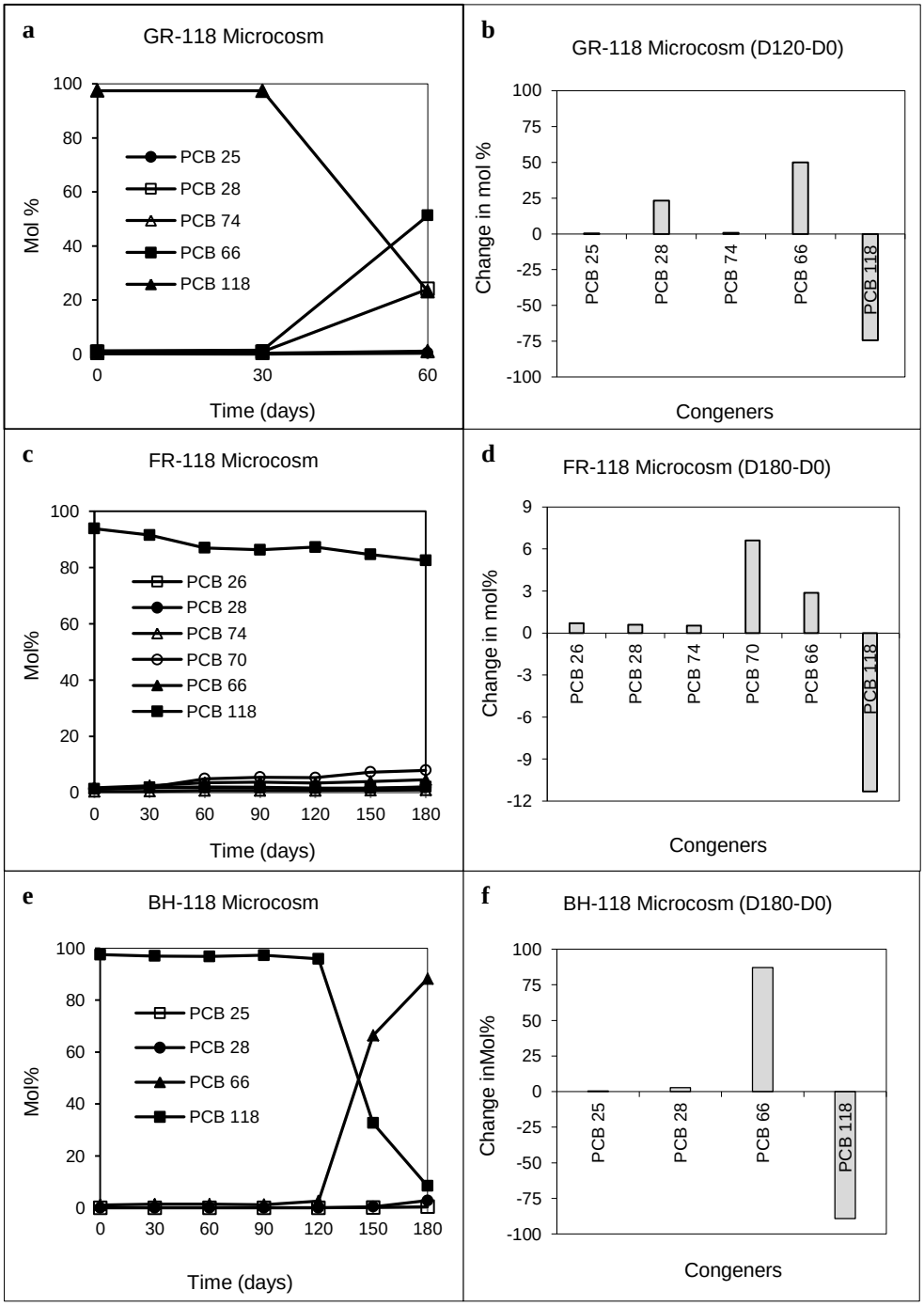


Figure 6.2. Mol% distributions of PCB 118 and its intermediate and end products of PCB 118 in microcosms: a and b) GR, c and d) FR, and e and f) BH, Congeners less than 0.1 mol% at any time point were excluded for the graph.

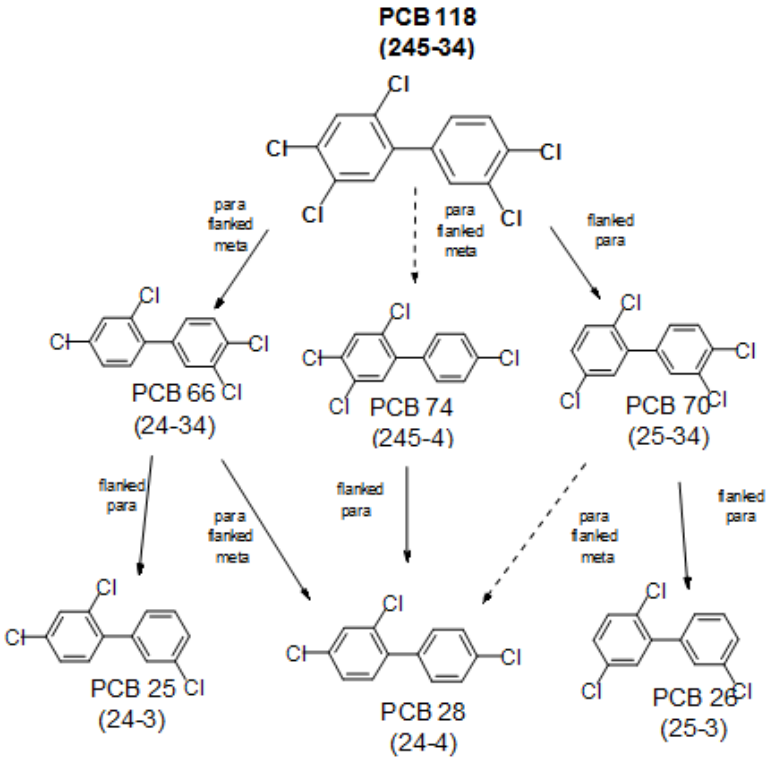


Figure 6.3. Dechlorination pathways of PCB 118 (245-34). Solid arrows indicate main pathways, while dashed arrows show minor pathways.

6.3.2. Aroclor 1254 dechlorination

Dechlorination of Aroclor 1254 in three sediment microcosms, GR, FR, BH, was investigated. Data showing *ortho*, *meta*, and *para* dechlorination, as well as removal of total chlorines per biphenyl by the three sediment microcosms are presented in Figure 6.4. A 30-day lag period was observed in GR and FR sediment microcosms while a 60-day of lag was observed with BH sediment microcosms before the start of dechlorination of Aroclor 1254 (Figure 6.4a). As was seen with microcosms spiked with PCB118, the highest dechlorination rate of Aroclor 1254 was that of GR sediment microcosms, $17x10^{-03}$ Cl^-/day. It was followed by FR sediment microcosms at about $8x10^{-03}$ Cl^-/day. Interestingly, with Aroclor 1254, BH sediment microcosms showed the least activity indicated by a very low dechlorination rate of about $0.62x10^{-03}$ Cl^-/day (Figure 6.4a). This might indicate a higher diversity or abundance

of native PCB dechlorinating microorganisms in GR and FR sediment compared to BH sediment.

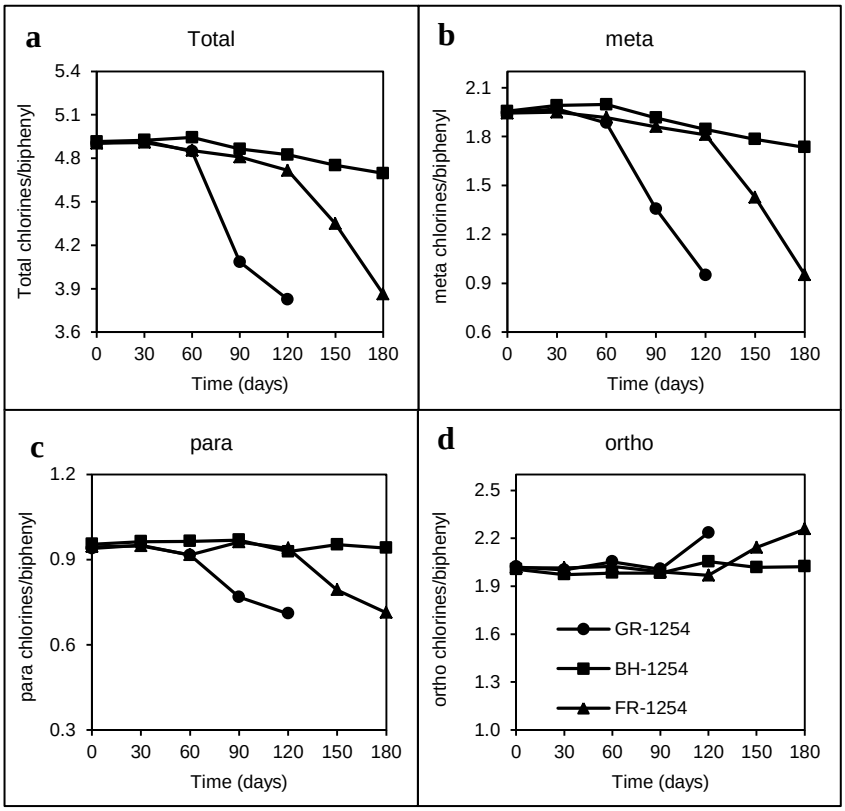


Figure 6.4. Dechlorination of Aroclor 1254 with three sediment microcosms (GR, FR, and BH) as a) total, b) meta, c) para and d) ortho chlorines/biphenyl within 120 days of incubation.

Overall 22%, 21.2% and 4.5% reductions in total chlorines of Aroclor 1254 were achieved by GR, FR and BH microcosms, respectively (Figure 6.4a). Dechlorination was predominantly through removal of the *meta* chlorines (Figure 6.4b) for all sediment cases: 51.2%, 51.1%, and 11.3% of *meta* chlorines were removed by GR, FR and BH sediment microcosms, respectively, (Figure 6.4b), while 24.4%, 24.6% and 1.4% of *para* chlorines (Figure 6.4c) were removed by GR, FR, BH microcosms, respectively. Even though the difference in % of para chlorine removal in GR and FR sediment microcosms was small, this observation along with the findings of PCB 118

dechlorination, suggests that the FR sediment microcosm first preference is para dechlorination. Also, about 72.3%, 81.7% and 46.7% of doubly flanked chlorines and by about 70.3%, 72.6% and 14.1% of single flanked chlorines were removed by GR, FR, and BH sediment microcosms, respectively, resulting an increase in unflanked congeners. None of the sediment microcosms showed *ortho* dechlorination during the Aroclor 1254 dechlorination (Figure 6.4d).

Figure 6.5 shows the homolog PCB distribution of GR, FR and BH sediment microcosms over the course of incubation period. As seen from Figure 6.5a, the GR sediment microcosms removed about 81%, 71%, 64%, and 48% of penta-, hexa-, hepta- and octa-chlorinated congeners, respectively. Interestingly, FR sediment microcosms removed 86%, 86%, 81%, and 55%, of penta-, hexa-, hepta-, and octa-chlorinated congeners, respectively (Figure 6.5b). On the other hand, BH sediment microcosms removed about 53%, 45%, and 32% of hepta- followed by hexa-, and octa-chlorinated congeners, respectively (Figure 6.5c). The dechlorination products of highly chlorinated congeners were generally unflanked tetra- and tri-chlorinated congeners in all three sediment microcosms at varying levels.

Figure 6.6 represents the changes in mol% of Aroclor 1254 congeners after 120 days (D120) of incubation in GR sediment microcosms. As seen from this figure, PCB congers such as PCB 93/95 (16 mol%), 110 (8 mol%), 101 (6 mol%), 87 (4.3 mol%), 44 (2.4 mol%), 153 (2.3 mol%), 99 (2.2 mol%), 70 (1.9 mol%), 97 (1.8 mol%), 85 (1.4 mol%), 141 (1.4 mol%), and 52/43 (1.4 mol%) were dechlorinated, resulting in the increase of congeners PCB 53, 15/17, 19, 16/32, 47, 49, 28, 51, 31, and 18 by about 20%, 10%, 7.5%, 6.6%, 5%, 4.5%, 3.4%, 2.2%, 1.8% and 1.7%, respectively. PCB 118, when present in Aroclor 1254, was not a main dechlorination substrate in GR microcosms.

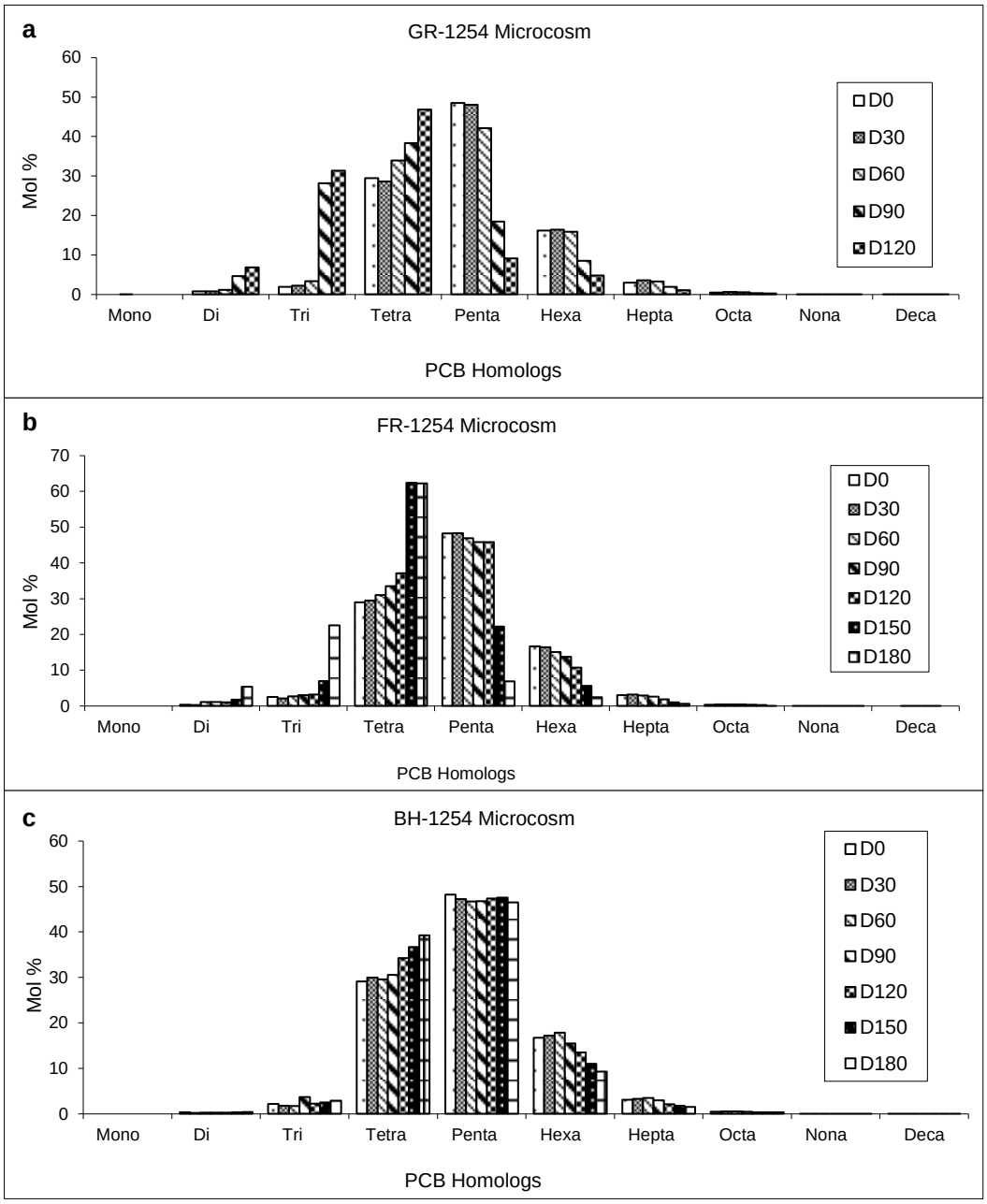


Figure 6.5 Homolog chlorine distributions of Aroclor 1254 in a) GR, b) FR, and c) BH microcosms (D#: sampling day).

The changes in mol% of Aroclor 1254 congeners after 180 days (D180) of incubation with Fox River sediment microcosms are presented in Figure 6.7. Congeners PCB 93/95, 110, 101, 81/87, and 44 were degraded by 16 mol%, 9 mol%, 7 mol%, 5 mol%, and 3 mol%, respectively. Terminal dechlorination products in FR

microcosms were PCB 53 15/17 47, 16/32, 49, 19, and 66 at about 24 mol%, 8.1 mol%, 7 mol%, 6 mol%, 6 mol%, 5 mol%, and 5 mol%, respectively (Table 6.2). PCB 118, when present in Aroclor 1254, was not a main dechlorination substrate in FR microcosms.

On the other hand, a different dechlorination profile was observed for Aroclor 1254 dechlorination in BH sediment microcosms (Figure 6.8). The changes in mol% of Aroclor 1254 congeners in BH sediment microcosms for top ten highly dechlorinated congeners, hence, as result highly accumulated final dechlorination byproducts PCB are summarized in Table 6.2. Congeners were PCB 81/87, 141, 85, 132/105/ 82/151, 153, and 110 for which 5 mol%, 1.4 mol%, 1.3 mol%, 1.2 mol%, 1 mol%, and 0.9 mol% decreased within 180 days of incubation, respectively. PCB 118, when present in Aroclor 1254, was not a main dechlorination substrate in BH microcosms (Table 6.2). Moreover, BH differs from GR and FR in that both much less mole% decrease (in value) is observed and also among the top 10 congeners decreasing, six of them such as PCB 141, 85, 132/105, 135/144/124/147, 156 and 158, do not coincide with that of GR and FR.

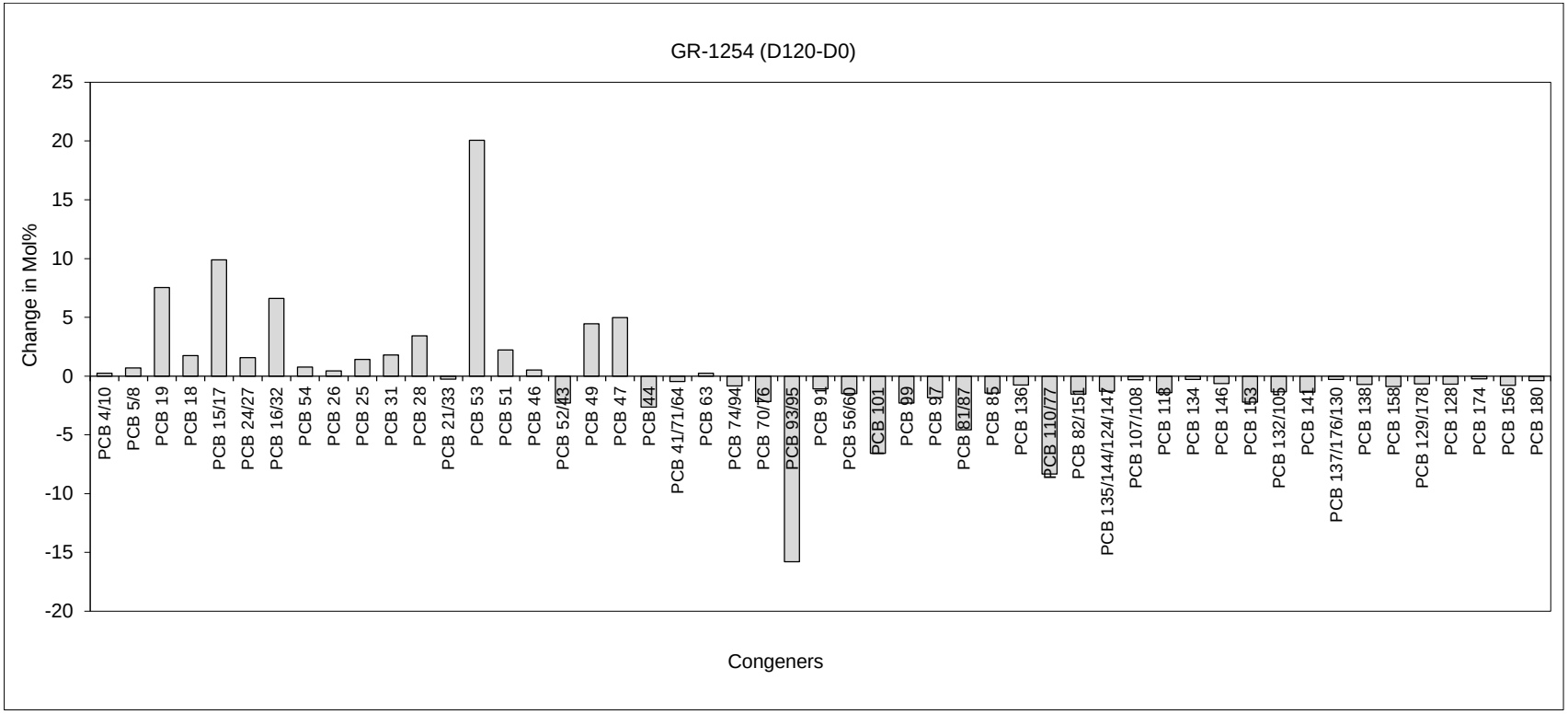


Figure 6.6. Changes in mol% of Aroclor 1254 congeners after 120 days (D120) of incubation in GR microcosms. Congeners with less than 0.2 mol% at any time point were excluded for the graph.

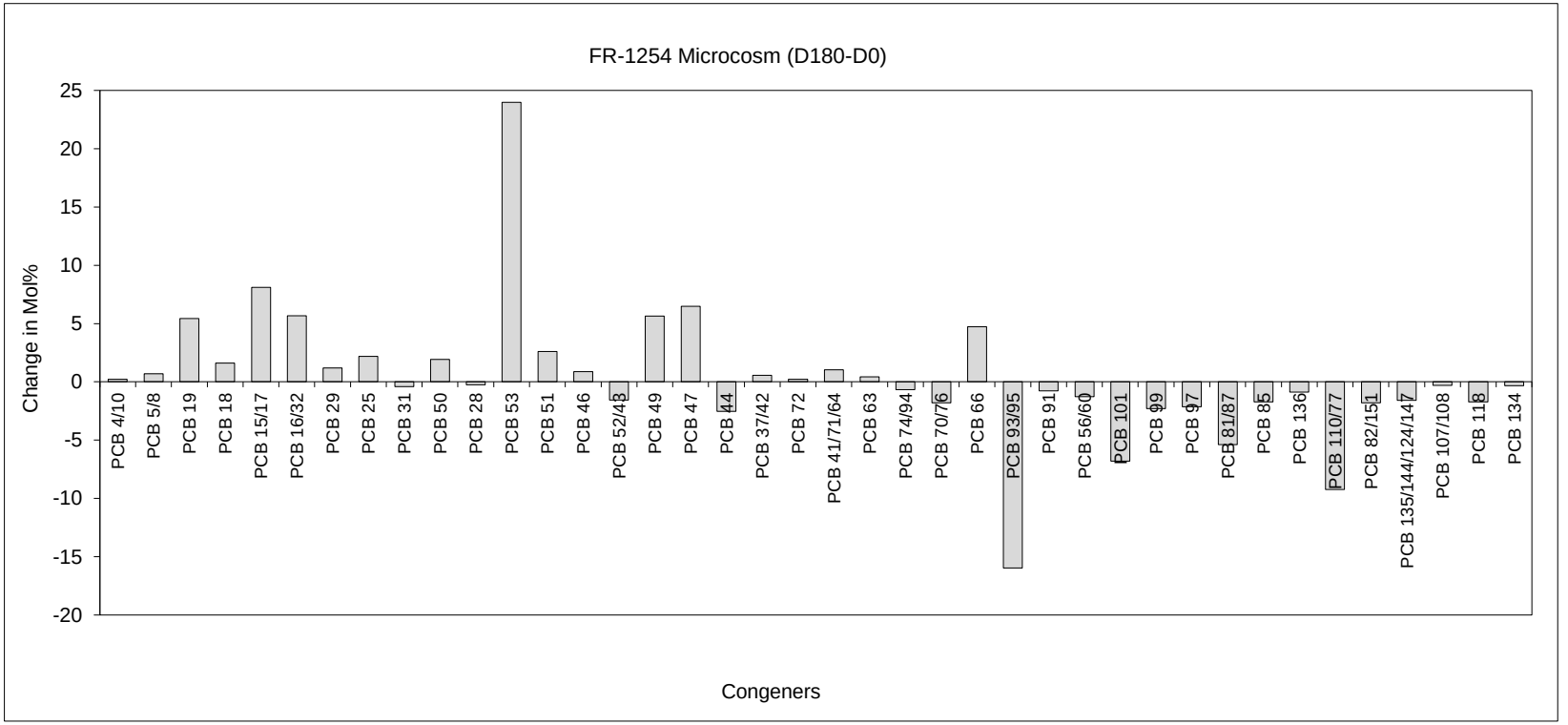


Figure 6.7. Changes in mol% of Aroclor 1254 congeners after 180 days (D180) of incubation in FR microcosms. Congeners with less than 0.2 mol% at any time point were excluded for the graph.

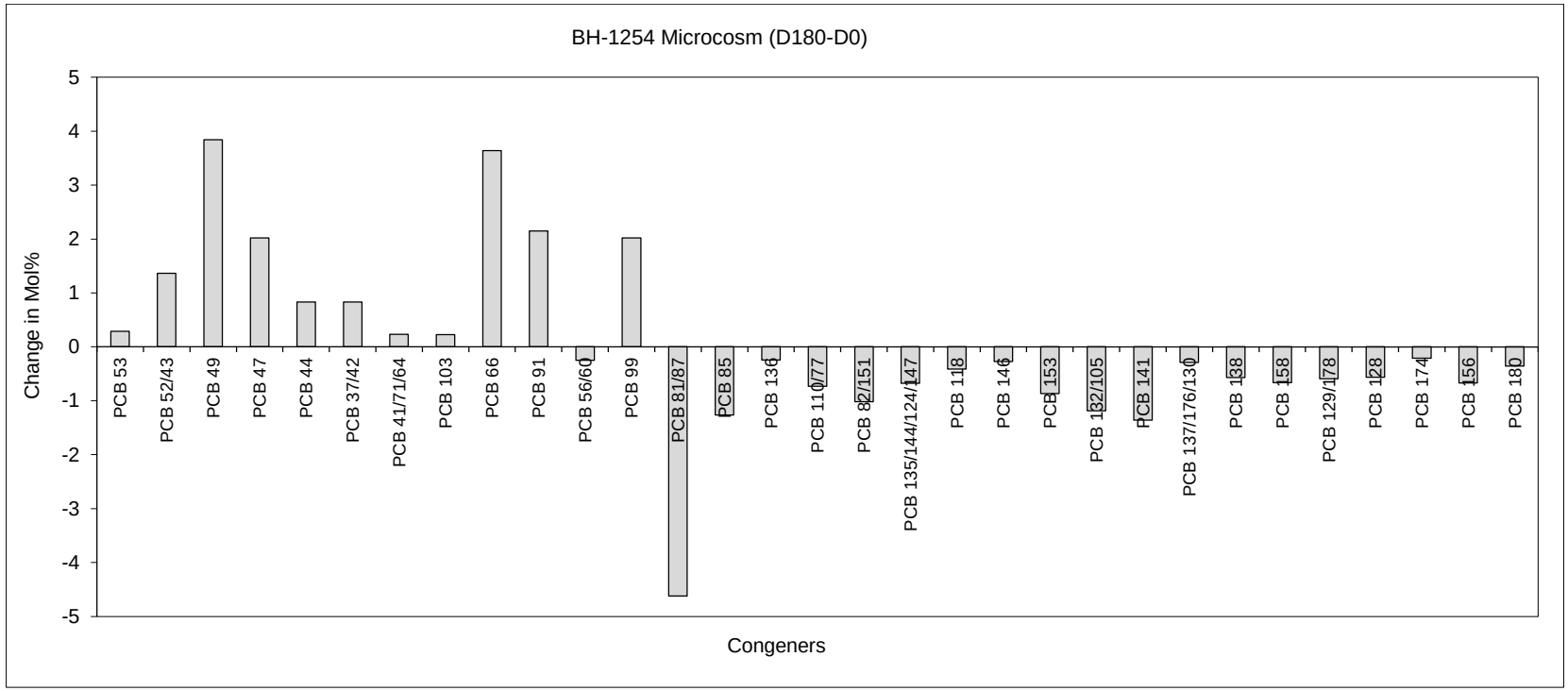


Figure 6.8. Changes in mol% Aroclor 1254 congeners after 180 days (D180) of incubation in BH microcosms. Congeners with lower than 0.2 mol% at any time point were excluded for the graph.

Table 6.2. A summary of highly dechlorinated and accumulated PCB congeners during Aroclor 1254 dechlorination with GR, FR, and BH microcosms.

Aroclor 1254 spiked sediment	Top 10 Congeners which were dechlorinated	Mol % decrease	Top 10 Congeners which accumulated	Mol % Increase
GR	PCB 93/95	-15.8	PCB 53	20.1
	PCB 110/77	-8.3	PCB 15/17	9.9
	PCB 101	-6.6	PCB 19	7.5
	PCB 81/87	-4.6	PCB 16/32	6.6
	PCB 44	-2.7	PCB 47	5.0
	PCB 99	-2.3	PCB 49	4.5
	PCB 52/43	-2.3	PCB 28	3.4
	PCB 153	-2.2	PCB 51	2.2
	PCB 70/76	-2.2	PCB 31	1.8
	PCB 97	-1.8	PCB 18	1.7
FR	PCB 93/95	-16.0	PCB 53	24.0
	PCB 110/77	-9.2	PCB 15/17	8.1
	PCB 101	-6.8	PCB 47	6.5
	PCB 81/87	-5.4	PCB 16/32	5.7
	PCB 44	-2.5	PCB 49	5.7
	PCB 99	-2.3	PCB 19	5.4
	PCB 97	-2.1	PCB 66	4.7
	PCB 70/76	-1.8	PCB 51	2.6
	PCB 82/151	-1.8	PCB 25	2.2
	PCB 118	-1.7	PCB 50	1.9
BH	PCB 81/87	-4.62	PCB 49	3.84
	PCB 141	-1.36	PCB 66	3.64
	PCB 85	-1.26	PCB 91	2.15
	PCB 132/105	-1.18	PCB 99	2.02
	PCB 82/151	-1.02	PCB 47	2.02
	PCB 153	-0.86	PCB 52/43	1.36
	PCB 110/77	-0.73	PCB 44	0.83
	PCB 135/144/124/147	-0.67	PCB 37/42	0.83
	PCB 156	-0.67	PCB 53	0.29
	PCB 158	-0.67	PCB 41/71/64	0.23

However, Wu *et al.* (1998) have reported better activity results with BH sediment culture for 800 ppm Aroclor 1260 dechlorination. Dechlorination of Aroclor 1260 resulted in extensive removal of *meta* and *ortho* chlorines from Aroclor 1260 in 180 days. Wu et al reported removal of 45% *meta* and 9% *ortho* chlorines from Aroclor 1260; while only11.3% *meta* and 1.4% *para* chlorine were removed from Aroclor 1254 in the current study. There was a lag of 60 days in BH sediment microcosm while the lag was longer (90 days) in the study of Wu *et al.* (1998). When they primed their microcosms initially with 350 mM 2, 3,4,5-CB or 2,3,5,6-CB, it stimulated the *meta* and *ortho* dechlorination of Aroclor 1260, even higher removals were achieved with a shortened lag time. Differences in Aroclor types and the concentrations might be the reasons of higher removal rates in that study; since it is known that the concentration of PCBs has been shown to be a factor in the dechlorination rate. As reported previously by Quensen III *et al.* (1988) that higher concentrations of Aroclor 1242 resulted in higher dechlorination rates, possibly because of higher concentrations resulted in higher solute concentration available for the microorganisms. Abramowicz *et al.* (1993) reported the highest dechlorination rates of Aroclor 1242 for the concentrations over 750 ppm, and that dechlorination rates were concentration dependent below 250 ppm. Also, Rhee *et al.* (1993) reported no dechlorination for the concentrations below certain "threshold" concentrations. These might be the case for BH sediment culture.

Another explanation for the activity differences of the sediment cultures might be related to contamination history of the sediments. All three sites are well known historically impacted sites. May *et al.* (2006) was stated that individual congeners can influence (either by stimulation or inhibition) the activity of individual PCB dechlorinating bacteria and later Kjellerrup *et al.* (2008) suggested that different congener profiles can lead to a selection of different dechlorinating communities. As it is known that GR was contaminated with Aroclor 1248 for more than four decades (USEPA, 2005) and the major PCB source to the Fox River sediments was Aroclor 1242 (Imamoglu, 2004). On the other hand, it was previously reported that BH sediment PCB profile resembles Aroclor 1260 (Payne *et al.*, 2011). As seen from

Table 6.3, analysis of PCB homologues of GR and FR sediments showed that their homologue profiles are very close to each other, whereas BH is significantly different from the two. This may help to understand the preference of BH sediment culture which dechlorinated selectively highly chlorinated (hepta-, hexa- and octa-chlorinated) PCB congeners during Aroclor 1254 dechlorination different than other sediment cultures and the dechlorination rate was very low compared to that of GR and FR. Both these results together with historical contamination of the BH sediments with Aroclor 1260 most probably enabled the development of bacterial culture capable of dechlorinating highly chlorinated congeners such as hepta-, hexa- and octa-chlorinated biphenyls has a preference towards highly dechlorinated congeners.

Table 6.3. Analyses of PCBs in GR, FR, and BH sediment samples and commercial Aroclor mixtures.

Parameter	GR[1]	FR	BH[2]	A 1016[1]	A 1242[1]	A 1248[1]	A 1254[1]	A 1260[1]
Total PCB concentration (ng/g)	6824	2021	1334	-	-	-	-	-
Average no. of Cl atoms	3.8	3.7	5.57	3.1	3.4	4.3	5.2	6.5
% of PCB with < 6 CL atoms	87	92	45	100	100	98	74	9
% of PCB with < 5 CL atoms	73	83	33	95	81	55	9	1

[1] Kjellerup *et al.* (2008). [2] Payne *et al.* (2011). A: Aroclor.

Moreover, when Table 6.2 is analyzed together with Table 6.3, it can be clearly seen that FR and GR had similar site and PCB contamination profiles, hence; their congener selection preference towards dechlorination was also similar. The same PCB congeners were dechlorinated by GR and FR sediment culture. which are generally the most abundant congeners in Aroclor 1254. It is more likely that PCB congener profiles and properties of sites promoted diversity, selection and enrichment of dechlorinating culture in these sediment microcosms. In addition,

Grasse River had higher total concentrations of PCB compared with the other two sites. Therefore, the concentration of PCB congeners accessible for dechlorination and the concentration of potential electron donors were higher in Grasse River favoring the enrichment of the PCB dechlorinating phylotypes. Moreover, the lag time exerted by BH sediment culture during the dechlorination of PCB 118 was very long compared to the other sites indicating the presence of slow and possibly different microbial communities. Microbial populations have an important effect on dechlorination and the dechlorination rates.

6.3.3. Enumeration of dechlorinating phylotypes

Microorganisms were enumerated in all microcosms for 3 time points (initial time, mid time and final time of incubation period) by quantitative PCR (qPCR) using a specific primer set (348F/884R) for microorganisms closely related to known PCB dechlorinators. Amplification efficiencies of purified DF-1 16S rRNA gene standards for all sets were 98.48±5.1% with the r^2=1 (Figure 6.9) and amplification efficiencies of samples were 94.95±6.5%

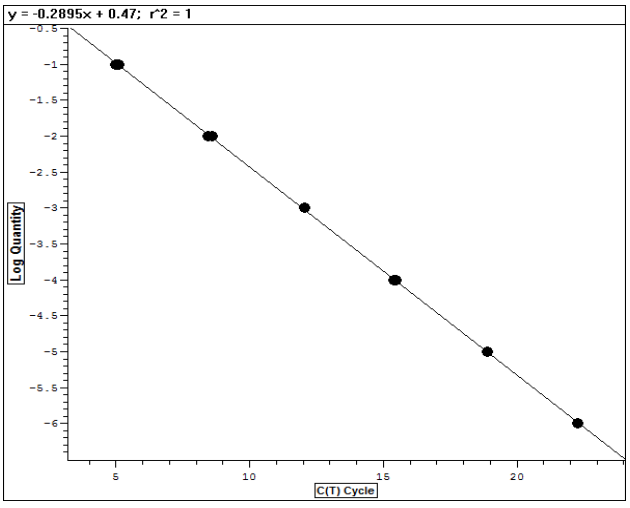


Figure 6.9. Standard curve used for enumeration of dechlorinating phylotypes in sediment microcosms.

Figure 6.10 shows the change in the number of 16S rRNA gene copies of putative dechlorinators in sediment microcosms over time. In all sediment microcosms the number of putative dechlorinators increased in all (Figure 6.10). The highest increase was seen in GR sediment microcosms amended with PCB 118 where the number of putative PCB dechlorinating phylotypes increased 38-fold (from $2.03x10^{+05}\pm5.73x10^{+04}$ to $7.63x10^{+06}\pm1.26x10^{+05}$), while the increase was about 25-fold observed during Aroclor 1254 dechlorination by GR microcosms. The increase in the number of putative dechlorinating bacteria of FR and BH sediment microcosms during PCB 118 dechlorination was 16.3 and 24-fold, respectively, while the increase in the number of putative dechlorinating bacteria during Aroclor 1254 dechlorination by FR and BH sediment microcosms was relatively low compared to that of GR sediment microcosms.

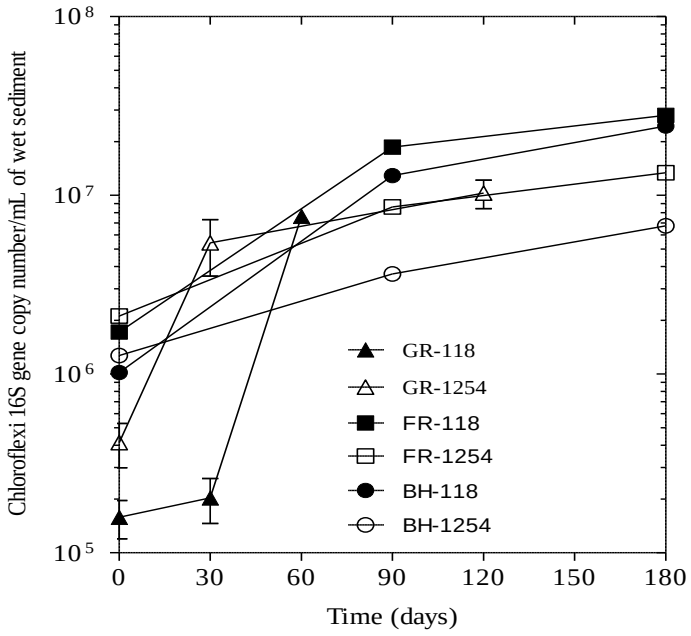
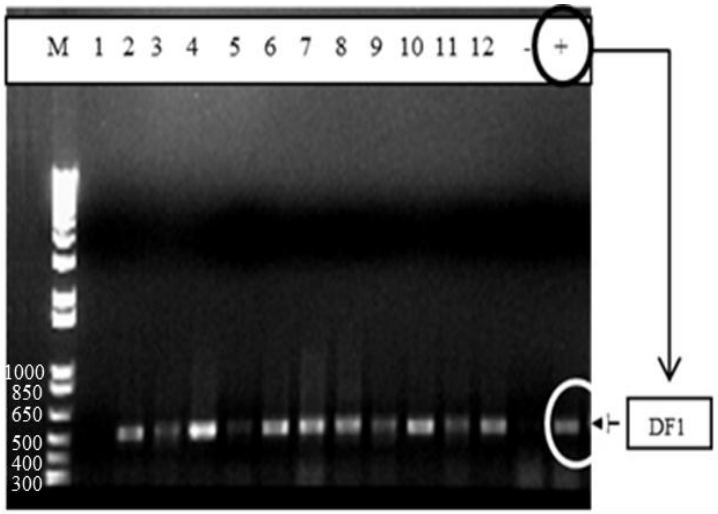


Figure 6.10. Changes in number of 16S gene copies over time in GR, FR, and BH microcosms.

In addition, day 0 and day final qPCR products of all three sediment microcosms were verified by gel electrophoresis. A pure culture of PCB dechlorinating bacterium, DF-1, was used as a positive control and sterile water template was used as a negative control during qPCR. As seen on the picture of the gel (Figure 6.11), DF-1 produced a band at about 500 bp, while the negative control yielded no product. Almost all samples produced the expected size band (Figure 6.11). Although gel electrophoresis is only semi-quantitative, the qPCR product at D0 was generally lighter than the D120, due to the initial low numbers of putative dechlorinating bacteria. Thus, it appears that the dechlorination of PCBs was growth-linked in all sediment microcosms.



Lane	Template type
M	1 kb plus DNA ladder
1	GR-118-D0
2	GR-118-D60
3	GR-1254-D0
4	GR-1254-D120
5	BH-118-D0
6	BH-118-D60
7	BH-1254-D0
8	BH-1254-D120
9	FR-118-D0
10	FR-118-D60
11	FR-1254-D0
12	FR-1254-D120
-	negative control (no DNA)
+	positive control (DF-1)

Figure 6.11. Agarose gel showing the qPCR performed for PCB dechlorinating bacterium DF-1 (positive "+" control), deionized sterile water (negative control "-") and slurry samples taken inoculated microcosms.

Surprisingly, enumeration of the putative dechlorinating bacteria in sediments of the three sites showed that higher numbers of putative dechlorinating bacteria initially do not always lead to a higher dechlorination activity. Specifically, the initial number of putative dechlorinating bacteria in Grasse River was about 10-fold lower than the other two sediments, but GR microcosms had the highest activity with a shorter lag

time and a greater extent of dechlorination than the others. A similar observation was reported previously by Kjellerup *et al.* (2008). They investigated the dechlorinating communities and dechlorination activity between the three PCB-impacted sites (Grasse, Anacostia and Buffalo Rivers) and determined that TOC (a measure of the electron donor availability) concentration in the sediments strongly correlated with the dechlorination activity in sediment microcosm studies. Although TOC was not measured in this study, GR microcosms had the highest dechlorination activity, suggesting similar situation as observed by Kjellerup *et al.* (2008).

6.3.4. Community analysis of PCB dechlorinating bacteria

The effect of PCB 118 and Aroclor 1254 on the microbial community in sediment microcosms was determined by DHPLC analysis of the 16S rRNA gene. Specific primers used in this study target putative dechlorinating bacteria within *Chloroflexi* since only these microorganisms have been confirmed to grow by dehalorespiration of PCBs (Cutter *et al.,* 2001; Wu *et al.,* 2002; Fennell *et al.,* 2004; Fagervold *et al.,* 2005, 2007, and 2011). Because dehalorespiring microorganisms typically represent a small fraction of the total bacterial community (Major *et al.* 2002; Amos *et al.* 2008) they often cannot be detected with universal 16s rRNA primers.

DHPLC analysis of all day zero and day final samples of the three sediment microcosms spiked with Aroclor 1254 and PCB 118 spike are given in Figure 6.12. In all DHPLC figures, the x-axis represents time (minutes) and the y-axis fluorescence (mV). Each peak theoretically is the rRNA gene amplified from a different microorganism among the population; the peak height semi-quantitatively shows the abundance of that rRNA gene in the population. PCR products of pure DF-1 and blanks (sterile water) were also analyzed.

In all of the microcosms the initially community diversity was high (about 6-10 apparent different organisms), and the community was distinct for each sediment. It can be seen from Figure 6.12a and 6.12b that microbial diversity of GR sediment

microcosms (PCB 118 or Aroclor 1254) changed from day 0 to day 120 (final day) and some of the phylotypes present initially were enriched. This suggests that the organisms enriched were the organisms responsible for the dechlorination observed.

Although DHPLC graphs of Aroclor 1254 microcosm and PCB 118 microcosm of GR sediment look similar, different phylotypes were enriched. For example, P4 (retention time at 3.5 min) became predominant in GR sediment with PCB 118, while a different phylotype became predominant in GR sediment with Aroclor 1254 (retention time at 5.1 min) (Figure 6.12b). This suggests that a subset of microorganisms (one or a few) in GR sediment dechlorinate PCB118 when it is added alone, and a different subset of microorganisms dechlorinate the congeners attacked in Aroclor 1254 (primarily 93/95, 110/77, 101, but significantly not PCB 118, Table 6.2).

Similarly, in FR sediment microcosm, the initial community was enriched for 1 or more dominant phylotypes by PCB 118 or Aroclor 1254. In FR microcosms but not GR microcosms, the same organisms are apparently enriched with either PCB 118 or Aroclor 1254. This suggests that the same subset of microorganisms (one or a few) in FR sediment dechlorinates PCB118 when it is added alone, and dechlorinate the congeners attacked in Aroclor 1254 (primarily 93/95, 110/77, 101, but significantly not PCB 118, Table 6.2).

