ABSTRACT

Title of Dissertation:	PREVENTION AND TREATMENT OF POLYCHLORINATED BIPHENYLS IN SEDIMENTS - SOURCES AND SOLUTIONS
	Ran Jing, Doctor of Philosophy, 2019
Dissertation directed by:	Associate Professor, Birthe Veno Kjellerup, Department of Civil and Environmental Engineering

PCBs are classified as one of the persistent organic pollutants (POPs) with high toxicity and have undesirable effects on the environment and on humans. Once released into the environment PCBs could bioaccumulate within the food chain, due to their high affinity for organic materials. Recently, studies indicated PCBs can potentially enter a wastewater treatment plant (WWTP) system and be discharged via wastewater effluents thereby further contaminating the downstream environments. This study evaluated the potential for bioremediation of polychlorinated biphenyls (PCBs) in the effluent from a large WWTP. It was found that the continuous effluent was responsible for the majority of the discharged PCB into the receiving river (1821 g for five years), while the intermittent discharge contributed 260 g over the five years. The average number of chlorine per biphenyl for the detected PCB congeners showed a 19% difference between the two types of effluent, which indicated a potential for organohalide respiration of PCBs during the continuous treatment. This

was further supported by a high level of tri-, tetra- and penta- chlorinated congeners accounting for 75% of the anaerobically respired PCBs. Potential for aerobic degradation and thus biomineralization of PCBs were identified for both effluents. In addition, the similarity of organohalide respiring (OHR) microbial populations in biosolids and intestinal human biofilms was determined by applying a bioinformatics approach. The OHR populations of the communities were analyzed from existing American and Chinese human intestinal microbiomes. The results of the biosolids analysis showed increased amounts of products from PCB respiration. Simultaneously, experiments with organohalide respiration of PCE in biosolids samples showed significant decreases in PCE concentration after 46 days (28-92%). Subsequently, it was evaluated if the OHR microbial populations in biosolids were similar to those present in intestinal human biofilms by applying a bioinformatic approach. The OHR populations of the communities were analyzed from existing American and Chinese human intestinal microbiomes. The overall groups *Proteobacteria, Bacteroides, Actinobacteria,* and *Firmicutes* phyla dominated the microbiomes in all datasets. The OHR groups in biosolids and intestinal biofilms included *Dehalogenimonas, Dehalobacter, Desulfitibacter, Desulfovibrio, Sulfurospirillum, Clostridium,* and *Comamonas*. The results of this study showed that several OHR phyla were present in all samples independent of origin. Wastewater and intestinal microbiomes also contained OHR phyla. Finally, biofilms made up by the OHR bacteria *Dehalobium chlorocoercia* DF-1 were inoculated on the surface of the pinewood biochar particles. The mole percent of the total PCE in the headspace decreased from 100% to 70.4%±17.6% for the rest of nine mesocosms which

suggested that the D. chlorocoercia DF-1 biofilm converted PCE to TCE. The gene copy numbers of DF-1 biofilm from nine mesocosms which are ranging from 1.95×10^{8} to 8.30×10^{8} gene copies/g pinewood biochar. The biochar-biofilms were subsequently applied to PCB contaminated sediment from the Grass River in Michigan, USA. The goal was to evaluate the organohalide respiration of the PCB contaminated sediments in the absence/presence of the biofilm and free-floating inoculum.

PREVENTION AND TREATMENT OF POLYCHLORINATED BIPHENYLS IN SEDIMENTS - SOURCES AND SOLUTIONS

by

Ran Jing

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Advisory Committee:
Dr. Birthe Veno Kjellerup, Chair
Dr. Alba Torrents
Dr. Allen Davis
Dr. Guangbin Li
Dr. Lance Yonkos, Dean's Representative

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Chapter 1: Introduction and Research Objectives

1.1 Introduction

1.1.1 Background

PCBs are classified as one of the persistent organic pollutants (POPs) with high toxicity and have undesirable effects on the environment and on humans (Lallas, 2001a). PCB molecules consist of two connected benzene rings and chlorine atoms attached to all or any of 10 different positions thereby resulting in 209 different congeners and 10 homologs (McFarland and Clarke, 1989). All congeners in the chemical group of PCBs share the chemical composition. PCBs have high octanol-water partition coefficients (K_{ow}) from $10^{4.10}$ (20°C) for mono-chlorobiphenyl to $10^{7.93}$ (20°C) for deca-chlorobiphenyl (Zhang et al., 2013). These chemicals have been used for industrial purposes since the 1920s (Lauby-Secretan et al., 2016). Their physical and chemical properties allow for a wide range of industrial applications such as oil used in transformers and capacitors, thermal insulation material, and oil-based paints (Jing et al., 2018). Properties such as electrically insulating and chemical persistence at high temperatures made PCBs commonly used components in cooling liquids for electronic equipment (Kajiwara et al., 2017). PCBs can cause numerous unwanted issues such as disruption of hormone function and the immune system (Aoki, 2001; Zheng et al., 2017) by bioaccumulation in the food chain due to their high affinity for organic material. They have been detected in tissues, blood, and breast milk due to exposure routes such as consumption of meat, fish, and dairy products (Stohs, 2016a). As a consequence, PCBs can cause immune system damage, decreased pulmonary functions and bronchitis as well as interfering with hormones in some cases causing cancer and reproductive issues (Schecter et al., 2006). Other identified toxic effects include sarcomas, non-Hodgkin lymphomas, and effect on serum lipids (Kelly et al., 2017).