In BH microcosms 1 or more phylotypes were enriched by amendment with PCB 118. This suggests that the enriched microorganisms were responsible for the dechlorination of PCB 188 and its intermediates. However, the presence of Aroclor 1254 did not apparently enrich members of the BH community. This observation is not surprising as the dechlorination activity of BH sediment microcosms amended with Aroclor 125 was very low compare to that seen in GR and FR sediment microcosms.

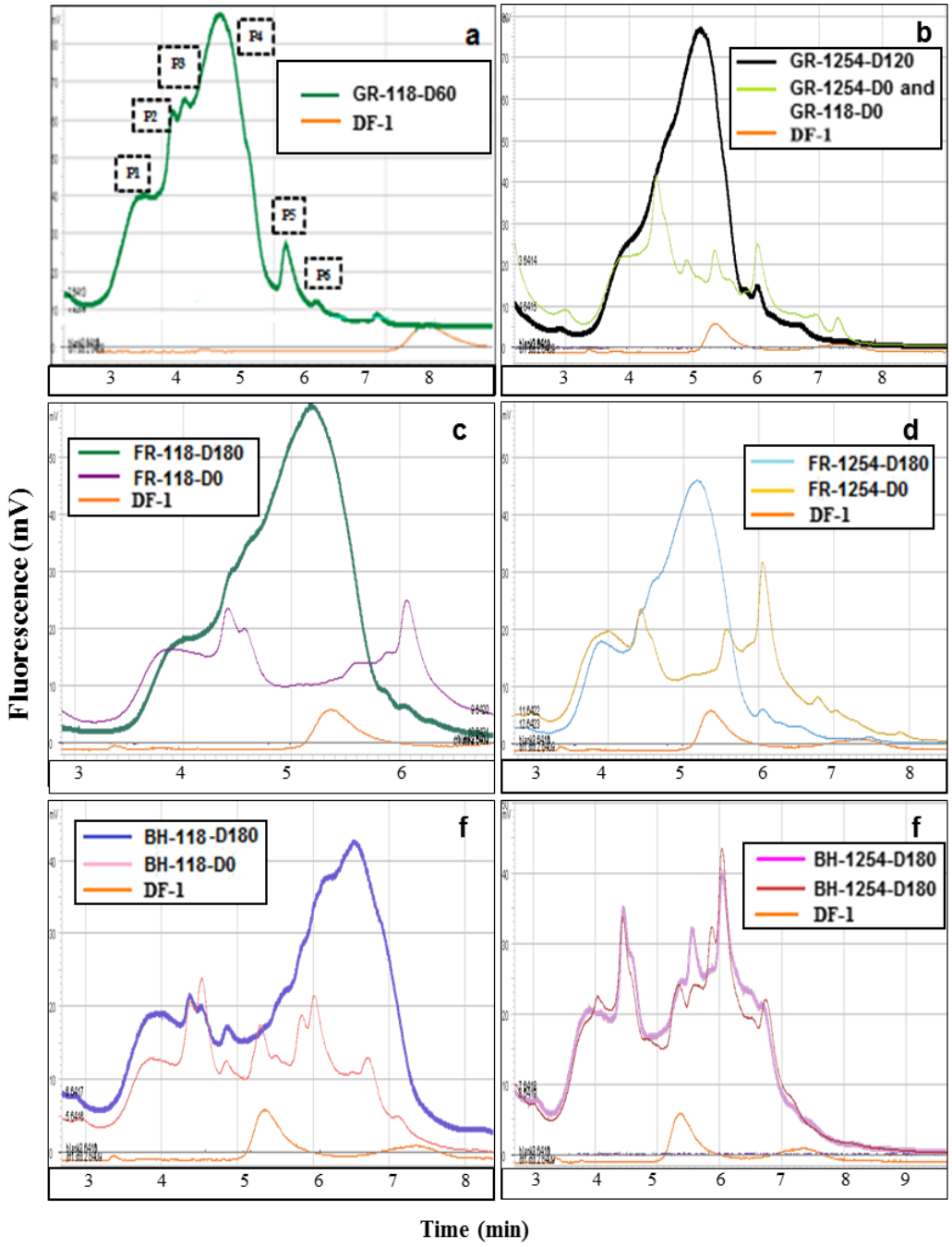


Figure 6.12. Results of DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in GR microcosm spiked with a) PCB 118 and b) Aroclor 1254, FR c) PCB 118 and d) Aroclor 1254, and BH e) PCB 118 and f) Aroclor1254 at day 0 and day final. The possible phylotypes are labeled as P1, P2, P3, P4, P5, and P6 in a) GR-118.

6.4. Conclusion

PCB 118 and Aroclor 1254 were reductively dechlorinated by three sediment microcosms to varying extents. The most active enrichment culture was made from GR sediment. This sediment provided higher activity and reduced lag time compared to FR and BH. The dechlorination of Aroclor 1254 results in removal of mostly *meta* followed by *para* chlorines. As a result, the abundance of highly chlorinated congeners decreased while that of lower chlorinated, *ortho* substituted, congeners increased by the preferential removal of flanked *meta* and *para* substituted chlorines. Since the *meta* and *para* chlorines are removed from the congeners, the coplanar structure, and hence, the dioxin-like toxicity of these congeners is reduced with the anaerobic dechlorination. In addition, the subsequent lower chlorinated congeners have greater potential to be degraded by aerobic bacteria, allowing for the complete biodegradation of PCBs into carbon dioxide and water via sequential anaerobic and aerobic microbial degradation processes. Molecular techniques were critical in order to detect and identify the active dechlorinators at PCB contaminated sites as well as to monitor the fate of both indigenous and augmented microorganisms. The application of molecular techniques will be crucial for the development of *in situ* treatment strategies.

CHAPTER 7

REDUCTIVE DECHLORINATION OF AROCLOR 1254 IN GRASSE RIVER SEDIMENT MICROCOSMS

7.1. Introduction

Starting from the late 1920s, Polychlorinated biphenyls (PCBs) were commercially produced as complex mixtures by a number of companies around the world. They were widely used in many industrial applications due to their stable properties, including chemical stability, low flammability, electrical insulating properties, low water solubility and vapor pressures. These properties made PCBs very useful for many industrial applications such as capacitor and transformer fluids. PCBs are one of the most persistent contaminants found today in the environment. Increase in the awareness of PCB toxicity and environmental contamination lead to the ban of PCB manufacturing, processing, distribution and use in 1979 in the US (Erickson, 1997). Even though they are no longer manufactured or used by industry, they continue to be encountered as contaminants of concern at hazardous waste sites and in the environment because they are persistent in the environment; able to accumulate in fatty tissues in the body; and they have toxic properties (USEPA, 2008).

Waste motor and transformer oils are considered as a main source of polychlorinated biphenyls (PCBs) emission into the environment (Jones *et al*., 2003). Approximately, 61% of PCBs are in electrical transformers that are still in use, another 12% in electrical capacitors and 27% in storage waiting for disposal. Large volumes of PCBs have been introduced into the environment by direct entry or leakage into sewers and streams; and by accidental spills or by possibly illegal disposal (Tagasuka *et al*.,

2006). Transformer oils were most commonly mixtures of chlorobenzenes with either Aroclor 1254 or 1260 (Erickson, 1997). Aroclor is a trade name for commercial mixtures of PCBs. Chlorines constitute an important fraction of Aroclor mixtures for example Aroclor 1254 is defined as being 54% chlorine by weight, Whereas Aroclor 1260 has 60% chlorine by weight (Field and Sierra-Alvarez, 2007). According to the Monsanto US (the clear global market leader, accounting for over half of worldwide PCB production) sales record between 1957 and 1974, Aroclor 1242 had the highest percentages of production/sold mixture, Aroclor 1254 was in the second place among the produced/sold Aroclor mixtures. Aroclor 1254 is one of the mixtures consisting of highly chlorinated PCB congeners, mostly penta- (49%) and hexa-chlorinated PCB congeners (about 28%) (Hansen, 1999).

Despite their recalcitrance to biodegradation, the microbial mediated anaerobic reductive dechlorination of PCBs was reported in a variety of anaerobic sediments (Bedard and Quensen III, 1995; Wiegel and Wu, 2000; Kjellerup *et al*., 2008; Fagervold *et al.*, 2005, 2007 and 2011). The process consists in the sequential reduction of highly chlorinated, toxic and bioaccumulable PCBs into lesser chlorinated congeners commonly having lower toxicity, lower bioaccumulation potential and higher susceptibility to undergo mineralization by aerobic bacteria (Bedard, 2003). Dechlorination under anaerobic conditions occurs due to dehalorespiration, where halogenated compounds are used as terminal electron acceptors. Consequently, an excess of a carbon source (electron donor) is required for efficient dechlorination (Tartakovsky *et al.*, 2000). The degree of chlorination as well as the position of chlorines in a congener is major factor that influence degradation potential of the compound (Borja *et al.*, 2005).

Also, microbial PCB dechlorination reduces the potential human exposure to PCBs by decreasing PCB bioaccumulation and persistence. Known correlations with octanol/water partitioning coefficients show that each chlorine loss decreases the log K_{ow} by 0.5 with corresponding reductions in bioaccumulation. In addition, dechlorination can also markedly decrease the persistence of PCBs. The depletion of

highly chlorinated congeners may reduce the exposure level of PCBs, thereby, reducing the potential carcinogenicity and bioaccumulation of PCBs (Abramowicz, 1995; Wiegel and Wu, 2000; Bedard and Quensen III, 1995; Fagervold, 2007; Payne *et al*., 2011). Due to all of these benefits of dechlorination of PCBs, which are widespread environmental pollutants, this degradation mechanism may have significant implications for risk assessment and remediation strategies (Bedard and Quensen III, 1995).

Microbial transformation of Aroclor 1254 by anaerobic reductive dechlorination has been shown in sediment, as well as laboratory microcosms (Quensen III *et al.*, 1990; Oefjord *et al*., 1994; Natarajan, 1998; Pakdeesusuk *et al.*, 2003, Zanaroli *et al.*, 2012) but in these studies either dechlorination was slow or not extensive.

In this study, sediment from Grasse River (GR), Massena, NY, was used for dechlorination of individual PCB congers selected from Aroclor 1254. It has been shown that GR was historically contaminated with high concentrations of Aroclor 1248 and had the shortest lag and greatest activity in dechlorination among the other sediments tested in the study of Kjellerup *et al*. (2008). Therefore, it is important to investigate the effects of using sediment enriched with the culture dechlorinating Aroclor 1248 on the dechlorination of Aroclor 1254. In this study, dechlorination pathways of 22 congeners chosen from Aroclor 1254 were investigated, which is the most extensive study about dechlorination pathway of 1254 with GR sediments to our knowledge.

7.2. Materials and Methods

7.2.1. Chemicals

All PCBs (99-100 % purity) were purchased from AccuStandard. Transformer oil is purchased from Ultra Scientific. PCE was purchased from Sigma-Aldrich. Hexane

(pesticide analyzed grade) was from Acros Organics. All other chemicals were reagent grade.

7.2.2. Sediment Location and Collection

The Grasse River site is located in the lower Grasse River (GR) in the Village of Massena, NY in the United States where ALCOA (Aluminum Company of America) has been producing aluminum since 1903. The GR is a U.S. Environmental Protection Agency (USEPA) Superfund site that contains high levels of PCBs. Historic disposal of production waste by-products into onsite landfills and lagoons, a practice that was common and widely accepted at the time, resulted in the release of PCBs into the lower GR from the 1930s to the 1970s. The PCBs were discharged to GR with the wastewater during routine operations through four permitted outfalls and resulted in contamination of a seven mile stretch of the river (Kjellerup *et al*., 2008). It has been reported that GR was contaminated with Aroclor 1248 that was used for aluminum production at this industrial site (EPA, 2005). Total of PCB concentration in GR sediments was found as 6829 ng/g (Kjellerup *et al*., 2008). In 2008, Kjellerup *et al.* showed that the GR PCB profile was most similar to the A1248 profile and the results of their analyses supported the historical information about the main sources of contamination for GR. GR sediments were collected during Spring 2008 with a ponar sampler from the top 30 cm of sediment from GR, NY (44°57.06 N 74°51.06 W). The sediment samples were stored anaerobically in Teflon lined sealed glass jars at 4°C in the dark prior to use. Sediments were black in color and had a sulfide odor indicative of reduced anoxic conditions.

7.2.3. Experimental Sets of GR

In this experiment (Table 7.1), the specific dechlorination pathways of Aroclor 1254 congeners were investigated. For this purpose, Aroclor 1254 and 22 individual PCB microcosms (PCB 44, 52, 70, 74, 85, 87, 91, 95, 97, 99, 101, 105, 110, 128, 132, 138, 141, 149, 153, 156, 163, and180) were prepared. Low-sulfate (0.3 mM) estuarine medium (E-Cl) prepared as described by Berkaw *et al*. (1996) was

anaerobically dispensed as 24-mL aliquots into 60-mL serum bottles for individual PCB congeners, while for Aroclor 1254, 50-mL E-Cl dispensed into 160-mL serum bottles. They were sealed under N_2/CO_2 (80:20) with 20-mm Teflon-coated butyl stoppers (West Co., Lionville, PA) secured with aluminum crimp seals.

The medium was autoclaved at 121°C for 20 minutes. The final pH was 6.8. All subsequent additions were performed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Michigan, USA) containing N_2:CO_2:H_2 (75:20:5). Aroclor 1254 microcosm was inoculated with wet GR sediment (10 mL) spiked with tetrachloroethylene (PCE) at a final concentration of 200 µM to stimulate growth of dehalorespiring bacteria. PCE was not added into individual PCB congener microcosms. These microcosms were inoculated by transferring 1-mL of homogenized slurry (culture) taken from Aroclor 1254 microcosms after seeing dechlorination activity on day 120 in the first study given in Chapter 6 and 0.5% (w/v) dry and sterile GR sediment. Sterile dry sediments were prepared by baking sediments at 115°C for 72 h, followed by autoclaving three times in a container sealed under N_2 for 60 min.

FA mixture (acetate, propionate, and butyrate) was supplied at a concentration of 2.5 mM as an electron donor and carbon source. Aroclor 1254 and individual PCB congeners dissolved in acetone were added to provide a final concentration of 100 mg/L and 50 mg/L (mL of acetone/ mL of effective volume of microcosm: 0.2% as v/v). After addition of all ingredients (E-Cl, dry-sterile sediments, active culture, FA, and PCBs), all microcosms were sealed in the anaerobic glove box with the stoppers secured using aluminum crimp seals and incubated statically at 30ºC in the dark until the following sampling. One control microcosm containing acetone without PCB was prepared. Sterile sets were prepared for only 3 congeners (PCBs 44, 87, 101). Sterile controls were also prepared identically except that the bottles were autoclaved twice for 1 h at 121°C on two consecutive days prior to PCB addition. All microcosms-except Aroclor 1254 microcosm were inoculated in triplicate. Aroclor 1254 microcosm was prepared in one replicate.

Table 7.1. Experimental setup of microcosms.

Microcosm label	Sterile	PCB type and concentration
GR-Aroclor 1254	No	Aroclor 1254: 100 mg/L
GR-44	No	PCB-44: 50 mg/L
GR-44-S	Yes	PCB-44: 50 mg/L
GR-52	No	PCB-52: 50 mg/L
GR-70	No	PCB-70: 50 mg/L
GR-74	No	PCB-74: 50 mg/L
GR-85	No	PCB-85: 50 mg/L
GR-87	No	PCB-87: 50 mg/L
GR-87-S	Yes	PCB-87: 50 mg/L
GR-91	No	PCB-91: 50 mg/L
GR-95	No	PCB-95: 50 mg/L
GR -97	No	PCB-97: 50 mg/L
GR-99	No	PCB-99: 50 mg/L
GR-101	No	PCB-101: 50 mg/L
GR-101-S	Yes	PCB-101: 50 mg/L
GR-105	No	PCB-105: 50 mg/L
GR-110	No	PCB-110: 50 mg/L
GR-128	No	PCB-128: 50 mg/L
GR-132	No	PCB-132: 50 mg/L
GR -138	No	PCB-138: 50 mg/L
GR-141	No	PCB-141: 50 mg/L
GR -149	No	PCB-149: 50 mg/L
GR-153	No	PCB-153: 50 mg/L
GR-156	No	PCB-156: 50 mg/L
GR -163	No	PCB-163: 50 mg/L
GR-180	No	PCB-180: 50 mg/L
GR-Ace	No	acetone without PCB

7.2.4. Analytical techniques

7.2.4.1. PCB extractions and analytical procedures

One mL samples from each triplicate bottle were extracted with 5 mL of hexane on a wrist shaker overnight for measurement of PCBs. The organic phase was passed through a copper/Florisil (1:4) column and PCB congeners were analyzed using a

Hewlett Packard 5890 series II GC equipped with ^{63}Ni electron capture detector as described previously (Fagervold *et al.,* 2005) and a DB-1 capillary column (30mx0.25mmx0.25μm). PCB congeners in a mixture containing 250 μgL^{-1} Aroclor 1232, 180 μgL^{-1} Aroclor 1248 and 180 $\mu g\ L^{-1}$ Aroclor 1262 (also known as ‘‘Mullin’s mix’’) (Swackhamer *et al.,* 1996) were used to make with a 10-point calibration curve with PCB 30 and PCB 204 used as internal standards.

Fifty-five additional congeners not present in the Aroclor mixture that were potential dechlorination products were also added to the PCB calibration table. A calibration mixture containing the internal and surrogate standards and Mullin’s mix was run with every batch of samples to determine the response factor of each peak relative to the internal standards and to correct/check shift in the retention times of peaks. PCB 166 was used as a surrogate compound and its recovery was about 85%. Congener concentrations were not corrected for surrogate recoveries, because of the possible bias in calculated mole fractions of lesser or more chlorinated congeners in the case of differential recoveries of the different surrogates. Peaks for the surrogate and internal standards and those that co-eluted with sulfur (which was sometimes not completely removed by the copper cleanup) were disregarded.

During analytical determination of PCBs, in the chromatograms, a peak may represent an individual congener or a group of congeners. The congeners that appear together in the same peak during chromatographic analysis are named as coeluting congeners. These coeluting congeners are designated by slashes separating their congener numbers. For data calculations, co-eluting congeners and homologues were assumed to be present in equal proportions. Dechlorination curves were made for all PCB congeners in 30-day intervals over the course of incubation. The PCB concentrations were measured as μg PCB/mL of microcosm slurry by GC/ECD and converted to mol% which was calculated as follows:

$$\text{mol }\% = \frac{\frac{C_i}{MW_i}}{\sum \frac{C_i}{MW_i}} .100 \qquad (7.1)$$

where MW_i is the molecular weight of each detected PCB congener (g/mol) and C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry). The extent of dechlorination was calculated from the weighted average number of chlorines ($Cl_{average}$) in samples before and after incubation. $Cl_{average}$ was reported as average number of Cl per biphenyl, and was calculated according to the formula below (Equation 6.2):

$$Cl_{average} = \frac{\sum C_i.n_i}{\sum C_i} \quad (7.2)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents.

The dechlorination rate was calculated within the linear slope of the dechlorination curve by dividing total chlorine removed per biphenyl with the time elapsed in days and calculated by using the formula below (Equation 6.3):

$$Rate = \frac{\left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{initial} - \left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{final}}{t} \quad (7.3)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents and t is the incubation time elapsed (days).The average rate and the standard deviation were calculated from triplicate cultures.

7.2.4.2. DNA extraction

DNA was extracted (in triplicate) from microcosms samples for three time points (time points are shown in text and/or on the graphs with letter “D” followed with time in days) and was used in downstream processes such as enumeration and community analysis of PCB dechlorinating bacteria. DNA was extracted by adding

0.25 mL of slurry samples from each microcosm to an individual Power Bead microfuge tube of a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc.) or to a 96-well bead beating plate for rapid and thorough homogenization. In the former, the Power Bead tubes were mixed by brief vortex prior to 30s of bead beating at speed "4.5" using a FastPrep120 (Q-Biogene, CA). In the latter, instead of FastPrep120 (Qiagen, Resch), a TissueLyser is used. The TissueLyser allows the usage of 96-well plate and like FastPrep120, it provides disruption of biological samples through high-speed shaking. In the DNA extraction process, cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the spin column. Total DNA was then isolated from the Power Bead tubes or 96-well plates according to the manufacturer's directions. DNA was eluted in 100 µL of Tris buffer (provided with kit) and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). All DNA samples were diluted to 2ng/µL in Tris buffer.

7.2.4.3. Enumeration of PCB dehalorespiring bacteria by quantitative polymerase chain reaction (qPCR)

The quantification of putative dechlorinating Chloroflexi in the microcosm samples was performed (in triplicate) by quantitative polymerase chain reaction (qPCR) using iQ SYBR green supermix (Bio-Rad Laboratories). DNA was amplified with primer Chl348F (5'-CGC CCG CCG CGC GCG GGA GGC AGC AGC AAG GAA-3') and Dehal884R (5'-GGCGGGACACTTAAAGCG-3') targeting the16S rRNA genes of the members within Chloroflexi including *Dehalococcoides* spp. (Fagervold *et al.*, 2005).

Each qPCR reaction volume (25-µL) contained 1x iQ SYBR green supermix, 400 nM forward and reverse primers and 1 µL of sample DNA. PCR amplification and detection were conducted in an iCycler (Bio-Rad Laboratories). qPCR conditions were as follows: initial denaturation for 15 min at 95°C followed by 35 PCR cycles

of 30 s at 95°C, then 30 s at 61°C, then 30 s at 72°C. One copy of the gene per genome was assumed based on the genomes of *Dehalococcoides mccartyi* strain 195 and *Dehalococcoides* sp, strain CBDB1 (Seshadri *et al.*, 2005: Kube *et al.*, 2005). qPCR data were analyzed with MJ Opticon Monitor Analysis Software v3.1 and compared to a standard curve of purified DF-1 348F/884R 16S rRNA gene product. The standard curve consisted of duplicate dilutions over 7 orders of magnitude. The specificity of qPCR amplification was verified by melting curve analysis followed by gel electrophoresis 1.2% (w/v) high-melt agarose gel.

Amplification efficiencies of dilutions of gel purified DF-1 16S rRNA gene PCR product used as standards for all sets were 90% ± 8% (r^2 = 0.999), and amplification efficiencies of samples were 94% ± 7%.

7.2.4.4. Community analysis of PCB dechlorinating bacteria by denaturing High Pressure Liquid Chromatography (DHPLC)

Denaturing high pressure liquid chromatography (DHPLC) analyses were performed using a WAVE 3500 HT system (Transgenomic, Omaha, NE) equipped with a florescence detector (excitation 490 nm, emission 520 nm). Primers 348F/884) were used both for DHPLC following PCR or qPCR (sometimes qPCR products were used) as described below.

PCR reactions were performed in 50 µL reaction volumes using the following GeneAmp reagents (PE Applied Biosystems, Foster City, CA): 10 mM Tris-HCl, a mixture of dNTPs (200 nM each), 1.5 mM $MgCl_2$, 100 nM of each primer, 0.8% dimethyl sulfoxide (DMSO), 2 units of Ampli *Taq* DNA polymerase, 200 nM of each primer, 1 µL of DNA template and 34.5 µL of nuclease-free water. Amplification was performed in a PTC200 thermal cycler (MJ Research, Watertown, MA) with the following cycle parameters: Initial denaturing (1 min at 95°C), 40 cycles of denaturation (45 s at 95°C), annealing (45 s at 61°C), and elongation (45 s

at 72°C), followed by a final extension (30 min at 72°C). PCR products of the correct length (about 500 bp) were confirmed by electrophoresis using a 1.2% high melting agarose gel prior to analysis by DHPLC. The 16S rRNA gene fragments were analyzed in a 30 μL injection volume by DHPLC with a DNASep® cartridge packed with alkylated non-porous polystyrene-divinyl benzene copolymer microspheres for high-performance nucleic acid separation (Transgenomic, Omaha, NE). The oven temperature was 63.0°C and the flow rate was 0.5 mL min^{-1} with a gradient of 55–35% Buffer A and 45–65% Buffer B from 0 to 13 min. The analytical solutions used for the analyses were such that Buffer A constituted 0.1 M triethyl ammonium acetate (TEAA) at pH 7, Buffer B was made of 0.1 M TEAA and 25% acetonitrile at pH 7, Solution D included 25% water and 75% acetonitrile. Syringe Wash Solution was supplied from Transgenomic (Omaha, NE).

Analysis was performed using the Wavemaker version 4.1.44 software. An initial run was used to identify individual PCR fragments and determine their retention times. Individual peaks were eluted for sequencing from a subsequent run and collected with a fraction collector based on their retention times. The fractions were collected in 96-well plates (Bio-Rad, Hercules, CA) and dried using a Savant SpeedVac system (Thermo Electron Corporation, Waltham, MA) followed by dissolution in 30 μL of nuclease-free water. Re-amplification was performed following the protocol described above. The PCR amplicons were electrophoresed in a 1.2% high melting agarose and the excised fragment was purified for sequencing using Wizard® PCR Preps DNA Purification Resin (Promega, Madison, WI) and then sequenced (BASLab, University of Maryland, IMET, Baltimore, USA).

7.2.4.5. Sequencing and analysis

Each DHPLC fraction was sequenced in the 5′ and 3′ direction with 100pM of primer 348F or 884R using the BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied

Biosystems, CA). Sequences were examined for errors and assembled using the software Finch TV software package (FinchTV, 2011). Alignments were then refined manually. Chimera formation was examined using Chimera Check (Cole *et al.*, 2003). The sequence alignments were done by using Ribosomal Database Project (RDP) produced by Cole *et al.* (2009). Phylogenetic tree was generated based on published Chloroflexi sequences over 1200 base pairs by the neighbor joining approach using default settings in RDP.

After comparative sequence analyses of DNA obtained from the DHPLC, 10 phylotypes (Figure 8.12) were sequenced and checked to find regions of local similarity between sequences by using Basic Local Alignment Search Tool (BLAST), which is available on the web on the National Center for Biotechnology Information (NCBI, USA) website. This program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Also, BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families (Altschul *et al.*, 1990).

DHPLC fractions was sequenced in the 5′ and 3′ direction with 100 pM of primer 348F or 884R using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA). Sequences were examined for errors and assembled using the software Finch TV software package (FinchTV, 2011). Alignments were then refined manually. Chimera formation was examined using Chimera Check (Cole *et al.*, 2003). The sequence alignments were done by using Ribosomal Database Project (RDP) produced by Cole *et al.* (2009). Phylogenetic tree was generated based on published Chloroflexi sequences over 1200 base pairs by the neighbor joining approach using default settings in RDP.

7.3. Results and Discussion

Firstly, Aroclor 1254 dechlorination was followed in GR-1254 that was operated for 120 days. After seeing an activity at day 120 in this microcosm, operation was ceased and it was sub-cultured for single congener microcosms. Dechlorinations of single congeners were followed for 180 days. The results of Aroclor 1254 and single congeners are given below under different subsections.

7.3.1. Dechlorination of Aroclor 1254

Changes in total chlorine per biphenyl values of Aroclor 1254 throughout the incubation time are presented in Figure 7.1a. As can be seen from this figure, dechlorination of Aroclor 1254, which lagged for 60-days, showed that the average number of Cl^- molecules per biphenyl was reduced from 4.9 to 3.9 within 120 days of incubation. A total of 21% of Aroclor 1254 was dechlorinated after 120 days. The dechlorination rate of Aroclor 1254 (Figure 7.1a) was $18.2x10^{-03}$ total Cl^-/day. Figure 7.1b shows the distribution of homolog chlorines observed in GR 1254 microcosms. Dechlorination of Aroclor 1254 was achieved by the removal of chlorines mostly from penta-, followed by hexa- and hepta-chlorinated congeners with about 81%, 71%, and 63%, respectively (Figure 7.1b). The dechlorination of highly chlorinated congeners resulted in increase in tetra- and tri-chlorinated congeners, in all microcosms at varying levels such as mole percent of tetra-chlorinated congeners increased from 29% to 47% and the mole percent of tri-chlorinated congeners increased from 2% to 31% during the incubation. The predominant dechlorination products of Aroclor 1254 after 120 days of incubation were PCB 53 at 20.1 mol%, PCB 17 at 9.9 mol% and PCB 19 at 7.5 mol%. Highly dechlorinated and accumulated top ten congeners products in first set of GR microcosms spiked with 1254 are shown in Figure 7.2 and summarized in Table 7.2.

7.3.2. Dechlorination of individual Aroclor 1254 congeners

The specific dechlorination pathways for Aroclor 1254 were determined in single congener microcosms developed with the 22 most predominant congeners in Aroclor 1254 and their resulting dechlorination intermediates, which included tetrachlorobiphenyl congeners (PCB 44, 52, 70, and 74), pentachlorobiphenyl congeners (PCB 85, 87, 91, 95, 97, 99, 101, 105, and 110), hexachlorobiphenyl congeners (PCB 128, 132, 138, 141, 149, 153, 156, and 163), and heptachlorobiphenyl congener (PCB 180). Congener selection was done by choosing the congeners which were highly dechlorinated compared to others in GR-1254 microcosm and which have a higher abundance in the Aroclor 1254 (Table 7.3). The complete composition of Aroclor 1254 is given in Table A2 (Appendix A).

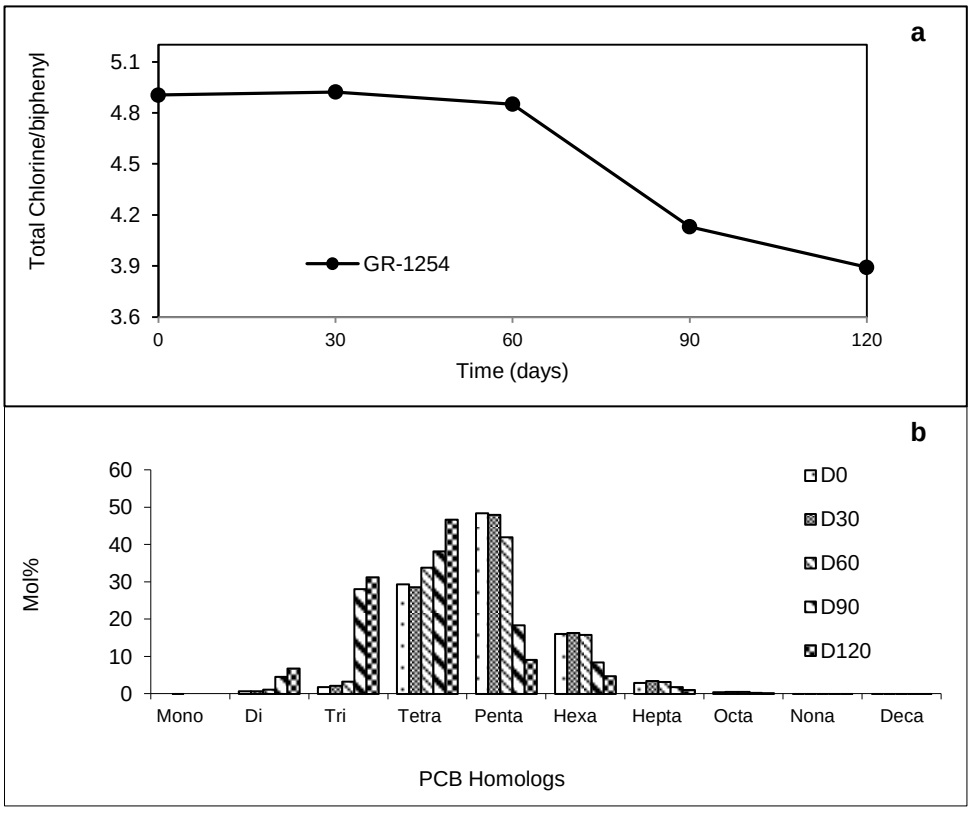


Figure 7.1. a) Changes in total chlorine per biphenyl values of first set of GR microcosms spiked with Aroclor 1254 and b) Homolog chlorine distributions of Aroclor 1254 in GR (D=day).

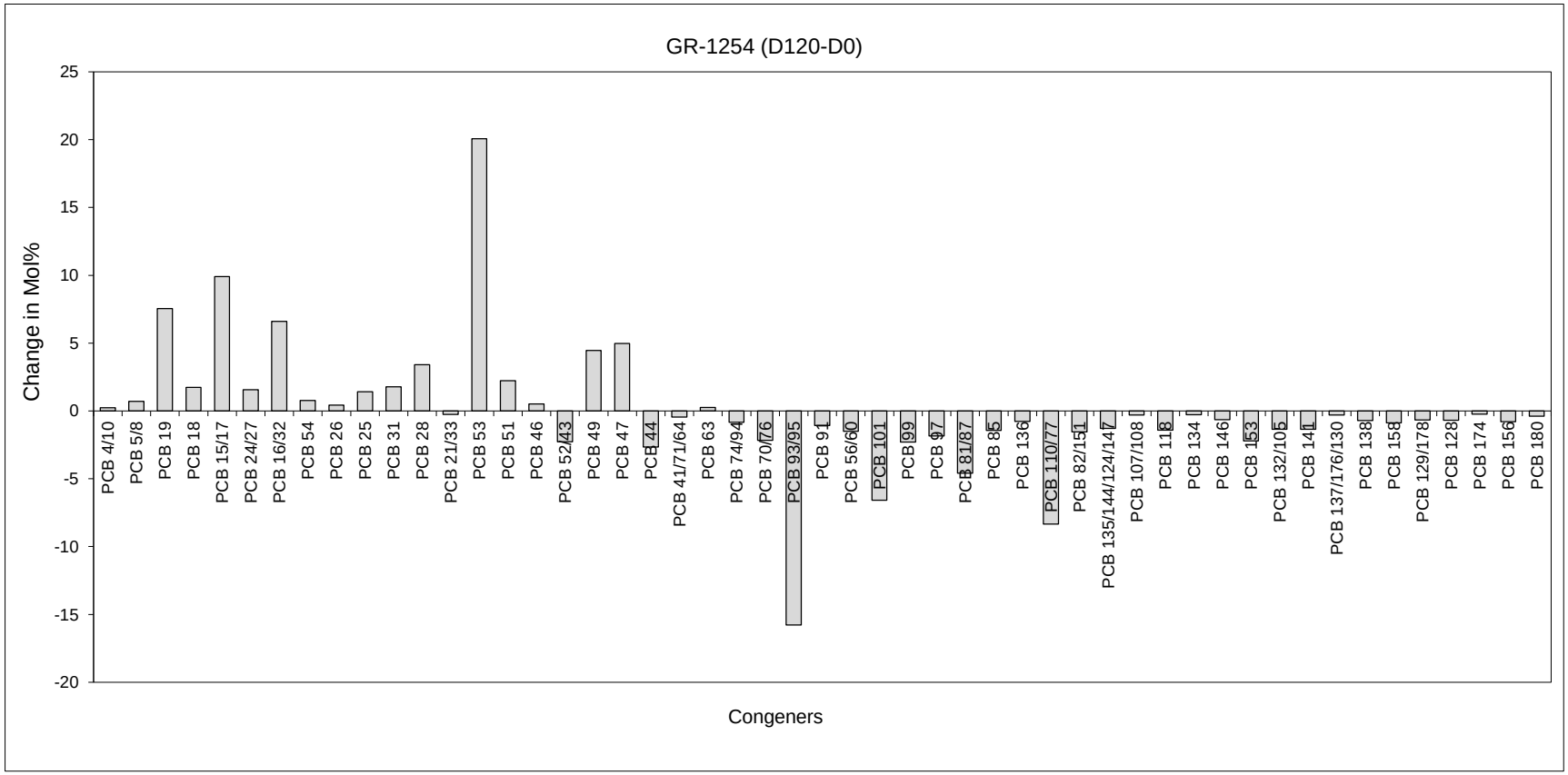


Figure 7.2. Changes in mol percent of Aroclor 1254 congeners after 120 Days (D120) of incubation in GR microcosms spiked with Aroclor 1254. Congeners of which the mol % was less than 0.2 % at any time point were excluded for the graph.

Table 7.2. Top ten congeners which were highly dechlorinated and the other top ten congeners which were highly accumulated in GR microcosms spiked with 1254.

Microcosm	Highly dechlorinated Top 10 Congeners	Mol % decrease	Highly accumulated Top 10 Congeners	Mol % Increase
GR-1254	PCB 93/95	-15.8	PCB 53	20.1
	PCB **110**/77	-8.3	PCB 15/**17**	9.9
	PCB 101	-6.6	PCB 19	7.5
	PCB 81/**87**	-4.6	PCB 16/**32**	6.6
	PCB 44	-2.7	PCB 47	5.0
	PCB 99	-2.3	PCB 49	4.5
	PCB 52/**43**	-2.3	PCB 28	3.4
	PCB 153	-2.2	PCB 51	2.2
	PCB 70/76	-2.2	PCB 31	1.8
	PCB 97	-1.8	PCB 18	1.7

Table 7.3. Weight % of selected congeners in Aroclor 1254 (Frame et al., 1996).

PCB Congener	Chlorine Substitution Pattern (IUPAC)	Weight % in Aroclor 1254
PCB 44	2,2',3,5'	(0.67-2.31)
PCB 52	2,2',5,5'	(0.83-5.38)
PCB 70	2,3',4',5	(6.83-3.49)
PCB 74	2,4,4',5	(2.19-0.84)
PCB 85	2,2',3,4,4'	(2.49-1.28)
PCB 87	2,2',3,4,5'	(3.41-3.99)
PCB 91	2,2',3,4',6	(0.53-0.93)
PCB 95	2,2',3,5',6	(1.84-6.25)
PCB 97	2,2',3,4',5'	(2.78-2.62)
PCB 99	2,2',4,4',5	(4.53-3.02)
PCB 101	2,2',4,5,5'	(5.49-8.02)
PCB 105	2,3,3',4,4'	(7.37-2.99)
PCB 110	2,3,3',4',6	(8.42-9.29)
PCB 128	2,2',3,3',4,4'	(1.71-1.42)
PCB 132	2,2',3,3',4,6'	(1.5-2.29)
PCB 138	2,2',3,4,4',5'	(5.95-5.8)
PCB 141	2,2',3,4,5,5'	(0.69-0.98)
PCB 149	2,2',3,4',5',6	(1.82-3.65)
PCB 153	2,2',4,4',5,5'	(3.29-3.77)
PCB 156	2,3,3',4,4',5	(1.13-0.82)
PCB 163	2,3,3',4',5,6	(0.31-0.4)
PCB 180	2,2',3,4,4',5,5'	(0.42-0.67)

7.3.2.1. Dechlorination of PCB 180 (2345-245)

PCB 180 is a di-*ortho* coplanar (having two *ortho*-substituted chlorine) PCB molecule, which is one of the toxic and environmentally prevalent PCB congeners (WHO, 2003; Johnson *et al*., 2005; McFarland and Clarke, 1989). It is also among the congeners which have been found relatively abundant in tissues together with PCB 87, 99, 101, and PCB 153 (McFarland and Clarke, 1989). The dechlorination of 22'344'55'-heptachlorobiphenyl (abbreviated as 2345-245 and/or PCB 180) with time is presented in Figure 7.3. PCB 180 dechlorination started with a rate of $1.56\pm0.19x10^{-03}$ total chlorines removed per biphenyl per day (from now on, rate unit will be given as Cl/day) between 0 and 90 days of incubation. As soon as PCB 180 started to dechlorinate, the mol% of PCB 153 (C153) and PCB 47 (C47) started to increase (Figure 7.3). After 60 days of incubation, PCB 49 started to accumulate as an indication of another dechlorination by-product. The dechlorination rate of PCB 180 increased to $4.37\pm0.2x10^{-03}$ Cl^-/day between day 90 and day 180 days. Figure 7.3 shows that 24.3 (±0.82) mol% of PCB 180 was dechlorinated at day 180 and this amount was distributed between its dechlorination products: 1.53±0.32 mol% increase in PCB 153, 1.86±0.70 mol% increase in PCB 99, 1.79±0.11 mol% increase in PCB 49, and PCB 180 dechlorination mostly ended in PCB 47 with 19.14±0.51 mol% increase. It can be seen from Figure 7.3a that PCB 180 (2345-245) was first dechlorinated to PCB 153 (245-245) via removal of double flanked *meta* (*d-f-meta*) chlorine removal, and then PCB 153 was dechlorinated to PCB 99 (245-24) through removal of *para* flanked *meta* (*p-f-meta*) chlorine removal from position 5. Then, the latter congener was mainly dechlorinated to PCB 47 (24-24) by removing chlorine from *p-f-meta* position. A minor pathway in the dechlorination of PCB 99 was also observed via removal of chlorine from *meta* flanked *para* (*m-f-para*) position to PCB 49 (25-24). These findings are in agreement with the findings of Fagervold *et al*. (2007), but the rate found this study is higher than that in their study. They also found PCB 180 dechlorinated via *d-f-meta* removal to PCB 153, together with another intermediate product, PCB 146 via removal of chlorine from double flanked *para* (*d-f-para*) position. In this study also there was very low amount of PCB 146,

indicating chlorine removal from *d-f- para* position, but it was excluded since the amount was lower than 0.2 mol%. The dechlorination rate of PCB 180 in the study of Fagervold *et al*. (2007) was 2.5 $\pm 0.2 \times 10^{-03}$ Cl^-/day. At the end of 400 days of incubation, almost 70 % of PCB 180 was dechlorinated in Baltimore Harbor (BH) sediment microcosms and the final products observed were PCB 47 (mostly) and PCB 49 (Fagervold *et al*., 2007), similar to the findings in this study. For a complete dechlorination of PCB 180 incubation time would need to be extended. Dechlorination of *ortho* and unflanked chlorines was not detected in PCB 180 microcosm, indicating that these enrichment microcosms selectively dechlorinated double- and single-flanked *meta* chlorines, with a lower extent of *meta* flanked *para* chlorine dechlorination. A dechlorination pathway of PCB 180 is given in Figure 7.34.

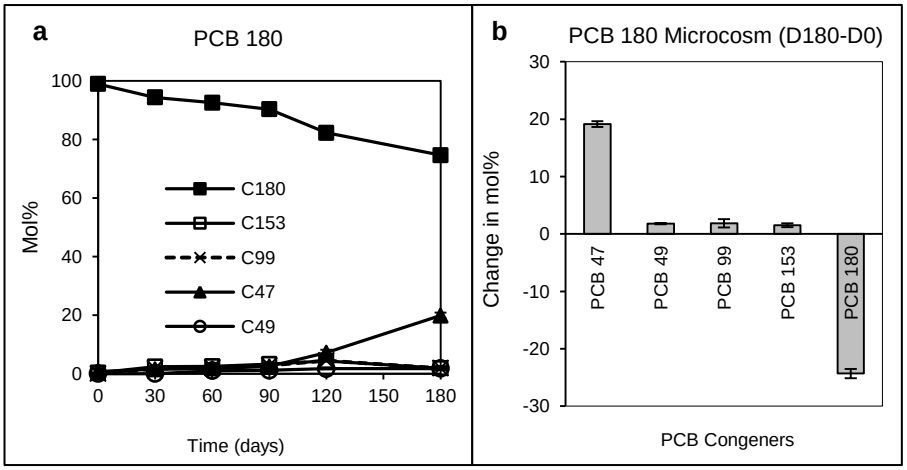


Figure 7.3 a) Mol% distributions of PCB 180 and its dechlorination products in GR-180 microcosm over180 days of incubation, b) Changes in mol percent of PCB180 and its dechlorination products in GR-180 microcosm after 180 days of incubation.

7.3.2.2. Dechlorination of PCB 163 (2356-34)

For PCB 163 microcosm, the dechlorination of PCB 163 over 180 days of incubation is presented in Figure 7.4. As it is obvious from this figure, PCB 163 was reductively

dechlorinated under anaerobic conditions. Dechlorination rate of PCB 163 was as low as $0.61\pm0.09x10^{-03}$ Cl^-/day in the first 90 days of incubation, but, with the increase in the number of 16S gene copies of PCB dechlorinating culture from $5.97x10^{+05}\pm5.06\ x10^{+05}$ to $5.89x10^{+06}\pm2.23\ x10^{+06}$ (Figure 7.5), the reduction in mol% of PCB 163 became more apparent and the dechlorination rate increased sharply to $5.07\pm0.7x10^{-03}$ Cl^-/day (Figure 7.4), hence, mol% decrease of PCB 163 was by 27.12 ±5.42 mol% between day 90 and day 180. Figure 7.5 also indicates that the dechlorination of PCB 163 was growth-linked.

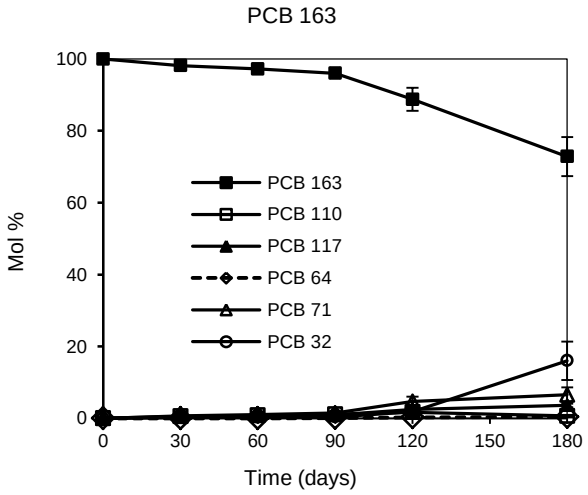


Figure 7.4. Mol % distributions of PCB 163 and its dechlorination products in 180 days of incubation

PCB 163 (2356-34) was dechlorinated via two pathways. The first way via *p-f-meta* removal to PCB 117 (2356-4) and the second way, which was the primary to PCB 110 (266-34) via removal of *ortho* flanked *meta* (*o*-f-*m*) from position 5. The high mol % of PCB 71 (26-34) found at the end of the 180 days of incubation indicates that PCB 163 dechlorinated mainly through PCB 110. Dechlorination pathway of PCB 163 strictly followed removal of *meta* chlorines resulting in the production of PCB 32 (26-4) as a terminal product with a total increase of 16.01±5.31 mol% of this

congener. No *ortho* and no further dechlorination was observed within the time course of the study. The dechlorination pathway of PCB 163 is shown in Figure 7.34.