1.1.2 PCBs in wastewater

Recently, studies reported that PCBs have been detected in the wastewater treatment plants (WWTP) via urban or agricultural runoff (Katsoyiannis and Samara, 2004; Rodenburg et al., 2010; Rodenburg et al., 2012). In a study performed by Darling et al. (2004), 14 low-chlorinated PCBs (mono- and di-chlorinated PCBs) were detected in surface water near wastewater treatment plant effluents in the cities of Harbor, Toronto, Canada (Darling et al., 2004). Katsoyiannis and Samara (2004) found that seven PCB congeners (PCB-28, 52, 101, 118, 138, 153, and 180) with a total concentration of 460 ng/L were present in wastewater samples from a wastewater treatment plant in Thessaloniki, northern Greece. Rodenburg et al., (2010) reported that PCBs were discharged into WWTPs via combined sewer overflows (Rodenburg et al., 2010). The concentrations of PCBs in 645 effluent samples from 40 different WWTPs ranged from 0.047 to 6800 ng/l. Balasubramani et al. (2014) investigated the concentration for all 209 congeners from16 municipal and industrial wastewater effluents in the Houston area (Balasubramani et al., 2014). The concentration of PCB congeners with low-chlorine numbers (<4) in the dissolved and suspended phases of the wastewater was 1.01 to 8.12 ng/l and 2.03 to 31.2 ng/l, respectively. This shows that clean-up of PCBs from wastewater and contaminated sites continues to be a big challenge (Abhilash et al., 2013; Meggo and Schnoor, 2013). Therefore, the PCBs from a municipal WWTP flowing into the surrounding aquatic environments (e.g., river or lake) need to be reduced. Legislation on the PCBs of municipal WWTP outflow is becoming stricter in the USA. The legislation criteria for PCBs are controlled by the national environmental jurisdictions (e.g., USEPA) and are enforced by the establishment of Total Maximum Daily Loads for discharge to the aquatic environment. The PCB TMDLs are usually expressed as average annual loads or average

daily loads (Bierman et al., 2009). For instance, the total PCB average annual TMDL for the District of Columbia Water and Sewer Authority to Potomac River was 30.2 g in 2016.

1.1.3 Bioremediation of PCBs in soil and sediments

In the US, the most common remediation technologies for PCB-contaminated soil or sediments are dredging and excavation followed by incineration or disposal in a landfill (Gomes et al., 2013). Incineration is mandatory for waste containing more than 500 ppm PCBs due to federal regulations (40 CFR 761) (Gomes et al., 2013). Dredging and capping are preferred remediation methods, but they are expensive technologies to apply, disruptive to existing ecosystems, and associated with several additional challenges. Dredging of sediment can cause resuspension and release of PCB-contaminated particles from the dredged sediments to rivers or nearby coastal zones thereby increasing the PCB exposure and thus the toxic levels. Other methods such as PCB degradation induced by chemical reagents are aggressive and are not commonly applied as a soil remediation technology due to the usage of high temperatures and strong acidic and alkaline compounds (Gomes et al., 2013). These strategies are also not commonly applied for PCB remediation due to most of these being disruptive and environmentally unsustainable (Agarwal et al., 2007).

In-situ microbial degradation of PCBs has experienced technological progress over the past decades. Evidence for biodegradation of PCBs in natural environments such as soil, sediment, groundwater and surface water has been well reported in various studies (Ashley and Baker, 1999; Feng et al., 1998; Martinez et al., 2010; Tu et al., 2011a). Biodegradation of PCBs by bacteria encompasses two different pathways: anaerobic organohalide respiration and aerobic degradation.

1.1.4 Anaerobic organohalide respiration of PCBs

Organohalide respiration (Figure 1.1) is a respiratory process, where a halogen-carbon bond of organic compound is broken and the halogen atom is released (Hug et al., 2013). Organohalide respiring (OHR) bacteria are capable of deriving energy for their growth from respiration with PCBs. As a result, the removal of chlorines from PCBs can impact their toxicity (Hug et al., 2013). Organohalide respiration of highly chlorinated PCB congeners (4> chlorines), (Lowry and Johnson, 2004) is a sole anaerobic process The PCB congeners serve as the terminal electron acceptor with three potential chlorine substituent positions; *para*, *meta*, and *ortho*. The substitution of chlorine preferentially occurs at a single or double flanked *para* and/or *meta* position (Agarwal et al., 2008).

Over the past decade, in-situ bioremediation technologies of PCB impacted soils and sediments based on organohalide respiration have been conducted (Sowers and May, 2013a; Tyagi et al., 2011). Two major types of bioremediation techniques include biostimulation and bioaugmentation. Some studies indicate that biostimulation by halo-priming with halogenated aromatic compounds can increase the activity of indigenous OHR bacteria and induce genes required for organohalide respiration (Sowers and May, 2013a). Biostimulation has also been achieved using electrochemical techniques to treat PCB contaminated groundwater or sediment (Chun et al., 2013; Yu et al., 2016). Voltage was applied to contaminated sediment from a Superfund site (Fox River, State) to stimulate the oxidative and reductive transformation of Aroclor 1242 with an overall 40-60% reduction of initial total weathered Aroclor concentration (Chun et al., 2013). In Guangdong, China, an application of bioanode stimulation resulted in organohalide respiration of 2,3,4,5-tetrachlorobiphenyl in an electronic waste recycling site by 42% after 110 days of incubation (Yu et al., 2016).

Bioaugmentation is a feasible in-situ strategy that is defined as the addition of bacteria to the contaminated site to accelerate the degradation rate of a contaminant (Tyagi et

al., 2011). May et al. (2008) reported that the isolated bacterium *Dehalobium chlorocoercia* DF-1 from Charleston Harbor sediment, SC, was capable of transforming Aroclor 1260 (containing double-flanked chlorines) to less chlorinated congeners (e.g., PCB-57, PCB-53, PCB-52, PCB-51, PCB-49, and PCB-32) during bioaugmentation experiments in the laboratory using PCB contaminated soil (May et al., 2008). Similarly, Payne et al (2011) found that *D. chlorocoercia* DF-1 enhanced the organohalide respiration of weathered Aroclor 1260 (Payne et al., 2011) in sediments (1.3 ppm) from Baltimore Harbor, MD.