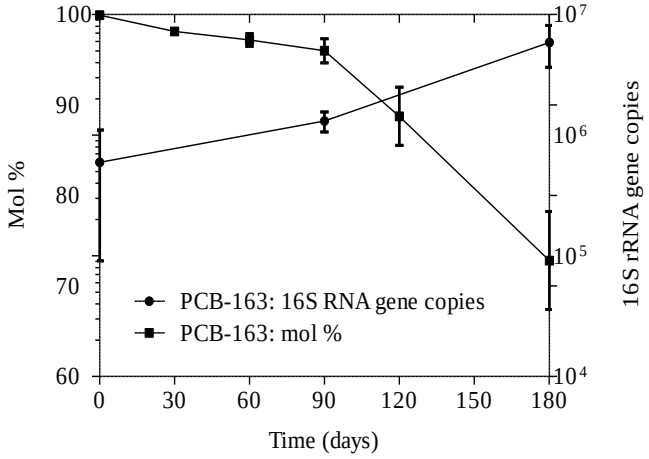


Figure 7.5. Mol% changes of PCB 163 in 180 days of incubation versus number of PCB dechlorinating phylotypes.

7.3.2.3. Dechlorination of PCB 156 (2345-34)

The dechlorination of PCB 156 (2345-34) with GR sediments is presented in Figure 7.6 for 180 days of incubation. From this figure, it can be seen that about 17±0.99 mol% of PCB 156 was dechlorinated mostly to PCB 118 (245-34) and PCB 107 (235-34) via removal of *d-f-meta* and *para* chlorines removal, respectively. The average dechlorination rate was 1.26±0.08 $x10^{-03}$ Cl^-/day, while the initial rate within the first 90 days was 1.45±0.06 $x10^{-03}$ Cl^-/day and the rate for the last 90 days was found as 1.08±0.09 $x10^{-03}$ Cl^-/day, resulting in an about 17 mol% decrease in PCB 156. The highly accumulated congeners found at the end of the 180 days of incubation were PCB 66 (24-34) at 5.97±0.50 mol%, which was dechlorinated from PCB 118 via removal of *o-f-meta* chlorine, and the final amount of PCB 118 was 3.20±0.20 mol%. It was thought that PCB 66 was further dechlorinated to PCB 28 (24-4), since there was a 2.37±0.47 mol% increase in PCB 28 at the end of the incubation. PCB 28 co-elutes with PCB 31 which might have possibly been

dechlorinated from PCB 63 (235-4) via *o-f-meta* chlorine removal. Moreover, as seen from Figure 7.6 (with the log 10 scale it would be easier to see), there was about 1 mol% increase in PCB 33 (2-34), indicating that there was also unflanked *para* chlorine removal from PCB 66. Also, PCB 25 (24-3) was detected at about 0.7 mol%, other possible daughter product of PCB 66, showing PCB 66 dechlorination through several pathways. As a result, in this microcosm dechlorination of PCB 156 occurred mainly and initially by *meta* chlorine removal, then dechlorination was followed with unflanked or flanked *para* chlorine removal, which is obvious from the increase in mol% of PCB 33 and PCB 25. A dechlorination pathway for PCB 156 is given in Figure 7.34.

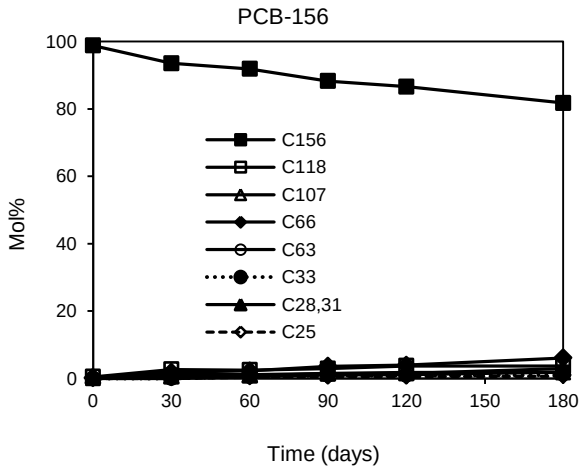


Figure 7.6. Mol % distributions of PCB 156 and its dechlorination products in 180 days of incubation,

7.3.2.4. Dechlorination of PCB 153 (245-245)

Figure 7.7 shows the dechlorination of PCB 153 and its dechlorination byproducts as well as mol % changes throughout the incubation time. The total dechlorination rate of PCB 153 was calculated as $0.83\pm0.04x10^{-03}$ Cl^-/day with a total 10.21±0.76 mol% decrease. PCB 47 (24-24) resulted from sequential removal of *p-f-meta* chlorine from

both rings of PCB 156 through an intermediate product, PCB 99 (245-24). At the end of the incubation period, the mol% of PCB 47 and PCB 99 were 8.27±0.2 mol% and 2.6±0.6 mol%, respectively. The dechlorination rate of PCB 153 was much lower than that of PCB 180 dechlorination. The increase in mol% of PCB 47, which is also the final product of PCB 180 dechlorination, was by 19.14±0.51 mol%. This was higher than that found during dechlorination of PCB 153, 8.27±0.2 mol%. This finding shows that dechlorination of PCB 153 is faster when PCB 180 was present. PCB 153 dechlorination pathway observed in this study with GR sediment culture was similar to that found in BH sediment microcosm study of Fagervold *et al.* (2007), while the dechlorination rate was much higher, $3.3\pm0.5x10^{-03}$ Cl^-/day, compared with this study. Also, at about 80 mol% of PCB 153 was dechlorinated within 400 days of incubation (Fagervold *et al*., 2007). A dechlorination pathway of PCB 153 is given in Figure 7.34.

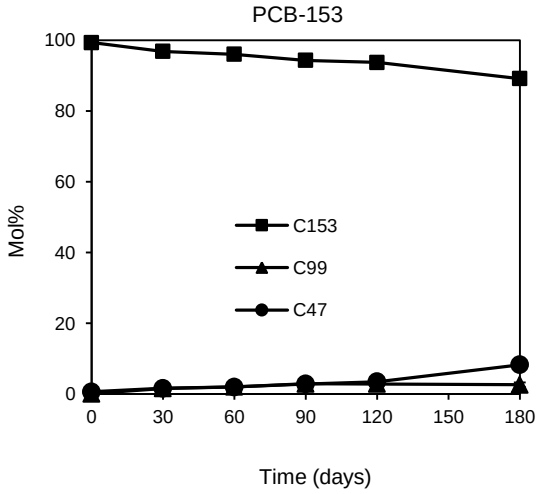


Figure 7.7. Mol% distributions of PCB 153 and its dechlorination products in 180 days of incubation

7.3.2.5. Dechlorination of PCB 149 (236-245)

PCB 149 can be dechlorinated initially through two pathways (Figure 7.34). Both pathways target *meta* chlorine. The first pathway involves *o-f-meta* dechlorination of PCB 149 to PCB 102 (26-245), which is further dechlorinated in the *p-f-meta* position to PCB 51 (26-24). The second pathway also resulted in the final product, PCB 51, but through a dechlorination from *p-f-meta* position to PCB 91 (236-24), which was followed by the removal of *o-f-meta* chlorine to PCB 51. At the end of the incubation period, there were 19.83±4.31 mol%, 9.63±1.19 mol%, and about 7.5 mol% increase in PCB 51, PCB 102, and PCB 91, respectively, resulting from dechlorination of PCB 149 by about 37 mol% (Figure 7.8). The initial dechlorination rate of PCB 149 was $2.71\pm0.57\text{x}10^{-03}$ Cl^-/day observed within the first 90 days and then it increased to $3.07\pm0.67\text{x}10^{-03}$ Cl^-/day within the last 90 days of incubation. These findings are in line with that of Fagervold *et al.* (2007), but the rate was higher, $6.7\pm0.7\text{x}10^{-03}$, in that study. The reason of higher rate in the prior study could be that since culture was transferred at least four times, it must be highly specific and enriched.

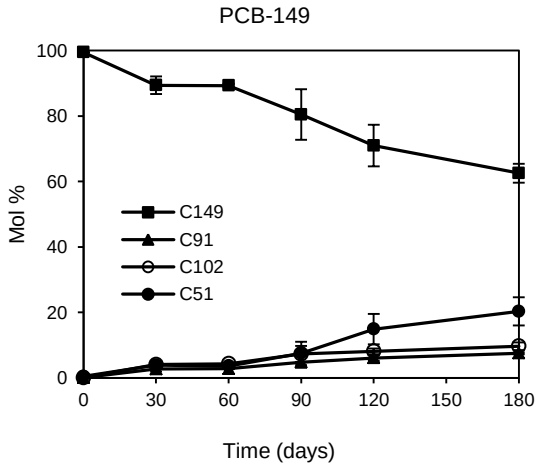


Figure 7.8. Mol% distributions of PCB 149 and its dechlorination products in 180 days of incubation

7.3.2.6. Dechlorination of PCB 141 (2345-25)

The dechlorination of PCB 141 in 180 days of incubation is presented in Figure 7.9. Initial dechlorination rate was $3.88\pm0.46x10^{-03}$ Cl^-/day in the first 90 days of incubation. The dechlorination rate decreased to $2.21\pm1.32x10^{-03}$ Cl^-/day after the first 90 days and then it reached to a plateau after 120 days, resulting in 29.92±12.16 mol% decrease in PCB 141 (Figure 7.9). The reason for observing such a high standard deviation at day 120 and day 180 was due to the higher dechlorination rate in one replicate of triplicate microcosms. Dechlorination of PCB 141 was through two pathways. The predominant pathway involved a *o-f-meta* dechlorination of PCB 149 (2345-25) to PCB 101 (245-25), which was further dechlorinated in the *p-f-meta* position to PCB 49 (24-25). The second pathway was through *para* chlorine removal resulting in PCB 92 (235-25), which might be further dechlorinated either through *meta* position to PCB 52 (25-25) or through unflanked *meta* position to PCB 43 (235-2). It could not be distinguished whether both or one of them was the end product, because PCB 52 co-elutes with PCB 43. Presence of relatively high amount of PCB 49 (25.75±10.99 mol %) was an indication that PCB 141 dechlorination happened preferentially through *o-f-meta* chlorine removal via PCB 101. A dechlorination pathway of PCB 141 is given in Figure 7.34.

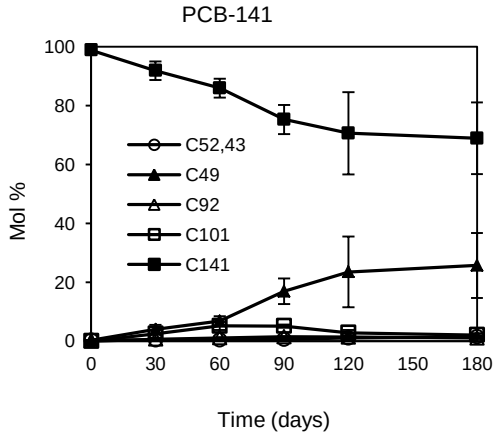


Figure 7.9. Mol% distributions of PCB 141 and its dechlorination products in 180 days of incubation

7.3.2.7. Dechlorination of PCB 138 (2345-25)

Dechlorination of PCB 138 over the course of the incubation is presented in Figure 7.10. Overall dechlorination rate of PCB 138 was $2.93x10^{-3}$ Cl^-/day and eventually about 36 mol% of PCB 138 was dechlorinated. Highest dechlorination rate was observed as $5.53x10^{-3}$ Cl^-/day between day 60 and day 120. PCB 138 was dechlorinated through sequential removal of *meta* chlorine and produced PCB 47. No unflanked *para* and *ortho* chlorine removal was observed within the incubation time. A dechlorination pathway of PCB 138 is given in Figure 7.34.

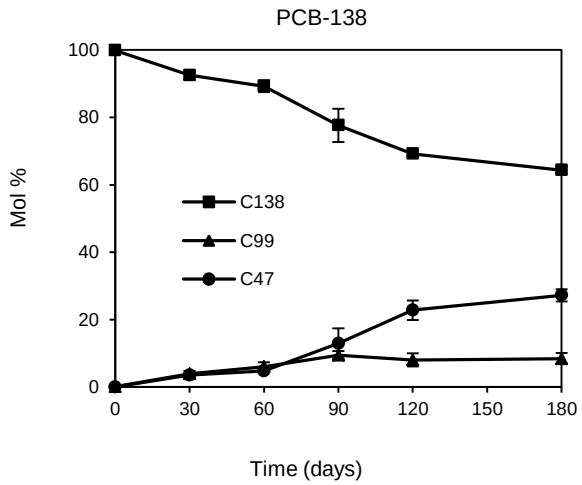


Figure 7.10. Mol % distributions of PCB 138 and its dechlorination products in 180 days of incubation.

7.3.2.8. Dechlorination of PCB 132 (234-236)

Figure 7.11 shows mol% change of PCB 132 together with its daughter products during 180 days of incubation. At the end of the incubation period, 28.5 mol% of PCB 132 resulted in about 21 mol% and 8 mol% increases in PCB 51 and PCB 91, respectively. The initial dechlorination rate was $1.87\pm0.09x10^{-3}$ Cl^-/day found for the first 90 days and it was increased to $2.89\pm0.22x10^{-3}$ Cl^-/day within the last 90 days of

incubation. PCB 132 was dechlorinated to PCB 51 through sequential flanked *meta* chlorine removal. A dechlorination pathway of PCB 132 is given in Figure 7.34.

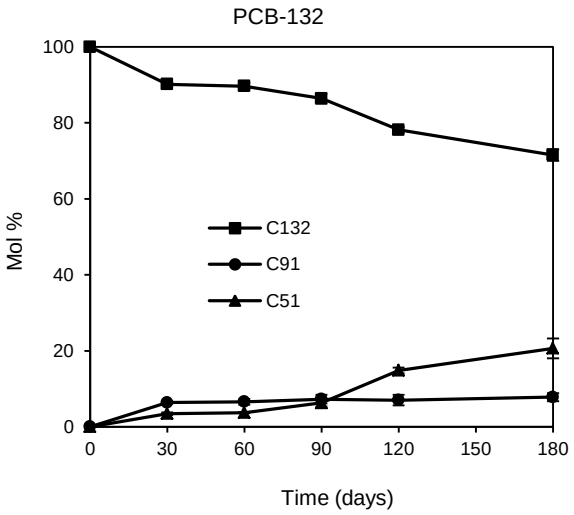


Figure 7.11. Mol% distributions of PCB 132 and its dechlorination products during 180 days of incubation.

7.3.2.9. Dechlorination of PCB 128 (234-234)

The dechlorination of PCB 128 is presented in Figure 7.12 for 180 days of incubation. About 12 mol% of PCB 128 was dechlorinated to PCB 85 and PCB 47 with an increase in their mol% by 1.69±0.23 mol% and 10.3±0.38 mol%, respectively. The dechlorination rate was $1.08\pm0.06\times10^{-03}$ Cl^-/day. PCB 128 (234-234) was dechlorinated to PCB 47 (24-24) via sequential *d-f-meta* chlorine removal over PCB 85 (234-234). A dechlorination pathway of PCB 128 is given in Figure 7.34.

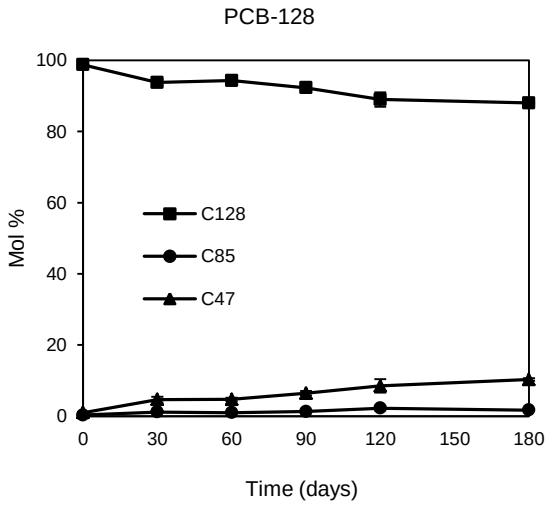


Figure 7.12. Mol% distributions of PCB 128 and its dechlorination products during 180 days of incubation.

7.3.2.10. Dechlorination of PCB 110 (236-34)

In 180 days of incubation, about 77 mol% of PCB 110 was dechlorinated with an initial rate of 7.42×10^{-3} Cl^-/day observed in the first 90 days of incubation and then with a lower rate at 5.98×10^{-3} Cl^-/day in the last 90 days of incubation period (Figure 7.13). PCB 110 (236-34) was mainly dechlorinated to PCB 71 (26-34) via *o-f-meta* chlorine removal followed by *p-f-meta* chlorine removal, and resulted in PCB 32 (26-4) as the terminal product. Also, PCB 59 (236-3) was detected at a very low amount, 0.87±2.06 mol%, indicating that PCB 110 was also dechlorinated through another pathway. In this second pathway, PCB 110 dechlorination was via *m-f-para* position to PCB 59, which was followed most probably by the removal *o-f-meta* chlorine resulting into PCB 27 (26-3). There is another possibility, which is not likely due to the findings of other GR sediment microcosms, that dechlorination of PCB 59 was followed by the removal of unflanked *meta* chlorine to PCB 24 (236-). The reason for the high standard deviation observed in PCB 24/27 (C24,27) at day 180 was due to the third replicate of this experiment in which about 62 mol% of PCB 110 dechlorinated to PCB 24/27 after 120 days of incubation. A dechlorination pathway of PCB 110 is given in Figure 7.34.

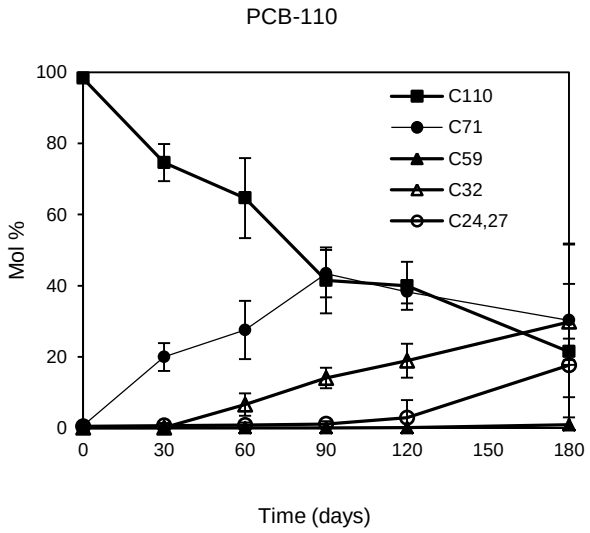


Figure 7.13. Mol% distributions of PCB 110 and its dechlorination products in 180 days of incubation.

7.3.2.11. Dechlorination of PCB 105 (234-34)

Change in mol % distributions of PCB 105 at final day of incubation (day 180) and its dechlorination products in GR-105 microcosm over180 days of incubation are presented in Figure 7.14. Within 180 days of incubation, about 15 mol% of PCB 105 was dechlorinated with an initial rate of $1.02\pm0.08\text{x}10^{-03}$ Cl^-/day observed in the first 90 days of incubation and then with a lower rate at $0.9\pm0.11\text{x}10^{-03}$ Cl^-/day in the last 90 days of incubation period. As it is shown in Figure 7.14, PCB 105 (234-34) was dechlorinated to PCB 28 (24-4) via sequential chlorine removal from the flanked *meta* position. A dechlorination pathway of PCB 105 is given in Figure 7.34.

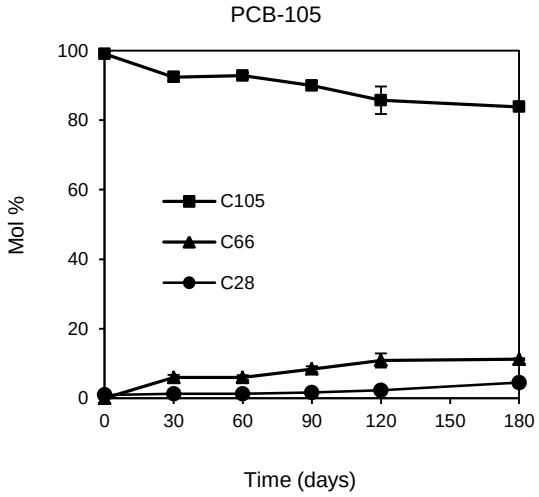


Figure 7.14. Mol% distributions of PCB 105 and its dechlorination products in 180 days of incubation.

7.3.2.12. Dechlorination of PCB 101 (245-25)

The dechlorination of PCB 101 is presented in Figure 7.15 for 180 days of incubation. It is very clear from this figure that in 180 days of incubation PCB 101 (245-25) was dechlorinated to PCB 49 (24-25) via *p-f-meta chlorine* removal. Overall, about 48 mol% of PCB 101 was dechlorinated with an initial rate of $4.52\pm0.87x10^{-03}$ Cl^-/day observed in the first 90 days and then dechlorination of PCB 101 almost ceased and the rate decreased to 1 ± 1.4 Cl^-/day in the last 90 days of incubation. No dechlorination by-products of PCB 101 were observed in sterile control of PCB 101 throughout the incubation period indicating dechlorination did not happen (Figure 7.15). A dechlorination pathway of PCB 101 is given in Figure 7.34.

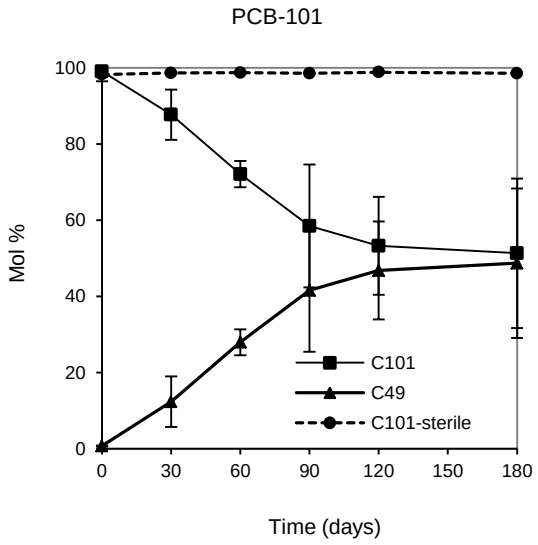


Figure 7.15. Mol% distributions of PCB 101 and its dechlorination products in 180 days of incubation

7.3.2.13. Dechlorination of PCB 99 (245-24)

Figure 7.16 presents change in mol% of PCB 99 (245-24) during its dechlorination in 180 days of incubation and the production of its daughter product, PCB 47 (24-24). At the end of the incubation period (Figure 7.16), there were 16.25±1.86 mol% increase in PCB 47 and decrease in PCB 99 with an initial dechlorination rate of $0.59\pm0.06\times10^{-03}$ Cl^-/day observed in the first 90 days. The rate increased to $1.04\pm0.11\times10^{-03}$ Cl^-/day within the last 90 days of incubation. Dechlorination of PCB 99 was again via removal of flanked *meta chlorine*. A dechlorination pathway of PCB 99 is given in Figure 7.34.

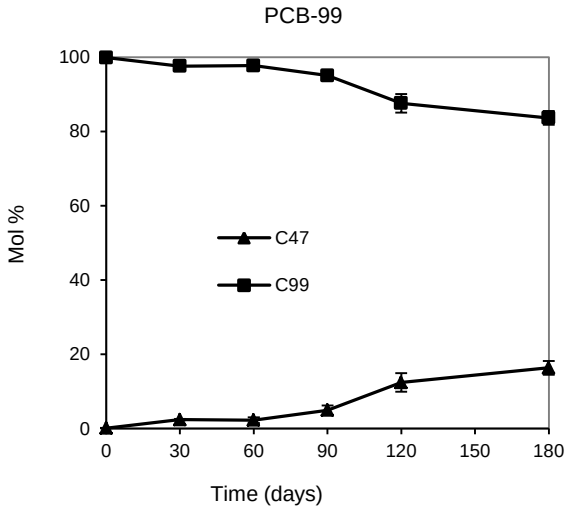


Figure 7.16. Mol% distributions of PCB 99 and its dechlorination products in 180 days of incubation

7.3.2.14. Dechlorination of PCB 97 (245-3

The change in mol% of PCB 97 (245-23) throughout the incubation is depicted in Figure 7.17. As seen from Figure 7.17, 58.51±4.88 mol% of PCB 97 was dechlorinated to PCB 42 (24-23) and PCB 17 (24-2) via flanked *meta* chlorine removal with an initial dechlorination rate of $4.48\pm0.24\text{x}10^{-03}$ Cl^-/day observed in the first 90 days. Then, dechlorination continued with a lower rate until the end of the incubation period. The dechlorination pathway of PCB 97 is given in Figure 7.34.

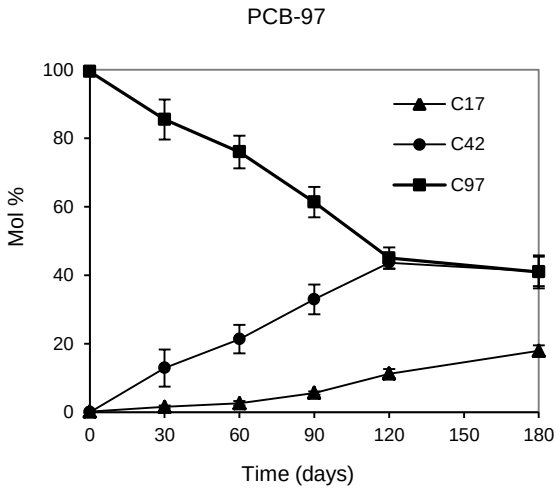


Figure 7.17. Mol% distributions of PCB-97 and its dechlorination products in 180 days of incubation.

7.3.2.15. Dechlorination of PCB 95 (236-25)

PCB 95 dechlorination over the incubation time is shown in Figure 7.18. The dechlorination of PCB 95 (236-25) occurred through the removal of flanked *meta* chlorine and resulted in the production of PCB 53 (26-25). The time course of the dechlorination showed a dramatic decrease in the mol% of PCB 95 and as a result an increase in mol% of PCB 53. A total of 61.81±1.32 mol% increase and decrease was observed in mol% of PCB 53 and PCB 97, respectively (Figure 7.18). The rate of the dechlorination was $4.21\pm0.11\times10^{-03}$ Cl^-/day observed in the first 90 days and later, the rate decreased to $2.2\pm0.07\times10^{-03}$ Cl^-/day within the last 90 days of incubation A dechlorination pathway of PCB 95 is given in Figure 7.34.

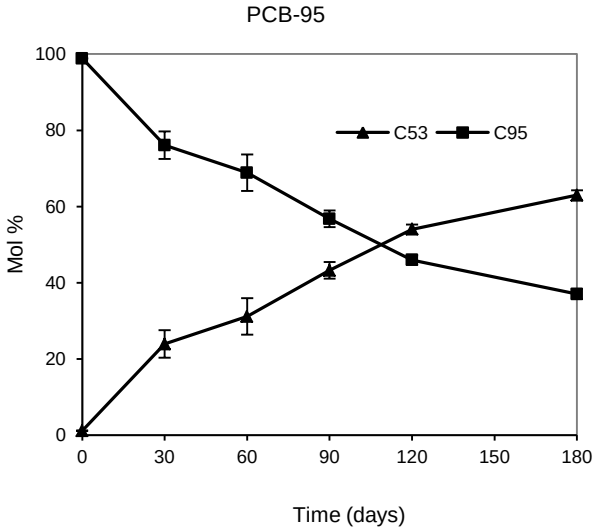


Figure 7.18. Mol% distributions of PCB-95 and its dechlorination products in 180 days of incubation

7.3.2.16. Dechlorination of PCB 91 (236-24)

Mol% distribution of PCB congeners in PCB 91 microcosm over the incubation time is presented in Figure 7.19. Within 180 days of incubation, about 34 mol% of PCB 91 was dechlorinated with an initial rate of $1.75\pm0.1x10^{-03}$ Cl^-/day observed in the first 90 days of incubation and dechlorination was continued with almost the same rate until the end of the incubation. All dechlorination occurred in a single step by flanked *meta* removal from PCB 91 (236-24) to PCB 51 (26-24). A dechlorination pathway of PCB 91 is given in Figure 7.34.

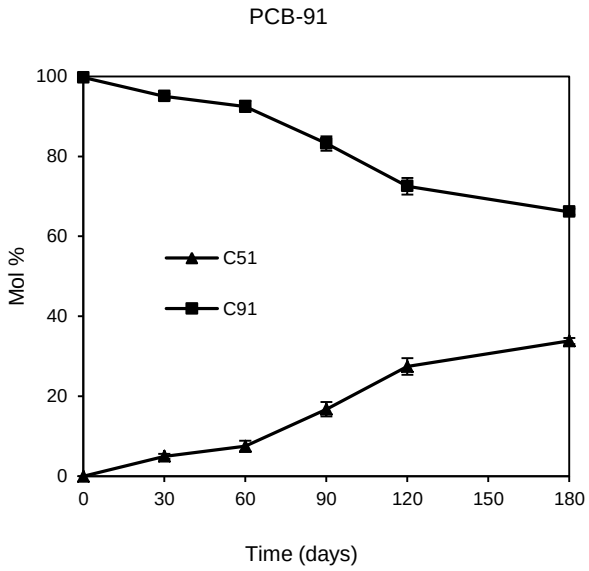


Figure 7.19. Mol% distributions of PCB 91 and its dechlorination products in 180 days of incubation

7.3.2.17. Dechlorination of PCB 87 (234-25)

As can be seen from Figure 7.20, 44.18±1.54 mol% of PCB 87 in live microcosm was dechlorinated to PCB 49 (24-25) via flanked *meta* chlorine removal at an initial dechlorination rate of $3.23\pm0.39\text{x}10^{-03}$ Cl^-/day within the first 90 days. Then, dechlorination continued with a lower rate until the end of the incubation period. Again, the target chlorine was flanked *meta* chlorine. No unflanked chlorine removal was detected throughout the incubation time. As depicted also from Figure 7.20, no dechlorination of PCB 87 was observed in sterile control of PCB 87 throughout the incubation period. The dechlorination pathway of PCB 87 is given in Figure 7.34.

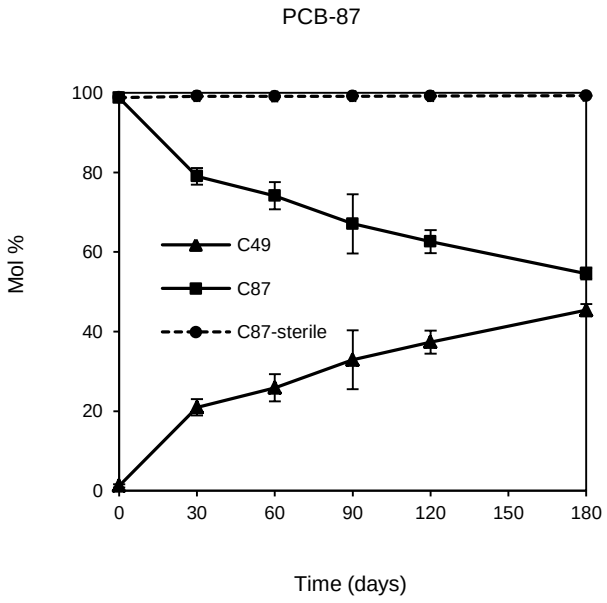


Figure 7.20. Mol% distributions of PCB 87 and its dechlorination products in 180 days of incubation

7.3.2.18. Dechlorination of PCB 85 (234-24)

Change in mol% distributions of PCB 85 and its dechlorination products in 180 days of incubation are presented in Figure 7.21. About 29 mol% of PCB 87 in total was dechlorinated with an initial rate of $1.49\pm0.08\text{x}10^{-03}$ Cl^-/day observed in the first 90 days of incubation. Dechlorination continued at the same rate, $1.44\pm0.17\text{x}10^{-03}$, until the end of the incubation period. During the incubation, PCB 85 (234-24) was dechlorinated via single step chlorine removal from flanked *meta* position to PCB 47 (24-24). A dechlorination pathway of PCB 85 is given in Figure 7.34.

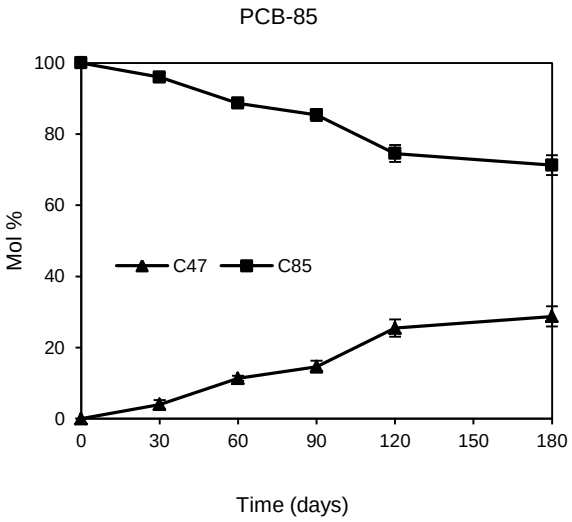


Figure 7.21. Mol% distributions of PCB 85 and its dechlorination products in 180 days of incubation

7.3.2.19. Dechlorination of PCB 74 (245-4)

As depicted in Figure 7.22, there was about a 90-day lag in the dechlorination of PCB 74. After the lag period, dechlorination started with a very low rate, about $0.04x10^{-03}$ Cl^-/day, and then a plateau was reached where no further decrease was observed in mol% of PCB 74. In 180 days of incubation, only about 2 mol% decrease was observed in PCB 74 (Figure 7.22) resulting into PCB 28/31. It could not be distinguished that whether the dechlorination was through flanked *meta* or *para* chlorine removal due to co-elution of two possible by-products of PCB 74, i.e. PCB 28 (24-4) and PCB 31 (25-4). A dechlorination pathway of PCB 74 is given in Figure 7.34.

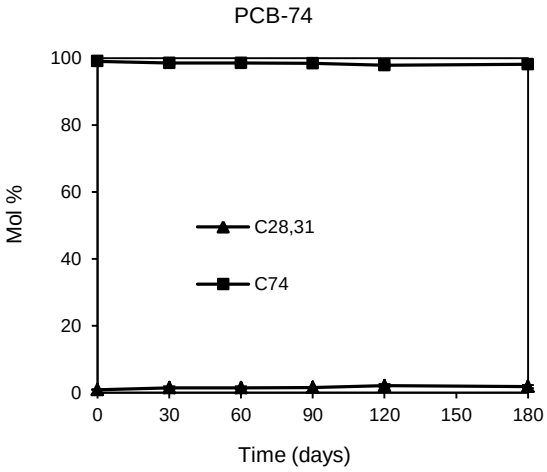


Figure 7.22. Mol% distributions of PCB 74 and its dechlorination products in 180 days of incubation

7.3.2.20. Dechlorination of PCB 70 (25-34)

Dechlorination profile of PCB 70 and production of its daughter products over the incubation time is given in Figure 7.23. PCB 70 can be dechlorinated initially through two pathways. The first pathway involves flanked *para* dechlorination to PCB 26 (25-3) and the minor, second, pathway involves flanked *meta* dechlorination to PCB 31 (25-4). At the end of the incubation period, 3 mol% and 0.90±0.34 mol% increases were observed in PCB 26 and PCB 31, (which co-elutes with PCB 28), respectively. During the 180 days of incubation, the decrease in mol% of PCB70 was 3.25±1.41 mol%. The initial dechlorination rate observed in the first 90 days was $0.27\pm0.04x10^{-03}$ Cl^-/day and the rate decreased to $0.07\pm0.17x10^{-03}$ Cl^-/day in the last 90 days of incubation. No further dechlorination was observed after day 120. The dechlorination pathway of PCB 70 is given in Figure 7.34.

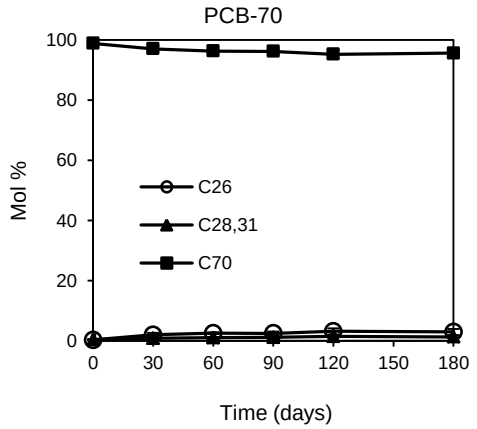


Figure 7.23. Mol% distributions of PCB 70 and its dechlorination products in 180 days of incubation.

7.3.2.21. Dechlorination of PCB 52 (25-25)

The dechlorination profile of PCB 52 (25-25), which has no flanked chlorine on its rings, is depicted over 120 days of incubation in Figure 7.24. Almost no dechlorination of PCB 52 was observed within the time course of the study, the decrease in mol% of PCB 52 was 0.12±0.13 mol%, with a high standard deviation, suggesting that GR sediment culture preferentially remove flanked chorines. The dechlorination pathway of PCB 52 is given in Figure 7.34.

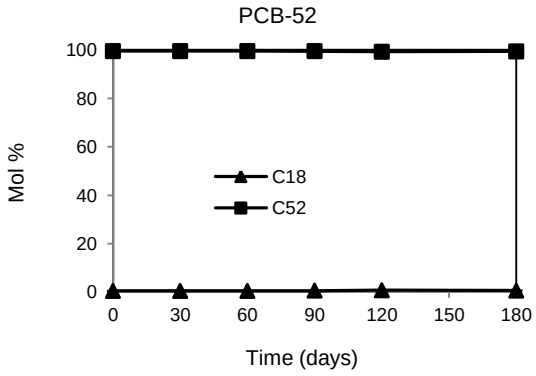


Figure 7.24. Mol% distributions of PCB 52 and its dechlorination products in 180 days of incubation

7.3.2.22. Dechlorination of PCB 44 (23-25)

PCB 44 (23-25) dechlorination over the incubation time is shown in Figure 7.25. The time course of the dechlorination showed a dramatic decrease in the mol% of PCB 44 (236-25) and increase in PCB 18 (2-25). A total of 75.58±0.67 mol% of PCB 44 dechlorinated to PCB 18 at an initial rate of $2.41\pm0.06x10^{-03}$ Cl^-/day within the first 90 days. After the first 90 days, the rate increased to $5.77\pm0.07x^{-03}$ Cl^-/day. Dechlorination of PCB 44 occurred via flanked *meta* chlorine removal from position 3. No dechlorination was observed in sterile control of PCB 44 (Figure 7.25). The dechlorination pathway of PCB 44 is given in Figure 7.34.

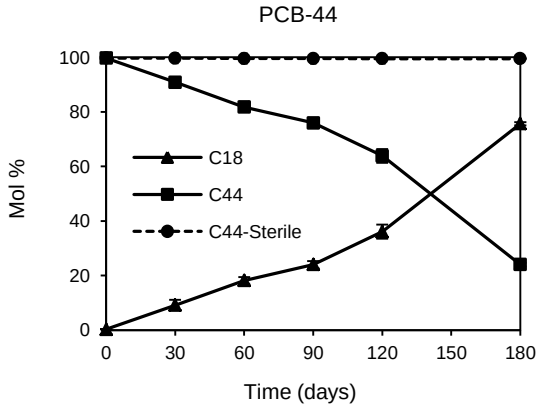


Figure 7.25. Mol% distributions of PCB 44 and its dechlorination products in 180 days of incubation

7.3.3. Enumeration of dechlorinating phylotypes in Aroclor 1254 and in each individual PCB congener microcosm

Microorganisms were enumerated in microcosms by quantitative PCR (qPCR) using specific primer sets (Fagervold *et al*., 2011) for phylotypes closely related to known PCB dechlorinators in order to determine whether dechlorination of Aroclor 1254 and its selected congeners is growth-linked. Amplification efficiencies of dilutions of

gel purified DF1 16S rRNA gene PCR product used as standards (in the range of 10^{-1}-10^{-6} for all sets were between 92.5±8.4% with the r^2=1. The amplification efficiencies of samples were between 88.1±4.6% and 90.8±7.1%, showing high reliability. The number of 16S rRNA gene copies of putative dechlorinators per mL microcosm slurry (seen as wet sediments in text or on figures) dechlorinating Aroclor 1254 shows an increase in numbers of putative dechlorinators as the dechlorination proceeded (Figure 7.26). The number of Chloroflexi 16S gene copies per mL wet sediment increased from $4.14x10^{+05}$ to $1.03x10^{+07}$ after 120 days, about 100-fold, showing successful enrichment of dechlorinating bacteria in Aroclor 1254 microcosm. Moreover, bands in lane 3 and 4 for time 0 (D0) and time final (D120) on Figure 7.27, respectively, shows the presence of dechlorinating bacteria in the final samples.

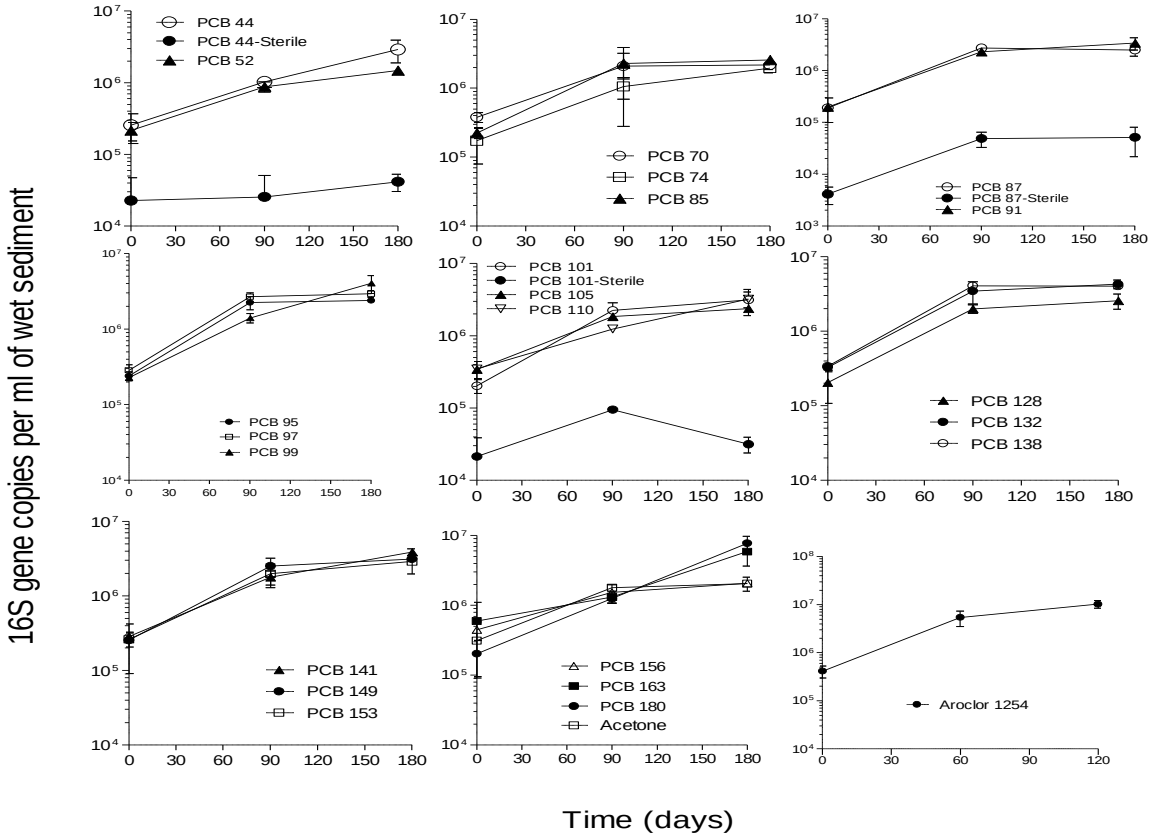


Figure 7.26. Changes of 16S gene copy number observed in the microcosms of individual PCB congeners and Aroclor 1254 (figures were randomly grouped for easy visualization).

The populations of PCB dechlorinating microorganisms, increased about 1-2 orders of magnitude when grown with the single congeners during the course of reductive dehalogenation, except for sterile controls. Generally, the extent of growth varied with different congeners (Figure 7.26). The highest increase was observed during the dechlorination PCB 180 (2345-245) with a 38-fold increase while the lowest increase occurred during the dechlorination of PCB 156 (2345-34) at about 4.6-fold. The control culture (named as GR-Ace) incubated with PCB-free acetone showed only a slight increase over 180 days (Figure 7.26), which could be accounted for by PCB that was carried over in the transfer from the Aroclor 1254 microcosm and also due to presence of PCBs in wet GR sediments. Amplicon identities were supported via agarose gel electrophoresis of PCR products performed for final day samples of individual PCB congener microcosms. As can be seen from Figure 7.28, all samples of live microcosms showed single bands approximating the expected size (about 516 bp=884R-348F, found from subtracting forward primer length (884) from the length of reverse primer (348)), indicating the presence of dechlorinating bacteria, while the results of sterile controls did not produce a band.