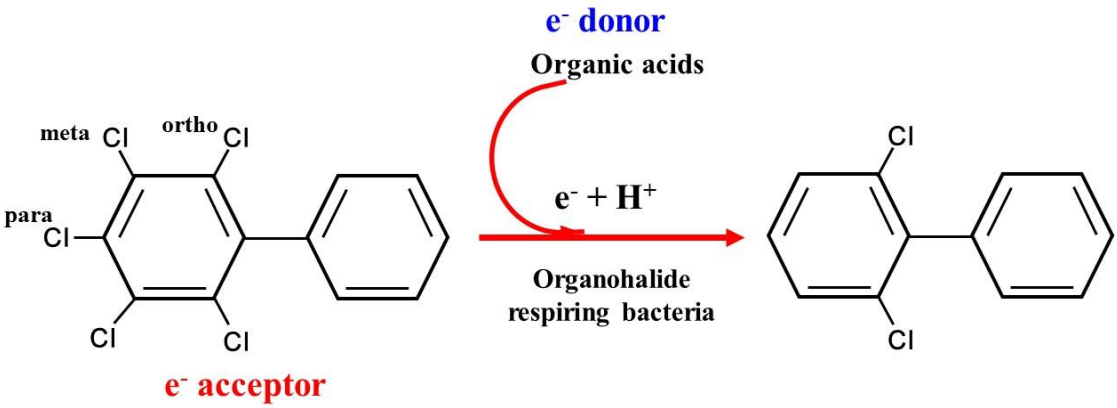

Figure 1.1 Reductive PCB dechlorination by OHR bacteria.

1.2 Current solutions

Removal of PCBs from sediments is a priority considering of their toxic and carcinogenic properties. PCB bioremediation generally includes two steps: first the highly chlorinated PCB congeners are reduced by the anaerobic organohalide respiring bacteria. For example, some dioxin-like PCBs (e.g., PCB-114, PCB-156, and PCB-157) can be transferred to lower chlorine content congeners by the anaerobic OHR bacteria such as *D. chlorocoercia* (Payne et al., 2017). After that, the lowly chlorinated PCB congeners (< 4 chlorines) are degraded by aerobic bacteria such as *Alcaligenes xylosoxidans*, *Pseudomonas stutzeri*, *Ochrobactrum anthropi*, and *Pseudomonas veronii* resulting in complete mineralization. This

study focused on PCB removal from the contaminated sediments by using anaerobic OHR bacteria as biofilm forming on the surface of an absorbent materials.

1.3 Objectives

The bottleneck of the overall PCB transformation is the anaerobic processes due to a low rate of the organohalide respiration. It is estimated that the dechlorination rate of 2,3,5,6-CB to 2,3,6-CB and 2,5-CB in the sediments collected from the Housatonic River was 9 to 10 umol/l day (Van Dort and Bedard, 1991). Many studies have documented the presence of organohalide respiration of PCBs and other organohalogen compounds in the natural environment (Ahmed and Focht, 1973; Krzmarzick et al., 2012; LaRoe et al., 2014; Lu et al., 2017; Su et al., 2015). However, it is uncertain *whether* WWTP processes have the ability to perform organohalide respiration with PCBs during the treatment process (Deblonde et al., 2011a; Kiedrzyńska et al., 2017; Syakti et al., 2012). The wastewater effluents from a WWTP are discharged into the downstream river, which will directly impact this ecosystem environments. Therefore, PCB removal from a WWTP and the downstream sediment is important.

The second chapter of this Ph.D. project aims to determine if organohalide respiring potential is present in the treatment process of a WWTP based on the analysis and comparison of the characteristics of PCBs present in by-pass and regularly treated effluents. In this study, a five-year PCB dataset of continuous and intermittent effluents was used to determine the differences in the average number of chlorine per biphenyl (an indication of organohalide respiration) for the 209 PCB congeners, the ratio of *ortho*-PCBs (indicators of organohalide respiration), PCB homologs distribution for intermittent versus continuous effluents. A relatively long-term (five years) PCB dataset statically showed differences

between continuous and intermittent effluents thereby reliably determining that PCB organohalide respiration could occur during the wastewater treatment process.

However, one research question still remained: what are the potential upstream sources of the OHR bacteria of the wastewater and biosolid samples from the WWTP? To assist in answering this question, the third chapter of this Ph.D. project focused on the presence of OHR bacteria in the WWTP and whether these bacteria are present in the human gut microbiome. Based on the analyses of the microbiomes from the human gut and from WWTP, the hypothesis was stated 1) organohalide respiration in the regular treatment process from the WWTP originates from the human gut bacterial community due to feces directly discharged into the WWTP through the sewage systems; 2) the OHR bacteria are present in the human gut due to the presence of natural halogenated compounds in food as well as the anthropogenic source of halogenated compounds that can contaminate the food chain. Until now, the relationship between OHR bacteria in the gut microbial community and in WWTP biosolid samples have rarely been reported (Cai et al., 2014a; Hughes et al., 2017; Newton et al., 2015). In this study, the bioinformatics profiles of OHR bacteria from WWTP biosolids were investigated by using molecular techniques such as next-generation sequencing. Moreover, the present OHR bacteria of the gut microbial community from the selected USA and Chinese human populations was determined through human fecal datasets obtained from the National Center for Biotechnology Information (NCBI) database.

The fourth chapter of this Ph.D. project aims to inoculate the biofilm of the existing OHR bacteria i.e., *D. chlorocoercia* DF-1 formed on the surface of absorbents. A hydrophobic surface of absorbents such as pinewood biochar particles can effectively sequester PCBs as well as the OHR bacteria from liquid cultures. Pinewood biochar is made from pine woodchip via pyrolysis processes, which can be used as a soil amendment (Essandoh et al., 2015). In addition, a high sorption capacity of the pinewood biochar can

influence the proximity of the PCB molecules that can be utilized by the OHR bacteria located in the biofilm. Therefore, the close proximity of a large number of PCB molecules to the OHR biofilm on the pinewood biochar particles will allow for enhanced organohalide respiration of PCBs. Recent studies indicated that some bacteria such as *D. chlorocoercia* DF-1 can survive in contaminated soil and were also able to dechlorinate the high-chlorinated PCBs (May and Sowers, 2016). Therefore, an important experiment in Chapter 5 was evaluate the biofilm formation of the existing OHR microorganisms i.e., *D. chlorocoercia* DF-1 on the surface of pinewood biochar particles and to evaluate its organohalide performance on the PCB contaminated sediments. In chapter 5 OHR bacterial biofilms formed on the surface of activated carbon materials to treat PCB contaminated sediments are being tested. Biofilms have a complex architecture in which microorganisms can exist in aggregates (Stoodley et al., 2002). Such a complex network can provide efficient access to nutrients. The polymeric matrix of a biofilm can increase the resistance to dramatic changes in environmental conditions such as pH and redox changes (Flemming and Wingender, 2010). In addition, another advantage is the ability of the polymeric matrix to concentrate the hydrophobic-organic contaminants such as PCBs. Containing a highly organic porous surface, the biochar has high affinities for the simultaneous attraction of biofilm-forming by OHR microorganisms and adsorption of PCBs. Both processes are essential components for the implementation of this dual approach in which the activated carbon as a foundation for biofilm formation and subsequent delivery systems for bioaugmentation of PCBs. Therefore, it is expected that the application of activated carbon particles covered with biofilm inoculum could enhance the PCB organohalide respiration.

The initial experiments focus on the enrichment of OHR microorganisms existing in the WWTP performing by the mesocosm study containing PCB contaminated wastewater and/or biosolid samples from the interest sites of the WWTP. Furthermore, this microbial

inoculum delivery system and a bioaugmentation system based on *D. chlorocoercia* DF-1 biofilms on activated carbon particles will be further applied for PCB remediation in sediments (Anyasi and Atagana, 2011). In addition, this approach can provide an efficient method for inoculating microorganisms for PCB bioaugmentation thereby increasing the potentials for long-term bioaugmentation.

Hypothesis 1: The characteristics of PCBs present in by-pass and regularly treated effluents are different in mass and composition due to a high OHR potential (organohalide respiring signal) in the regular treatment process.

Object 1: to evaluate the OHR potential from the by-pass and regularly treated effluents by investigating their characteristics of PCBs such as the composition of PCB homolog and congeners, annual PCB discharges, and chlorine/biphenyl for PCBs.

(**Alternative hypothesis 1:** The characteristics of PCBs present in by-pass and regularly treated effluents are different in mass and composition are not due to the organohalide respiring bioactivity in the regular treatment process.)

Hypothesis 2: the organohalide respiration occurring in the regular treatment process from the WWTP could potentially be from the human gut bacterial community due to feces directly discharged into the WWTP through the sewage systems and these OHR bacteria are presenting in the human gut due to the presence of natural halogenated compounds in food as well as the anthropogenic source of halogenated compounds that can contaminate the food chain.

Object 2: to identify the OHR potential via the presence of PCB dechlorination and determine the presence of OHR bacteria from wastewater and biosolid samples.

Object 3: to identify the OHR bacteria in gut microbial communities from selected American and Chinese human populations and compare them with the OHR bacteria profiles of the from

wastewater and biosolid samples.

Hypothesis 3: A bioaugmentation system based on the OHR bacterial biofilm on pinewood biochar particles can enhance PCB organohalide respiration in sediments.

Object 4: to inoculate the biofilm of the existing OHR bacteria i.e., *D. chlorocoercia* DF-1 formed on the surface of pinewood biochar.

Object 5: to evaluate the organohalide performance of the DF-1 biofilm covered pinewood biochar on bioremediation of PCB contaminated sediments and compare it with that of the liquid DF-1 inoculum.

Chapter 2: Distribution of polychlorinated biphenyls in effluent from a large municipal wastewater treatment plant: Potential for bioremediation?

(Note: This Chapter was published in Journal of Environmental Science. The paper was directly incorporated into the thesis.)

ABSTRACT:

This study involved an evaluation of the potential for bioremediation of polychlorinated biphenyls (PCBs) in the effluent from a large municipal wastewater treatment plant. The study was focused on the presence of PCBs in two types of effluents: the continuous effluent present during dry weather conditions and the intermittently present effluent that was present during wet weather due to incoming stormwater. The annual discharge of PCBs for both types of effluent was calculated based on a five-year dataset (2011-2015). In addition, the toxicity and bioremediation potential of the PCBs in the effluent were also assessed. It was found that the amounts of PCB discharged via the effluent exceeded the established level for Total Maximum Daily Load (TMDL) at 30.2 g/year. The continuous effluent was responsible for the majority of the discharged PCB into the receiving river (1821 g for five years), while the intermittent discharge during contributed 260 g over the five years. The average number of chlorine per biphenyl for the detected PCB congeners showed a 19% difference between the two types of effluent, which indicated a potential for organohalide respiration of PCBs during the continuous treatment. This was further supported by a high level of tri-, tetra- and penta- chlorinated congeners accounting for 75% of the anaerobically respired PCBs. Potential for aerobic degradation and thus biomineralization of PCBs was identified for both

effluents. Furthermore, toxicity of 12 dioxin-like PCBs showed that normal operation of the wastewater reduced the toxicity throughout the wastewater treatment plant.