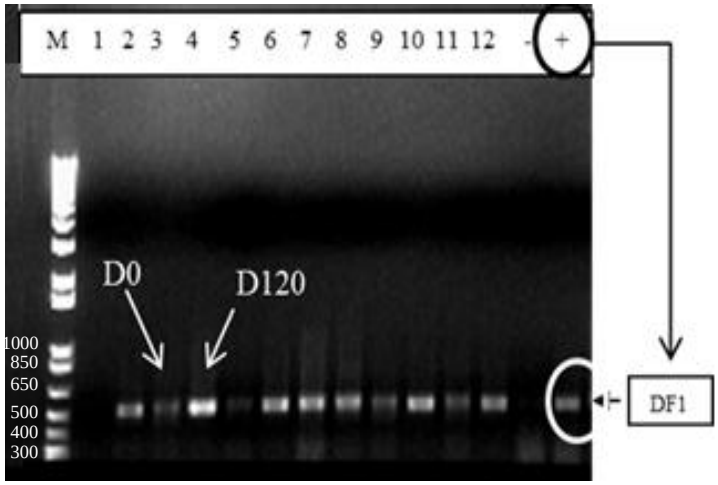


Figure 7.27. Agarose gel showing the qPCR performed for pure cultures of PCB dechlorinating bacterium DF-1 (“+” control), no DNA (“- ”), slurry samples of Aroclor 1254 microcosm for Day 0 and Day 120 (lane 3 and 4) and M: 1kb plus marker (DNA ladder).

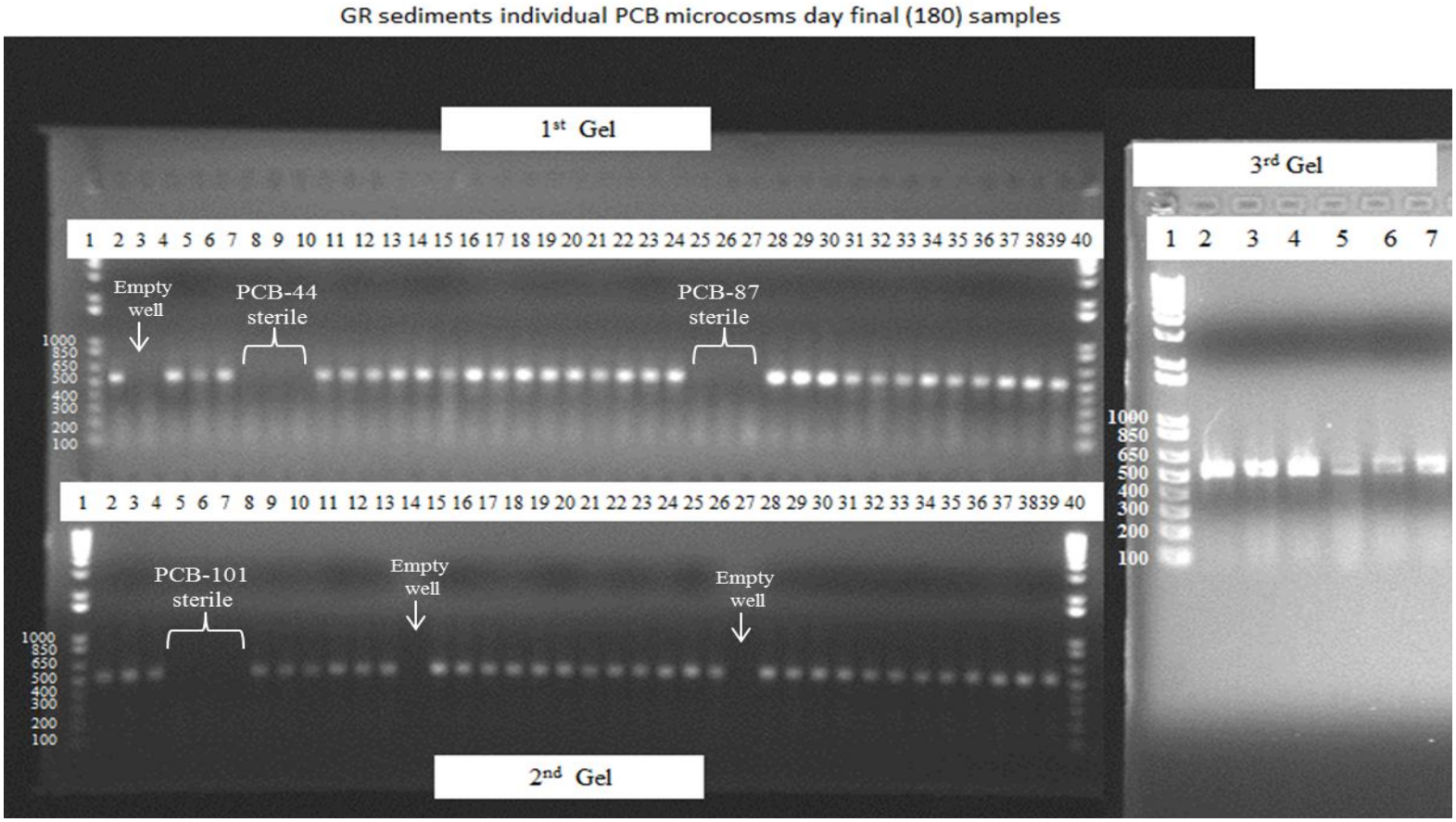


Figure 7.28. Agarose gel showing the qPCR performed for Grasse River sediment individual PCB microcosm samples (sample labels) with respect to the lanes of gel are given in Table 7.4 and 7.5)

Table 7.4. Labels of GR sediments day final (180) samples run on 1st and 2nd agarose gel with respect to gel lane numbers.

1st gel lane no	product/sample	2nd gel lane no	product/sample
1 and 40	1kb plus marker (DNA ladder)	1 and 40	1kb plus marker (DNA ladder)
2	DF1(positive control)	2	PCB-101 sample 1
3	empty	3	PCB-101 sample 2
4	PCB-44 sample 1	4	PCB-101 sample 3
5	PCB-44 sample 2	5	PCB-101 sterile sample 1
6	PCB-44 sample 3	6	PCB-101 sterile sample 2
7	PCB-44-sterile sample 1	7	PCB-101 sterile sample 3
8	PCB-44-sterile sample 2	8	PCB-105 sample 1
9	PCB-44-sterile sample 3	9	PCB-105 sample 2
10	PCB-52 sample 1	10	PCB-105 sample 3
11	PCB-52 sample 2	11	PCB-110 sample 1
12	PCB-52 sample 3	12	PCB-110 sample 2
13	PCB-70 sample 1	13	PCB-110 sample 3
14	PCB-70 sample 2	14	Empty
15	PCB-70 sample 3	15	PCB-128 sample 1
16	PCB-74 sample 1	16	PCB-128 sample 2
17	PCB-74 sample 2	17	PCB-128 sample 3
18	PCB-74 sample 3	18	PCB-132 sample 1
19	PCB-85 sample 1	19	PCB-132 sample 2
20	PCB-85 sample 2	20	PCB-132 sample 3
21	PCB-85 sample 3	21	PCB-138 sample 1
22	PCB-87 sample 1	22	PCB-138 sample 2
23	PCB-87 sample 2	23	PCB-138 sample 3
24	PCB-87 sample 3	24	PCB-141 sample 1
25	PCB-87-sterile sample 1	25	PCB-141 sample 2
26	PCB-87-sterile sample 2	26	PCB-141 sample 3
27	PCB-87-sterile sample 3	27	Empty
28	PCB-91 sample 1	28	PCB-149 sample 1
29	PCB-91 sample 2	29	PCB-149 sample 2
30	PCB-91 sample 3	30	PCB-149 sample 3
31	PCB-95 sample 1	31	PCB-153 sample 1
32	PCB-95 sample 2	32	PCB-153 sample 2
33	PCB-95 sample 3	33	PCB-153 sample 3
34	PCB-97 sample 1	34	PCB-156 sample 1
35	PCB-97 sample 2	35	PCB-156 sample 2
36	PCB-97 sample 3	36	PCB-156 sample 3
37	PCB-99 sample 1	37	PCB-163 sample 1
38	PCB-99 sample 2	38	PCB-163 sample 2
39	PCB-99 sample 3	39	PCB-163 sample 3

Table 7.5. Labels of GR sediments day final (180) samples run on 3rd agarose gel with respect to gel lane numbers

3rd gel lane no	product/sample
1	1kb plus marker (DNA ladder)
2, 3, 4	PCB-180 sample 1, 2, and 3, respectively
5, 6, 7	GR-Ace microcosm sample 1, 2, and 3, respectively

7.3.4. Community analysis of PCB dechlorinating bacteria by denaturing high pressure liquid chromatography (DHPLC)

Polymerase chain reaction (PCR)-based 16S rRNA gene assays have been described for monitoring indigenous dehalogenating communities within the phylum Chloroflexi, including *Dehalococcoides* species (Hendrickson *et al*., 2002; Fagervold *et al*., 2005; Kjellerup *et al*., 2011). Therefore, to characterize microbial consortia in PCB dechlorinating culture in the GR sediment microcosms, molecular screening of the 16S rDNAs from the total community of genomic DNAs was used. To determine whether there was a relationship between differences in the dechlorination activity or congener distribution and the composition of indigenous dechlorinating bacterial communities, DNA was extracted from the sediments and analysed by DHPLC to characterize the community profiles of putative dechlorinating phylotypes. Specific primers used in this study target only putative dechlorinating bacteria within *Chloroflexi* since only these phylotypes have been confirmed up to date for reductive dechlorination of PCBs (Cutter *et al*., 2001; Wu *et al*., 2002; Fennell *et al*., 2004; Fagervold *et al*., 2005, 2007, and 2011: Payne *et al*, 2011) and also because dehalogenators occur in low numbers and often cannot be detected with "universal primers".

Initial screening results obtained from DHPLC analysis for day zero and day 120 (final) of Aroclor 1254 microcosms are given from Figure 7.29. In this figure, every hump on the DHPLC graph may possibly represent a group of dechlorinating organisms/phylotypes. As presented in this figure, initial diversity of phylotypes detected by DHPLC was high and the number of dechlorinating bacteria was low which was inferred indirectly from the observance of low absorbance, which was confirmed with qPCR results that the number of dechlorinating bacteria was 100 times lower than that was observed at the final day (Figure 7.26-GR-1254 figure). As can be inferred from the day final (D120) curve (Figure 7.29), some dechlorinating phylotypes was enriched during the incubation of Aroclor 1254 with GR sediments.

A number of studies suggest that *Dehalococcoides* spp. and closely related to well-defined species play role in dechlorination of PCBs (Cutter *et al*., 2001; Fagervold *et*

al., 2005, 2007; Bedard *et al*., 2006, 2007; Yan *et al*., 2006a-b; Bedard, 2008; Field and Sierra-Alvarez, 2008; Kjellerup *et al*., 2008; Adrian *et al*., 2009). Since the aim was to determine the enriched phylotypes which are responsible for the dechlorination, after the initial screening of initial and final samples through DHPLC, only day final samples were sequenced. For this purpose, PCR-amplified 16S rRNA gene fragments of Aroclor 1254 microcosm, as shown with numbers between 20 and 29 in Figure 7.30, were collected from DHPLC. They were run on agarose gel to decide which fragments to be sequenced. As shown in Figure 7.30 on agarose gel pictures, fragment 20, 21, 22, 23, and 24 (indicated on agarose gel picture also as 20, 21, 22, 23, and 24, respectively) gave bright bands indicating high number/presence of dechlorinating phylotypes. Even though fragments 25 and 26 gave bands, but fade, they could not be sequenced, due to the very low number of dechlorinating bacteria or these bands being PCR artifacts, as repeated attempts to sequence them were unsuccessful. Since fragments between 27-29 (F27, F28, and F29) did not show any indication for the presence of dechlorinating bacteria, as no clear peak on DHPLC figure (Figure 7.30) and no band on agarose gel was present for such fragments, no attempt was performed for sequencing of these fragments.

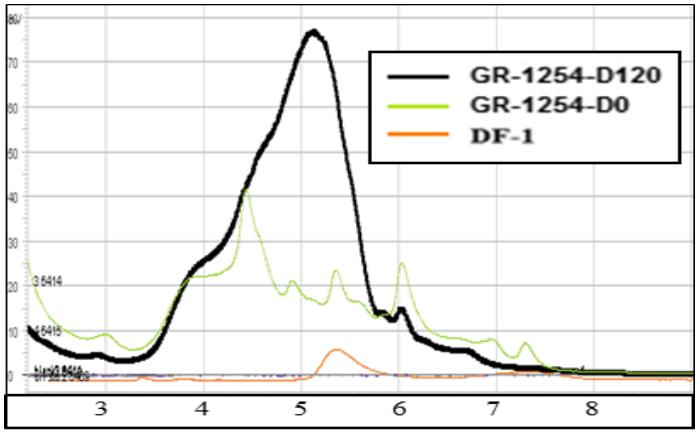


Figure 7.29. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in Aroclor 1254 microcosm incubated with GR sediments (y-axis shows absorbance between 0-80 mv, x-axis shows min between 0-9 min).

The fragments which gave bright bands, F20-F24, were excised, purified, and sequenced. Sequences were checked to find regions of local similarity between sequences by using basic local alignment search tool (BLAST), which is available on the National Center for Biotechnology Information (NCBI, USA) website. This program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Also, BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families (Altschul *et al*., 1990). Blast results showing the highest matches with the publicized organisms are given in Table 7.6.

Table 7.6. DHPLC phylotypes identified in at the final day (day 120) of GR-1254 microcosm spiked with Aroclor 1254 initially.

Band[1,2]	**% identity (nucleotides, base pair)**	**BLAST Match (Gene bank accession number)**
G1	100 (515)	Dehalococcoides sp. JN18_V108_B 16S ribosomal RNA gene, partial sequence (EF059530.1)
G2	100 (515)	Dehalococcoides sp. CBDB1 16S ribosomal RNA gene (AF230641.1) and also Dehalococcoides sp. FL2 16S ribosomal RNA gene(AF357918.2)
G3	100 (515)	Dehalococcoides sp. H10 16S ribosomal RNA gene, partial sequence (AY914178.1)
G4	99 (515)	Dehalococcoides sp. enrichment culture clone GD-B-1 16S ribosomal RNA gene, partial sequence (HQ122956.1)
G5	100 (450)	Dehalococcoides sp. enrichment culture clone GD-B-1 16S ribosomal RNA gene, partial sequence (HQ122956.1)

[1]The sequence from all bands were 100% or 99% identical to several clones identified in reductive dehalogenation of trichloroethene to ethane and similar resulted was reached with NCBI blast.
[2]The sequences from all bands were also 99% identical to the clone identified in a study called "Development and Characterization of an Anaerobic Microbial Sediment-Free Culture that Reductively dechlorinates meta- and *ortho* -Chlorines of Aroclor 1260" (HQ122956.1)

Although not all of the putative dechlorinating bacteria identified are necessarily involved in PCB dechlorination (Kjellerup *et al*., 2008) the identified phylotypes have high 16S rRNA sequence similarity to all of the known PCB dechlorinating bacteria (Table 7.6). The sequences which were related to *Dehalococcoides* with sequence similarities ranging between 99%-100% were detected in the indigenous dechlorinating phylotypes of GR sediments spiked with Aroclor 1254.

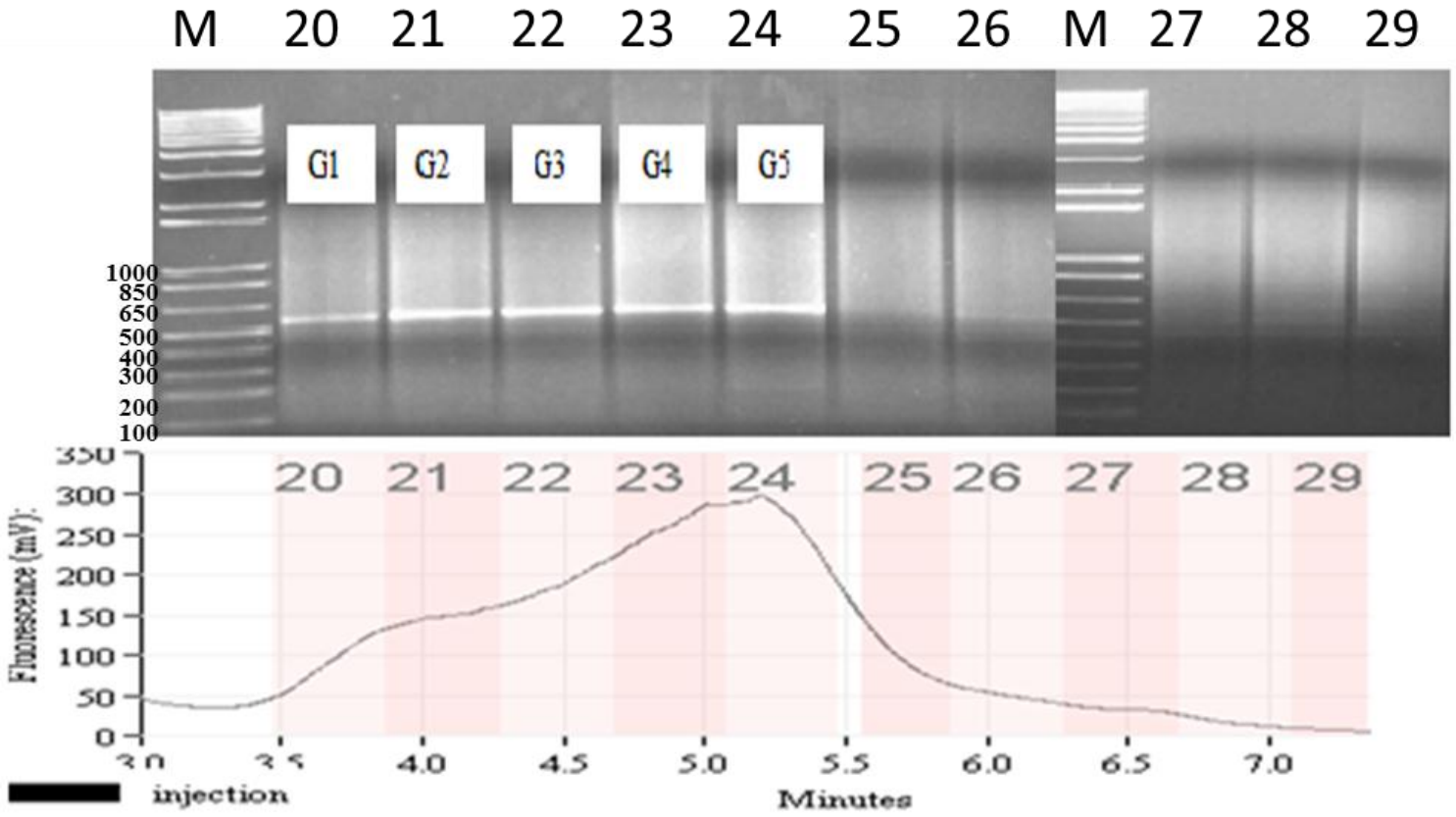


Figure 7.30. Agarose gel showing the PCR performed on Aroclor 1254 Day final (120) samples which were also run on DHPLC

For individual PCB congeners, DHPLC analyses for time zero and time final was also performed. Time 0 results are presented in Figure 7.31. As can be seen from this figure, all curves of different PCB congeners were the same, indicating the same phylotypes were present as expected since all individual PCB congener microcosms were inoculated with active GR sediment culture transferred from Aroclor 1254 microcosm after detection of dechlorination.

The results of DHPLC community analysis for putative dechlorinating Chloroflexi 16S rRNA genes in individual PCB congener microcosms for time final also revealed very similar curves. DHPLC results of PCB 52 and 91 microcosms for time 0 (D0) and time final (D180) is given Figure 7.32, which is a chosen representative for all PCB congeners to present a clear view, otherwise due to large number of congeners, it would be very crowded. As can be seen from this figure, day final DHPLC curves of PCB 52 and PCB 91 were almost the same, indicating same phylotypes were enriched in both microcosms of these congeners (Figure 7.32). It can be seen from Figure 7.32 that diversity of phylotypes detected by DHPLC increased from day 0 to day 180 (final day) and some phylotypes were highly enriched, which were not detected in day 0 analysis, possibly because they were below the detection limit.

After DHPLC screening for day final samples of all individual PCB congeners, fragments starting with numbers from 36 to 49, as shown in Figure 7.33, were collected and sequenced for community analysis of PCB dechlorinating bacteria. Blast results showing the highest matches with the publicized organisms are given in Table 7.7. The identified phylotypes showed high 16S rRNA sequence similarities to *Dehalococcoides* with sequence similarities ranging mostly between 95%-100% (Table 7.7) except for fragment 36, which did not correspond to any peak on DHPLC curve either (Figure 7.33). Some fragments such as 41, 44, 46 and 47, did not produce any band and thus, no sequencing was done for them. In addition, fragments for 50 and 51 were not collected due to absence of DHPLC peaks for these fragments which possibly indicated either very low number or absence of dechlorinating bacteria.

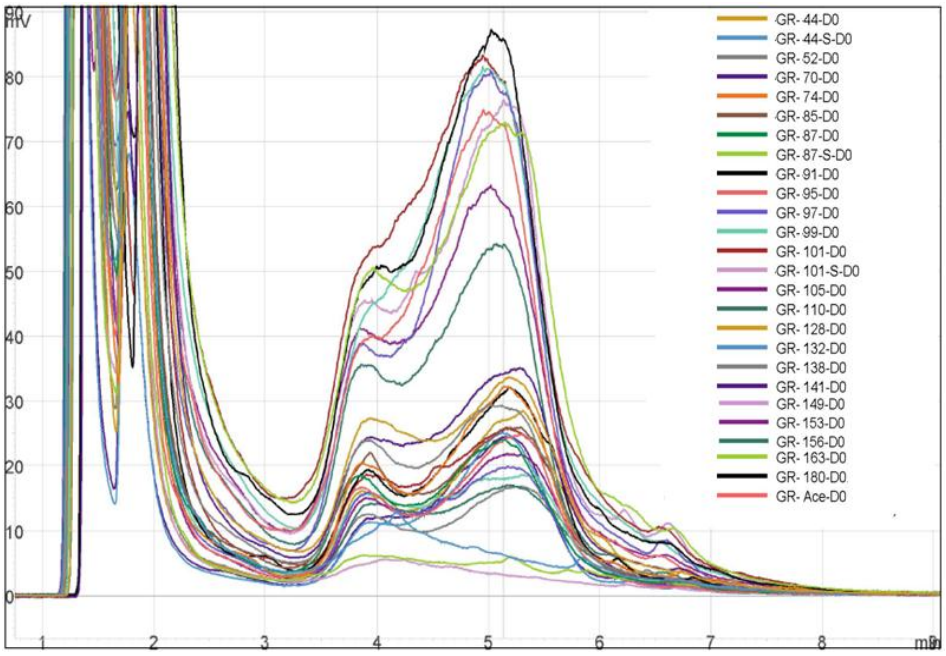


Figure 7.31. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in GR individual PCB congeners at day 0.

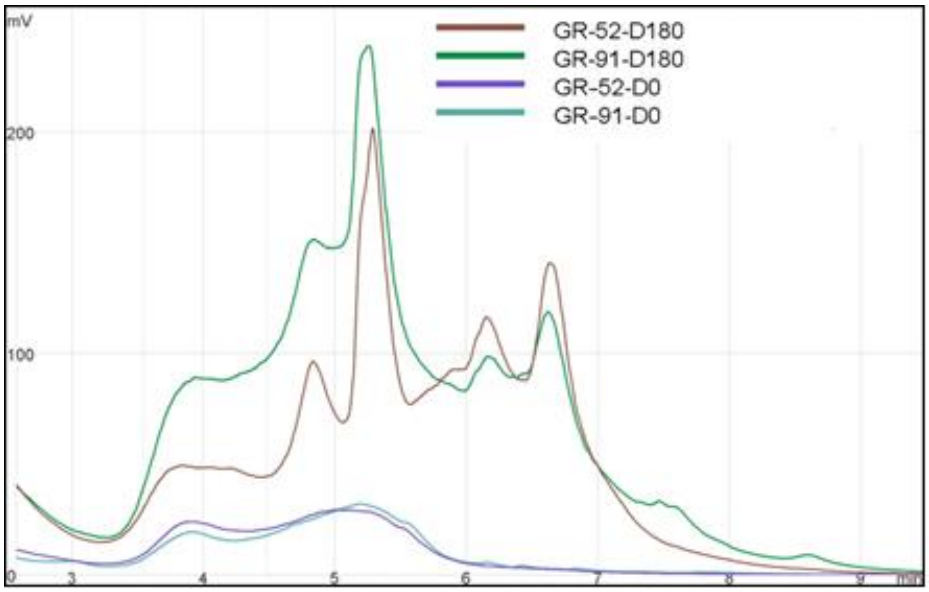


Figure 7.32. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in GR individual PCB congeners (D0 and D180 (final) samples of PCB 52 and 91 inoculated microcosms as a representative).

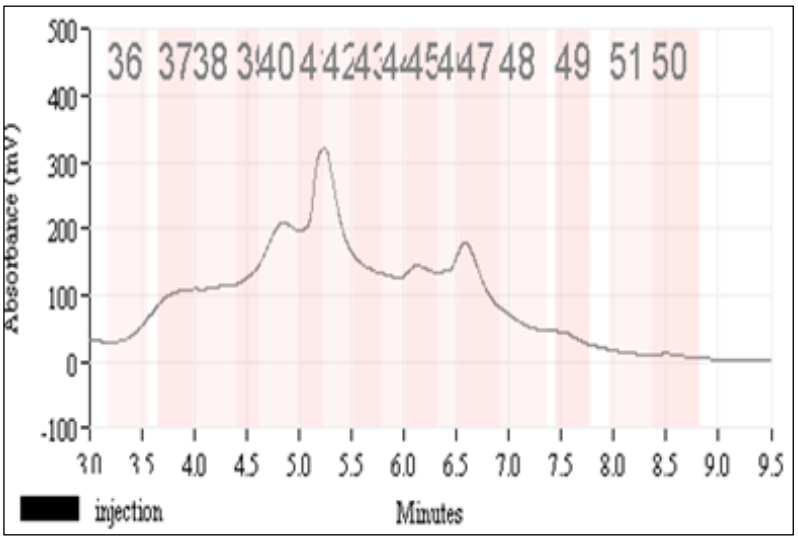


Figure 7.33. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in PCB 91 spiked microcosms at day 180. The phylotypes of putative dechlorinating Chloroflexi labeled as P1-P10.

Table 7.7. DHPLC phylotypes identified in at the final day (day 180) of GR-91 microcosm spiked with PCB-91 1254 initially.

DHPLC fragment	**% identity (nucleotides, bp)**	**BLAST Match (Gene bank accession number)**
36	86(449)	Uncultured bacterium clone J2A2 16S ribosomal RNA gene, partial sequence (GU139270.1)
37	99 (520)	Dehalococcoides sp. CBDB1 16S ribosomal RNA gene(AF230641.1)
38	98 (515)	Dehalococcoides sp. 11a5 16S ribosomal RNA gene, partial sequence (HM138520)
39	97 (493)	Uncultured Dehalococcoides sp. clone DEH10 16S ribosomal RNA gene, partial sequence (DQ021869.1)
40	99 (406)	Dehalococcoides sp. enrichment culture clone CT2 16S ribosomal RNA gene, partial sequence (JF698642.1)
42	100 (493)	Dehalococcoides sp. enrichment culture clone GD-B-1 16S ribosomal RNA gene, partial sequence (HQ122956.1)
43	100 (516)	Dehalococcoides sp. JN18_V108_B 16S ribosomal RNA gene, partial sequence (EF059530.1)
45	96(500)	Uncultured bacterium clone Er-MS-19 16S ribosomal RNA gene, partial sequence (EU542435.1)
48	95 (502)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1) and 95% identical to Uncultured bacterium clone Er-MS-19 16S ribosomal RNA gene, partial sequence (EU542435.1)
49	95 (505)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1) and 95% identical to Uncultured bacterium clone Er-MS-19 16S ribosomal RNA gene, partial sequence (EU542435.1)

7.4. Discussion and Conclusion

Sediment from GR was used for dechlorination of individual congers selected from Aroclor 1254. It has been shown that GR was historically contaminated with high concentrations of Aroclor 1248 and had the shortest lag and greatest activity in dechlorination among the other sediments tested in the study of Kjellerup et al. (2008). Therefore, it was important to investigate the possibility of using sediment enriched with the culture dechlorinating Aroclor 1248 for the dechlorination of Aroclor 1254. In this study, dechlorination pathways of 22 congeners chosen from Aroclor 1254, which included tetrachlorobiphenyls 44, 52, 70, 74, pentachlorobiphenyls 85, 87, 91, 95, 97, 99, 101, 105, 110, hexachlorobiphenyls 128, 132, 138, 141, 149, 153, 156, 163, and heptachlorobiphenyl 180, were revealed. In this context, to our knowledge this study is the most extensive study about dechlorination pathway of 1254 with GR sediments reported so far.

Findings showed that about 21% of Aroclor 1254 was dechlorinated after 120 days at a rate of $18.2x10^{-03}$ total Cl-/day. Dechlorination of Aroclor 1254 was achieved by the removal of chlorines mostly from penta-, followed by hexa- and hepta-chlorinated congeners by about 81%, 71%, and 63%, respectively (Figure 7.1b). The dechlorination of highly chlorinated congeners resulted in an increase in tetra- and tri-chlorinated congeners, in all microcosms at varying levels such as mole percent of tetra-chlorinated congeners increased from 29% to 47% and the mole percent of tri-chlorinated congeners increased from 2% to 31% during the incubation. The Aroclor removal percentage observed in this study is slightly higher and much faster than that reported by Zanaroli *et al.* (2012). In the latter study, they investigated the reductive dechlorination of Aroclor 1254 PCBs by a coplanar PCB-dechlorinating microbial community enriched from an actual site contaminated marine sediment of the Venice lagoon, Italy. These cultures dechlorinated about 75% of the penta- through hepta-chlorinated biphenyls to tri- and tetra-chlorinated congeners in 30 weeks, while it just took 16 weeks to dechlorinate 81% of penta-chlorinated congeners in our study. Table 7.8, 7.9 and 7.10 present the positions of the target chlorines, the rate of each reaction, and the final mol% for each of the starting congeners, intermediate, and

daughter products at day 180. Table 7.9, 7.10 and 7.11 were prepared separately according dechlorination step numbers for a better view. Figure 7.34 shows the dechlorination pathways from each of the starting congeners to the final products. The dechlorination rates of the parent compounds were higher than the dechlorination rates of the daughter compounds, with one exception; PCB 141 (2345-25) was dechlorinated less rapidly than PCB 101 (245-25).

Table 7.8. Pathways, end mol% and maximum reductive dechlorination rates for PCB congeners (PCB 180, 163 and PCB 156).

Parent	End Mol %	1st products	End Mol %	2nd products	End Mol %	3rd products	End Mol %*	Rate ($x10^{-3}$) (Cl/day)
PCB 180 *(2345-245)*	74.6±0.8	PCB 153 *(245-245)*	1.9±0.3	PCB 99 *(245-24)*	1.9±0.7	PCB 49 *(25-24)*	1.9±0.1	4.4±0.2
						PCB 47 *(24-24)*	**19.9±0.5**	
PCB 163 *(2356-34)*	72.8±5.4	PCB 117 *(2356-4)*	3.9±0.5	PCB 64 *(236-4)*	0.4±0.1	PCB 32 *(26-4)*	**16.0±5.3**	5.1±0.7
		PCB 110 *(236-34)*	0.7±0.3	PCB 71 (26-34)	6.5±2.1			
PCB 156 *(2345-34)*	81.8±1.0	PCB 118 *(245-34)*	3.7±0.2	PCB 66 (24-34)	6.1±0.5	PCB 25 *(24-3)*	0.7±0.2	1.5±0.1
						PCB 33 *(2-34)*	1.04±0.2	
		PCB 107 *(235-34)*	1.8±0.1	PCB 63 *(235-4)*	1.9±0.2	PCB 28 *(24-4)*+ PCB 31 *(25-4)*	3.0±0.4	

**congeners with end mol% higher than 20 mol% are boldfaced*

Although some examples of double flanked *para* dechlorination were observed in this study the results showed that dechlorination in GR microcosms more often occurred in the double flanked *meta* position. This was especially confirmed within the PCB 180 (2345-245) spiked microcosms, where all the dechlorination occurred in the double-flanked *meta* position and for PCB 156 (2345-34) and PCB 141 (2345-25), where about 80% and 91% of total dechlorination resulted from chlorine

removal of the double flanked *meta* position and about 20% and 9% of the dechlorination occurred in the double flanked *para* position, respectively.

Table 7.9. Pathways, end mol% and maximum reductive dechlorination rates for PCB congeners (PCB 153, 149, 141, 138, 132, 128, 110, 105, and 97).

Parent	End Mol %	1st products	End Mol %	2nd products	End Mol %*	Rate ($x10^{-3}$) (Cl/day)
PCB 153 *(245-245)*	89.1±0.8	PCB 99 *(245-24)*	2.6±0.6	PCB 47 *(24-24)*	8.3±0.2	1.0±0.1
PCB 149 *(236-245)*	62.5±2.8	PCB 102 *(26-245)*	9.7±1.2	PCB 51 *(26-24)*	**20.3±4.3**	3.1±0.7
		PCB 91 *(236-24)*	7.5±0.3			
PCB 141 *(2345-25)*	68.9±12.2	PCB 101 *(245-25)*	2.2±0.6	PCB 49 *(25-24)*	**25.8±11**	3.9±0.5
				PCB 52 *(25-25)*+ PCB 43 (235-2)	1.5±0.6	
		PCB 92 *(235-25)*	1.1±0.3			
PCB 138 *(234-245)*	64.4±1.1	PCB 99 *(245-24)*	8.4±1.7	PCB 47 *(24-24)*	**27.2±1.8**	3.6±0.4
PCB 132 *(234-236)*	71.5±1.5	PCB 91 *(236-24)*	7.8±1.1	PCB 51 *(26-24)*	**20.7±2.6**	2.9±0.2
PCB 128 *(234-234)*	88.0±0.2	PCB 85 *(234-24)*	1.7±0.2	PCB 47 *(24-24)*	10.3±0.4	1.1±0.1
PCB 110 *(236-34)*	21.6±3.7	PCB 71 *(26-34)*	30.2±21.6	PCB 32 *(26-4)*	**29.8±10.7**	7.4±0.7
				PCB 27 *(26-3)*+ PCB 24 (236-)	17.6±34	
		PCB 59 *(236-3)*	0.9±2.1			
PCB 105 *(234-34)*	83.8±0.8	PCB 66 *(24-34)*	11.2±0.3	PCB 28 *(24-4)*	4.5±0.7	1.0±0.1
PCB 97 *(23-245)*	41±4.8	PCB 42 *(24-23)*	41.1±4.3	PCB 17 *(24-2)*	17.9±1.6	4.5±0.2

**congeners with end mol% higher than 20 mol% are boldfaced.*

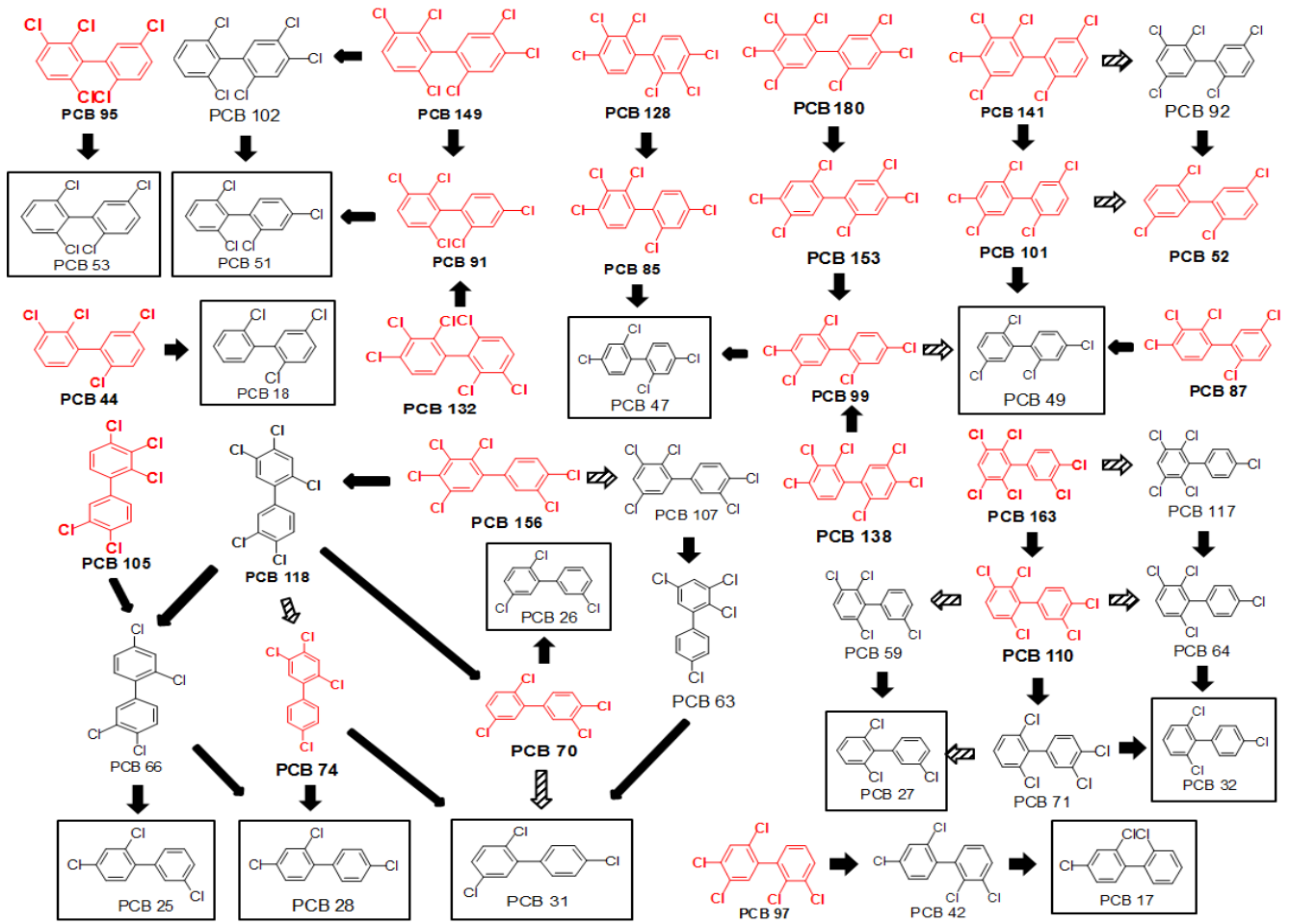


Figure 7.34. PCB dechlorination pathways of the predominant PCB congeners in Aroclor 1254. Parent congeners are shown in red. Black arrows indicate main pathway and hatched arrows indicate minor pathways. The predominant end products are boxed.

Table 7.10. Pathways, end mol % and reductive dechlorination rates for PCB congeners (PCB 101, 99, 95, 91, 87, 85, 74, 70 52, and 44) for 180 days of incubation.

Parent	End Mol %	Product	End Mol %*	Rate ($x10^{-3}$) (Cl/day)
PCB 101 (245-25)	51.3±19.6	PCB 49 (25-24)	**48.7±19.6**	4.5±0.9
PCB 99 (245-24)	83.6±1.9	PCB 47 (24-24)	16.4±1.9	1.0±0.1
PCB 95 (236-25)	37.0±1.3	PCB 53 (26-25)	**63.0±1.3**	4.2±0.1
PCB 91 (236-24)	66.1±0.8	PCB 51 (26-24)	**33.9±0.8**	1.8±0.1
PCB 87 (234-25)	54.6±1.5	PCB 49 (25-24)	**45.4±1.5**	3.2±0.4
PCB 85 (234-24)	71.3±2.8	PCB 47 (24-24)	**28.7±2.8**	1.5±0.1
PCB 74 (245-4)	98.1±0.5	PCB 28 (24-4) + PCB 31 (25-4)	1.9±0.5	0.1±0.0
PCB 70 (25-34)	95.7±1.3	PCB 31 (25-4)	1.4±0.3	0.3±0.0
		PCB 26 (25-3)	3.0±1.0	
PCB 52 (25-25)	99.3±0	PCB 18 (25-2)	0.7±0	0.01±0.0
PCB 44 (23-25)	24.04±0.7	PCB 18 (25-2)	**75.7±0.6**	6.7±0.1

**congeners with end mol% higher than 20 mol% are boldfaced.*

Based on the observations of the individual congeners, the general sequence of dechlorination in GR microcosms is; double flanked *meta* and to a very less extent double flanked *para* of 2345-substituted chlorophenyl rings; double flanked *meta* of 234-substituted chlorophenyl rings; *ortho* -flanked *meta* of 236-substituted chlorophenyl rings; *ortho* -flanked *meta* of 23-substituted chlorophenyl rings; *para*-flanked *meta* of 245-substituted chlorophenyl rings; *para*-flanked *meta* of 34-substituted chlorophenyl rings and *ortho* -flanked *meta* of 235-substituted chlorophenyl rings.

The predominant dechlorination products observed within 180 days of incubation in single PCB congener experiments were PCB 53 (26-25), PCB 51 (24-26), PCB 47 (24-24), PCB 49 (24-25), PCB 32 (26-4), PCB 18 (25-2), PCB 17 (24-2), PCB 27 (26-3) (which co-elute with PCB 24 (236-), which are in agreement with the results

of Aroclor 1254 mixture experiment. Basically, in all of the individual PCB microcosms, the end products of the dechlorination processes were unflanked congeners. Unflanked or *ortho* chlorine removals were not observed in any of the microcosms. Sterile controls did not show detectable dechlorination.

The pattern of Aroclor 1254 dechlorination in Grasse River sediment microcosms resembles Process N and Process H' (Quensen III *et al.*, 1990). Process N was first identified in Aroclor 1260 microcosms from Silver Lake sediments (Quensen III *et al*., 1990) and described as exclusive dechlorination in the *meta* position from both flanked and double flanked positions with a characteristically high accumulation of 2,2',4,4'-CB (PCB 47) (Bedard and Quensen III, 1995). This pattern has been previously observed in sediment microcosms from several PCB impacted freshwater sources, including Woods Pond (Bedard and May, 1996), Hudson River (Rhee *et al.*, 1993), from sediment-free microcosms developed from the Housatonic River (Bedard *et al.*, 2006), and from Baltimore Harbor sediment microcosms inoculated with Aroclor 1260 (Fagervold *et al.*, 2007). Also, several observations were consistent with the dechlorination pathways for PCB 101, 132, 138, 153, and 180 in Process N, anticipated in a comprehensive review by Bedard and Quensen III (1995).

Process H' is the removal of both *meta* and *para* chlorines, but only if these chlorines are flanked. Alder *et al.* (1993) reported dechlorination pattern H' for preexisting Aroclor 1242 PCBs in New Bedford Harbor sediments. Other studies have also reported pattern H/H' as being a frequent dechlorination pattern (Imamoglu *et al.*, 2004; Zanaroli *et al.*, 2006 and 2012).

All 21 major parent congeners, which account for about 85 weight% of Aroclor 1254, were dechlorinated each to a different extent. The dechlorination rates of PCB 70 and 74 (Table 7.11) were among the congeners with lowest rates. PCB 91, 99, and 105 which showed slightly better dechlorination rates than PCB 70 and 74 were the other congeners with low rates when they were incubated alone with GR sediment. The reason of observing higher dechlorination for such congeners in Aroclor 1254 mixture might be due to the presence of other congeners in the mixture, which is

known as “priming”, and the requirement of other congeners by the culture playing role in the dechlorination. Previous findings have shown that the presence/addition of some congeners had stimulatory effects on the dechlorination congener specificity by PCB-dehalogenating microorganisms (Nies and Vogel, 1991; Bedard and Quensen III, 1995; May *et al.,* 2006).

Among the studied PCB congeners, only PCB 52 did not dechlorinate. Interestingly, one explanation for this situation could be explained from the study of Cho *et al* (2003). In that study, they investigated the dechlorination kinetics of PCB congeners in Aroclor 1248 at threshold concentrations. Below threshold values (the minimum concentrations for the growth of dechlorinating microorganisms or consortia) active metabolism ceases for PCB congeners in sediment microcosms from the Aroclor 1248-contaminated St. Lawrence River. They also worked with the same PCB congeners (PCB 70, 74, 91, 99, and 105) which showed low dechlorination rates when compared the other congeners of this present study. PCB 52 was classified under group B that dechlorinated only at high concentrations above 60 ppm. As a result, they proposed that an accumulation of other utilizable congeners by the same microorganism may maintain the total effective concentration above the threshold even when the final concentration of a particular congener is lower than its threshold value. Therefore, when incubated alone, the absence of other congeners caused the threshold concentrations to have a negative impact on the reaction dynamics of the microcosms. The effective concentration at which these researchers studied were lower than the trace concentration for those congeners and that was explained to be the reason of seeing lower dechlorination for such congeners in this study. The final concentration of single congeners in the microcosms of this study was 50 ppm, while it was stated in the study of Cho *et al.* (2003), no dechlorination was observed below 60 ppm for this congener. So, the studied concentration for this congener might be low for observing dechlorination. Besides, throughout in this study there was not any dechlorination of the congeners with unflanked chlorines, indicating GR sediment culture preference is towards flanked chlorines and the most possible reason of not seeing any dechlorination activity for PCB 52 since it has unflanked *meta* congeners.

The threshold values and the specific dechlorination rates may not be universally applicable to all contaminated sediments because they may also be a function of physical, chemical, and biological factors such as the sediment composition, age of contamination, and/or sediment microbial community. For example, even for a given Aroclor or a congener, the threshold level could change with the organic content and particle size distribution (Cho *et al.,* 2003). Indeed, it has been shown by Kjellerup *et al.* (2008) that organic factors such as physical sediment characteristics, PCB availability and the number of dechlorinating bacteria were not the reason of the differences observed in the dechlorinating communities and activities at studied sites of Anacostia, Buffalo and Grasse Rivers. In fact, higher concentrations of PCBs and total organic carbon (TOC) were found to be closely related to a more active PCB dechlorinating population in case of Grasse River.

Additionally, it has been stated that the congener-specific kinetic constants changes if different PCB congeners, even non-PCB halo aromatic compounds, are also present (Cho *et al.,* 2003). Dehalogenating enzymes have broad substrate specificities (Cho *et al.,* 2003), which have been previously stated by Nies and Vogel (1991), Rhee *et al*. (1993) and Wu *et al.* (2000), and later by May *et al.* (2006). The pattern of Cl substitution on the biphenyl ring appears to be a factor underlying the specificity of PCBs (Rhee *et al*., 1993; Wu *et al*., 2000). Similarly, addition of certain congeners such as brominated biphenyls or chlorophenols, chlorobenzenes, or chlorobenzoates to PCB-contaminated sediments (this process is known as priming) have been shown to have a stimulatory effect on both the rate and the dechlorination patterns in Aroclor microcosms (Bedard *et al*., 1998; Cho *et al*., 2002). Therefore, it is possible that the threshold concentration of a specific congener may change in the presence of co-contaminants. In this current study, the stimulating effect of specific or multiple congeners in the Aroclor 1254 dechlorinating microcosm likely promoted the reductive dechlorination of some congeners. Besides, PCE was added to stimulate growth of culture, which linked to dechlorination.

Also, the observed differences in reaction rates has been attributed to the steric properties of the individual congeners (Brown *et al*., 1987), which could affect the

specific activities of individual reductive dehalogenases. As stated earlier by Williams (1994), the reactivity of a specific PCB chlorine is dependent upon both the chemical properties of the congener and catalytic properties of the microbes.

Another significant finding of this study is that when the growth curve of each congener (Figure 7.26) was analyzed together with their dechlorination curves, it can be seen there is a direct correlation of growth of dechlorinating microorganisms and the dechlorination rate. As can be seen from Figure 7.26, the number of 16s gene copies of steeply increased as it is most obvious for PCB 180, 163, 110, and 44. When the growth curve of these four congeners reached to a plateau, at the same time their dechlorination curves were also reached to a plateau after 90 days of incubation, indicating that the close relationship between microorganisms growth and dechlorination rate. Interestingly, the dechlorination rates found for PCB 180, 163 (Table 7.9), 110 (Table 7.10), and 44 (Table 7.11) were among the highest ones and these congeners were still dechlorinating since the dechlorination curve of these congeners did not level-off at the end of 180 days of incubation. There were two exceptional congeners, PCB 141 and PCB 99 which did not show the same trend; their dechlorination slowed down after 120 days of incubation but the growth of dechlorinating organisms for these congeners was still showing an increasing trend. Due to his observation, it can be said there is a direct relationship between microorganisms growth and dechlorination rate.

Cho *et al*. (2003) also stated that there may be two different kinds of threshold level: one is a threshold concentration for growth of dechlorinating microorganisms or consortia below which they cannot grow. This was confirmed with their previous findings in which they found that the threshold concentration required for the growth of dechlorinating microorganisms for Aroclor 1248 was the same as the threshold for dechlorination. These findings meant that the dechlorination threshold is linked to the growth of dechlorinating microorganisms (Rhee *et al*., 2001). Cho *et al*. (2002) and Kim and Rhee (1997) also found that there is a significant correlation between the maximum level of dechlorination and the population size. The other threshold is defined for active metabolism, below which dechlorinating organisms die or remain

inactive. Between this two threshold levels, dechlorinating microorganisms may still be able to utilize PCBs although their growth has ceased and the population may decrease (Cho *et al*., 2003). These statements may explain the findings of this study about the growth of dechlorinating bacteria and dechlorination of PCB congeners such that due to chemical properties of some congener and catalytic properties of the microbes as mentioned earlier.

All in all, the single congeners-except for PCB 52 and Aroclor 1254 incubated with GR sediment exhibited reductive dechlorination preferentially in *meta* position and to lower extent in *para* position, as observed during the dechlorination of PCBs 180, 156, 141, and 110. Dechlorination of *ortho* and unflanked *meta* chlorines was not detected, indicating that these enrichment microcosms selectively dechlorinated double- and single-flanked *meta* chlorines and *para* chlorines. This is in agreement with the findings of Kjellerrup *et al*. (2008) in which the analysis of GR sediments in terms of chlorine positions showed a higher level of *ortho* -chlorinated congeners in Grasse River and a higher level of the unflanked chlorines at the *ortho* position, indicating the removal of chlorines from *meta* and *para* positions. The same pattern of *meta* and *para* dechlorination was also previously reported for dechlorination of Aroclor 1242 (Morris *et al*., 1992) and Aroclor 1248 (Klasson *et al*., 1996) in Hudson River sediment and Aroclor 1254 in Venice Lagoon (Zanaroli *et al*., 2012).

The abundance of highly chlorinated congeners decreases while those of lower chlorinated, *ortho*-substituted, congeners increases by the preferential removal of *meta*- and *para*-substituted chlorines. Since the *meta* and *para* chlorines are removed with the anaerobic dechlorination, the coplanar structure, and hence, the dioxin-like toxicity of them is reduced (Kimbrough and Goyer, 1985; Abramowicz, 1995; Quensen III *et al*., 1998). Moreover, the resulting lower chlorinated congeners can be degraded by aerobic bacteria, allowing for the complete biodegradation of PCBs into carbondioxide and water via sequential anaerobic and aerobic microbial degradation processes (Bedard and Quensen III, 1995).

CHAPTER 8

EFFECTS OF TRANSFORMER OIL ON ANAEROBIC REDUCTIVE DECHLORINATION OF PCBS IN CONTAMINATED SEDIMENTS AND SEWAGE SLUDGE

8.1. Introduction

Polychlorinated biphenyls (PCBs) are members of the halogenated aromatic group of environmental pollutants that have been identified worldwide in diverse environmental matrices (Safe, 1994). Health concerns arose from PCBs suspected toxic and carcinogenic properties, as well as its endocrine disruptive effects (USEPA, 2008). Due to their persistence and lipophilicity (Brown and Wagner, 1990), their residence time in the environment is very long (Decastro *et al.*, 2006). Although their commercial production was banned since the late 70s (Fagervold et al., 2005), they are still on Earth due to existing transformers, capacitors, heat exchangers and hydraulic equipment. It is estimated that about 10 million tons, which is equivalent to one-third of the total worldwide production of PCBs, had been released into the environment (Eisenreich, 1987) due to inappropriate disposal practices, accidents and leakages from industrial facilities.

Waste motor and transformer oils are considered as the main source of PCBs into the environment. Approximately, 61% of PCBs in electrical transformers are still in use. Another 12% in electrical capacitors and 27% in storage are waiting for disposal (Jones *et al.*, 2003). Large volumes of PCBs have been introduced into the environment by direct entry or leakage into the sewers and streams; and by accidental spills or by possibly illegal disposal (Tagasuka *et al.*, 2006).

Health concerns arose from PCBs due to their suspected toxic and carcinogenic properties, as well as possible effect as endocrine disrupters. PCBs are known to affect the immune system, reproductive system, nervous system, and endocrine system (USEPA, 2008). PCBs also bioaccumulate in the food chain, with concentrations increasing by several orders of magnitude at succeeding trophic levels (Jacobson and Jacobson, 1996).

Sewage sludge contains many chemicals of natural and synthetic origin at varying concentrations. PCBs have been reported extensively in sewage sludge (Moreda *et al.*, 1998; Stevens *et al.*, 2001; Nakhla *et al.*, 2002; Patureau and Trably, 2006; Harrison *et al.*, 2006; Benabdallah El-Hadj *et al.*, 2007). A typical PCB content of l–10 mg/kg has been detected with an elevated portion of highly chlorinated PCBs (Benabdallah El-Hadj *et al.*, 2007). The detection of PCBs in sludge has raised a health concern. This concern has led the European Union to regulate the PCB contents in sewage sludge before spreading it on land by setting limits for the future common EU Sewage Sludge Directive (Patureau and Trably, 2006). The proposed limit values are of 0.2 mg/kg for each PCBs no. 28, 52, 101, 118, 138, 153, and 180. The current limit value in Turkey is of 0.8 mg/kg.dw for sum of the seven PCBs (Patureau and Trably, 2006).

Disposal of waste containing PCBs constitutes a major problem. The biological degradation of PCBs under anaerobic conditions has been observed in a variety of contaminated matrices, such as sediments (Berkaw *et al.*, 1996; Fagervold *et al.*, 2011; Payne *et al.*, 2011) and some anaerobically digested activated sludge (Ye *et al.*, 1992; Phelps *et al.*, 1996; Chang *et al.*, 2002; Fava *et al.*, 2003). Hence, such an elimination strategy for PCBs involving biological methods may prove to be useful. Anaerobic digestion is a commonly applied treatment option for waste activated sludge (WAS) stabilization because of its abilities to transform organics into biogas (60-70 vol% of methane, CH_4). At the same time digestion reduces the amount of organics and hence the final sludge solids for disposal, destroys most of the pathogens present in the sludge and controls the odor. The European Union has put an objective to increase the amount of energy obtained from renewable sources from

the 2005 level of 8.5% to 20% in 2020 (Mottet *et al.,* 2010). In this context, anaerobic digestion of PCB contaminated sewage sludge may contribute to reaching this objective.

Dechlorination under anaerobic conditions occurs due to dehalorespiration, where halogenated compounds are used as terminal electron acceptors. Consequently, an excess of a carbon source (electron donor) is required for efficient dechlorination (Tartakovsky *et al.,* 2000). The degree of chlorination and the position of chlorines in a congener are major factors influencing degradation potential of the compound (Borja *et al.,* 2005). PCBs contaminating the environment typically exist as mixtures of congeners in waste motor and transformer oils. Aroclor 1254 is one of the most widely used mixtures in transformer oil formulations. Microbial transformation of Aroclor 1254 by anaerobic reductive dechlorination has been shown in sediment, as well as in laboratory microcosms (Quensen III *et al.,*1990; Oefjord *et al.,* 1994; Natarajan, 1998; Pakdeesusuk *et al.,* 2003; Zanaroli *et al.,* 2012) but in these studies either dechlorination was slow or not extensive and in none of them sludge is used as a carbon source during PCB dechlorination. Besides, in the dechlorination studies it may be critical to use acclimated culture to increase the extent of dechlorination.

In this study, sediment from Grasse River was used for dechlorination of PCB 118 and Aroclor 1254. Grasse River was historically exposed to PCBs, and PCB dechlorinating activity has been reported previously in these sediments (Kjellerup *et al.,* 2008). PCB 118 is the most abundant congener found in Aroclor 1254 and one of the congeners that is listed in EU Sewage Sludge Directive and Turkey's Regulation on The Use of Domestic and Urban Sludge in the Soil. Also, sludge digestion together with PCB dechlorination was investigated as well as the effect of transformer oil doses in this study.

8.2. Materials and Methods

8.2.1. Chemicals

All PCBs (99-100 % purity) were purchased from AccuStandard. Transformer oil (TO) was purchased from Ultra Scientific. Hexane (pesticide grade) and acetone was purchased from Acros Organics. All other chemicals were reagent grade.

8.2.2. Sediment Location and Collection

The Grasse River (GR) site is located in the lower Grasse River in the Village of Massena, NY in the United States where ALCOA (Aluminum Company of America) has been producing aluminum since 1903. The GR is a U.S. Environmental Protection Agency (USEPA) Superfund site that contains high levels of PCBs. Historic disposal of production waste by-products into onsite landfills and lagoons, a practice that was common and widely accepted at the time, resulted in the release of PCBs into the lower GR from the 1930s to the 1970s. PCBs were discharged to the GR with wastewater during routine operations through four permitted outfalls and resulted in contamination of a seven mile stretch of the river (Kjellerup *et al.*, 2008). It has been reported that GR was contaminated with A1248 that was used for aluminum production at this industrial site since the 1930s (USEPA, 2005). Total of PCB concentration detected in GR sediments was 6829 ng/g (Kjellerup *et al.*, 2008). Indeed, in 2008, Kjellerup *et al.* showed that the GR PCB profile was most similar to the A1248 profile and the results of their analyses supported the historical information about the main sources of contamination in the river. GR sediments were collected in spring 2008 with a ponar sampler from the top 30 cm of sediment from GR, NY (44°57.06 N 74°51.06 W). These were used as inocula in microcosms supplemented with waste activated sludge (WAS) in this study. The sediment samples were stored anaerobically in glass jars sealed with Teflon-line lids at 4°C in the dark prior to use. Sediments were black in color and had a sulfide odor indicative of reduced anoxic conditions.

8.2.3. Microcosm Setup

Ten mL aliquots of E-Cl medium were anaerobically dispensed into 60-mL serum bottles and sealed under N_2/CO_2 (80:20) with 20-mm Teflon-coated butyl stoppers (West Co., Lionville, PA) secured with aluminum crimp seals. The medium was autoclaved at 121°C for 20 minutes. The final pH was 6.8. All subsequent additions into E-Cl medium containing microcosms were performed in an anaerobic glove box (Coy Laboratory Products, Ann Arbor, Michigan, USA) containing N_2:CO_2:H_2 (75:20:5) and the phases of this study and preparation details of microcosms are described below.

This study consists of two consecutive phases: Phase one microcosms were prepared to investigate the effect of varying doses of transformer oil (TO) on the dechlorination of PCB 118 and Aroclor 1254 in the presence of an easily degradable carbon source, a fatty acid (FA) mixture. A mixture of active culture transferred from a previous set of GR and original GR sediment were used as acclimated culture/inoculum. In order to investigate effect of TO on PCB dechlorination rate and pathways, two different doses of TO were chosen as 1% oil and 10% oil meaning that 1% and 10% of working (effective) volume of microcosms were filled with TO, respectively. For this purpose, 0.6 mL and 6 mL TO was added to 1% oil and 10% oil into microcosms containing E-Cl medium, respectively. One mL of active GR culture taken from previous enrichment microcosms and 10 mL of original wet GR sediment was added to the microcosms as an inoculum. FA mixture (acetate, propionate, and butyrate) was supplied at a concentration of 2.5 mM as an electron donor and carbon source. PCB 118 and Aroclor 1254 were dissolved in acetone (about 0.2% v/v) and separately added to microcosms at a final concentration of 50 mg/L and 100 mg/L, respectively. The final initial concentrations of PCB 118 in TO phase can be calculated as 5000 mg/L and 500 mg/L as low level (1%) and high level of oil (10%), respectively. The concentrations of Aroclor 1254 in TO phase can be calculated as 10000 mg/L and 1000 mg/L low level (1%) and high level of oil (10%), respectively. Oil control microcosms spiked with either PCB 118 or Aroclor 1254 was also prepared identically, but without oil. Phase one microcosms were labeled as

GR and presented in Table 8.1. Phase one microcosms labeled as GR (Table 8.1) with PCB 118 or Aroclor 1254 were operated for 210 or 180 days, respectively.

Phase two microcosms were prepared to investigate the dechlorination potential of PCB 118 and Aroclor 1254 in simulated small scale digesters. Also, the effect of one dose of TO, which was determined in phase one, on PCB 118 and Aroclor 1254 dechlorination in the presence of WAS and on anaerobic sludge digester performance was investigated. A mixture of active culture transferred from phase one and original wet GR sediment was used as an acclimated culture/inoculum by using a mixture of active culture obtained from phase one and original GR sediment. In this phase, sludge was used as an electron donor and carbon source, no FA was supplied and microcosms were labeled as WAS (Table 8.1). The WAS microcosms were prepared similar to phase one' in the anaerobic chamber by mixing 1 mL active culture and 2 mL original wet GR sediment with 12 mL of WAS collected from the return line of secondary sedimentation tank of *Little Patuxent Water Reclamation Plant* (Baltimore, USA). PCB 118 and Aroclor 1254 were solubilized in acetone (about 0.2% v/v), and separately added to triplicate microcosms to a final concentration of 50 mg/L and 100mg/L, respectively. Since this set was planned according to the result of first phase and negligible dechlorination activity was observed in microcosms with 10% oil in the first phase, only 1% oil was tested in this phase. Accordingly, 1% TO was added to certain microcosms of phase two indicated in Table 8.1 to investigate its effect on PCB 118 and Aroclor 1254 dechlorination. This way, the concentrations of PCB 118 and Aroclor 1254 in the oil phase were 5000 mg/L and 10000 mg/L, respectively. Control microcosms with solvent only and solvent plus 1% oil were also prepared separately in order to check the effect of acetone and oil on methane production. One sterile control was prepared without oil, which was prepared identical to the live microcosm except that the bottles were autoclaved twice for 1 h at 121°C on 2 consecutive days followed by addition of Aroclor 1254 at a final concentration of 100 mg/L. All microcosms were prepared in triplicate and operated for 120 days.

After the preparation, all microcosms were sealed with stoppers and mixed. They were then sampled for day zero analyses and incubated statically at 30ºC in the dark until the next sampling. Sampling of microcosms was done in 30-day intervals in an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI) until their termination.

Table 8.1. Experimental setup

Phase	Microcosm label	Sterile	WAS	Spiked PCB type and concentration (mg/L)	TO
Phase 1	GR-118-No oil	-	-	PCB 118 : 50 mg/L	-
	GR-118-1% oil	-	-	PCB 118 : 50 mg/L	+
	GR-118-10% oil	-	-	PCB 118 : 50 mg/L	+
	GR-1254-No oil	-	-	Aroclor 1254: 100 mg/L	-
	GR-1254-1% oil	-	-	Aroclor 1254: 100 mg/L	+
	GR-1254-10% oil	-	-	Aroclor 1254: 100 mg/L	+
Phase 2	WAS-Ace-No oil	-	+	no PCB spike, but Acetone	-
	WAS-Ace-1% oil	-	+	no PCB spike, but Acetone	+
	WAS-118-No oil	-	+	PCB 118 : 50 mg/L	-
	WAS-118-1% oil	+	+	PCB 118 : 50 mg/L	+
	WAS- 1254- sterile	+	+	Aroclor 1254: 100 mg/L	-
	WAS-1254-No oil	-	+	Aroclor 1254: 100 mg/L	-
	WAS-1254-1% oil	-	+	Aroclor 1254: 100 mg/L	+

8.2.4. Analytical techniques

8.2.4.1. PCB extractions and analytical procedures

One mL samples from each triplicate bottle were extracted with 5 mL of hexane on a wrist shaker overnight for measurement of PCBs. The organic phase was passed through a copper/Florisil (1:4) column and PCB congeners were analyzed using a Hewlett Packard 5890 series II GC equipped with ^{63}Ni electron capture detector as described previously (Fagervold *et al.*, 2005) and a DB-1 capillary column

(30mx0.25mmx0.25μm). PCB congeners in a mixture containing 250 μgL^{-1} Aroclor 1232, 180 μgL^{-1} Aroclor 1248 and 180 $\mu g\ L^{-1}$ Aroclor 1262 (also known as ‘‘Mullin’s mix’’ (Swackhamer *et al.*, 1996) were used to make with a 10-point calibration curve with PCB 30 and PCB 204 used as internal standards.

Fifty-five additional congeners not present in the Aroclor mixture that were potential dechlorination products were also added to the PCB calibration table. A calibration mixture containing the internal and surrogate standards and Mullin’s mix was run with every batch of samples to determine the response factor of each peak relative to the internal standards and to correct/check shift in the retention times of peaks. PCB 166 was used as a surrogate compound and its recovery was about 85%. Congener concentrations were not corrected for surrogate recoveries, because of the possible bias in calculated mole fractions of lesser or more chlorinated congeners in the case of differential recoveries of the different surrogates. Peaks for the surrogate and internal standards and those that co-eluted with sulfur (which was sometimes not completely removed by the copper cleanup) were disregarded.

During analytical determination of PCBs, in the chromatograms, a peak may represent an individual congener or a group of congeners. The congeners that appear together in the same peak during chromatographic analysis are named as coeluting congeners. These coeluting congeners are designated by slashes separating their congener numbers. For data calculations, co-eluting congeners and homologues were assumed to be present in equal proportions. Dechlorination curves were made for all PCB congeners in 30-day intervals over the course of incubation. The PCB concentrations were measured as μg PCB/mL of microcosm slurry by GC/ECD and converted to mol% which was calculated by using the formula below (Equation 8.1):

$$mol\% = \frac{\frac{C_i}{MW_i}}{\sum \frac{C_i}{MW_i}} .100 \qquad (8.1)$$

where MW_i is the molecular weight of each detected PCB congener (g/mol) and C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry). The extent of dechlorination was calculated from the weighted average number of chlorines ($Cl_{average}$) in samples before and after incubation. $Cl_{average}$ was reported as average number of Cl per biphenyl, and was calculated according to the formula below (Equation 8.2):

$$Cl_{average} = \frac{\sum C_i.n_i}{\sum C_i} \quad (8.2)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents.

The dechlorination rate was calculated within the linear slope of the dechlorination curve by dividing total chlorine removed per biphenyl with the time elapsed in days and calculated by using the formula below (Equation 8.3):

$$Rate = \frac{\left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{initial} - \left(\frac{\sum C_i.n_i}{\sum C_i}\right)_{final}}{t} \quad (8.3)$$

Where C_i is the concentration of each detected PCB congener (µg PCB/mL of microcosm slurry) and n_i is its number of Cl substituents and t is the incubation time elapsed (days).The average rate and the standard deviation were calculated from triplicate cultures.

8.2.4.2. DNA extraction

DNA was extracted (in triplicate) from microcosms samples for three time points (time points are shown in text and/or on the graphs with letter “D” followed with time in days) and was used in downstream processes such as enumeration and community analysis of PCB dechlorinating bacteria. DNA was extracted by adding 0.25 mL of slurry samples from each microcosm to an individual Power Bead microfuge tube of a Power Soil DNA Isolation Kit (MOBIO Laboratories, Inc.) or to

a 96-well bead beating plate for rapid and thorough homogenization. In the former, the Power Bead tubes were mixed by brief vortex prior to 30 s of bead beating at speed "4.5" using a FastPrep120 (Q-Biogene, CA). In the latter, instead of FastPrep120 (Qiagen, Resch), a TissueLyser is used. The TissueLyser allows the usage of 96-well plate and like FastPrep120, it provides disruption of biological samples through high-speed shaking. In the DNA extraction process, cell lysis occurs by mechanical and chemical methods. Total genomic DNA is captured on a silica membrane in a spin column format. DNA is then washed and eluted from the spin column. Total DNA was then isolated from the Power Bead tubes or 96-well plates according to the manufacturer's directions. DNA was eluted in 100 µL of Tris buffer (provided with kit) and quantified with a NanoDrop 1000 Spectrophotometer (ThermoScientific). All DNA samples were diluted to 2ng/µL in Tris buffer.

8.2.4.3. Enumeration of PCB dehalorespiring bacteria by quantitative PCR

The quantification of putative dechlorinating Chloroflexi in the microcosm samples was performed (in triplicate) by quantitative polymerase chain reaction (qPCR) using iQ SYBR green supermix (Bio-Rad Laboratories). DNA was amplified with primer Chl348F (5'-CGC CCG CCG CGC GCG GGA GGC AGC AGC AAG GAA-3') and Dehal884R (5'-GGCGGGACACTTAAAGCG-3') targeting the16S rRNA genes of the members within Chloroflexi including *Dehalococcoides* spp. (Fagervold *et al.*, 2005).

Each qPCR reaction volume (25-µL) contained 1x iQ SYBR green supermix, 400 nM forward and reverse primers and 1 µL of sample DNA. PCR amplification and detection were conducted in an iCycler (Bio-Rad Laboratories). qPCR conditions were as follows: initial denaturation for 15 min at 95°C followed by 35 PCR cycles of 30 s at 95°C, then 30 s at 61°C, then 30 s at 72°C. One copy of the gene per genome was assumed based on the genomes of *Dehalococcoides mccartyi* strain 195 and *Dehalococcoides* sp, strain CBDB1 (Seshadri *et al.*, 2005; Kube *et al.*, 2005). qPCR data were analyzed with MJ Opticon Monitor Analysis Software v3.1 and compared to a standard curve of purified DF-1 348F/884R 16S rRNA gene product.

The standard curve consisted of duplicate dilutions over 7 orders of magnitude. The specificity of qPCR amplification was verified by melting curve analysis followed by gel electrophoresis 1.2% (w/v) high-melt agarose gel.

Amplification efficiencies of dilutions of gel purified DF-1 16S rRNA gene PCR product used as standards for all sets were 90% ± 8% (r^2 = 0.999), and amplification efficiencies of samples were 94% ± 7%.

8.2.4.4. Community analysis of PCB dechlorinating bacteria by denaturing High Pressure Liquid Chromatography

Denaturing high pressure liquid chromatography (DHPLC) analyses were performed using a WAVE 3500 HT system (Transgenomic, Omaha, NE) equipped with a florescence detector (excitation 490 nm, emission 520 nm). Primers 348F/884) were used both for DHPLC following PCR or qPCR (sometimes qPCR products were used) as described below.

PCR reactions were performed in 50 μL reaction volumes using the following GeneAmp reagents (PE Applied Biosystems, Foster City, CA): 10 mM Tris-HCl, a mixture of dNTPs (200 nM each), 1.5 mM $MgCl_2$, 100 nM of each primer, 0.8% dimethyl sulfoxide (DMSO), 2 units of Ampli *Taq* DNA polymerase, 200 nM of each primer, 1 μL of DNA template and 34.5 μL of nuclease-free water. Amplification was performed in a PTC200 thermal cycler (MJ Research, Watertown, MA.) with the following cycle parameters: Initial denaturing (1 min at 95°C), 40 cycles of denaturation (45 s at 95°C), annealing (45 s at 61°C), and elongation (45 s at 72°C), followed by a final extension (30 min at 72°C). PCR products of the correct length (about 500 bp) were confirmed by electrophoresis using a 1.2% high melting agarose gel prior to analysis by DHPLC. The 16S rRNA gene fragments were analyzed in a 30 μL injection volume by DHPLC with a DNASep® cartridge packed with alkylated non-porous polystyrene-divinyl benzene copolymer microspheres for high-performance nucleic acid separation (Transgenomic, Omaha, NE). The oven temperature was 63.0°C and the flow rate was 0.5 mL min^{-1} with a

gradient of 55-35% Buffer A and 45–65% Buffer B from 0 to 13 min. The analytical solutions used for the analyses were such that Buffer A constituted 0.1 M triethyl ammonium acetate (TEAA) at pH 7, Buffer B was made of 0.1 M TEAA and 25% acetonitrile at pH 7, Solution D included 25% water and 75% acetonitrile. Syringe Wash Solution was supplied from Transgenomic (Omaha, NE. Analysis was performed using the Wavemaker version 4.1.44 software. An initial run was used to identify individual PCR fragments and determine their retention times. Individual peaks were eluted for sequencing from a subsequent run and collected with a fraction collector based on their retention times. The fractions were collected in 96-well plates (Bio-Rad, Hercules, CA) and dried using a Savant SpeedVac system (Thermo Electron Corporation, Waltham, MA) followed by dissolution in 30 μL of nuclease-free water. Re-amplification was performed following the protocol described above. The PCR amplicons were electrophoresed in a 1.2% high melting agarose and the excised fragment was purified for sequencing using Wizard® PCR Preps DNA Purification Resin (Promega, Madison, WI) and then sequenced (BASLab, University of Maryland, IMET, Baltimore, USA).

8.2.4.5. Sequencing and analysis

Each DHPLC fraction was sequenced in the 5′ and 3′ direction with 100pM of primer 348F or 884R using the BigDye® Terminator v3.1 (Applied Biosystems, Foster City, CA) kit per the manufacturer's instructions. Sequencing of purified DNA was performed on an ABI 3130 XL automated capillary DNA sequencer (Applied Biosystems, CA). Sequences were examined for errors and assembled using the software Finch TV software package (FinchTV, 2011). Alignments were then refined manually. Chimera formation was examined using Chimera Check (Cole *et al.*, 2003). The sequence alignments were done by using Ribosomal Database Project (RDP) produced by Cole *et al.* (2009). Phylogenetic tree was generated based on published Chloroflexi sequences over 1200 base pairs by the neighbor joining approach using default settings in RDP.

After comparative sequence analyses of DNA obtained from the DHPLC, 10 phylotypes (Figure 8.12) were sequenced and checked to find regions of local similarity between sequences by using Basic Local Alignment Search Tool (BLAST), which is available on the web on the National Center for Biotechnology Information (NCBI, USA) website. This program compares nucleotide or protein sequences to sequence databases and calculates the statistical significance of matches. Also, BLAST can be used to infer functional and evolutionary relationships between sequences as well as help identify members of gene families (Altschul *et al.*, 1990).

8.2.4.6. Methane analysis

Methane analysis was done only in phase two microcosms. Methane was measured by taking 100 µl gas with a gas tight syringe (SGE) and injecting into a GC (Hewlett Packard 5890A) equipped with an FID. The column was 0.32- by 182.88-cm stainless steel packed with silica gel (80/100 mesh; Supelco). The column oven was operated at 110°C, and He was the carrier gas. Purified methane (Matheson) was used as a standard (Sowers and Ferry, 1983). A calibration curve was prepared before every analysis by injecting a high purity methane gas.

8.2.4.7. Chemical oxygen demand (COD) analysis

COD contents of the reactors were measured at day 0 and at the end of the operation period according to a USEPA approved reactor digestion method (for a COD range of 0-15000 mg/L) (Jirca and Carter, 1975) by using Hach DR2000 spectrophotometer at 620 nm as given in Hach Water Analysis Handbook (1989). For COD analysis, Hach Spectrophotometer (Model 45600-02, Cole Parmer Instrument Co., USA) and vials were used according to manufacturer's directions.

8.2.4.8. Analysis of solids

VS, TS, and TSS of all microcosms were measured at day 0 and at the end of the operation period. VSS analysis was only done initially to show initial status of WAS. TS and TSS determinations were carried out as described in Standards Methods

(Method 2540 B and 2540 D, respectively) and VS and VSS measurements were done according to Method 2540E (APHA, 2005).

8.2.4.9. Statistical Evaluation

All statistical evaluations were performed through Student t test with 95% confidence (a statistical significance of $p<0.05$) by using Graphpad Prism 5 software.

8.3. Results and Discussion

8.3.1. Results of Phase 1

8.3.1.1. Effect of TO on PCB 118 dechlorination

Figure 8.1 shows changes in total chlorine per biphenyl values of PCB 118 dechlorination in GR sediment microcosms without oil, 1% oil and 10% oil cases for 210 days of incubation. As can be seen from this figure, PCB 118 dechlorination rate was the highest in no oil microcosm, in which dechlorination rate was $12.6x10^{-3}$ Cl^-/day, while in 1% oil microcosms PCB 118 dechlorination rate was about one fifth ($2.57x10^{-3}$ Cl^-/day) of the no oil microcosm. The dechlorination of PCB 118 in 10% oil microcosm was negligible, as its rate was very low, $0.086x10^{-3}$ Cl^-/day (Figure 8.1). As it is obvious from the difference observed in the dechlorination rates of PCB 118 in GR microcosms, the effect of presence oil as well its dose on the PCB118 dechlorination was very significant and it was also statistically confirmed ($p<0.05$).

Distribution of PCB 118 and its intermediate and terminal byproducts in GR-118 microcosm without oil and with 1% oil are presented in Figure 8.2. In the no oil microcosm, about 74% of PCB 118 was dechlorinated in 210 days. About 63% of this decrease occurred in the first 90 days of incubation. As PCB 118 started to dechlorinate an increase in mol% of PCB 66 (24-34) and PCB 28 (24-4)/31 (25-4) (which co-elute) started immediately. The mol% of PCB 28/31 remained the same after day 60, while after that time PCB 25(24-3) and PCB 26 (25-3) was detected (Figure 8.2a)

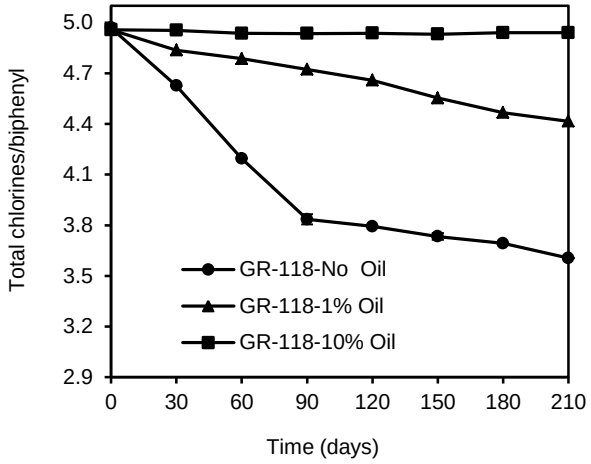


Figure 8.1. Changes in total chlorine per biphenyl values in GR microcosms spiked with PCB 118 with no oil, 1% oil and 10% oil over 210 days of incubation.

From these findings, it was observed that PCB 118 dechlorinated to PCB 66 and eventually PCB 66 dechlorinated to PCB 28/31, most probably to PCB 28 (not PCB 31) via sequential removal of *para* flanked *meta* chlorine. Since the mol% of PCB 66 decreased to below 1 mol% after day 60 and simultaneously mol% of PCB 28 leveled off, it was thought that possibly the dechlorinated congener was PCB 66 leading to the formation of PCB 28/31. Another possibility source of PCB 28/31 may be either through flanked *meta* and/or *para* dechlorination of PCB 74 (245-4) to PCB 28 and/or PCB 31, respectively. The reason for this mechanism to be suggested is the mol% of PCB 74 fluctuated throughout 210 days of incubation. The mol% of PCB 74 increased up to about 1 mol% at day 30 and after which day it started to decrease to 0.02 mol%, (even though it may not be considerable), suggesting the production and subsequent consumption of this congener. The possible high rate of production and consumption during dechlorination might have prevented the accumulation of this congener, making it difficult to decide if PCB 74 was the intermediate byproduct of PCB 118 and parent congener for PCB 28/31.

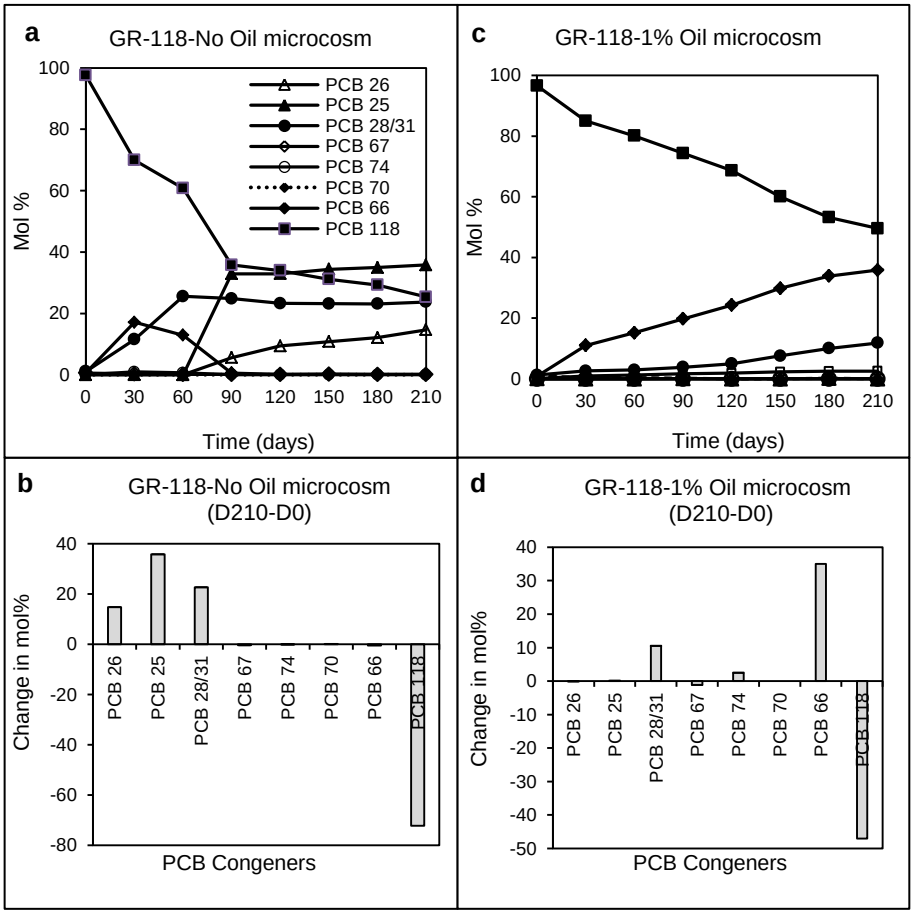


Figure 8.2. Mol% distributions of PCB 118 and its intermediate and end products observed in GR-118 microcosm with absence and presence of 210 days of incubation.

By looking at distribution throughout the incubation period, an interesting observation was the accumulation of PCB 26 (25-3) and PCB 25 (24-3) after day 60 and the simultaneous accumulation of PCB 70 (25-34) despite the low decrease of PCB 118. This finding implies that PCB 118 might have been possibly dechlorinated via sequential *para* chlorine removal to PCB 70 and then to PCB 26. The same idea about PCB 74 can be valid for PCB 70 such that the dechlorination rate was so high that PCB 70 could not accumulate, and as soon as it was dechlorinated from PCB 118 via removal of flanked *para* chlorine, it was immediately dechlorinated to PCB 26 (25-3) via the same dechlorination process. Another possibility might be that PCB

25 and PCB 26 were dechlorinated from PCB 67 (245-3) via removal of flanked *meta* and flanked *para* chlorines, respectively.

For the no oil microcosm, it can be concluded that PCB 118 first dechlorinated to PCB 66 by removal of *para* flanked *meta* chlorine and this congener further dechlorinated possibly through two pathways: one pathway through *para* flanked *meta* chlorine removal to PCB 28 and the other one through *meta* flanked *para* chlorine removal to PCB 25. As a result, end products observed and their mol% at day 210 are shown in Figure 8.2b. At the final day of the incubation, mol% of PCB 25, PCB 28, and PCB 26 were 35.8 mol%, 23.7 mol%, and 14.7, respectively. At the same time PCB 118, the mother congener, decreased to 25.3 mol%. There were negligible amounts of intermediate products, such as PCB 66 and PCB 67 (at 0.136 and 0.103 mol%, respectively). Dechlorination was achieved through flanked *meta* and/or *para* chlorine removal. There was no *ortho* or unflanked chlorine removal throughout the incubation period.

It is obvious that dechlorination of PCB118 was affected by the presence of oil (Figure 8.1). Although no lag was observed during the dechlorination, the rate was slow compared to no oil microcosm as stated earlier. As can be seen from Figure 8.2c, there was a gradual decrease in PCB 118 with an increase in mol% of PCB 66 and PCB 28/31 and also in mol% of PCB 74. In PCB 118 microcosms with 1% oil, obviously PCB 118 was dechlorinated to PCB 66 via chlorine removal from *para* flanked *meta* position as was also observed in no oil microcosm. PCB 66 was dechlorinated to PCB 28. All dechlorination products show that dechlorination of PCB 118 followed sequential removal of *para* flanked *meta* chlorine. PCB 70, 26 and 25 were not observed in 1% oil microcosm (Figure 8.2d). At the end of the incubation period, there was about 47 mol% decrease, 35 mol% increase, 10.5 mol% increase, and 2.5 mol% increase of PCBs 118, 66, 28/31, and 74, respectively (Figure 8.2d). As a result, addition of 1% oil to GR sediment microcosm affected the dechlorination rate of PCB 118 and its extent. About 25% less dechlorination occurred in 1% oil microcosm compared to the case of no oil.

Since a negligible amount of dechlorination was observed in microcosms with 10% oil no further data/graph is presented for this microcosm. The amount of PCB 118 dechlorination in 10% oil microcosm was 2 mol% resulting in an increase of PCB 66 and PCB 74 by 1.8 mol% and 0.2 mol%, respectively within 210 days of incubation.

8.3.1.2. Effect of TO on Aroclor 1254 dechlorination

The removal of chlorines/biphenyl during the Aroclor 1254 dechlorination from *meta*, *para*, and *ortho* positions, as well as removal of total chlorines per biphenyl, are shown in Figure 8.3 for no oil, 1% and 10% oil cases. There was no lag in the dechlorination of Aroclor 1254 without oil (Figure 8.3a), but its dechlorination rate ($0.9x10^{-03}$ total Cl^-/day) was very low compared to that of PCB 118 without oil ($12.5x10^{-03}$ total Cl^-/day). The dechlorination rate of Aroclor 1254 without oil was about 13 times higher than that of 1% oil microcosm ($0.03x10^{-03}$ total Cl^-/day). Therefore, similar to the PCB 118 case, the presence of oil reduced the dechlorination rate. Dechlorination in Aroclor 1254 with 1% oil was negligible compared to that of no-oil microcosm and similarly, no dechlorination was observed in 10% oil microcosm, indicating negative effect of oil on the dechlorination of Aroclor 1254.

In no oil microcosm, total reduction of *meta* chlorines during Aroclor 1254 dechlorination was similar to the reduction of total chlorines (Figure 8.3a and b), indicating dechlorination to be mostly through the removal of *meta* chlorine. The overall removal percent of *meta* chlorine was higher than that of *para* chlorines in both Aroclor 1254 with 1% and without oil. In the no oil microcosm, about 8.27% of *meta* chlorines and 3.73% of *para* chlorines were removed, which were mostly from removal of doubly flanked chlorines, about 11.6%. While in 1% oil microcosm, *meta* and *para* chlorines were about 0.64% and 0.41% (Figure 8.3), respectively, and removal of doubly flanked chlorines was about 3.41%.

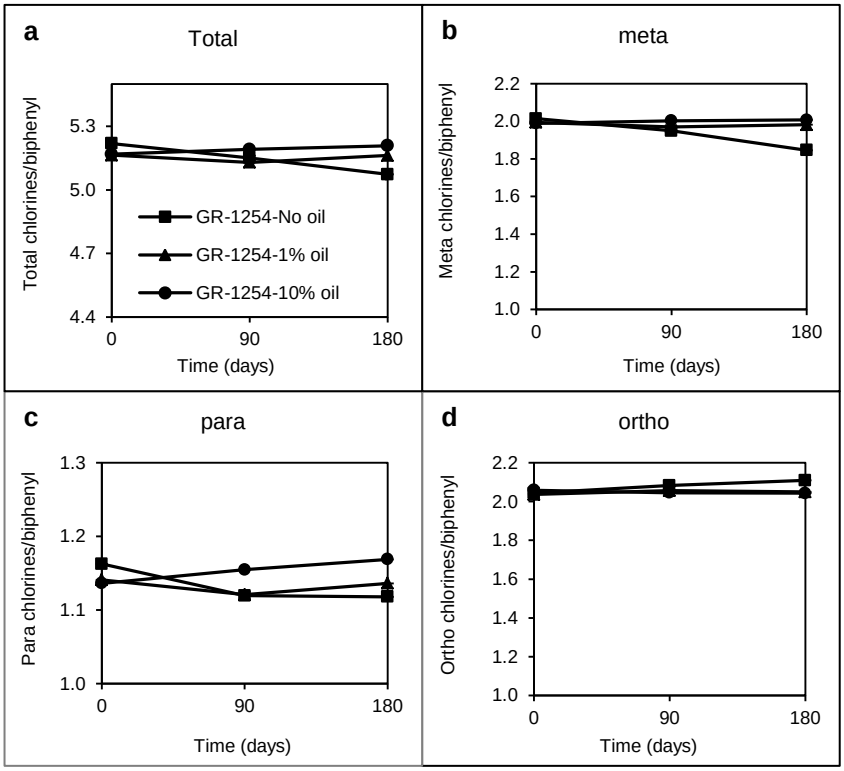


Figure 8.3. Dechlorination of a) total, b) *meta*, c) *para* and d) *ortho* chlorines per biphenyl in Aroclor 1254 microcosms with GR sediment with different treatments in 180 days of incubation.