2.1 Introduction

Polychlorinated biphenyls (PCBs) are a group of persistent organic pollutants (POPs) with 10 homologs and 209 congeners (Focant et al., 2004). They have globally been used for industrial purposes since the 1920s (Abbas et al., 2014). Many studies have over time shown that PCBs are highly toxic with undesirable effects on the environment and humans (Cheng and Hu, 2010; Lallas, 2001b). They can bioaccumulate in tissue, blood, and breast milk through environmental exposure routes (e.g., consumption of dairy products) thereby also causing chronic health effects (Stohs, 2016b). Among the 209 PCB congeners, 12 coplanar PCBs (also called "Dioxin-like PCBs") share a similar activity and toxicity with tetra-chlorodibenzo-p-dioxin (Bruner-Tran and Osteen, 2010). Studies show that high-level exposure to dioxin-like PCBs can cause cancer and malignant melanoma (Gallagher et al., 2011).

PCBs can attach to particles and solid phases due to high octanol-water partition coefficients (K_{ow}) ranging from $10^{4.10}$ to $10^{7.93}$ (Passatore et al., 2014). A conventional type of municipal wastewater often contains about 220 mg/l of suspended solids (Ellis, 2004). As a result, PCBs can potentially enter a wastewater treatment plant (WWTP) via the sewer system and/or stormwater (in areas with combined sewer and stormwater systems) and be discharged via wastewater effluent. PCBs from wastewater effluent with a large volume discharging into an aquatic environment can result in bioaccumulation through the food chain and thus expose aquatic organisms to PCBs with toxicity as a result (Kulkarni et al., 2008b; North, 2004).

WWTPs are subject to effluent quality requirements, which are often described by the regulatory concept “Total maximum daily load” (TMDL). This allows the regulatory authority a tool to assess the effect of the pollutants in the effluent on a localized waterbody. In the case of a large WWTP in this study, this also includes a TMDL for the total discharged mass of PCBs. The criteria of the PCB impairment being addressed by the TMDL may include human health, aquatic life, and wildlife parameters (Keller and Cavallaro, 2008). The environmental jurisdictions (e.g., USEPA) usually lack information concerning the discharge quality from smaller municipal and permitted industrial facilities that potentially are discharging PCBs. Therefore, the TMDL of PCBs was established to apply a consistent approach to all WWTP effluents.

However, a TMDL is not an operational tool for the assessment of toxicity and bioremediation potential of PCBs. Biodegradation of PCBs can take place under aerobic conditions that can lead to biomineralization or during anaerobic conditions, where organohalide respiration can take place (Krzmarzick et al., 2012; LaRoe et al., 2014; Zanaroli et al., 2015a). PCBs have traditionally been applied in industrial mixtures of congeners referred to as Aroclor in the USA, Phenoclor in France and Kenechlor in Japan (Voogt and Brinkman, 1989). Each of the congeners can during anaerobic organohalide respiration transform to different products with reduced chlorine content and potentially changed toxicity depending on the pathway. One way to assess PCB toxicity is the Toxic Equivalency (TEQ) that can be calculated by applying Toxic Equivalency Factors (TEFs) that have been defined in comparison to the most toxic dioxin, 2,3,7,8-tetrachlorodibenzodioxin (2,3,7,8-TCDD) (Van den Berg et al., 2006). However, the calculation of TEQs is limited to the toxicity of the 12 dioxin-like PCB congeners, since TEFs do not exist for the remaining 197 PCB congeners (Baars et al., 2004). Numerous studies have documented the presence of organohalide respiration with PCBs in the natural environment (Ahmed and Focht, 1973; Krzmarzick et al.,

2012; LaRoe et al., 2014; Lu et al., 2017; Su et al., 2015). However, it is currently uncertain whether WWTP processes have the ability to perform organohalide respiration with PCBs or aerobically mineralize PCBs during treatment (Deblonde et al., 2011a; Kiedrzyńska et al., 2017; Syakti et al., 2012). The total amount of PCBs discharged in the effluent and their toxicity can potentially be controlled by aerobic and anaerobic processes that transform the PCBs. The toxicity of the highly chlorinated PCB congeners can be reduced by anaerobic organohalide respiring bacteria. Some dioxin-like PCBs (e.g., PCB-114, PCB-156, and PCB-157) can be reduced to lower chlorinated congeners by anaerobic organohalide respiring bacteria such as *D. chlorocoercia* DF-1, *Desulfitobacterium dehalogenans*, *Desulfomonile tiedjei*, and *Dehalococcoides mccartyi* (Ahmed and Focht, 1973; Kranzioch et al., 2013; Payne et al., 2017b). Subsequently, these products containing less than four chlorines per molecule can be degraded by aerobic bacteria (e.g., *Burkholderia xenovorans* strain LB400, *Alcaligenes xylosoxidans*, *Pseudomonas stutzeri*, *Ochrobactrum anthropi*, and *Pseudomonas veronii*) (Correa et al., 2010; Murínová et al., 2014; Passatore et al., 2014). As a result, their toxicity will be eliminated due to biodegradation into chlorobenzoic acid as well as other degradation products and eventually into carbon dioxide (Chang et al., 2013; Furukawa et al., 1978; Pieper, 2005).

This paper will present an assessment of the annual PCB amount discharged from effluents originating from an intermittent (wet weather) discharge and a continuous discharge from a large municipal WWTP. The amount of total PCBs was compared with the TMDL that has been in place since 2011 as a part of the overall discharge permit for PCBs. In addition, the toxicity and bioremediation potentials of PCBs will be discussed based on an evaluation of the proportion of PCBs that can be anaerobically or aerobically transformed. The number of chlorines on the biphenyl rings as well as the positions affects the toxicity of

the PCBs thus the discharge of PCBs will influence the potential health effects downstream of the discharge area.