Figure 8.4 shows the distribution of homolog chlorines observed in no oil microcosms, showing dechlorination was achieved by mostly through the removal of chlorines from penta-, (about 22%) in no oil case. Since hepta-chlorinated congeners were dechlorinated about 10%, there was 0.92% increase in hexa-chlorinated congeners of GR-1254-No oil microcosm. This increase was lower than the decrease observed in hepta-chlorinated congeners, the difference can be explained through the analysis of changes in mol% of lower chlorinated congeners such as tetra-chlorinated congeners, which was increased by about 36% and tri-chlorinated congeners with doubled mol%, indicating the dechlorination of hexa-chlorinated congeners ended up in these lower-chlorinated congeners via fast sequential dechlorination. Since the dechlorination was very low in 1% and no dechlorination was observed in 10% oil microcosms, no further data is presented for these microcosms.

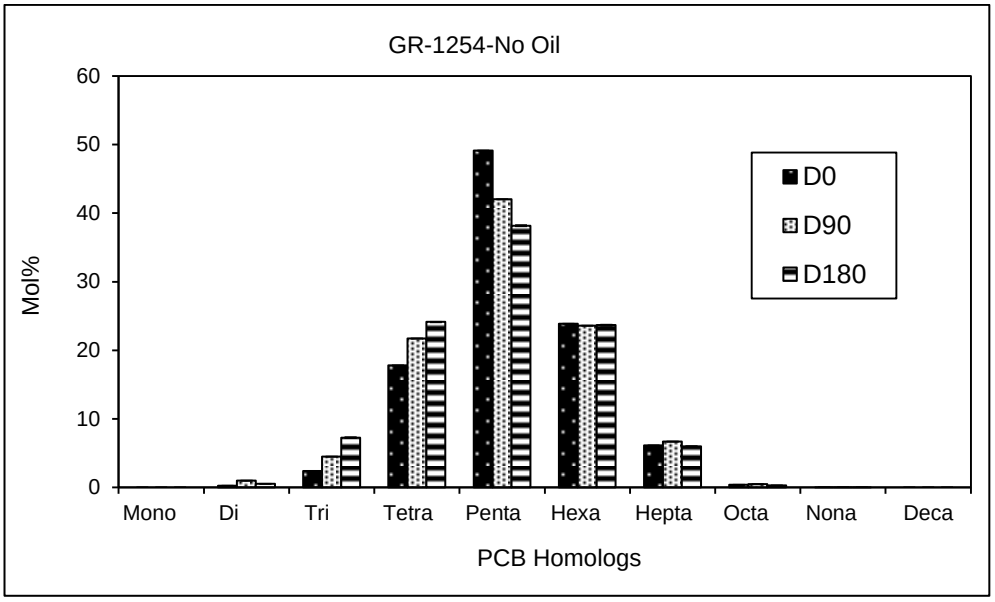


Figure 8.4. Homolog distributiona of Aroclor 1254 in GR sediment microcosms without oil in 180 days of incubation.

Figure 8.5 shows the changes in mol % distributions of Aroclor 1254 congeners in no oil microcosm after 180 days. PCB110 (2.21 mol%), 105 (0.85 mol%), 101 (2.19 mol%), 99 (0.51 mol%), 97 (0.70 mol%), 95/66/88/121 (1.19), 87(1.02 mol%), 82 (0.85mol%), 85 (0.51 mol%), 70 (0.95 mol%), 52/43 (0.70 mol%), and 44 (0.89 mol%) were highly dechlorinated congeners in this microcosm. Dechlorination of these congeners resulted in the accumulation of PCB 53/33/20 (25-26) by 3.21 mol%, PCB 49 (24-25) by 2.62 mol%, PCB 71 (26-34) by 1.90 mol%, PCB 51 (24-26) by 1.04 mol% and PCB 46 (23-26) by 1.09 mol%. Dechlorination of Aroclor 1254 resulted in a deposition of lower chlorinated congeners; however some of these congeners had flanked chlorines in meta and/or para positions such as PCB 71 and PCB 46, indicating incomplete dechlorination since in previous sets, all end products of dechlorination were with unflanked chlorines (data not shown).

Dechlorination rate of Aroclor 1254 was very low when compared to that of PCB 118. The reason might be related to the dechlorinating culture since active culture used in Aroclor 1254 and PCB 118 microcosms of this study were transferred from different microcosms of previous set (enrichment), which were initially prepared

with the same original wet GR sediment and separately spiked with Aroclor 1254 and PCB 118, respectively. The initial activity observed in the enrichment set was also different for Aroclor 1254 and PCB 118 case such as there was about 60 days lag with Aroclor, while the lag was 30 days for the case of PCB 118. Aroclor 1254 and PCB 118 microcosms in the enrichment set were operated for 120 days and 60 days, respectively. The reason of observing very low dechlorination rate in Aroclor 1254 microcosm of this set (phase one) is not clear, but, it might be due to the activity loss of the dechlorination during the culture transfer from Aroclor 1254 enrichment microcosm to this set of GR-1254 microcosms.

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8.3.1.3. Enumeration of dechlorinating phylotypes

In order to determine whether dechlorination of PCBs was growth-linked, microorganisms were enumerated by performing qPCR using specific primers for PCB dechlorinating phylotypes on the samples taken from each microcosm for three time points as day 0, 90 and 180. The number of 16S rRNA gene copies of putative dechlorinators per mL of wet sediment shows an increase in the number of putative dechlorinators (Figure 8.6). The number of PCB dechlorinating phylotypes increased almost 10-fold in all microcosms of this phase and statistical analyses showed the effect of TO was insignificant, although a decrease in the number of 16S rRNA gene copies of putative dechlorinators per mL of wet sediment was observed in the microcosm with 10% oil after day 90.

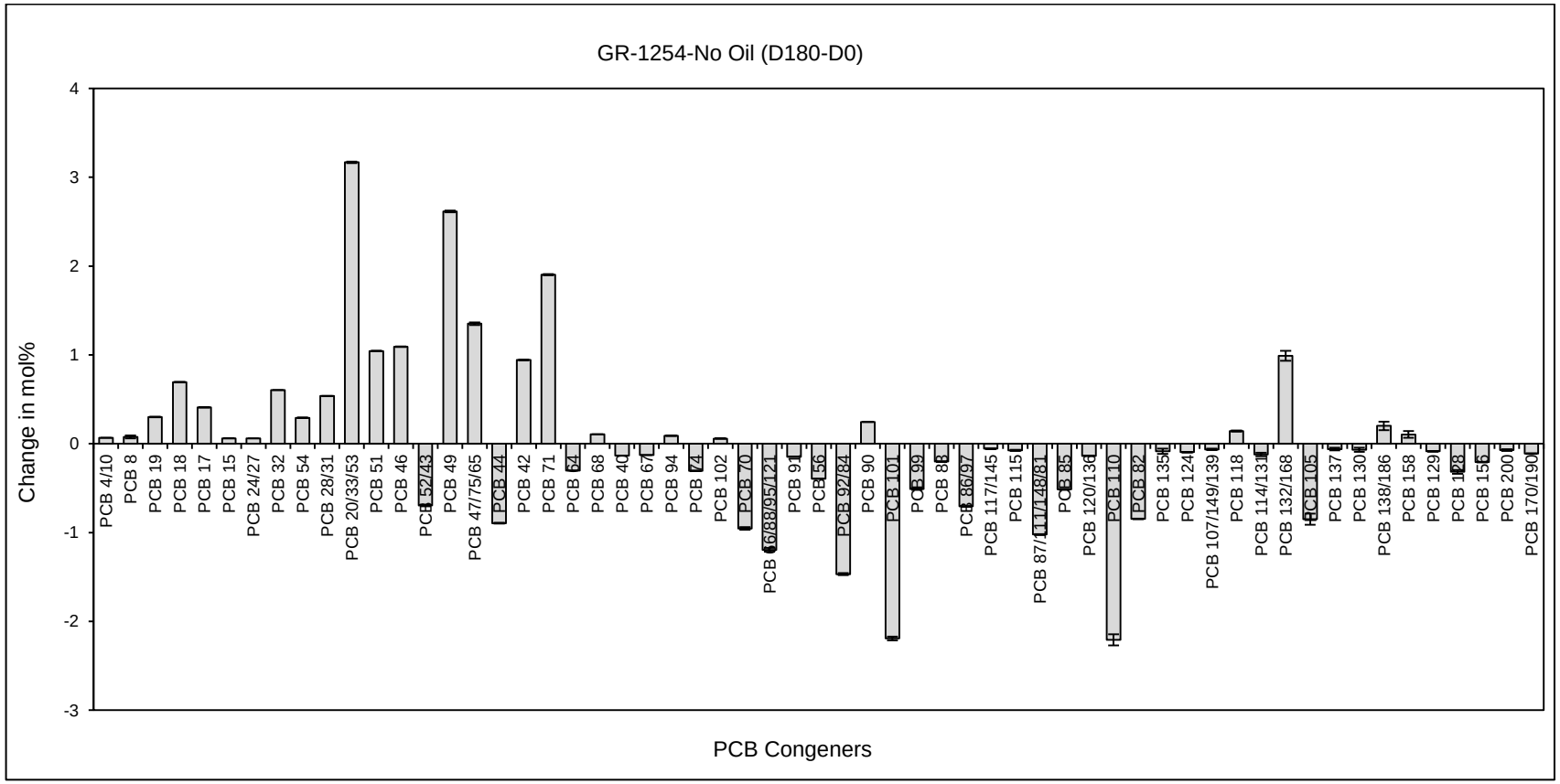

Figure 8.5. Changes in mol% distributions of Aroclor 1254 congeners in GR-1254 microcosm without oil after 180 days (D180).

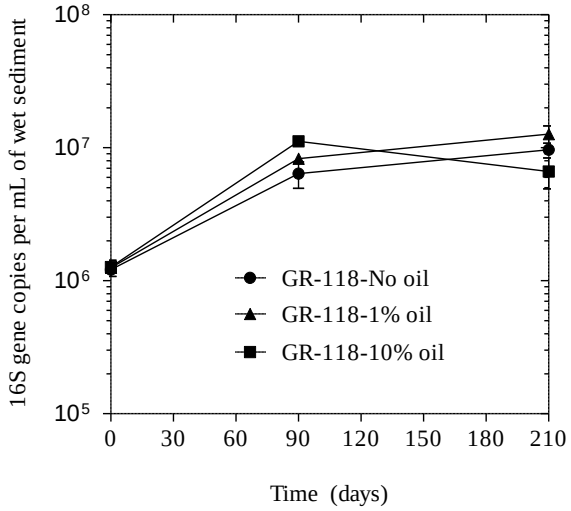

Figure 8.6. Changes in number of putative dehalogenating Chloroflexi 16S gene copies per mL wet sediment in GR microcosms in 210 days of incubation.

The number of 16S rRNA gene copies of putative dechlorinators per mL of wet sediment (or sample) shows an increase in the number of putative dechlorinators in Aroclor microcosms (Figure 8.7). The number of PCB dechlorinating phylotypes increased almost 10-fold in no oil microcosm while the increase was about 5- and 4-fold compared to that the number of day zero in 1% oil and 10% oil microcosms, respectively. This indicates a relationship of higher growth with higher dechlorination. Statistical analyses showed that the effect of oil on the growth was not significant ($p>0.05$).

As a result of occurrence of lower dechlorination in Aroclor 1254 microcosms, lower increase was observed in the number of dechlorinating phylotypes in Aroclor 1254 compared to that of PCB 118 microcosms, indicating dechlorination was growth linked.

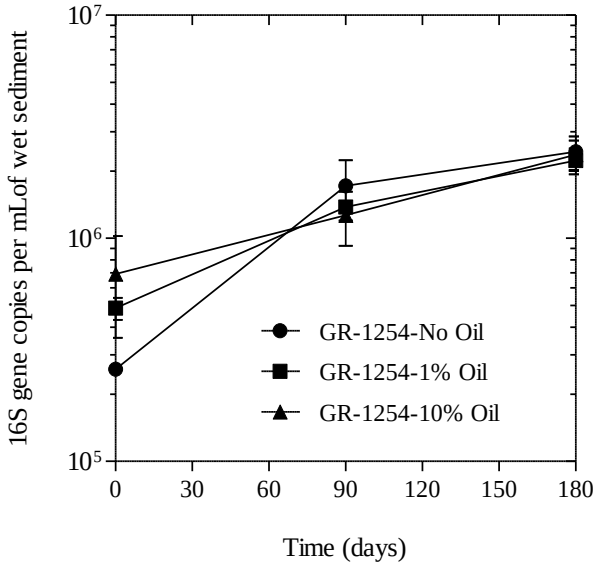

Figure 8.7. Changes in number of putative dehalogenating Chloroflexi 16S gene copies per mL wet sediment in GR microcosms in 180 days of incubation.

8.3.2. Phase 2

Since one of the main objectives of this phase was to investigate whether preexisting anaerobic sludge digesters can be used for dechlorination of PCBs or can contaminated sewage be treated in preexisting anaerobic digesters, this set was prepared to address these issues. Towards this end, PCB 118 and Aroclor 1254 dechlorination was investigated under anaerobic conditions in BMP bottles with the presence of WAS and TO. TS, VS, TSS, and VSS concentrations of WAS were determined before adding it into the reactors. The results (mean ± standard deviation) are presented in Table 8.2.

Table 8.2. Characteristics of WAS used in the WAS set.

TS mg/L	VS mg/L	TSS mg/L	VSS mg/L
21,027±31	17,047±114	20,400±132	16,450±100

The overall objective of anaerobic sludge treatment is the efficient reduction of organic matter. TS and VS are commonly used as indicators of organic matter reduction. Therefore, in this study, TS, VS, COD contents, biogas composition as well as extent of the PCB dechlorination were investigated.

8.3.3. Results of Phase 2

8.3.3.1. Sludge reduction and methane production

Figure 8.8 shows cumulative CH_4 productions of Phase 2 microcosms operated with WAS throughout the 120 days of incubation. Biogas production occurred in all of the biologically active microcosms, starting from the beginning of the incubation. About 85-90% of total cumulative methane production occurred within the first 46 days of incubation in all microcosms with the highest total methane production happening in the control microcosm without PCB or oil (WAS-Ace-No oil) as 437.6±0.4 mL (Table 8.3).

Addition of TO affected the production of methane significantly ($p=0.0036<0.05$). There was about 6% lower methane production in the control microcosm with oil (WAS-Ace-1% Oil) compared to control reactor without oil (WAS-Ace-No oil). When the total methane production of PCB and/or Aroclor microcosms with oil was compared to those without oil, it is seen that the addition of oil reduced the cumulative methane production in all cases (Figure 8.8). This finding was confirmed to be statistically significant at 95% confidence level ($p=0.0002<0.05$).

The comparison of methane production results of WAS-118-No oil with those of WAS-Ace-No oil is expected to reveal the effect of PCB 118 on methane production. From this comparison, it can be seen that there was 56 mL more methane production in microcosm without PCB (Table 8.3), indicating that the presence of PCB reduces the methane production by about 15% in this case. Statistical evaluations performed by using Student's t test showed that the effect of presence of PCB or Aroclor on the methane production was significant at 95% confidence level ($p<0.0001<0.05$). Even

though Aroclor 1254 and PCB 118 are different compounds, another statistical evaluation carried out between methane productions of WAS-118-No oil in which PCB 118 was added at a concentration of 50 mg/L and methane productions of WAS-1254-No oil in which Aroclor 1254 was added at as 100 mg/L might give information about the effect of PCB concentration and/or the presence of other PCB congeners over the methane productions. *P* value of that evaluation was found higher than *0.05*, indicating insignificant effect. However, this effect became much more apparent when oil was included, which might have had a synergistic effect together with PCBs. The effect, this time was significant and in a negative way; such that in the presence of TO a higher concentration of PCB and/or the presence of other PCB congeners caused lower methane production ($p=0.012<0.05$).

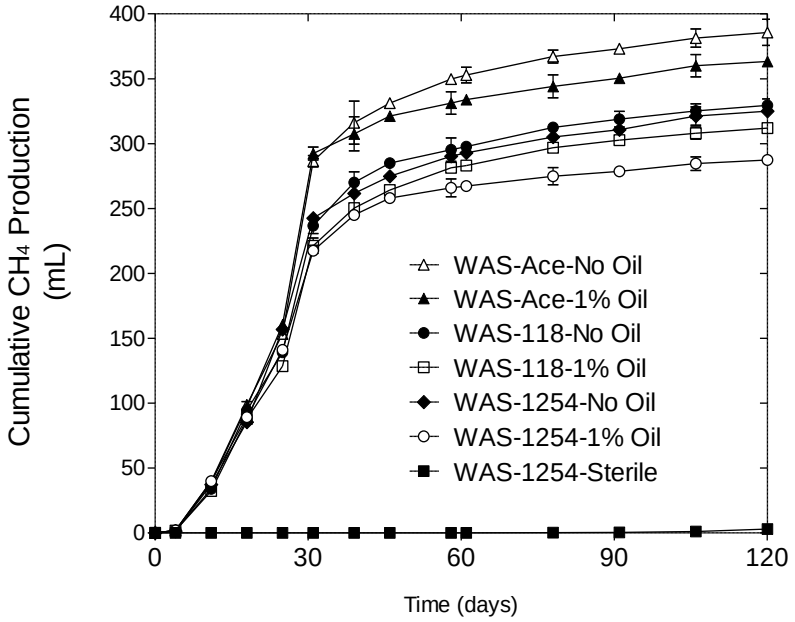

Figure 8.8. Variation of the cumulative CH_4 productions of Phase 2 microcosms operated with WAS throughout the 120 days of incubation.

Table 8.3. TS, VS, COD, total cumulative CH_4 and CH_4 yield values of WAS set microcosms.

Microcosms	**İnitial**			**Final**			**Total cumulative CH_4 volume mL**	**mL CH_4/gVS removed**
	VS mg/L	**TS mg/L**	**COD mg/L**	**VS mg/L**	**TS mg/L**	**COD mg/L**		
WAS-Ace-No Oil	18016±400	43850±50	32083±381	8079±220	31031±337	15100±200	385±0	1116.8
WAS-Ace-1% Oil	19383±300	44766±76	42083±803	10883±57	33449±132	25000±173	363±2	1059.0
WAS-118-No Oil	18516±175	44349±278	33500±250	10787±114	33196±214	18800±100	329±0	1021.0
WAS-118-1% Oil	19766±600	45349±86	42250±1089	12083±381	35766±104	26700±264	311±0	925.4
WAS-1254-No Oil	18633±236	44750±132	33666±877	11825±787	34711±249	19733±208	324±0	1041.3
WAS-1254-1% Oil	19816±732	45550±180	42833±1181	13082±112	36900±62	27866±57	287±2	883.4
WAS-1254-Sterile	18533±57	44149±327	33333±629	18133±236	43633±332	32440±69	3±3	13.4

In order to explain the reason of such occurrence, it is better first to have a look at the composition of TO, which is a highly-refined mineral oil. Mineral oils are composed of straight and branched chain paraffinic, naphthenic, and aromatic hydrocarbons with 15-40 carbons in a complex mixture (Aluyor and Ori-jesu, 2009). Molecular structure and solubility of the compound in the aqueous medium containing the microorganism are known to be important parameters for biodegradation. The inherent characteristics of the TO, which is hydrophobic and a long-chain fatty acid compound (LCFA), might have prevented microorganisms from accessing water-soluble degradable wastes to produce methane. Also, it is reported that the biodegradability of long-chain carbon compounds increases with their decreasing carbon chain lengths and increasing degree of unsaturation of carbon chains (Chipasa and Medrzycka, 2006). Moreover, LCFA are well-known inhibitors of various microorganisms at milimolar concentrations and, consequently cause some serious problems in anaerobic treatment systems (Rinzema, 1988). LCFA are mostly β-oxidized (Weng and Jeris, 1976) to acetic acid and hydrogen which in turn are further converted to methane gas. It is reasonable to expect that both methanogens and acetogens, the two related consortia to β-oxidation, suffer greatly from LCFA inhibition (Roy *et al.*, 1986). Adsorption of the surface active LCFA onto cell wall/membrane leading to the damage of transport function or protective function are reported mechanisms of LCFA toxicity (Galbraith and Miller, 1973). Also, the effect of LCFA toxicity is dependent on the concentration but not on the concentration: biomass ratio (Koster and Cramer, 1987; Angelidaki and Ahring, 1992; Rinzema *et al.*, 1994). Thus, one of the main reasons for observing decreased methane production in oil containing microcosms of this study could be the high concentration of the oil. Indeed, the concentration of the oil used in this study was about 8900 mg/L, which is greater than the minimum inhibition concentration of LCFA on methanogenesis, which was reported as 401 mg/L (Chipasa and Medrzycka, 2006).

Moreover, the ability of lipids to form floating aggregates limits the biological treatment of lipid-rich wastewater. Gujer and Zehnder (1983) demonstrated that low density of the floating aggregates slows the biodegradation of lipids. In order to

improve good contact between bacteria and lipids in the anaerobic digester, rigorous mixing is needed. Since, microcosms in this study were incubated statically, floatation of solids with oil was observed during the incubation period, which might have been caused a low contact of bacteria and materials. As a result, inherent characteristics of mineral oil, high concentration, and no mixing might be the possible reasons for observing lower methane productions in oil receiving microcosms.

A similar effect was observed in the other anaerobic digester (microcosm) operating parameters such as VS, TS and COD reduction (Figure 8.9). The presence of oil in the microcosm reduced the loss of TS, VS and COD in most of the microcosms and the effect of oil on the performance parameters was statistically significant ($p<0.05$). In addition, the effect of the presence of PCB 118 /Aroclor-1254 and presence of mixture of PCB congeners and/or total dose of PCBs was also significant with $p<0.05$.

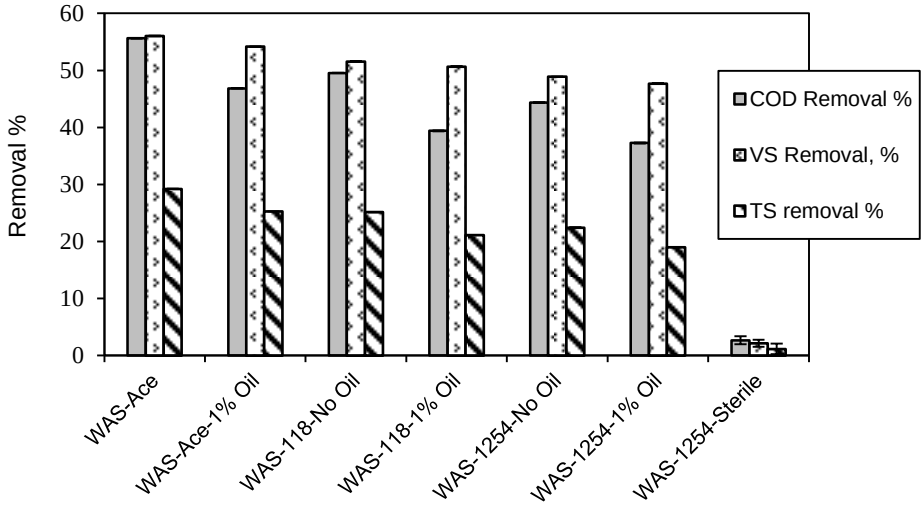

Figure 8.9. Comparison of TS, VS and COD removals of WAS set microcosms (if not seen, standard deviations are very low).

To sum up, addition of TO has affected methane productions to a certain degree and presence of PCBs and/or concentration (50 vs. 100 mg/L) and/or presence of different type of congeners (Aroclor 1254 vs. PCB 118) have a significant negative effect on the digester performances. But, still, the removal percentage range of two important digester performance parameters, COD and VS were between 35-53% and 34-55%, respectively, depending on the presence of oil and PCBs. The results of control microcosms and most of the other PCB/Aroclor microcosms were in line with the typical sludge digester ranges found in the literature 40-60% (Benefield and Randall, 1980).

In addition, the methane yield for an anaerobic digestion process is commonly expressed as a function of either VS or COD reduction. For this study, it was evaluated as function of VS destroyed and the specific gas production was in the range of 883-1116 mL CH_4/g of VS destroyed (Table 8.3) varying with the presence of oil and/or PCBs. These results were also in line with the literature, in which the typical ranges are stated as 750-1120 mL CH_4/g of VS destroyed (Tchobanoglous, and Burton, 2003; Qasim, 1999). In contrast, no biological activity was observed in the sterile reactors. Since no biogas was produced and solid reduction rates were non-significant, it was concluded that the controls were well sterilized and were representative of the abiotic losses.

8.3.3.2. Effect of oil on dechlorination of PCB 118

From Figure 8.10, it can be seen that there was a 30-day lag period in the microcosm containing PCB 118 without oil, while this lag extended up to 90 days in the microcosm with oil. After this lag period, PCB118 started to dechlorinate at a max rate of $18.47\pm0.86\text{x}10^{-03}$ Cl^-/day without oil, which is calculated from the decrease in the total chlorine per biphenyl observed between day 30 and day 60. However, the rate of PCB 118 dechlorination in microcosm with oil was max $2\pm0.9\text{x}10^{-03}$ Cl^-/day, between day 90 and day 120. An obvious decrease in the dechlorination due to presence of oil was observed.

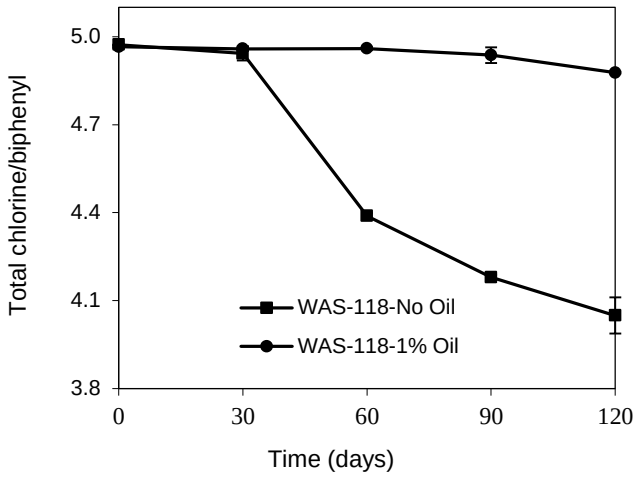

Figure 8.10. Changes in chlorine per biphenyl in WAS-118 microcosm with no oil and 1% oil throughout the 120 days of incubation.

In the microcosm without oil, about 52 mol% of PCB 118 (Figure 8.11a) was dechlorinated first to PCB 70 (25-34) and PCB 66 (24-34) and these intermediate products were further dechlorinated to PCB25 (24-3), PCB 26 (25-3), and PCB 28 (24-4)/31(25-4) throughout the 120 days of incubation (Figure 8.11c). In the microcosm with oil, only about 9.4 mol% of PCB 118 (Figure 8.11b), was dechlorinated to PCB 66, 70, and 74 (245-4). The latter congener was not detected in microcosm without oil. One possibility for detecting PCB 74 in the WAS-118-1% oil microcosm could be the fact that as soon as PCB 74 dechlorinated from PCB 118, it was immediately dechlorinated to PCB 31 (25-4) (Figure 8.11d), which was detected in both of the microcosms.

The lower overall rates observed in oil microcosm supports this conclusion. By using findings of dechlorination by-products analysis, dechlorination pathways observed in PCB 118 microcosms was determined and is presented in Figure 8.12. Analysis of pathways together with the presence of flanked/*para* dechlorination by-products of PCB 118 shows that PCB 118 microcosms exhibited reductive dechlorination either in flanked *meta*- or *para*- position or both.

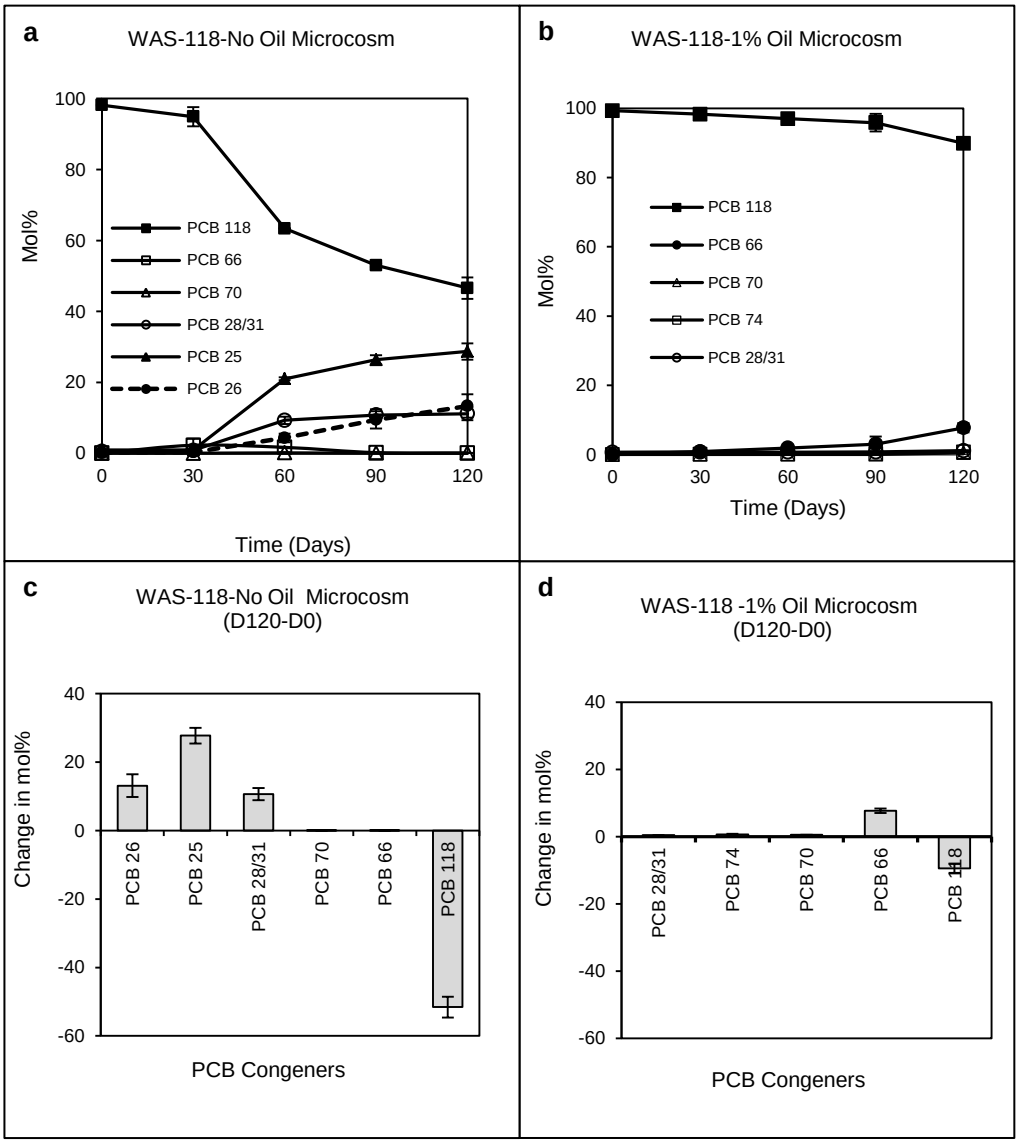

Figure 8.11. Dechlorination of PCB 118 and occurrence of by products throughout the 120 days of incubation in a) WAS-118-no oil and b) WAS-118-1% oil microcosm and Changes in mol % of PCB 118 congeners after 120 days of incubation in c) WAS-118-no oil and d) WAS-118-1% oil microcosm.

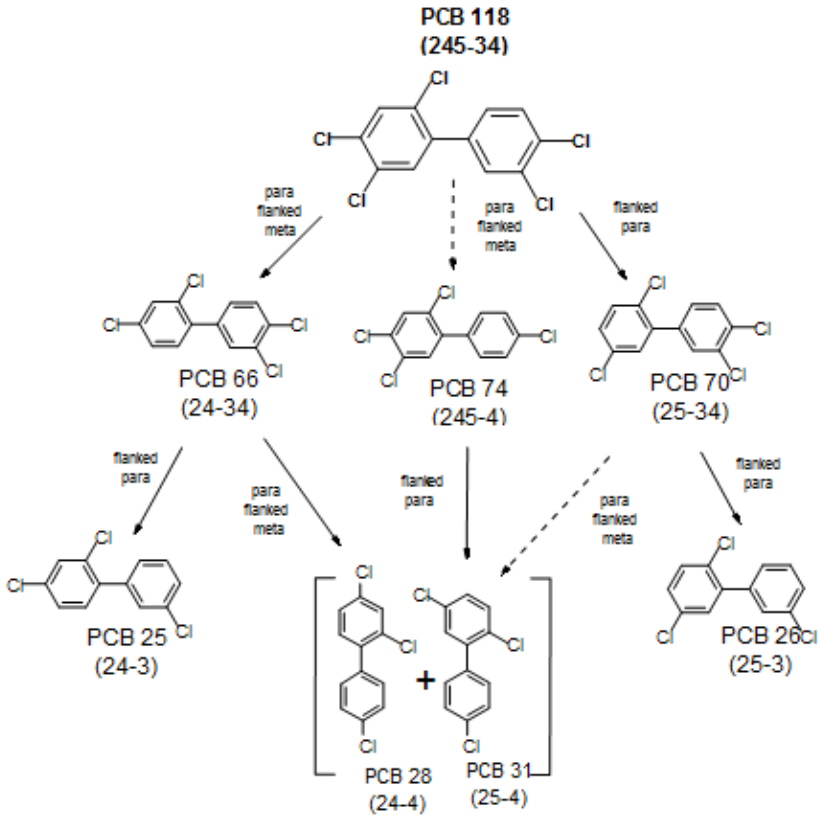

Figure 8.12. Dechlorination pathways of PCB 118 (245-34). Black solid arrows indicate main pathway, while dashed arrow shows minor pathways.

8.3.3.3. Effect of oil on dechlorination of Aroclor 1254

Dechlorination of Aroclor 1254 in waste activated sludge with or without oil was investigated. The removal of chlorines at the *ortho*, *meta*, and *para* positions, as well as removal of total chlorines per biphenyl, shown as the average number of chlorines per biphenyl residue in the GR cultures with the presence of WAS spiked with Aroclor 1254, is illustrated in Figure 8.13. No lag was observed in the dechlorination of Aroclor 1254 without oil. In contrast, the dechlorination rate was very low during the first 30 days of incubation in the presence of oil (Figure 8.13a), indicating a lag.

The highest dechlorination rate, 18.8×10^{-03} total Cl^-/day was observed within the first 30 days of incubation in the microcosm without oil, while it was much lower in the

microcosm with oil, $1.7x10^{-03}$ total Cl^-/day, indicating a negative effect of oil on dechlorination similar to PCB 118 case. After the first 30 days of incubation, the overall dechlorination rate of Aroclor 1254 with oil reached that of the no oil microcosm, about $8.3x10^{-03}$ total Cl^-/day and $8.7x10^{-03}$ total Cl^-/day, respectively. The initial average chlorination degree of Aroclor 1254 PCBs was reduced by about 25% (i.e., from 5.24 to 3.95 total chlorines/biphenyl) in no oil microcosms and 16% (i.e., from 5.24 to 4.40 chlorines per biphenyl) in 1% oil microcosm during 120 days of incubation (Figure 8.13a).

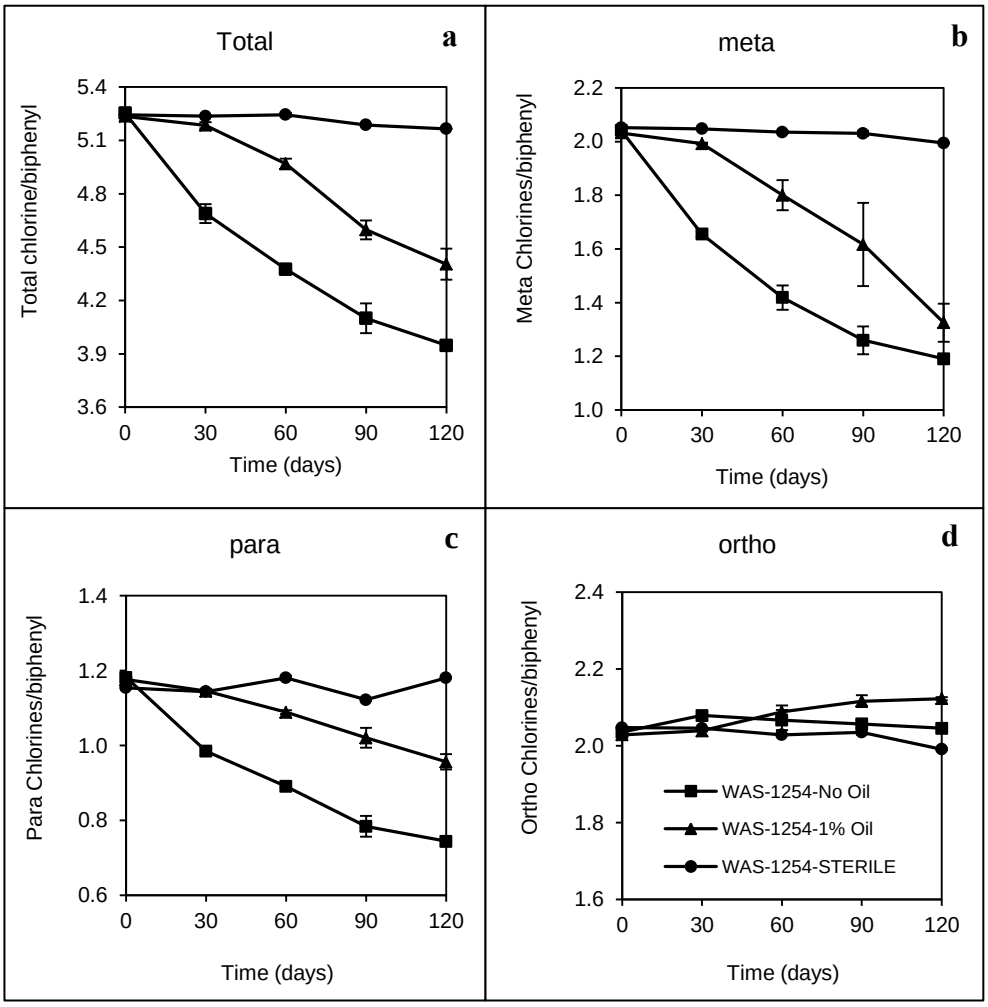

Figure 8.13. Dechlorination of Aroclor 1254 with different treatments calculated as a) total, b) meta, c) para and d) ortho chlorines per biphenyl throughout the 120 days of incubation.

Dechlorination was mostly through removal of the *meta* chlorines (Figure 8.13b). The overall removal percentages of *meta* chlorine were very close to that of *para* chlorines in both of the biotic microcosms. About 41% of *meta* chlorines and 37.5% of *para* (Figure 8.13c) chlorines were removed in the no oil microcosm. Also, by about 80% of doubly flanked chlorines and by about 74% of single flanked chlorines were removed in no oil microcosm of Aroclor 1254. On the other hand, about 35% of *meta* chlorines and 19% of *para* chlorines were removed in 1% oil microcosm. About 53% of doubly flanked and about 54% of singly flanked chlorine removals were achieved in oil receiving microcosms of Aroclor 1254 (Figure 8.13). Dechlorination resulted in the accumulation of unflanked congeners. There was no significant *ortho* dechlorination in the biotic microcosms, while there was negligible amount of *ortho* dechlorination (2.7%) in sterile controls after about 90 days of lag (Figure 8.13d), which was statistically insignificant ($p>0.05$).

Figure 8.14 shows the distribution of homolog chlorines observed in oil and no oil microcosms, showing dechlorination was achieved by the removal of chlorines mostly from penta-, followed by hexa- and hepta-chlorinated congeners at about 81%, 77%, and 77%, respectively in no oil microcosm (Figure 8.14a). On the other hand, in the 1% oil microcosm, dechlorination was achieved mostly from penta- followed by hexa-chlorinated congeners, at 67% and 48%, respectively (Figure 8.14b). The dechlorination products of highly chlorinated congeners were tetra- and tri-chlorinated congeners in both microcosms at varying levels.

Since dechlorination of highly chlorinated PCB congeners results in the production of less chlorinated congeners, the biological dechlorination rate for the lightest PCBs cannot be easily examined in Aroclor spiked microcosms due to simultaneous production and degradation. Similar observations were made by others in the literature (Brown *et al.*, 1987; Quensen III *et al.*, 1990; Wu *et al.* 1998). Moreover, Figure 8.15 shows the dechlorination trend in WAS set in all live Aroclor 1254 microcosms based on single congener analysis. Since this figure shows the differences between the beginning and the end of incubation, highly dechlorinated congeners and resultant congeners of dechlorination can be seen from Figure 8.15.

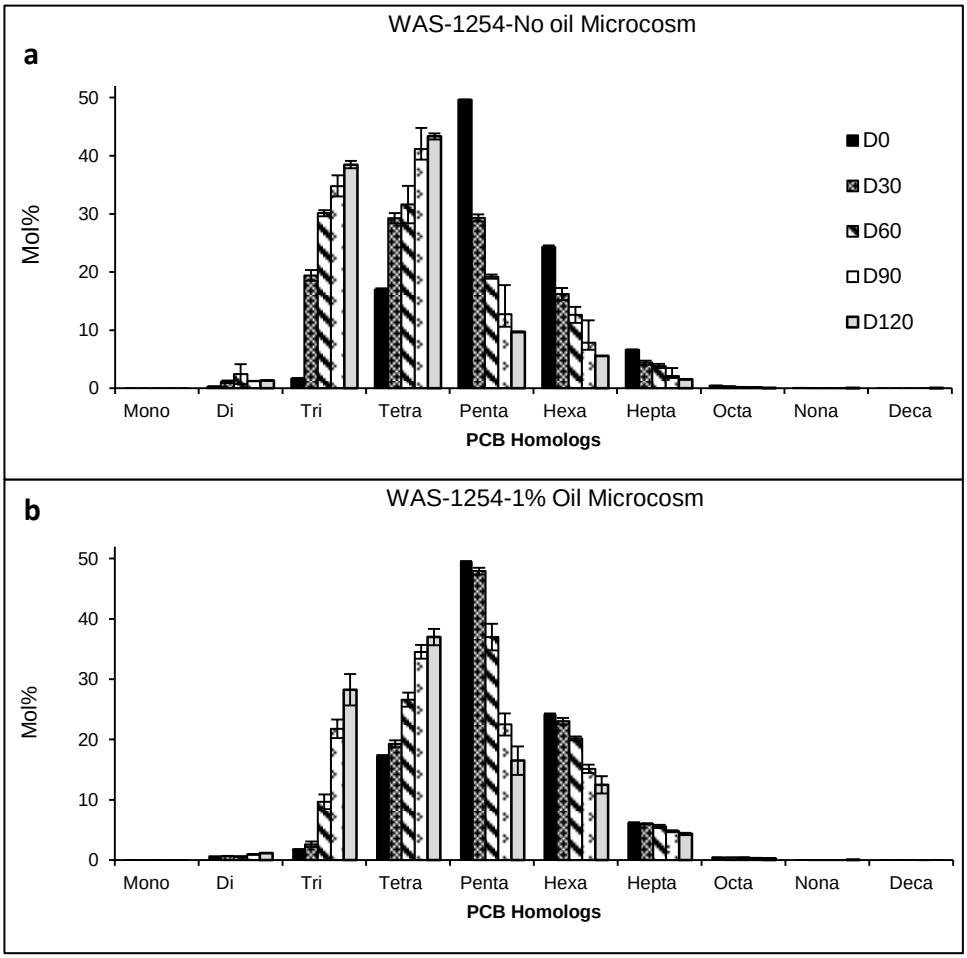

Figure 8.14. Homolog distributions of a) WAS-1254-No Oil and b) Was-1254-1% Oil microcosm throughout the 120 days of incubation

Additionally, Table 8.4 and Table 8.5 were prepared in order to show the net trend in Aroclor 1254 microcosms and to summarize highly dechlorinated PCB congeners and their removal percentages and highly accumulated by-products, respectively. Clearly, almost the same congeners were involved in the dechlorination of Aroclor 1254, whether oil was present or not, but, the dechlorination extent of these congeners was slightly different. For example, the highly chlorinated congener couple, PCB 180/193, decreased by 0.6 mol% in microcosms without oil, which is equivalent to 74.3±4.6% removal of this congener within the 120 days of incubation. The decrease observed for the same congener was 0.1 mol% with oil, which is equivalent to 15.2±4.1% removal in the same time course. In contrast, some of the

less chlorinated congeners were dechlorinated to a greater extent. For example, PCB110, 101 and 118 were the top three congeners, which were highly dechlorinated in both microcosms by 87.2±3.2, 85.7±3.5, and 75.2±5.9 mol%, respectively without oil, while the removal observed for the same congeners with oil were 86.7±5.8, 77.3±9.3, and 64±12 mol%, respectively. The predominant dechlorination products of Aroclor 1254 after 120 days of incubation were PCB 47 (24-24) at 11.2±0.2 mol%, and PCB 49 (24-25) at 10.6±0 mol% in microcosm without oil. In contrast PCB 49 was at 9.7±0.6 mol% and was PCB 28/31 at 7.3±1.1 mol% in microcosm with oil.

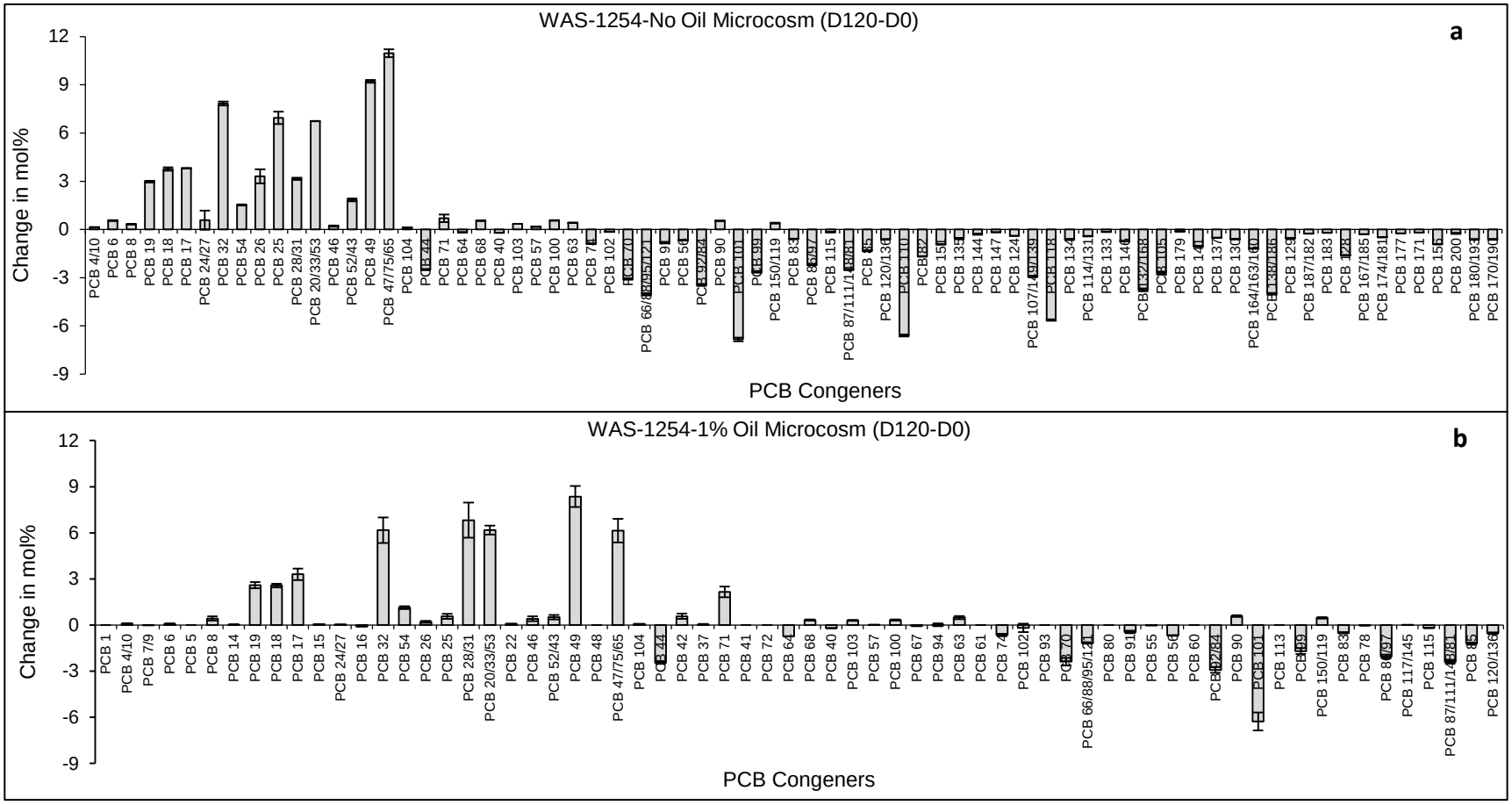

Figure 8.15. Changes in mol percent of Aroclor 1254 congeners after 120 days (D120) of incubation in WAS microcosm spiked with Aroclor 1254 in the absence of oil throughout the 120 days of incubation.

Table 8.4. Highly dechlorinated congeners in Phase 2 live microcosms with Aroclor 1254.

WAS-1254-No Oil				WAS-1254-1% Oil			
Highly Dechlorinated Congeners	**Initial mol%**	**Final mol%**	**Removal %**	**Highly Dechlorinated Congeners**	**Initial mol%**	**Final mol%**	**Removal %**
PCB 101	7.8±0	1.1±0.2	85.7±3.5	PCB 110	7.2±0	0.9±0.3	86.7±5.8
PCB 110	7.4±0	0.9±0.1	87.2±3.2	PCB 101	8.1±0	1.8±0.5	77.3±9.3
PCB 118	7.2±0	1.7±0.3	75.2±5.9	PCB 118	7.1±0	2.5±0.5	64±12
PCB 66/88/95/121	4.6±0	0.6±0	85.9±3.2	PCB 92/84	4.2±0	1.3±0.1	68.1±6.5
PCB 138/186	5.2±0	1.3±0.2	73.9±5.5	PCB 105	2.9±0	0.4±0.1	86.4±4.9
PCB 132/168	5.2±0	1.5±0.2	69.7±6.2	PCB 44	2.5±0	0.1±0	94.2±3
PCB 92/84	4.1±0	0.7±0	81.8±2.8	PCB 87/111/148/81	2.6±0	0.2±0.1	91±4.8
PCB 70	3.3±0	0.2±0	91±2.8	PCB 70	3.3±0	0.9±0.2	70.7±10.4
PCB 107/149/139	3.7±0	0.8±0.1	76.7±4.6	PCB 132/168	5.1±0	3±0.2	41.2±13.7
PCB 105	3±0	0.3±0.1	87.2±4.7	PCB 107/149/139	3.6±0	1.5±0.2	56.9±10.2
PCB 99	3.2±0	0.6±0	78.9±3.6	PCB 86/97	2.3±0	0.3±0.1	85.8±6.1
PCB 44	2.6±0	0.1±0	95.5±2.1	PCB 138/186	4.2±0	2.2±0.1	47.4±10.5
PCB 87/111/148/81	2.7±0	0.2±0	89.9±2.8	PCB 99	3.3±0	1.6±0.1	50.2±11
PCB 86/97	2.4±0	0.3±0	86.9±2.9	PCB 82	1.7±0	0±0	94.9±3.6
PCB 82	1.7±0	0.1±0	93±2.3	PCB 85	1.3±0	0.1±0	86.8±5.9
PCB 128	1.9±0	0.3±0	81±3.9	PCB 128	1.9±0	0.7±0.1	60.5±9.6
PCB 85	1.4±0	0.1±0	89.2±2.7	PCB 66/88/95/121	4.7±0	3.6±0	24.3±3.6
PCB 164/163/160	1.5±0	0.3±0	75.1±5.2	PCB 64	0.7±0	0±0	100±0.8
PCB 141	1.2±0	0.2±0	78.9±5.8	PCB 56	0.7±0	0±0	95.7±3.9
PCB 156	1.1±0	0.2±0	80.4±4	PCB 164/163/160	1.5±0	0.8±0	42.3±12.5
PCB 74	0.9±0	0.1±0	89±2.6	PCB 74	1±0	0.3±0	63.9±12.2
PCB 56	0.6±0	0±0	96.8±1.8	PCB 141	1.2±0	0.6±0	46.4±14
PCB 180/193	0.8±0	0.2±0	74.3±4.6	PCB 180/193	0.8±0	0.7±0	15.2±4.1

The pattern of Aroclor 1254 dechlorination with GR sediment culture resembles process N and process H/H' (Quensen III *et al.*, 1990; Bedard and Quensen III, 1995; Bedard, 2003). Process N was first identified in Aroclor 1260 microcosms from Silver Lake sediments (Quensen III *et al.*, 1990) and described as exclusive dechlorination in the *meta* position from both flanked and doubly flanked positions with a characteristically high accumulation of 2,2',4,4'-CB (PCB 47) (Bedard and Quensen III, 1995). This pattern has been previously observed in sediment microcosms from several PCB impacted freshwater sources, including Woods Pond

(Bedard and May, 1996), Hudson River (Rhee *et al.*, 1993), sediment-free microcosms developed from the Housatonic River (Bedard *et al.,* 2006), and Baltimore Harbor sediment microcosms inoculated with Aroclor 1260 (Fagervold *et al.*, 2007).

Table 8.5. Highly accumulated congeners in Phase 2 live microcosms with Aroclor 1254 set.

WAS-1254-No Oil			WAS-1254-1% Oil		
Accumulated congeners	Initial mol%	Final mol %	Accumulated congeners	Initial mol%	Final mol %
PCB 47/75/65	0.2±0	11.2±0.2	PCB 49	1.37±0	9.7±0.6
PCB 49	1.4±0	10.6±0	PCB 28/31	0.48±0	7.3±1.1
PCB 32	0±0	7.8±0.1	PCB 20/33/53	0.36±0	6.5±0.2
PCB 25	0.1±0	7.1±0.3	PCB 32	0.06±0	6.2±0.8
PCB 20/33/53	0.3±0	7.1±0	PCB 47/75/65	0.25±0	6.4±0.7
PCB 17	0±0	3.9±0	PCB 17	0.09±0	3.4±0.3
PCB 18	0.2±0	4±0	PCB 19	0.05±0	2.6±0.2
PCB 26	0±0	3.3±0.4	PCB 18	0.25±0	2.8±0.1
PCB 28/31	0.4±0	3.6±0	PCB 71	0.25±0	2.4±0.3
PCB 19	0±0	3±0	PCB 54	0.02±0	1.1±0
PCB 52/43	4.5±0	6.4±0	PCB 90	0.29±0	0.8±0
PCB 54	0±0	1.5±0	PCB 42	0.25±0	0.8±0.1

Process H’ is the removal of both *meta* and *para* chlorines, but only if these chlorines are flanked. Alder *et al.* (1993) reported dechlorination pattern H’ towards the preexisting Aroclor 1242 PCBs in New Bedford Harbor sediments. Also, in the study of Ye *et al.* (1992) it was reported that methanogenesis occurred concurrently with process H (*meta*-, *para*-) dechlorination of Aroclor 1242. Other studies have also reported pattern H/H’ as being a frequent dechlorination pattern (Imamoglu *et al.*, 2004; Zanaroli *et al.*, 2006 and 2012; Christensen *et al.*, 2008). The findings of this study are in line with the previous studies.

Oefjord *et al.* (1994) studied Aroclor 1254 dechlorination in semi-continuously electron donor (chitin)-fed anaerobic marine sediment cultures. Aroclor concentration was the same with this study, 100 mg/L, but feeding was different. Chitin was fed once a week at a loading rate averaging 0.1 g/L.d, but without liquid replacement. They had 4 months of lag after which about 14-15% *meta* and 9-10 %of *para* chlorines were removed over a period of 1 year with the accumulation of mainly tetrachlorobiphenyls. The extent of dechlorination was 7-8%. While in this study, much higher removals were achieved in much shorter time with no lag period such as about 41% of *meta* chlorines (Figure 8.13b) and 37.5% of *para* (Figure 8.13c) chlorines were removed in the no oil microcosm. Also, by about 80% of doubly flanked chlorines and by about 74% of single flanked chlorines were removed in no oil microcosm of Aroclor 1254. While about 35% of *meta* chlorines and 19% of *para* chlorines were removed in 1% oil microcosm, the dechlorination extent was about 25% in no oil microcosms and 16% within 1% oil microcosms during the 120 days of incubation. The reason for the removal differences achieved in this study and that of Oefjord *et al.* (1994) could be due to the supply of different electron donors and inoculum despite that the use of enriched culture in both studies. In addition, they incubated their microcosms at 20ºC while in this study, it was 30ºC since it is known that temperature has a significant effect on the growth and the physiological activity including uptake and enzymatic dehalogenation of PCB congeners (Wiegel and Wu, 2000).

In a recent study, Zanaroli *et al.* (2012) achieved about 74% removal of the overall initial amount of penta-, hexa and hepta-chlorinated PCBs of Aroclor 1254, while we observed penta-, followed by hexa- and hepta-chlorinated congeners at about 81%, 77%, and 77%, respectively in no oil microcosms (Figure 8.14a). The overall dechlorination extent of the study of Zanaroli *et al.* (2012), 20%, was also lower than that of this study. By products of both studies were about the same with but there were some final PCB congeners with flanked *para* or *meta* chlorines accumulated in the study of Zanaroli *et al.* (2012) which was not the case in this study. Also, in another study, 59.4-83.5% and 33.0-58.0% total PCB removal (PCBs 28, 52, 101, 138, 153, and 180) in anaerobic sludge digester under thermophilic and mesophilic

conditions, respectively, was reported. In that study, total initial PCB concentration was 0.5 ppm (Benabdallah El-Hadj *et al.*, 2007).

An interesting result was reported by Nakhla *et al.* in 2002. They investigated the application of aerobic and anaerobic treatment for removal of PCBs in sludges generated from a groundwater treatment system. PCB concentration was 520 ppm, with Aroclors 1254 and 1260 accounting for 26 and 74%, respectively, as well as total organic carbon (TOC), and oil and grease concentrations of 108, 500 and 18,600 mg/L, respectively. They reported by 73.4 to 88.0% (under aerobic conditions with different treatment) and 64.7 to 80% (under anaerobic conditions with different treatments) reduction in PCB concentrations on a volumetric and solids basis, respectively, over a period of 60 days. Furthermore, their aerobic PCB removal efficiency was as high as 88.7% for both Aroclors 1254 and 1260. They found that anaerobic PCB removal efficiencies were generally 12 to 24% lower than the best aerobic treatment. No analysis for intermediate metabolites of PCBs was carried out in that study and the initial TSS concentration was 68,800 mg/L (more than 3 times of TSS of this study). The degradation reported in the study of Nakhla *et al.* (2002) together with no dechlorination byproduct and extremely high solid concentration as well as TOC might indicate adsorption, rather than reductive dechlorination as the dominant PCB removal mechanism. Also, Chang *et al.* (1999) have previously shown that higher solid concentration causes higher rate of PCB adsorption in sludge.

To sum up, in this study, about 41% of *meta* chlorines and 38% of *para* chlorines were removed in the no oil microcosm, while about 35% of *meta* chlorines and 19% of *para* chlorines were removed in 1% oil microcosm. Therefore, the abundance of highly chlorinated congeners decreased while that of lower chlorinated, *ortho* substituted congeners increased by the preferential removal of flanked *meta* and *para* substituted chlorines. Since the *meta* and *para* chlorines were removed from the congeners, the coplanar structure, and hence, the potential dioxin-like toxicity of the congeners in Aroclor 1254 was reduced (Abramowicz, 1995; Quensen III *et al.*, 1998). In addition, the subsequent lower chlorinated congeners can be degraded by

aerobic bacteria, allowing for the complete biodegradation of PCBs into carbon dioxide and water via sequential anaerobic and aerobic microbial degradation processes (Bedard and Quensen III, 1995).

8.3.4. Enumeration of dechlorinating phylotypes in Phase 2 microcosms

Microorganisms were enumerated in WAS microcosms by quantitative PCR (qPCR) using a specific primer set for phylotypes closely related to known PCB dechlorinators in order to determine whether dechlorination of PCB 118 and Aroclor 1254 was growth-linked. Amplification efficiencies of dilutions of gel purified DF1 16S rRNA gene PCR product used as standard for all sets were 95.1±7.2% with the r^2=0.999 (Figure 8.16) showing high reliability. Amplification efficiencies of samples were 87.4±4.1%.

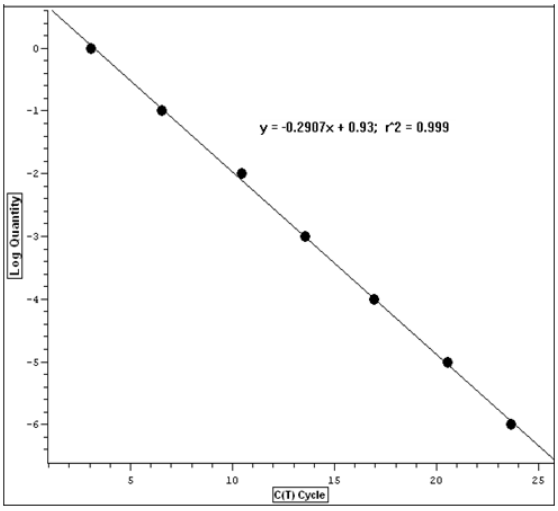

Figure 8.16. Standard curve used for enumeration of dechlorinating phylotypes in Phase 2 microcosms.

The number of 16S rRNA gene copies of putative dechlorinators per mL of microcosm slurry from WAS showed that the number of putative dechlorinators increased in all microcosms of WAS set, except for sterile control, as time proceeded

(Figure 8.17). After 120 days of incubation, the number of putative PCB dechlorinating phylotypes increased 49-fold (from $5.10x10^{+05}\pm3.40x10^{+04}$ to $2.46x10^{+07}\pm1.26x10^{+06}$) in microcosms spiked PCB 118 with no oil compared to time zero. On the other hand, there were about 21-fold increase in microcosms with oil. For Aroclor containing microcosm, the trend was the same; the numbers increased linearly for both no oil and oil containing microcosms with 80-folds (from $6.29x10^{+05}\pm6.09x10^{+04}$ to $5.02x10^{+07}\pm9.70x10^{+06}$) and 88-fold (from $5.55x10^{+05}\pm 2.25x10^{+05}$ to $4.88x10^{+07}\pm3.82x10^{+07}$) during 120 days of incubation, respectively. To determine whether presence of oil has a significant effect on the numbers, a statistical evaluation was performed by using Student's *t*-test and the results showed that the effect of 1% oil on the number of PCB dechlorinating phylotypes was insignificant ($p>0.05$).

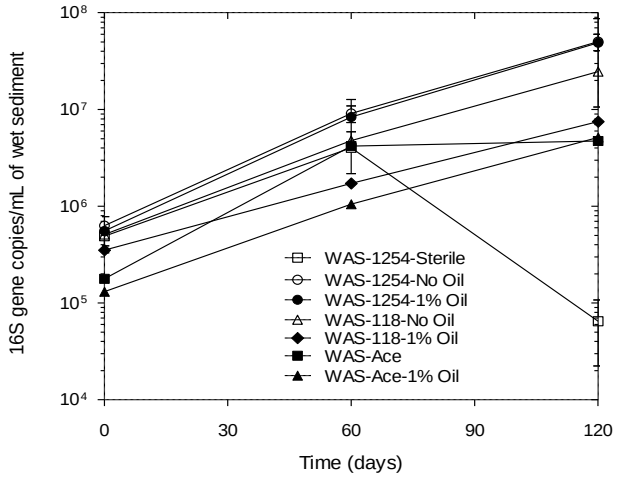

Figure 8.17. Enumeration of PCB dechlorinating phylotypes in Phase 2 microcosms.

There was a low increase in their numbers of putative dehalogenators in no-PCB, acetone spiked control microcosms (Figure 8.17), but this is likely since the original wet GR sediments were also used as inoculum in these microcosms and since GR sediment was historically contaminated with PCBs. Hence, traces of PCB co-transferred/the presence of PCBs coming from sediment into no-PCB controls during inoculation might be the possible reason.

8.3.5. Community analysis of PCB dechlorinating bacteria in Phase 2 microcosms by denaturing High Pressure Liquid Chromatography

Numerous studies have described PCR-based 16S rRNA gene assays for monitoring indigenous dehalogenating communities within the phylum *Chloroflexi*, including *Dehalococcoides* sp. (Hendrickson *et al.*, 2002; Fagervold *et al.*, 2005). A unique feature of the genus *Dehalococcoide*s and other species within the phylum *Chloroflexi* is the capability to respire by reductive dehalogenation of PCBs as well as other polychlorinated aromatic compounds. *Dehalococcoides mccartyi* strain 195, *Dehalobium chlorocoercia* DF-1, *Dehalococcoides sp.* strains CBDB1, BAV1 and FL2 are among them which are well known and characterized. *D. mccartyi* clusters within the Cornell clade. It is a pure bacterial strain which uses PCE, TCE and *cis* -DCE as metabolic electron acceptors (Major *et al.*, 2002) and it has been this strain that dechlorinated 2,3,4,5,6-pentachlorobiphenyl and other aromatic organochlorines (Fennell *et al.*, 2004). *D. chlorocoercia* (DF-1) is also a pure strain which grow by respiratory dechlorination of several PCB congeners (Wu *et al.*, 2002; May *et al.*, 2008*)*). Strain DF-1 is also a member of the dechlorinating *Chloroflexi*, as are *Dehalococcoides* species. Also, higher rates of dechlorination of some PCB congeners were found in enriched cultures containing strains o-17 (Cutter *et al.*, 2001) and DF-1 (Wu *et al.*, 2002). *Dehalococcoides sp.* strains CBDB1, BAV1 and FL2 cluster within the Pinellas clade and it has been shown that they have an active role on the dechlorination of a wide range of chloroorganic compounds (Fennell *et al.*, 2004; Kube *et al.*, 2005; Liu and Fennell, 2008). Recently, Adrian *et al.* (2009) reported that *Dehalococcoides* sp. strain CBDB1 dechlorinated a wide range of PCB congeners with three to eight chlorine substituents in Aroclor 1260 mixture through process H.

Therefore, to characterize microbial consortia in PCB dechlorinating culture of the sludge microcosms inoculated with GR sediments, molecular screening of the 16S rDNAs from the total community of genomic DNAs was used. To determine whether there was a relationship between differences in the dechlorination activity or congener distribution and the composition of indigenous dechlorinating bacterial

communities, DNA was extracted and analysed by DHPLC to characterize the community profiles of putative dechlorinating phylotypes. Specific primers used in this study target only putative dechlorinating bacteria within *Chloroflexi* since only these phylotypes have been confirmed up to date for reductive dechlorination of PCBs (Wu*et al.*, 2002; Fennell*et al.*, 2004; Fagervold*et al.*, 2005, 2007, and 2011; Payne *et al*, 2011). Also because dehalogenators typically represent a small fraction of the total bacterial community (Major *et al.* 2002; Lendvay *et al.* 2003; Amos *et al.* 2008) and they often cannot be detected with "universal primers" the aforementioned specific group was targeted.

DHPLC analysis was conducted for time zero and time final of the WAS microcosms. Initial screening results for these samples are shown in Figure 8.18. In all microcosms the initial diversity of phylotypes detected by DHPLC was low, but after 120 days of incubation diversity increased (Figure 8.18a). It can be seen from Figure 8.18 that microbial diversity has changed from day 0 to day 120 (final day). Some phylotypes were enriched and others appeared which were not detected at day 0, possibly because they were below the detection limit. Almost the same DHPLC profiles were observed for different treatments of this set, for example, for microcosms with no oil, the DHPLC profiles of PCB 118 inoculated microcosm (Figure 8.18b as WAS-118-Noil-D120) and Aroclor 1254 (WAS-1254-Noil-D120) were exactly the same, indicating same phylotypes were enriched in both microcosms. Although DHPLC profiles observed at the final day of incubation (d120) for PCB 118 without oil (green line) and PCB 118 with oil (red line) looked different between 4^{th} min and 6^{th} min (Figure 8.18a), it was concluded they were possibly the same phylotypes since both of the microcosms were inoculated with the same culture, but the presence of oil interfered during the PCR amplification and reduced the detection of some phylotypes.

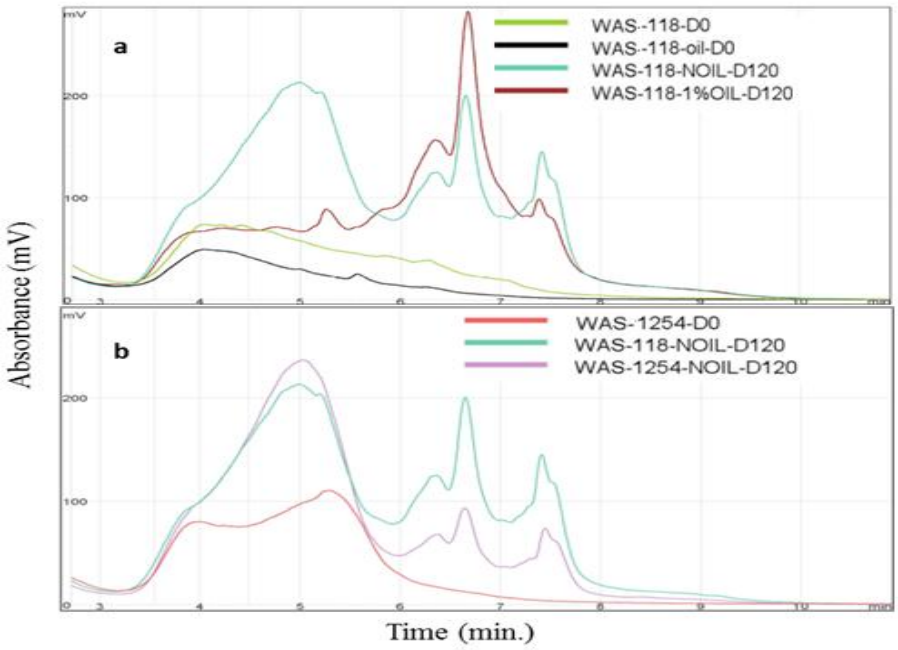

Figure 8.18. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in WAS set a) PCB 118 microcosm and b) Aroclor 1254 microcosms results both no oil and oil cases at day 0 and day 120. In figure b, D0 and D120 profile of WAS-1254-1 % oil is not given, but it was exactly the same to that of WAS-1254-No oil (shown as WAS-1254-D0 and WAS-1254-Noil-D120 in the legend, respectively).

Bacterial community analysis of PCR-amplified 16S rRNA gene fragments revealed different phylotypes in the microcosms (Figure 8.18). Since the aim was to determine the enriched phylotypes which are responsible for the dechlorination, after the initial screening of initial and final samples through DHPLC, fragments to be collected were selected and PCR-amplified 16S rRNA gene fragments collected from DHPLC for day final. Day final DHPLC fragments, which were collected and further processed for sequencing, are shown in Figure 8.19.

Blast results of sequences showing the highest matches with the publicized organisms are given in Table 8.6. Sequences obtained from these ten fragments were used in the phylogenetic tree (Figure 8.20) together with the well-defined organisms to show the relationships between the phylotypes of this study and the closest dechlorinating species within the dechlorinating Chloroflexi group.

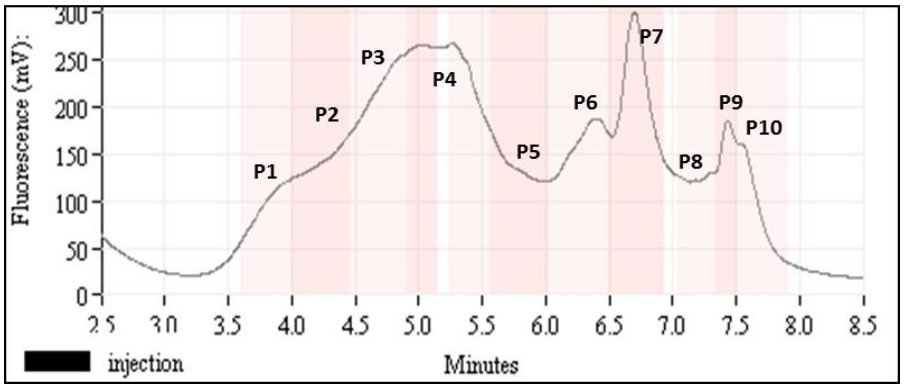

Figure 8.19. DHPLC community analysis of putative dechlorinating Chloroflexi 16S rRNA genes in WAS set microcosms at day final. The phylotypes of putative dechlorinating Chloroflexi labeled as P1-P10

Table 8.6 DHPLC phylotypes identified in sludge microcosms inoculated with GR sediment culture. BLAST match with greatest identity is shown

DHPLC Phylotype name	**% identity (nucleotides, base pair)**	**BLAST Match (Gene bank accession number)**
P1	99 (513)	Dehalococcoides sp. JN18_V108_B 16S ribosomal RNA gene, partial sequence (EF059530.1)
P2	99 (513)	Dehalococcoides sp. CBDB1 16S ribosomal RNA gene(AF230641.1) and also Dehalococcoides sp. FL2 16S ribosomal RNA gene(AF357918.2)
P3	97 (445)	Dehalococcoides sp. enrichment culture clone GD-B-1 16S ribosomal RNA gene, partial sequence (HQ122956.1) and Uncultured Dehalococcoides sp. clone DEH10 16S ribosomal RNA gene, partial sequence (DQ021869.1
P4	98 (515)	Dehalococcoides sp. JN18_V12_B 16S ribosomal RNA gene, partial sequence (EF059528.1)
P5	100 (501)	Dehalococcoides sp. JN18_V12_B 16S ribosomal RNA gene, partial sequence (EF059528.1), and it also matches to all the others above.
P6	96 (503)	Uncultured bacterium clone Er-MS-19 16S ribosomal RNA gene, partial sequence (EU542435.1)
P7[1]	98 (432)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1)
P8[1]	99 (493)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1)
P9[1]	98 (491)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1)
P10[1]	98 (492)	Uncultured Chloroflexi bacterium clone JBS_E336 16S ribosomal RNA gene, partial sequence (EU702900.1)

[1] These phylotypes also were matched to uncultured bacterium clone Er-MS-19 16S ribosomal RNA gene, partial sequence (EU542435.1) with sequence similarities ≥ 96%.

The sequences which were related to both the *Dehalococcoides* and DF-1/*o*-17 clade with sequence similarities ranging between 96%-100% were detected in the indigenous dechlorinating communities of WAS microcosm inoculated with GR sediments. The first five of ten phylotypes of this study (P1-P5) were closely related to *Dehalococcoides* (Figure 8.20) with sequence similarities ≥97% (Table 8.6). The identified phylotypes had high 16S rRNA sequence similarity to all of the known PCB dechlorinating bacteria, even though not all of the putative dechlorinating bacteria identified were necessarily involved in PCB dechlorination. These findings are in agreement with the findings of Kjellerup *et al.* (2008); they also reported 5 phylotypes closely related to *Dehalococcoides* in GR sediments.

However, the remaining five phylotypes of this study (P6-P10) were not closely related to either *Dehalococcoides spp.* or o-17/DF-1 clade, since the distance of these five phylotypes was far from these groups as shown in phylogenetic tree (Figure 8.20), but, they were within the phylum of Chloroflexi as *Dehalococcoides* are. Also, the sequences of these phylotypes were closely related to an uncultured bacterium clone (Er-MS-19 16S ribosomal RNA gene) with high sequence similarities (≥96%), which was identified during the dechlorination of coplanar PCBs in sediment slurries. This indicates that these phylotypes (P6-P10) possibly had a role in PCB dechlorination of the GR sediment inoculated sludge microcosms of this study. These results indicated that active PCB dechlorinators were present in the microcosms of this study and were closely related to previously identified PCB dechlorinators.

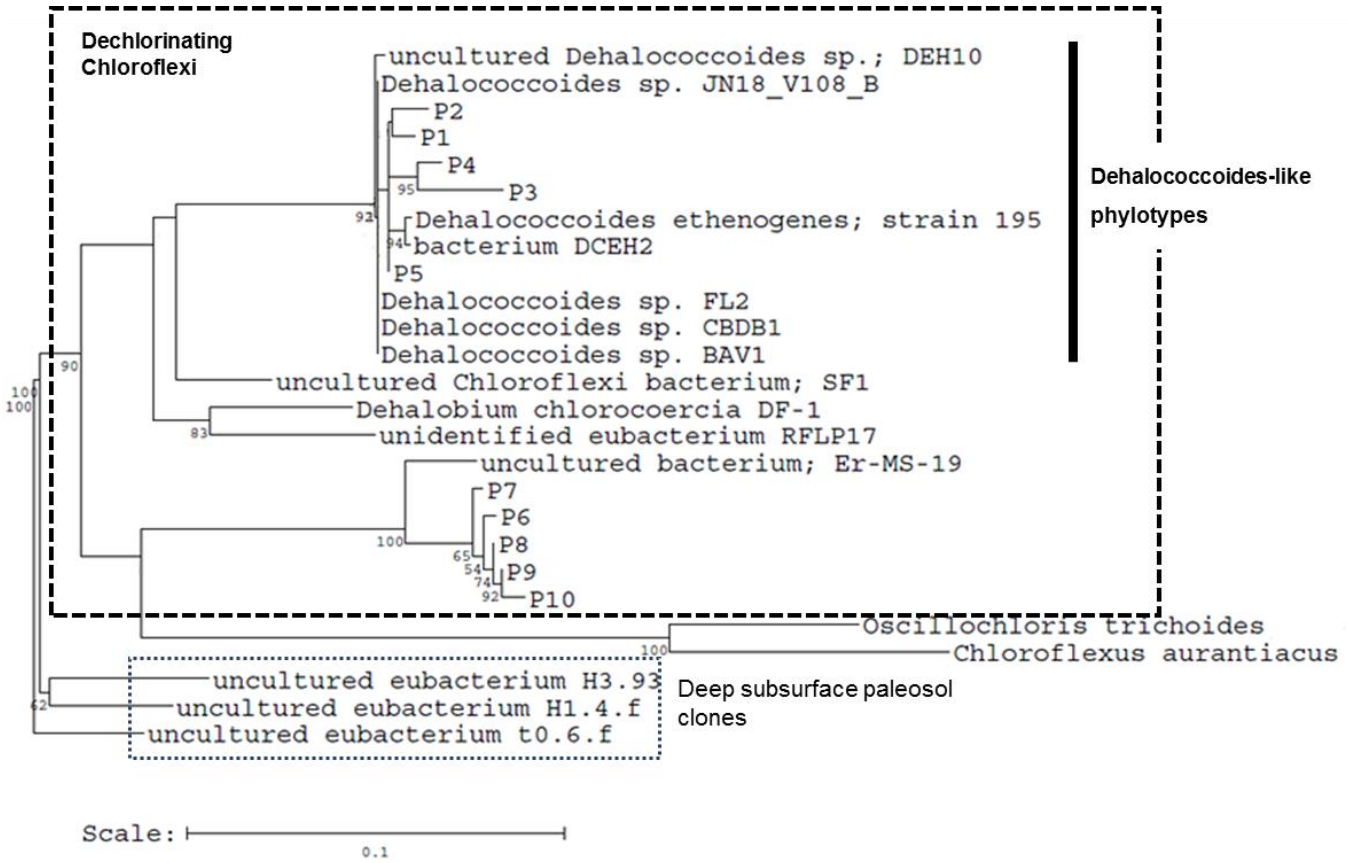

Figure 8.20. Phylogenetic tree showing the relationships between the phylotypes identified in Grasse (GR) River sediments inoculated in WAS set and the closest dechlorinating species within the dechlorinating Chloroflexi group. The tree was generated by the neighbor joining method supported by Ribosomal Database Project (http://rdp.cme.msu.edu/). The scale bar indicates 10 substitutions per 100

8.4. Conclusions

The results showed that the presence of transformer oil had a negative effect on dechlorination of both PCB 118 and Aroclor 1254. This finding was valid for both microcosms inoculated with GR sediment in both of the phases of this study. Transformer oil dose of 10% as total liquid volume completely inhibited PCB dechlorination, while 1% oil resulted in a significant decrease in PCB dechlorination. Oil decreased dechlorination rate and extent, with no significant effect on dechlorination pathways. In no oil PCB 118 microcosm of Phase 1, about 74% of PCB 118 was dechlorinated in 210 days; where 63.3% of this decrease was observed in the first 90 days of incubation. At the end of incubation period, there was about 50% of PCB 118 dechlorination in 1% oil PCB 118 microcosms of Phase 1, about 25% less dechlorination occurred compared to the no oil microcosm. In the WAS microcosm without oil, about 52 mol% of PCB 118 was dechlorinated in 120 days. On the other hand, there was by about 9.4 mol% PCB 118reduction in 1% oil PCB 118 microcosms of Phase 2 (WAS microcosm). In all microcosms of PCB 118, the intermediate products of PCB 118 were similar as PCB 66, 70, and 74 was observed and the final dechlorination products were PCB 25, 26, and PCB 28/31. Analysis of pathways together with the presence of flanked/*para* dechlorination by-products of PCB 118 shows that PCB 118 microcosms exhibited reductive dechlorination either in flanked *meta*- or *para*- position or both PCB.

The overall dechlorination extent of Aroclor 1254 decreased by addition of 1% oil into microcosms from 25% to 16% observed in sludge microcosms. The dechlorination was selectively through mostly flanked *meta* and *para* chlorine removal such as about 41% of *meta* chlorines and 37.5% of *para* chlorines were removed in the no oil microcosm, by about 80% of doubly flanked chlorines and by about 74% of single flanked chlorines were removed in the same Aroclor 1254 microcosms without oil. While about 35% of *meta* chlorines and 19% of *para* chlorines were removed in 1% oil case. Moreover, about 69-92 PCB congeners (changing co-elution assumptions) were dechlorinated and dechlorination was mostly from penta-, followed by hexa- and hepta-chlorinated congeners at about 81%, 77%,

and 77%, respectively in no oil microcosm of sludge set. On the other hand, in the 1% oil microcosm, dechlorination was achieved mostly from penta- followed by hexa-chlorinated congeners, at 67% and 48%, respectively. The dechlorination products of highly chlorinated congeners resulted in the accumulation of unflanked tetra- and tri-chlorinated PCB congeners in both microcosms at varying levels. Almost the same congeners were involved in the dechlorination of 1254, whether oil was present or not, but, the dechlorination extent of these congeners was slightly different. The predominant dechlorination products of Aroclor 1254 after 120 days of incubation were mostly PCB 47, PCB49, PCB 53, PCB 28/31, PCB 32, PCB 17, and PCB 19. No *ortho* dechlorination or unflanked chlorine removal was observed. The pattern of Aroclor 1254 dechlorination with GR sediment culture resembled process N and process H/H'. Dechlorination was growth-linked and the culture dechlorinated. PCB 118, a dioxin-like coplanar PCB congener, as well as Aroclor 1254, with rate and extent higher than those reported so far in the literature. The abundance of highly chlorinated congeners decreased while that of lower chlorinated, *ortho* substituted, congeners increased by the preferential removal of flanked *meta* and *para* substituted chlorines, hence, reducing the dioxin-like toxicity of with the anaerobic dechlorination.

Moreover, the presence and amount of transformer oil showed negative effect on sludge digestion in terms of methane production. Methane production of control reactor was decreased by about 6% with the addition of oil. The addition of PCB 118 resulted in by about 15% reduction in methane production. Other anaerobic digester (sludge microcosm) performance parameters such as VS, TS and COD reduction were also negatively affected. The presence of PCBs and their concentration (50 vs. 100 mg/L) as well as the presence of different type of congeners (Aroclor 1254 vs. PCB118) had an important negative effect on the digester performances.

But, still, the removal percentage range of two important digester performance parameters, such as COD and VS were between 35-53% and 34-55%, respectively, for all sludge microcosms of this study varying with the presence of oil and/or PCBs.

Nevertheless, most of these removal percentages were in line with the typical sludge digester ranges found in the literature 40-60% (Benefield and Randall, 1980).

These results of PCR-based 16S rRNA gene assays for monitoring indigenous dehalogenating communities within the phylum *Chloroflexi*, including *Dehalococcoides species,* together with high rate of dechlorination indicated that active PCB dechlorinators were present in the microcosms of this study and were closely related to previously identified PCB dechlorinators. Despite the unknown metabolic function of the microorganisms, their detection shows that this group has a significant role in the reductive dechlorination of PCBs in sediments.

Findings of this study show that anaerobic digestion is able to dechlorinate single congener and mixture of PCBs. Knowing that PCB concentrations in urban wastewater and sludge ranges between non-detectable to considerably high levels, anaerobic digesters in WWTPs can be considered as a potential step for their removal. Additionally, despite the negative effect of oil, this study was successful in showing significant dechlorination of Aroclor 1254 and PCB 118 along with good methane productions together with acceptable removal rates of TS, VS and COD in sludge microcosms inoculated with GR sediment at high PCB doses. Therefore, for the cases where anaerobic digesters for industrial or sludge treatment is available, preexisting digesters may be used for dechlorination and digestion of already contaminated sludge and/or for dechlorination of PCB contaminated transformer oils. Furthermore, an aerobic treatment following the anaerobic digester can be proposed to further dechlorinate the resultant lightly chlorinated PCB congeners of anaerobic digester to achieve a complete dechlorination

CHAPTER 9

CONCLUSIONS

This study aimed to investigate the degradation of PCBs in the presence of TO under varying experimental components including different electron donors (sludge or fatty acids), inocula (unacclimated or acclimated culture) and the doses of PCB and TO under anaerobic conditions. In the study where five groups of tasks were undertaken, the following conclusions were obtained.

The toxicity of increasing doses of PCB 118 and transformer oil (TO) on anaerobic sludge digestion was investigated in five different sets of ATA reactors operated at four different concentrations of PCB 118 (1, 10, 20, and 30 mg/L) and three different concentrations of TO (0.38, 0.76, 1.52 g/L). It was found that methanogenesis was negatively affected by the increase in PCB 118 dose, while the dechlorination was favored. The presence of TO was found to negatively affect the methane formation, whereas, the increasing oil dose seemed to have no further effect. The effect of TO within the range of this study (0-1.52 g/L) remained unclear. The methane yield range lied between 265-365 mL CH_4/gVS added as a function of PCB dose. Yield significantly decreased with the increase in PCB 118 dose, indicating inhibition to varying extents. No toxicity of PCB-118 at 1 mg/L was observed, while a notable inhibition was observed mostly at 30 mg/L. Also, a negative influence of PCB 118 and TO was observed on COD and solids removal. A maximum of 26.5% PCB 118 removal was attained.

In laboratory scale batch digesters, it was found that digester performance was negatively affected by the presence of PCBs. All the results of anaerobic digester performance parameters were in accordance and provided evidence that the presence of PCB 118 and increase in its dose affected anaerobic digester performance

negatively. The cumulative CH_4 production showed consistent decrease as PCB 118 receiving dose was increased and hence, the highest methane production was observed in PCB-free control reactor with the total production of 10427.4±55.4 mL in 159 days. There was 22% decrease in methane production when PCB 118 dose was 1 mg/L, while 45% decrease was observed in methane productions of laboratory scale reactors with 20 mg/L PCB 118 compared to that of control reactor without PCB 118. Also, as the PCB concentration increased from 1 mg/L to 20 mg/L, indicating the negative effect of PCB 118 presence and its dose. So, as the higher the PCB dose was the lower the methane production obtained.

Considerable tCOD reduction was observed in all biotic reactors, with the highest tCOD removal achieved in PCB free-biotic control at about 57%. This was followed in a decreasing order by 1 mg/L and then by 20 mg/L PCB-118 receiving reactor with about 47% and 39% reductions, respectively. PCB-118 removal was attained at most in 20 mg/L PCB applied reactor by about 22%. These results showed that it was possible to remove PCB 118 in unacclimated cultures, but the performance was limited and could be improved.

To enhance dechlorination performance, enrichment cultures capable of anaerobically dechlorinating PCB 118, and Aroclor 1254 were developed. From the three previously investigated exposed sediments (GR, BH and FR), the highest dechlorination rate of Aroclor 1254 was that of GR sediment microcosms, with $17x10^{-03}$ Cl^-/day. GR was followed by FR sediment microcosms at about $8x10^{-03}$ Cl^-/day. BH sediment microcosms showed the least activity indicated by a very low dechlorination rate of about $0.62x10^{-03}$ Cl^-/day. This might indicate a higher diversity or abundance of native PCB dechlorinating microorganisms in GR and FR sediment compared to BH sediment. An overall 22%, 21.2% and 4.5% reduction in total chlorines of Aroclor 1254 was achieved by GR, FR and BH microcosms, respectively. The dechlorination of Aroclor 1254 resulted in removal of mostly *meta* followed by *para* chlorines.

Further GR sediment cultures were tested for specific dechlorination rates of 22 single PCB congeners and for inferring clear parent-daughter congener relationships. The results of this experiment showed that all, the single congeners, except for PCB 52 and Aroclor 1254 incubated with GR sediment exhibited reductive dechlorination preferentially in *meta* position and to lower extent in *para* position. Dechlorination of *ortho* and unflanked *meta* chlorines was not detected, indicating that these enrichment microcosms selectively dechlorinated doubly- and singly-flanked *meta* chlorines and *para* chlorines.

When FA was used as electron donor in GR sediment microcosm, 74% of PCB 118 was dechlorinated with dechlorination rate at $12.6x10^{-3}$ Cl^-/day in 210 days. About 63.3% of this decrease occurred in the first 90 days of incubation, while in 1% oil microcosms, PCB 118 dechlorination rate was $2.57x10^{-3}$ Cl^-/day with a total of 47 mol% decrease in PCB 118 in 210 days. The effect of 10% oil on the dechlorination of PCBs was detrimental only 2 mol% decrease in PCB 118 was achieved in GR sediment microcosm with 10% oil in 210 days.