2.2 Materials and Methods

2.2.1 Wastewater treatment processes

The WWTP that was studied has a daily treatment capacity of above 200 million gallons of wastewater. The sewer collection system in the service area is a combined sewer system that covers one-third of the area, while separate sanitary sewer systems cover the remaining area. Under normal operational conditions when the influent flow is below the treatment capacity of the plant, two separately primary influents drain directly into the WWTP, where large particles from untreated wastewater are initially removed using screens and gravity settling basins. The organic matter present in the effluent is then consumed through biological treatment (secondary treatment) with aeration. After this step, the microorganisms convert ammonia into nitrate and nitrite through a nitrification process. These compounds are subsequently converted to nitrogen gas. As a final step, the wastewater passes through a filtration unit made by sand and anthracite. Before discharge to the river the effluent is disinfected by sodium hypochlorite-based chlorination and the residual chlorine is removed before the treated wastewater is discharged into the river. During periods of rain, the combined sewer systems in the area will also transport stormwater thus increasing the required volume for treatment above the capacity of the WWTP. In this case, the volume that cannot be treated as described above will pass through the primary processes, but the effluent will be discharged into the river without any biological treatment. Disinfection takes place to comply with the requirements for discharge (Gray, 2004).

2.2 Wastewater sampling and PCBs congener analysis

In this study, 10 PCB homologs and 209 PCB congeners were evaluated in effluent discharge during normal treatment and in case of stormwater overflow during a five-year study period (2011-2015). The principal wastewater treatment flowchart is shown in Figure 2.1. The samples evaluated in this study were collected from two wastewater discharge points: One was a bypass effluent (Intermittent stormwater overflow) after the primary treatment process, while the other was from regular treatment effluent (Continuous treatment). The samples from the bypass effluent were collected during rain events as grab samples. The sample was pumped from the dechlorination tank to the sample bottle after the pump had been running for at least 15 minutes prior to sampling to flush the stale volume out of the pipe. At this point the effluent was flushed out for another five minutes, then the flow was reduced to allow for sampling. The influent flow was required to be approximately 40% higher than the daily capacity for sampler initiation from this effluent discharge. Composite samples were collected from the normally treated effluent during planned events twice per quarter or as required by EPA using a Sigma 900Max #006 composite sampler (HACH Company, Colorado, USA). Teflon-lined Tygon (American Durafilm Co. Inc, Holliston, USA) tubing with a strainer attached to the pump tubing with a stainless steel connector and two hose clamps at each end of the connector. The sampling bottles were placed into a double-bagged drum liner bag in case of leakage and the tubing from the sampler head was carefully placed into the sample bottle. Trace rainfall amounts were not reported 72 hours prior to these sampling events. Deionized water was applied as field blank samples for each sampling batch (EPA, 2008). All sampling operations were performed with gloves and the samples were handled in a biological safety cabinet to prevent exposure to humans and contamination of the samples.