Finally when the effectiveness of WAS was tested as an electron donor in comparison to FA in GR sediment microcosms, about 52 mol% of PCB 118 was found to be dechlorinated in 120 days in the absence of TO. On the other hand, there was about 9.4 mol% PCB 118 reduction in the presence of 1% TO. The overall dechlorination extent of Aroclor 1254 decreased from 25% to 16% by the addition of 1% TO into the microcosms. The use of acclimated cultures improved the effectiveness of dechlorination. The dechlorination was through mostly flanked *meta* and *para* chlorine removal such as about 41% of *meta* chlorines and 37.5% of *para* chlorines were removed in the no oil microcosm, by about 80% of doubly flanked chlorines and by about 74% of single flanked chlorines were removed in the same Aroclor 1254 microcosms without TO. While about 35% of *meta* chlorines and 19% of *para* chlorines were removed in 1% TO case.

During the investigations, molecular tools including quantitative polymerase chain reaction (qPCR) and denaturing high pressure liquid chromatography (DHPLC) were

used to reveal the relationship between the indigenous putative dechlorinating bacteria and dechlorination. These results of PCR-based 16S rRNA gene assays for monitoring indigenous dehalogenating communities within the phylum *Chloroflexi,* including *Dehalococcoides species,* together with high rate of dechlorination indicated that active PCB dechlorinators were present in the microcosms of this study and were closely related to previously identified well characterized PCB dechlorinators.

Based on these conclusions, it is postulated that anaerobic digestion is able to dechlorinate single congener and mixture congeners of PCBs. Knowing that PCB concentrations in urban wastewater and sludge ranges between non-detectable to considerably high levels, anaerobic digesters in WWTPs can be considered as a potential step for their removal. Additionally, despite the negative effect of TO, this study was successful in showing significant dechlorination of Aroclor 1254 and PCB 118 along with good methane productions together with acceptable removal rates of TS, VS and COD in sludge microcosms inoculated with GR sediment at high PCB doses. Therefore, this thesis has proved that pre-existing anaerobic sludge digesters has the potential to be developed as an effective and economic mean for the dechlorination and digestion of already contaminated sludge and/or for dechlorination of PCB contaminated transformer oils.

CHAPTER 10

RECOMMENDATIONS

Regarding the results and the conclusion of this study, the following points can be recommended as future work:

- Since, in the present study, the enrichment microcosms selectively dechlorinated doubly- and singly-flanked *meta* chlorines and *para* chlorines and neither *ortho* nor unflanked *meta* chlorines dechlorination was detected, further research on the removal of unflanked chlorine and/or *ortho* chlorine removal is needed for higher PCB dechlorination efficiency and/or complete dechlorination. Also, aerobic treatment after anaerobic digester might be applied for the complete PCB dechlorination.

- More research is needed to understand whether TO was also degraded during the incubation, since, in the current study, TO degradation was not investigated. Moreover, since the effect of 10% oil on the dechlorination of PCBs was detrimental due to deposition of PCBs in the oil phase which prevented bioavailability of PCBs, therefore, surfactant addition and optimization of its dose might be beneficial to increase bioavailability and solubility of PCBs.

- The kinetics of PCB dechlorination by GR sediment microorganisms is needed to be investigated in anaerobic sludge digesters towards the use pre-existing anaerobic digesters. The effect of substrate, WAS, concentration/dose on the PCB dechlorination might be examined. Further investigation on the effect of continuous addition of PCBs in to anaerobic digesters is also needed.

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APPENDIX A

LIST OF PCB CONGENERS

Table A1. Structures of PCBs with IUPAC numbers.

#	Structure	#	Structure	#	Structure	#	Structure
MonoCB		31	25-4	63	235-4	95	236-25
1	2-	32	26-4	64	236-4	96	236-26
2	3-	33	34-2	65	2356-	97	245-23
3	4-	34	35-2	66	24-34	98	246-23
DiCB		35	34-3	67	245-3	99	245-24
4	2-2	36	35-3	68	24-35	100	246-24
5	23-	37	34-4	69	246-3	101	245-25
6	2-3	38	34-5	70	25-34	102	245-26
7	24-	39	35-4	71	26-34	103	246-25
8	2-4	**TetraCB**		72	25-35	104	246-26
9	25-	40	23-23	73	26-35	105	234-34
10	26-	41	234-2	74	245-4	106	2345-3
11	3-3	42	23-24	75	246-4	107	235-34
12	34-	43	235-2	76	345-2	108	234-35
13	3-4	44	23-25	77	34-34	109	2346-3
14	35-	45	236-2	78	345-3	110	236-34
15	4-4	46	23-26	79	34-35	111	235-35
TriCB		47	24-24	80	35-35	112	2356-3
16	23-2	48	245-2	81	345-4	113	236-35
17	24-2	49	24-25	**PentaCB**		114	2345-4
18	25-2	50	246-2	82	234-23	115	2346-4
19	26-2	51	24-26	83	235-23	116	23456-
20	23-3	52	25-25	84	236-23	117	2356-4
21	234-	53	25-26	85	234-24	118	245-34
22	23-4	54	26-26	86	2345-2	119	246-34
23	235-	55	234-3	87	234-25	120	245-35
24	236-	56	23-34	88	2346-2	121	246-35
25	24-3	57	235-3	89	234-26	122	345-23
26	25-3	58	23-35	90	235-24	123	345-24
27	26-3	59	236-3	91	236-24	124	345-25
28	24-4	60	234-4	92	235-25	125	345-26
29	245-	61	2345-	93	2356-2	126	345-34
30	246-	62	2346-	94	235-26	127	345-35

Table A1. (Continued)

#	Structure	#	Structure	#	Structure	#	Structure
HexaCB		151	2356-25	174	2345-236	197	2346-2346
128	234-234	152	2356-26	175	2346-235	198	23456-235
129	2345-23	153	245-245	176	2346-236	199	2345-2356
130	234-235	154	245-246	177	2356-234	200	23456-236
131	2346-23	155	246-246	178	2356-235	201	2346-2356
132	234-236	156	2345-34	179	2356-236	202	2356-2356
133	235-235	157	234-345	180	2345-245	203	23456-245
134	2356-23	158	2346-34	181	23456-24	204	23456-246
135	235-236	159	2345-35	182	2345-246	205	23456-345
136	236-236	160	23456-3	183	2346-245	**NonaCB**	
137	2345-24	161	2346-35	184	2346-246	206	23456-2345
138	234-245	162	235-345	185	23456-25	207	23456-2346
139	2346-24	163	2356-34	186	23456-26	208	23456-2356
140	234-246	164	236-345	187	2356-245	**DecaCB**	
141	2345-25	165	2356-35	188	2356-246	209	23456-23456
142	23456-2	166	23456-4	189	2345-345		
143	2345-26	167	245-345	190	23456-34		
144	2346-25	168	246-345	191	2346-345		
145	2346-26	169	345-345	192	23456-35		
146	235-245	HeptaCB		193	2356-345		
147	2356-24	170	2345-234	OctaCB			
148	235-246	171	2346-234	194	2345-2345		
149	236-245	172	2345-235	195	23456-234		
150	236-246	173	23456-23	196	2345-2346		

Table A2. Aroclor 1254 Composition (Weight %) (Frame et al., 1996)

Congener Number	*Chlorine Substitution Pattern (IUPAC)*	*Aroclor Composition (Wt %)*		*Congener Number*	*Chlorine Substitution Pattern (IUPAC)*	*Aroclor Composition (Wt %)*	
		A1254a	*A1254g*			*A1254a*	*A1254g*
1	2	0.02		**113**	2,3,3',5',6	0.01	
4	2,2'	0.02	0.06	**114**	2,3,4,4',5	0.5	0.18
6	2,3'	0.01	0.02	**115**	2,3,4,4',6	0.37	0.2
8	2,4'	0.05	0.13	**117**	2,3,4',5,6	0.19	0.23
15	4,4'	0.01	0.03	**118**	2,3',4,4',5	13.59	7.35
16	2,2',3	0.02	0.09	**119**	2,3',4,4',6	0.12	0.08
17	2,2',4	0.02	0.08	**122**	2,3,3',4',5'	0.25	0.1
18	2,2',5	0.08	0.25	**123**	2,3',4,4',5'	0.32	0.15
22	2,3,4'	0.02	0.04	**124**	2,3',4',5,5'	0.47	0.29
28	2,4,4'	0.06	0.19	**125**	2,3',4',5',6	0.03	0.02
31	2,4',5	0.11	0.28	**126**	3,3',4,4',5	0.02	trace
32	2,4',6	0.01	0.05	**128**	2,2',3,3',4,4'	1.71	1.42
33	2,3',4'	0.05	0.16	**129**	2,2',3,3',4,5	0.39	0.38
37	3,4,4'	0.01	0.07	**130**	2,2',3,3',4,5'	0.5	0.6
40	2,2',3,3'	0.15	0.12	**131**	2,2',3,3',4,6	0.14	0.19
41	2,2',3,4	0.02	0.01	**132**	2,2',3,3',4,6'	1.5	2.29
42	2,2',3,4'	0.09	0.15	**133**	2,2',3,3',5,5'		0.11
44	2,2',3,5'	0.67	2.31	**134**	2,2',3,3',5,6	0.2	0.37
45	2,2',3,6	0.02	0.05	**135**	2,2',3,3',5,6'	0.28	0.61
47	2,2',4,4'	0.07	0.14	**136**	2,2',3,3',6,6'	0.24	0.7
48	2,2',4,5	0.05	0.12	**137**	2,2',3,4,4',5	0.52	0.42
49	2,2',4,5'	0.26	1.1	**138**	2,2',3,4,4',5'	5.95	5.8
52	2,2',5,5'	0.83	5.38	**139**	2,2',3,4,4',6	0.14	0.15
53	2,2',5,6'	0.04	0.12	**141**	2,2',3,4,5,5'	0.69	0.98
56	2,3,3',4'	1.7	0.55	**144**	2,2',3,4,5',6	0.12	0.24
59	2,3,3',6	0.01	0.02	**146**	2,2',3,4',5,5'	0.45	0.67
60	2,3,4,4'	0.95	0.18	**147**	2,2',3,4',5,6	0.02	0.1
63	2,3,4',5	0.07	0.02	**149**	2,2',3,4',5',6	1.82	3.65
64	2,3,4',6	0.36	0.59	**151**	2,2',3,5,5',6	0.22	0.69
66	2,3',4,4'	3.56	1.01	**153**	2,2',4,4',5,5'	3.29	3.77
67	2,3',4,5	0.01		**154**	2,2',4,4',5,6'	0.02	0.04
70	2,3',4',5	6.83	3.49	**156**	2,3,3',4,4',5	1.13	0.82
71	2,3',4',6	0.11	0.15	**157**	2,3,3',4,4',5'	0.3	0.19
74	2,4,4',5	2.19	0.84	**158**	2,3,3',4,4',6	0.9	0.81
76	2,3',4',5'	0.03	0.02	**163**	2,3,3',4',5,6	0.7	1.03
77	3,3',4,4'	0.2	0.03	**164**	2,3,3',4',5',6	0.31	0.4
81	3,4,4',5	trace		**166**	2,3,4,4',5,6	0.05	0.05
82	2,2',3,3',4	1.53	1.11	**167**	2,3',4,4',5,5'	0.35	0.27
83	2,2',3,3',5	0.56	0.48	**170**	2,2',3,3',4,4',5	0.35	0.52
84	2,2',3,3',6	1.58	2.32	**171**	2,2',3,3',4,4',6	0.08	0.14
85	2,2',3,4,4'	2.49	1.28	**172**	2,2',3,3',4,5,5'	0.03	0.07
86	2,2',3,4,5	0.1	0.06	**174**	2,2',3,3',4,5,6'	0.14	0.34
87	2,2',3,4,5'	3.41	3.99	**176**	2,2',3,3',4,6,6'	0.01	0.04
89	2,2',3,4,6'	0.11	0.09	**177**	2,2',3,3',4,5',6'	0.08	0.2
91	2,2',3,4',6	0.53	0.93	**178**	2,2',3,3',5,5',6		0.03
92	2,2',3,5,5'	0.57	1.29	**179**	2,2',3,3',5,6,6'	0.02	0.1
94	2,2',3,5,6'	0.01	0.02	**180**	2,2',3,4,4',5,5'	0.42	0.67
95	2,2',3,5',6	1.84	6.25	**183**	2,2',3,4,4',5',6	0.09	0.18
96	2,2',3,6,6'	0.01	0.04	**187**	2,2',3,4',5,5',6	0.09	0.25
97	2,2',3,4',5'	2.78	2.62	**189**	2,3,3',4,4',5,5'	0.01	0.01
99	2,2',4,4',5	4.53	3.02	**190**	2,3,3',4,4',5,6	0.05	0.07
101	2,2',4,5,5'	5.49	8.02	**193**	2,3,3',4',5,5',6		0.03
102	2,2',4,5,6'	0.09	0.15	**194**	2,2',3,3',4,4',5,5'		0.01
103	2,2',4,5',6		0.03	**199**	2,2',3,3',4,5,5',6'		0.01
105	2,3,3',4,4'	7.37	2.99	**203**	2,2',3,4,4',5,5',6		0.02
107	2,3,3',4',5	0.78	0.37	**206**	2,2',3,3',4,4',5,5',6	0.03	0.03
110	2,3,3',4',6	8.42	9.29	**208**	2,2',3,3',4,5,5',6,6'	0.01	0.01

APPENDIX B

MEDIUMS AND CALIBRATION CURVES

Table B1. Basal medium components (Speece, 1996)

Chemical	Concentration in the Reactor (mg/L)	Chemical	Concentration in the Reactor (mg/L)
NH_4Cl	400	NH_4VO_3	0.5
$MgSO_4.7H_2O$	400	$CuCl_2.2H_2O$	0.5
KCl	400	$ZnCl_2$	0.5
$Na_2S.9H_2O$	300	$AlCl_3.6H_2O$	0.5
$CaCl_2.2H_2O$	50	$NaMoO_4.2H_2O$	0.5
$(NH_4).2HPO_4$	80	H_3BO_3	0.5
$FeCl_2.4H_2O$	40	$NiCl_2.6H_2O$	0.5
$CoCl_2.6H_2O$	10	$NaWO_4.2H_2O$	0.5
KI	10	Na_2SeO_3	0.5
$(NaPO_3).6\ H_2O$	10	Cysteine	10
$MnCl_2.4H_2O$	0.5	$NaHCO_3$	6000

Table B2. Composition of 0.36 ECL Medium (Berkaw *et al.*, 1996)

Final Volume	**500 mL**	**1000 mL**	**1500 mL**	**2000 mL**
H_2O	498 mL	996 mL	1494 mL	1992 mL
NaCl	4.21 g	8.4 g	12.62 g	16.83 g
$MgSO_4 * 7H_2O$	2.4 g	4.8 g	7.2 g	9.6 g
KCl	0.14 g	0.27 g	0.41 g	0.55 g
$CaCl_2 * 2H_2O$	0.025 g	0.05 g	0.075 g	0.10 g
NH_4Cl	0.25 g	0.5 g	0.75 g	1.0 g
Resazurin (1000x)	0.5 mL	1.0 mL	1.5 mL	2.0 mL
Trace minerals solution (1000x)	0.5 mL	1.0 mL	1.5 mL	2.0 mL
Vitamin solution (1000x)	0.5 mL	1.0 mL	1.5 mL	2.0 mL
HCl, concentrated	0.25 mL	0.5 mL	0.75 mL	1.0 mL
$Na_2HPO_4 * 7H_2O$	0.56 g	1.12 g	1.68 g	2.24 g
cysteine	0.125 g	0.25 g	0.375 g	0.5 g
Na_2CO_3	1.5 g	3.0 g	4.5 g	6.0 g

Preparation details of E-Cl Medium:

1. Add 1/2 the total volume of H_2O to a round bottom flask containing the special stir bar
2. Dissolve all the ingredients in water up to the vitamin solution. Use some of the remaining H_2O to wash the powder off the walls of the flask.
3. Insert a gassing stone and gas solution with nitrogen:carbon dioxide (80%:20%, flow rate 3sec:12sec), while stirring for 15 min.
4. Add concentrated HCl.
5. Add $Na_2HPO_4 * 7H_2O$ and cysteine and dissolve completely.
6. Slowly add Na_2CO_3 while stirring and gassing and dissolve completely. Use the remaining H_2O to wash powder off the walls of the flask.
7. Adjust final pH to 6.8 with concentrated HCl (dropwise, very slowly) while degassing. Complete degassing for approx. 30-60 min for 1 Liter or 60-120 min for 2 Liter.
8. Insert a gassing cannula into the flask. Turn off the stir plate, and then remove the gassing stone.
9. Dispense medium slowly under N_2:CO_2.
10. After dispensing medium into tubes or bottles, crimp septa and autoclave for 20 minutes under fast exhaust (gravity). Bottles should be placed in a tray filled with approximately 1 cm water. Note: Precipitates may form during autoclaving, but may redissolve by shaking the medium after it has cooled to redissolve the CO_2.

Trace minerals and vitamins from:
Wolin, E.A., M.J. Wolin, and R.S. Wolfe. 1963 Fromation of methane by bacterial extracts. J Biol Chem. 238:2882-2886

* Or 3.66 g of MgCl for 1000 mL

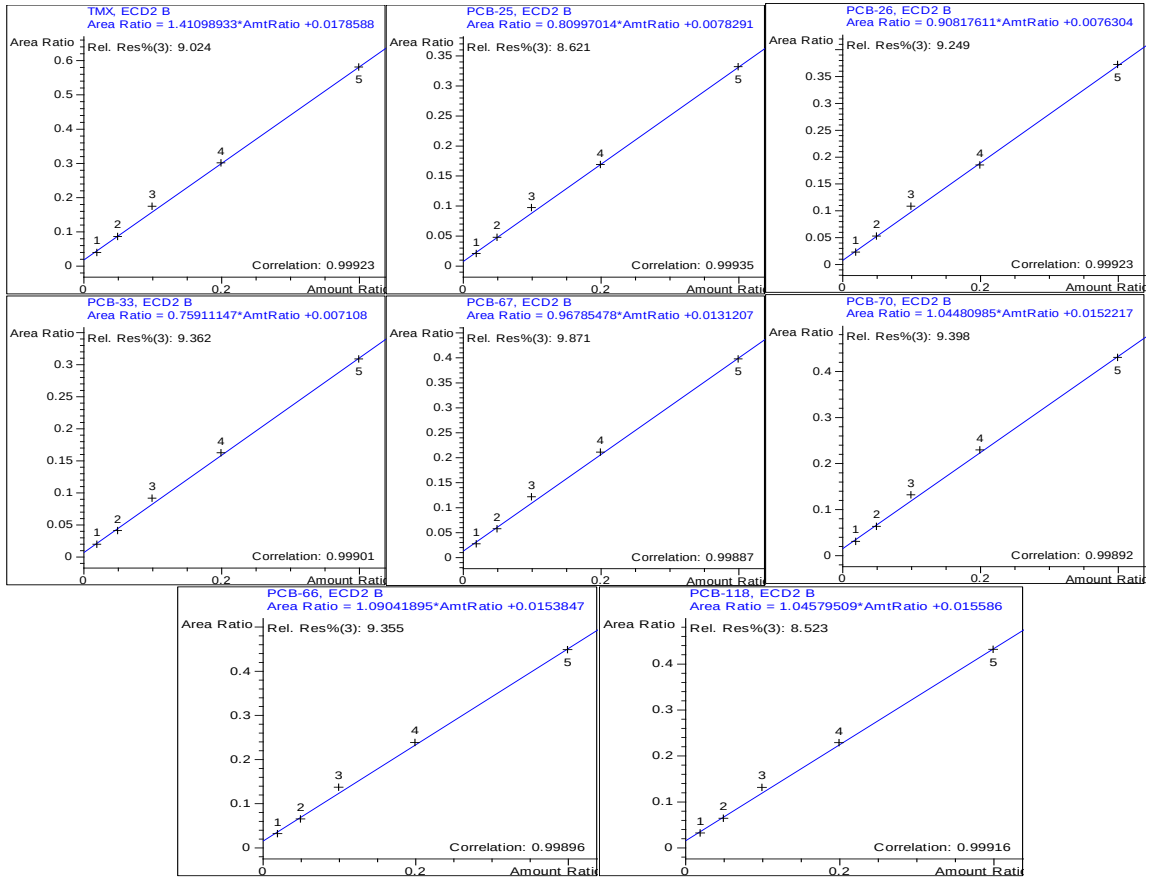

Figure B1. Low range PCB Calibration curves

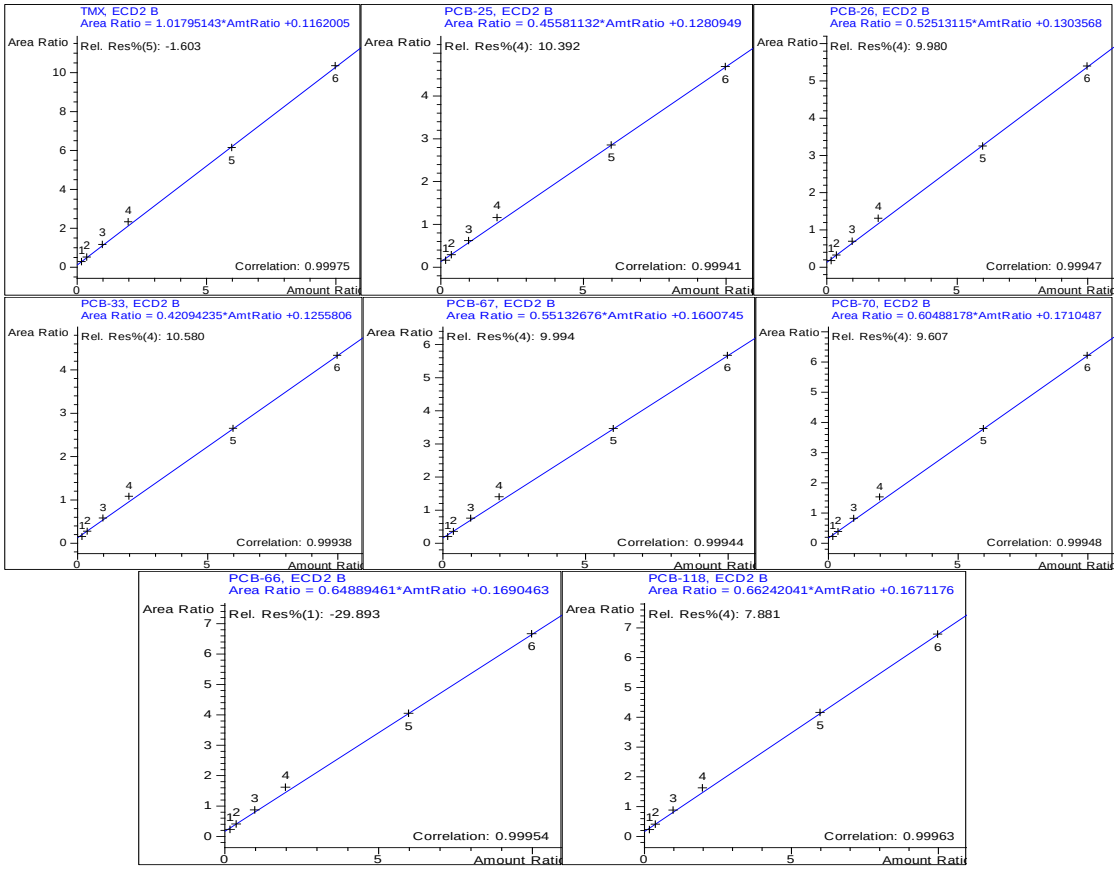

Figure B2. Medium range PCB calibration curves

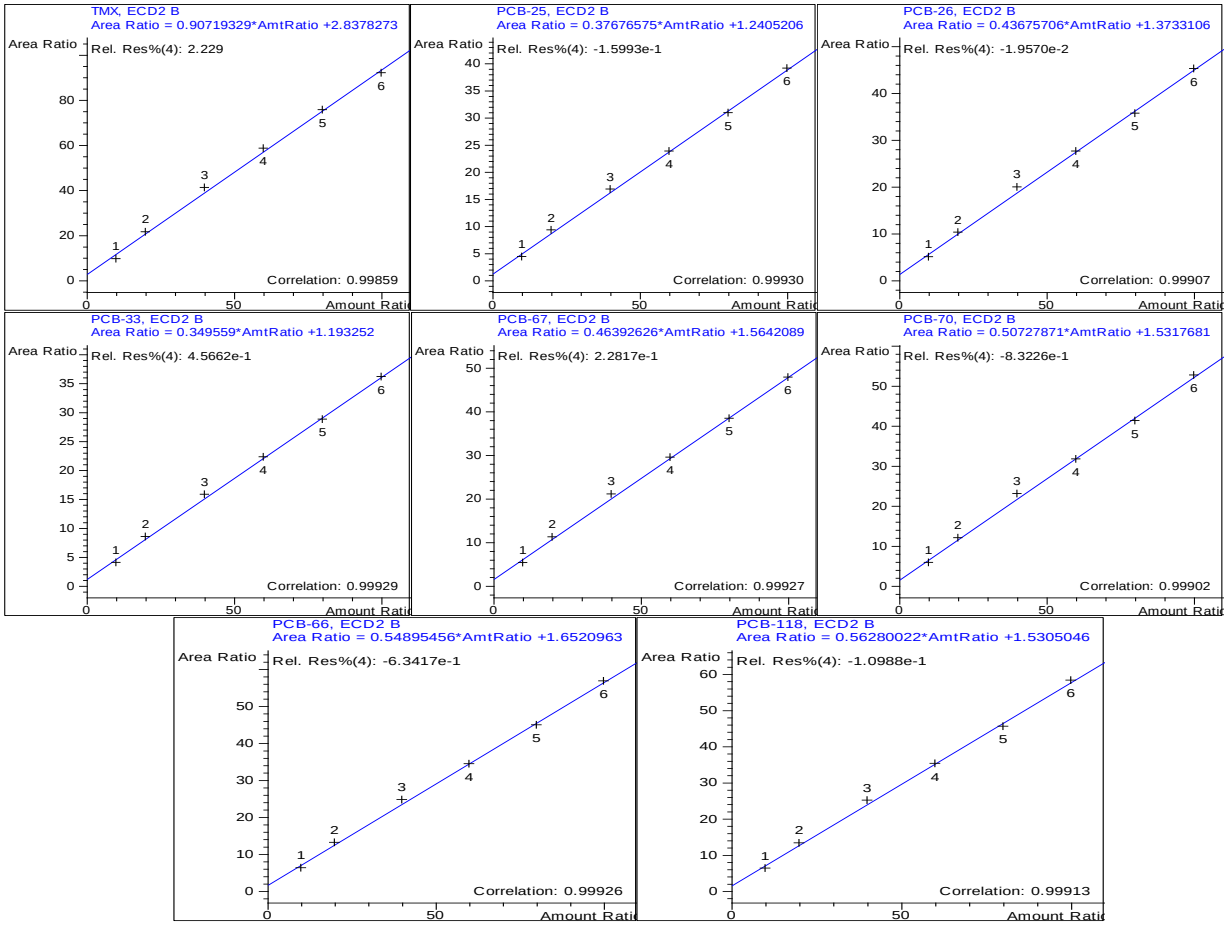

Figure B3. High range calibration curves

Table B3. GC conditions of previous studies for PCB analysis

References	**Lowry *et al.*, 2004.**	**Bauer, 1991.**	**Xu, 2006.**	**Davenport, 1999.**	**Wu, 1996.**	**Chen et al, 2001.**	**Wong and Wong, 2006.**	**Shiriringam, 2007.**	**TSE, 2003.**	**İmamoğlu, 2001.**
Instrument	GC/µECD	GC/FID	GC/MS	GC/ECD	GC/ECD	GC/ECD	GC/ECD	GC/µECD	GC/ECD	GC/ECD
Column	HP-5 (60m x 0.25mm x 0.25µm)	DB-5 (30m)	HP-5 MS (30mx0.25 mmx 0.25µm)	DB-1 (30m x 0.25mm x 0.25 µm)	DB-1 (30m x 0.25mm x 0.25 µm)	DB-5 (30m x 0.53mm x 1.5µm)	HP-5 MS	DB-5 (30m x 0.53 mm x1.5µm)	Not specified	DB-5 (30m x 0.32mm x 0.25µm)
Carrier gas (pressure / rate)	He (20 psi)	He (10 mL/min)	He (35 cm/sec)	He (1.5mL/min)	Not specified	N_2 (27.3 cm/s)	He (1.4 mL/min)	He (6 mL/min)	He (1 mL/min), 270 kPa	He
Detector make-up gas (rate)	Ar/(5%) CH_4 (60 mL/min)	N_2 (25 mL/min)	Not specified	N_2 (6 mL/min)	Not specified	N_2 (27.3 cm/s)	N_2 (30 mL/min)	Ar-Metane (P-5) (40 mL/min)	20-40 mL/min	Not specified
detector temp.	325°C	250°C	Not specified	320°C	300°C	300°C	300°C	300°C	300-350°C	325°C
Inlet mode (temp. / press) and inj. Vol.	splitless (250°C/20 psi) 1µL	splitless (220°C) 1µL	splitless (250°C) 1µL	250°C	250°C	280°C split:15:1	splitless (280 °C) 1µL	splitless (225°C) 2 µL	Not specified	splitless (250°C) 1µL
Oven temperature program	115°C for 3 min, 5°C/ min to 230°C, 15 °C/min to 300°C for 10 min.	70°C for 3 min, 10°C/min 250°C for 5 min for Aroclor 1242-1248 and 270°C for 6 min for Aroclor 1254-1260	50 °C for 1 min,25°C/min to 100 °C, 5°C/min to 300°C for 5 min	130 °°C to 20°C/min 160 °C for 37 min, 2°C/min to 200°C, 8°C/min to 260 °C for 8 min.	40 °C to 160 °C at 20 °C/min for 3 min, 2°C/min to 200 °C, 8°C/min to 250 for 18 min.	170°C for 2 min, 3°C/min to 260°C for 18 min	70°C for 2 min, 20°C/min to 190°C, 1°C/min to 200°C, 30°C/ min to 300°C for 2 min.	140°C to 265°C	50°C for 1 min, 50°C/min to 168°C, 4°C/min to 310°C for 10 min. ; 70°C for 1 min, 40°C/min to 130°C, 2.5°C/ min to 290°C for 5 min.	50°C for 2 min, 3°C / min to 290°C for 10 min.

Table B4. PCB extraction details of previous studies

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Fava et al., 2003.	32 mL serum bottles (25.5 mL sediment slurry, final reaction volume: 30 mL)	slurry, 0.3 mL	N.S.	900 ul of anhydrous diethyl ether was added along with 10 ul of a 40-mg/L stock. OCN and 150 ul of elemental mercury	1.5 mL	18 h, 28 C	horizontally shaken on a rotary shaker at 250 rpm	GC vials were horizontally shaken on a rotary shaker. Then centrifuged, placed at -20C for 30 min and then the liquid ether phase was analyzed.
Palaker et al., 2003.	150 mL slurry in a 250 mL serum bottle sealed with a teflon-lined butyl rubber stopper and an aluminum crimp cap.	slurry, 2-5 mL	A slurry sub-sample was removed for analysis at day 0, 30, 90, 180, and 360.	16 ml acetone/hexane (1:1 v/v) or 5 ml ethyl acetate	30 ml glass tube	24 h	shaker table at 200 strokes/ min	For acetone/hexane extracted samples, the organic layer was transferred into separatory funnel and extracted with hexane-washed water to remove residual acetone. Repeated concentrated sulfuric acid and copper applications to the hexane extract removed hydrolysable impurities and elemental sulfur, respectively. The final eluent (10 ml) was concentrated down to 1 ml under gently flowing high purity nitrogen gas and transferred to GC vials.
Mcdonough, and Dzombak, 2005.	100-mLglass serum bottles with rubber septa and aluminum seals, to observe gas generation and sample headspace gas	Semi-permeable membrane device, water	N.S.	3x10 ml of acetone/hexane (1:1 v/v)	12 mL vial	24 h	shaking end over end	PCBs were extracted from a synthetic sediment slice by adding acetone/hexane solution to the sediment in a vial and shaking end over end for 24 h. Preliminary laboratory work showed that this technique yielded better extraction efficiencies for synthetic sediment than the ultrasonication method. All extracts were cleaned with fluorisil (EPA Method 3620B) and analyzed on a gas chromatograph with a micro-ECD detector (EPA Method 8082).

N.S.: Not Specified.

Table B4. (Continued)

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Hadnagy et al., 2007.	40 mL VOA vials (10 mL batches) or in 125 mL Erlenmeyer flasks (100 mL batches)	pure solvent system made up methanol/distilled water solution and mixture of distilled water and Mg/Pd.	Samples were taken at different time intervals, ranging from 1 minute to 2 hours.	hexane, 5 ml	N.S.	3 × 30 sec.	vigorously shaken by hand	Manual liquid-liquid extraction was used. Hexane was added to batch solution and the mixture was vigorously shaken by hand for 3 × 30 seconds and vented in between. To measure the amount of contaminants adsorbed to the Mg/Pd, the bimetal was first filtered using vacuum filtration, then extracted by ultrasonic extraction. The filtered solution was extracted manually by liquid-liquid extraction
Bauer, 1991.	N.S.	40 mL of treated aqueous suspensions	N.S.	acetone, 4 mL	Cartridge was used.		Cartridge was used.	Treated aqueous suspensions were passed through SEP-PAK C13 Plus solid phase cartridges. The adsorbed PCBs were then eluted from the solid phase with acetone and collected into a chromatographic vial. By eluting with only 4 mL, a 10-fold concentration was directly incorporated into PCB-work-up procedure.
Cedzynska, and Sobiecka, 2004.	N.S.	Slurry sample	N.S.	acetone/hexane (1:3)	N.S.	12 h	rotary shaker	Organic solvent mixture was added to the mineral medium and shaken strongly for 5 min. Then the samples were mixed on the rotary shaker during 12 h. T. J. Baker separate discs and Bakerbond columns with special phase were used for extract and purify the PCB congeners.

N.S.: Not Specified.

Table B4. (Continued)

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Kuipers et al., 1999.	25-mL Balch tubes	Sediment slurry		3 x 2 ml acetone, 3 x 2 ml hexane	N.S.	N.S.	N.S.	The sediment was extracted twice with acetone and then twice with hexane. Extracts were pooled and evaporated down to approximately 0.5 mL, eluted through a Fluorisil column, and made up to 1 mL with hexane. Analyzed by GC.
Master et al., 2002.	For anaerobic: 125-mL serum bottles, for aerobic: 6 additional slurries were prepared in 125-mL serum bottles by mixing 15 g of the above soil and 10mLof mineral medium.	Slurry. 2-g samples from each aerobic treatment, 8 ml slurry from anaerobic treatment.	For aerobic: 0, 3, 7, 14, and 28 days of incubation. For anaerobic: after inoculation and after 3 and 4 months of incubation.	Acetone and hexane.	N.S.	N.S.	Shaking and vortexing.	The slurries were extracted by shaking and vortexing soil twice with acetone and then twice with an equivalent volume of hexane. Tubes were centrifuged (2 min at 1500g) between extractions. The extracts were pooled and evaporated to approximately 0.5 mL using nitrogen gas and then passed through a Pasteur pipet packed with hexane washed Fluorisil topped with sodium sulfate.
Bedard et al., 2005.	N.S.	Aliquots, 1 mL	Sampled at intervals of 7-10 days up to 152 days	Anhydrous diethyl ether, 5 mL	8 mL	16 h	Horizontal shaking on a platform shaker	Aliquots of each microcosm were aseptically removed under a stream of sterile, O_2-free, N_2 gas and transferred to a glass vials fitted with teflon-lined screw caps. Halogenated biphenyls were extracted with anhydrous diethyl ether by vigorous horizontal shaking on a platform shaker for a minimum of 16 h. Acid-reduced copper filings (50 mg) were added to each extract to remove sulfur.

N.S.: Not Specified.

Table B4. (Continued)

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Quensen III et al., 1990.	28-mL tube: with 1 g of Sediments and 160-mL bottles: with 25 g of Sediments	2 ml of the sediment slurry	N.S.	Hexane: acetone (9:1, v:v), 3x 10 mL	N.S.	N.S..	Shaking	Samples were extracted by shaking once with acetone containing OCN as ISTD and twice more with hexane-acetone (9:1). Extracts were combined, and the acetone was extracted with 2% NaCl in deionized water. The remaining hexane extract was extracted with concentrated sulfuric acid, rinsed again with NaCl, and then dried over anhydrous Na2SO4. Further cleanup was performed on a Fluorisil-copper powder column. The sample was eluted from the column with hexane.
Natarajan et al., 1998.	158 ml serum vials, contained 50 ml medium or sediment slurry	2-3 mL of aliquots which contained both solid and liquid contents	N.S.	Not specified.	N.S.	N.S.	N.S.	Serum vials were transported and opened in the anaerobic glove box using a decrimper. Aliquots were sampled from each vial with a wide-mouth pipet. PCBs were extracted from the samples in pressure tubes according to the method described by Quensen et al. (1990). OCN was used as an ISTD. Glass columns packed with Fluorisil-copper powder were used to clean the extracted samples. The samples were analyzed using a GC.
ASTM, 2003.	N.S.	For liquid samples: 3 mL. For solids, 3 g of samples	N.S.	For liquid: 27 mL acetone/hexane, for solids: 30 mL acetone/hexane.	40 mL	30 s	vortex	Sample was taken into a vial and DCB surrogate and acetone/hexane were added. Vortexed for at least 30s. If sediment is visible centrifuge the extract to separate sediment. If required apply clean-up (acid clean-up, Silica Gel Clean-up, etc.)

N.S.: Not Specified.

Table B4. (Continued)

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Kuo et al., 1998.	120-ml anaerobic microcosms	0.25 mL of activated sludge samples	at the 7th day of the experiment, then monthly during the subsequent 24 weeks of incubation and at the end of the study	900 ul of anhydrous diethyl ether was added along with 10 ul of a 40-mg/L stock OCN and 150 ul of elemental mercury	1.5 mL	18 h, 28 C	Horizontally shaken o a rotary shaker at 250 rpm	The head-space gas was quantified by aseptically connecting the microcosm head-spaces to a Mariotte system, then 0.5 ml sample of the head-space gas were taken from each serum bottle and injected into the GC. Activated sludge samples collected from microcosms was subjected to extraction with anhydrous diethyl ether.
Lowry and Johnson, 2004.	12 mL amber serum bottles (2g iron + 10 mL of PCB solution)	7 mL aliquot of aqueous phase	N.S.	0.5 mL of hexane containing 1 mg/L IS	12 mL vial		centrifuge and then mixing on a vortex orbital mixer	Serum bottle was centrifuged for 5 min at 3500 rpm to separate solid iron from the water/suspended iron. Then aliquot of the aqueous phase was withdrawn from the reactor and then extracted wit 0.5 mL hexane by mixing. 100 ul of the hexane extract was taken and analyzed by GC. Extractions are conducted in a reactor bottles without removing the septa.
Wu, 1996.	N.S.	1 mL of slurry	From 2 days for highly active samples to more than 2 months for samples with extremely low dechlorination activities	diethyl ether, 5 mL	8 mL vial	24 h	G24 environmental incubator shaker	After vigorous shaking, aliquots (1 mL) of the slurries were sampled using in an anaerobic chamber. PCBs were extracted by vigorously shaking the samples on shaker at 200 strokes/min for 24 h with diethyl ether (5mL) in 8 mL vials. OCN (IS) was added to the ether. After centrifugation organic phase of each sample was transferred from the ether/sediment mixture into a fresh vial and shaken with elementary mercury for 30 min. to remove sulphur.

N.S.: Not Specified.

Table B4. (Continued)

References	Reactor volume	Sample type and volume	Sampling Frequency	Extraction solvent type and volume	Vial volume	Extraction time and temp.	Shaker type	Extraction Procedure
Kim, 2000.	N.S.	1 mL of wet sludge	N.S..	2x4 mL hexane, 2x2mL acetone	sample tube	2-3 h	tube rotator	Wet sludge taken, OCN (IS), hexane and acetone were added to the sample tube. Bottles were shaken on a rotator. Solvent layer decanted into a cylindrical separatory funnel. An additional solution of hexane and acetone was added to the sample bottles and shaken by hand for 1 min. upper layer combined with the previous one. Acetone was extracted by NaCl solution. Concentrated sulfuric acid were added to the hexane and shaken to degrade non-PCB org. after shaking aqueous phase discarded and hexane was washed again with the same amount of NaCl. Further clean-up of extract was performed by filtering through a column containing fluorisil, reduced copper and anhydrous sodium sulfate.
Supelco app. 67, 1998.	N.S.	0.2 g oil diluted 100 ml with hexane from this 10 ml is taken.	N.S.	5 mL concentrated sulfuric acid	40 mL	1 min	vigorously shaken by hand	Shake for 1 min. Allow the layers to settle, then transfer the upper (organic) layer to a second vial.
Davenport, 1999.	Tube:28 mL Bottle: 160 mL	From bottles 1 mL sediment slurry, for tubes all contents.	N.S.	3x 2.5 mL hexane – acetone (1:1)	N.S.	overnight	shaker	PCBs were extracted by shaking overnight with hexane:acetone containing the surrogate in vials. Then, centrifugation was applied, process was repeated two more times with hexane:acetone. The solvents after centrifugation were combined and some samples were further cleaned up with acid-reduced copper fillings (0.5 g). OCN (IS) was added.

N.S.: Not Specified.

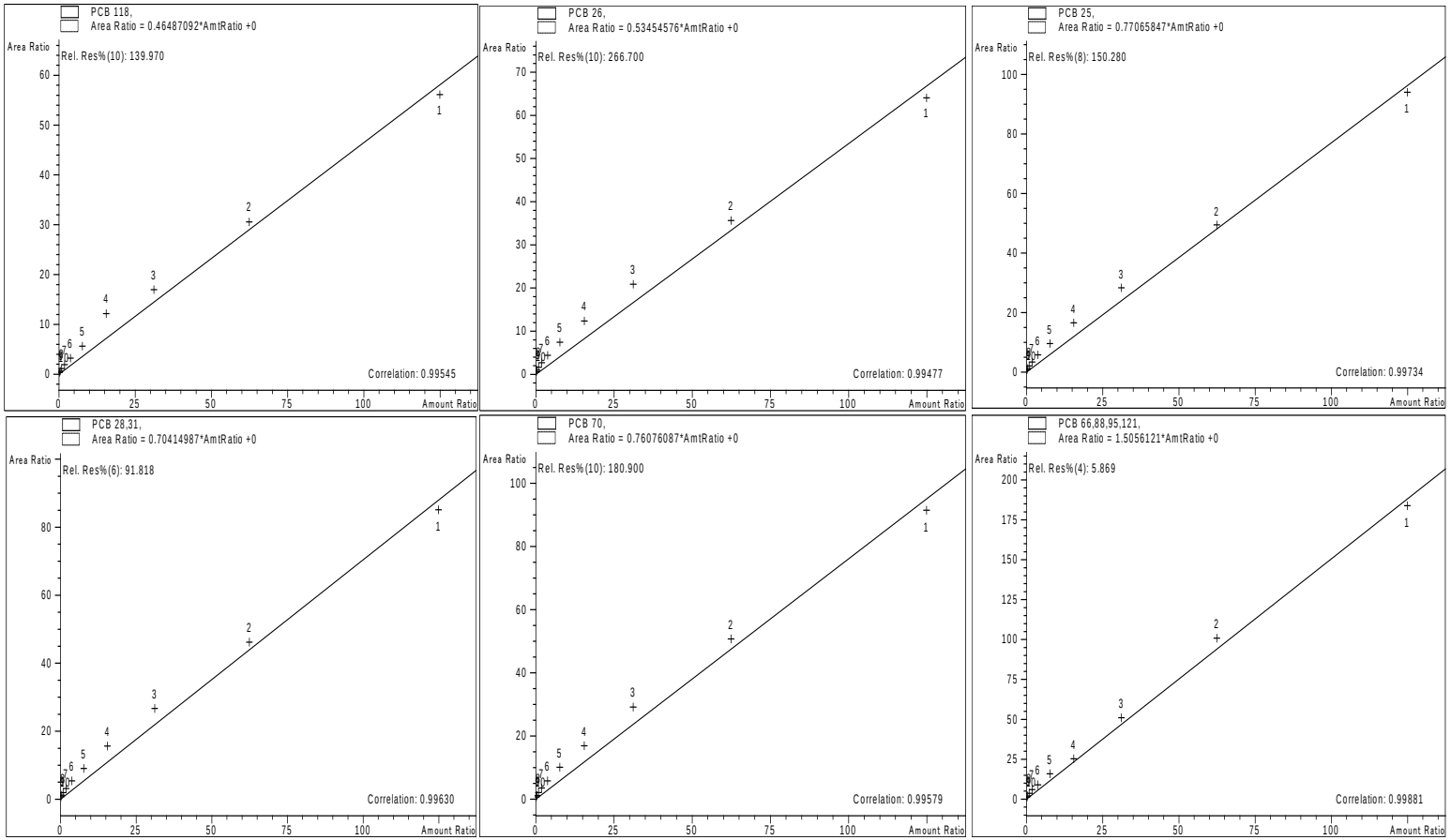

Figure B4. Sample calibration curves for PCB 25, 28/31, 66, 70 and 118 used in second PCB analysis method.

CURRICULUM VITAE

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PUBLICATIONS

Journal Papers

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