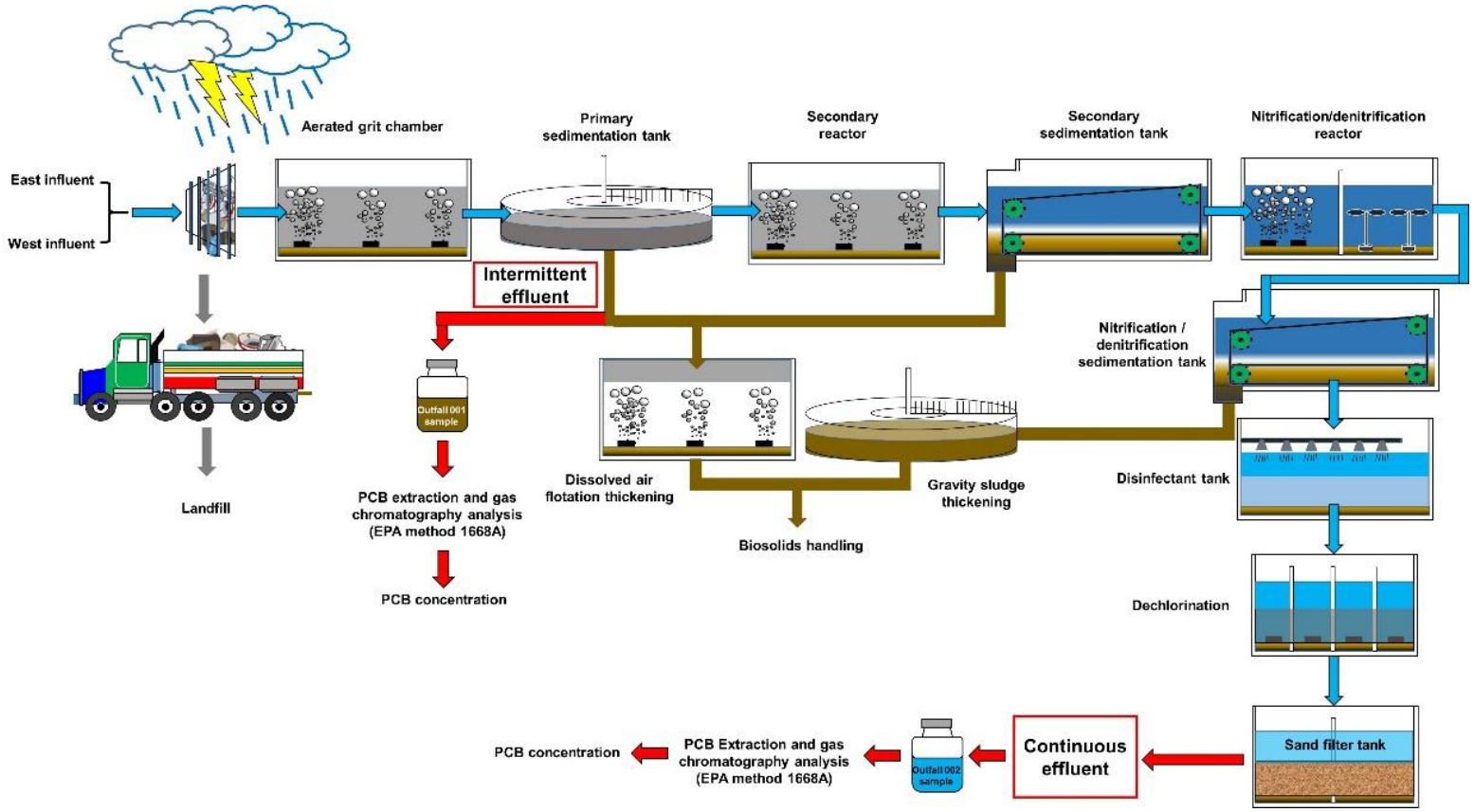

Figure 2.1 Principal flow diagram showing the major wastewater treatment processes at the waste water treatment plant that was analyzed in this study.

Extraction of PCBs from the samples was performed using solid-phase extraction (SPE) or a continuous liquid/liquid extraction (CLLE) (EPA, 2008) by SGS AXYS Analytical Services Ltd., British Columbia, Canada. After extraction, a cleanup standard was spiked into the extracts and additional clean up using back-extraction with sulfuric acid and Florisil chromatography was performed. The PCB extracts were concentrated to 20 ul and internal standards were injected into each extract prior to analysis by gas chromatography (GC) and high-resolution mass spectrometer (MS). All 209 PCB congeners were analyzed at a certified laboratory by using EPA method 1668B as discussed above (EPA, 2008; Rushneck et al., 2004). The analytical results were corrected by the a rinsate blank (Equipment Blank) and a method blank prior to any future calculation and data analysis (Johnson et al., 2008; Keith, 1996).

2.3 Calculation of annual PCB discharge

The collection of effluent samples from continuous treatment occurred approximately 11 times per year, while collection of grab samples from the stormwater overflow took place 6-7 times per year over the five year study period. Missing data points between the sampling events were estimated based on the existing sampling data. The calculation of the total PCB mass distribution and contribution from PCB homologs was based on the concentration from the analyzed samples and converted into mass by multiplying with the effluent flowrate (Equation S1 and S2).

2.4 Analysis of toxicity equivalent (TEQ) for 12 dioxin-like PCBs

The TEQ concentration for each data point was calculated by multiplying the concentration of each of the 12 dioxin-like PCB congener with the corresponding toxicity

equivalent factor (TEF) thus obtaining a total TEQ for each sample (Van den Berg et al., 2006). In this study, TEFs from the WHO (Van den Berg et al., 2006) were applied. The calculated total TEQ concentrations represent the equivalent amount of toxicity that 2,3,7,8-TCDD would have impacted the environment with.

2.5 Analysis of chlorine/biphenyl for PCBs

The number of chlorines per biphenyl is a measure for the weathering of PCBs throughout the sewer and wastewater processes. This was calculated based on the total mass (g) of each PCB homolog, the number of chlorines and the molecular weight (g/mol) for each PCB homolog. The number of chlorine per biphenyl ring for each day then was calculated by using the calculated mole of chlorine number for 10 PCB homologs divided by the their total moles. Finally, the number of chlorine per biphenyl ring for each day was calculated by adding the average number of chlorine/biphenyl for each year divided by the WWTP operation days (365 days) (Equation S3 and S4).

2.6 Estimated aerobic and anaerobic biodegradation potential

Two biological processes can be responsible for transformation of PCBs: aerobic oxidation and anaerobic organohalide respiration (Abramowicz, 1995; Nyholm et al., 2010). The total mass (g) of PCB homologs with four or less chlorine atoms (i.e., mono-, di-, tri- and tetra- chlorobiphenyls) were firstly calculated by their mass concentration (g/l) from both effluents for 365 days and their corresponding flowrates (l/day). Aerobic biodegradation potential of PCBs was calculated by total mass (g) of PCB homologs divided by the annual total mass (g) of all PCB homologs. The calculation of the anaerobic biodegradation potential was calculated by adding the mass of the homologs with five to ten chlorine atoms and

subtracting the mass (g) of PCB congeners, where chlorine atoms were solely placed in *ortho* positions and divide by the total mass of (g) of PCBs. The congeners with *ortho* chlorinated positions were subtracted since multiple studies have shown that organohalide reducing bacteria cannot utilize these chlorines as electron acceptors (Sun et al., 2000; Wu et al., 2002; Zhang et al., 2015) (Equation S5 and S6).

2.7 DNA extraction and molecular analysis

DNA was extracted from ten locations at the WWTP: Primary sedimentation tank (PST), Nitri/denitrification reactor (NDR), Primary effluent (PE), Anaerobic digestion reactor (Digested biosolids: DB), Final product biosolids (FPB), Nitri/denitrification sedimentation (NDS), Secondary Reactor (SR), Secondary Sedimentation tank (SST), Centrifuge pre-dewatering (Liquid and biosolid:LB), East primary influent (EPI). DNA was extracted using the MoBIO PowerSoil DNA Isolation Kits (Qiagen Inc., Germantown, USA) (Krzmarzick et al., 2012). The extracted DNA from the nine samples was analyzed on the nano-drop (Thermo Fisher Scientific, Waltham, USA) to measure the concentration of the DNA and the purity. Polymerase chain reaction (PCR) was performed by using DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, USA) and primers set 348F/884R specific for the 16S rRNA gene of *Chloroflexi* (Chun et al., 2013; Kjellerup et al., 2012b). The PCR products were evaluated via gel electrophoresis and bands were compared to the amplicon length for the positive control (536 bp) and a molecular ladder (Genprice Inc., San Jose , USA) (Figure S1).

2.3 Results

2.3.1 Annual PCB discharge

After the production of PCBs was banned in the 1970s in the USA, the amount of PCBs present in the environment was expected to decrease, since existing PCB sources would be eliminated (Zhang et al., 2004). The results from this study show that current sources still exist such as discharged effluent from a WWTP. The continuous effluent contributed to the major PCB discharge (80.7% - 94.7%) of total PCB discharge from both intermittent effluent and continuous effluent over the five years period (Figure 2.2). A significantly ($p < 0.01$) larger contribution (1821.37 g) came from the continuous effluent discharge compared to the stormwater overflow (260.32 g) thus showing that the sources of PCBs in the effluent originate from the wastewater and not from the stormwater contribution in the combined sewer system (Table 2.1). A similar result of total PCBs discharge (1.65 g PCB/day) during dry weather was observed from a WWTP located in Philadelphia North East discharging to the Delaware River (Hansler et al., 1998). This PCBs discharge was estimated to 602.3 g/year, which was comparable to the results of this study.

The distribution of PCB homologs for the two types of effluent showed that tri-, tetra-, penta-, hexa-, and hepta- chlorinated PCBs were the most abundant congeners in the stormwater overflow with penta- and hexa- chlorinated congeners having the highest abundance (Figure 2.3). The contributions from these five homologs ranged on average for 49.5-58.1 % of the total annual PCBs (Table 2.2). For the continuous effluent, di-, tri-, tetra-, penta-, and hexa- chlorinated congeners were most abundant (Figure 2.3). Here, tetra- and penta- chlorinated congeners accounted for annual mass discharge ranging from 47.8-51.1% of the total PCBs over the five year period.

The implemented TMDL for the PCB discharge, stated that the discharged mass should be below 30.2 g/year, which has been considered to be safe for aquatic and human

health. Based on the results from the five year investigation the TMDL was exceeded during this period thus actions should be taken to limit the contribution of PCBs from the WWTP effluent.

Table 2.1. Summary of total PCBs estimated based on the annual PCB loading model.

	Intermittent effluent	Continuous effluent
Year	Total Mass (g)	Total Mass (g)
2011	46.34 (± 0.90)	426.85 (± 0.68)
2012	73.78 (±2.42)	428.19 (±0.63)
2013	42.49 (±0.77)	307.31 (±0.24)
2014	79.48 (±1.61)	331.59 (±0.28)
2015	18.23 (±0.36)	327.43 (±0.30)
Average	52.06 (±24.96)	364.27 (±58.30)
Total	260.32 (±1.43)	1821.37 (±0.49)

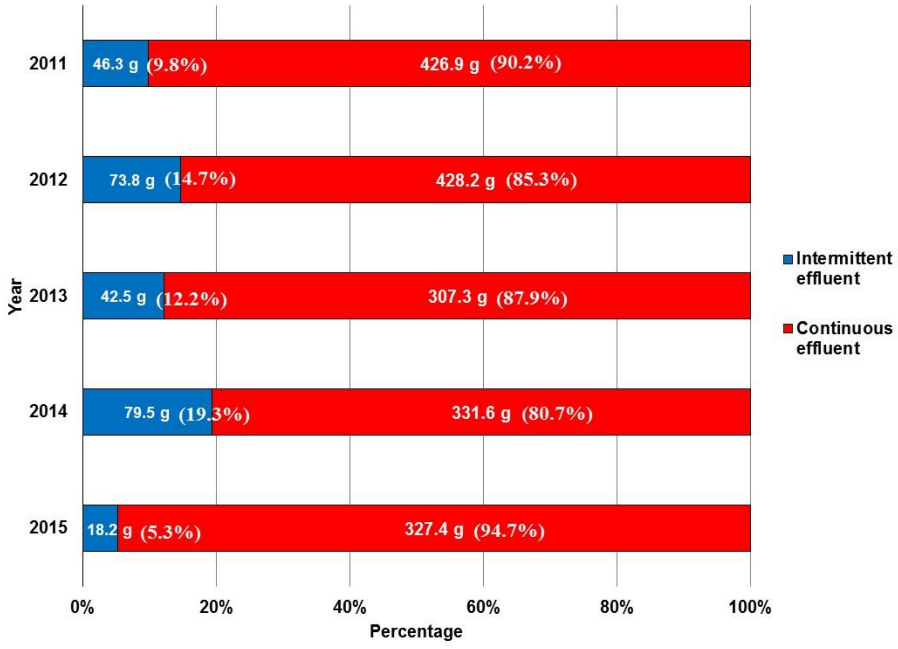

Figure 2.2 Comparison of estimated annual PCBs discharged from the intermittent and continuous effluents during 2011 to 2015. More than 90% of the total influent flow received complete treatment thus the large treated volume resulted in a large PCB mass despite lower concentrations in the treated WW compared to the intermittent effluent.

2.3.2 Toxicity equivalent (TEQ) for 12 Dioxin-like PCBs

The toxicity of PCBs depends on the number of chlorine atoms, but also the positions of the chlorines on the biphenyl rings (Barbalace, 2003b). Non-*ortho* PCB is generally considered to be "dioxin-like" and they are more toxic than the rest of the congeners, since the chlorine atoms will line up in a single plane as coplanar, when there is one or no chlorines in the *ortho* position (Barbalace, 2003a; Van den Berg et al., 2006). The arithmetic mean of calculated TEQs over five-year periods for four non-*ortho* and eight mono-*ortho* dioxin-like PCBs in the two effluents are summarized in Table 2.3. The maximum contaminant level in water for TCDD established by USEPA is 0.03 ng/l (Rodriguez et al., 2008). None of the mean value for the 12 dioxin-like PCBs observed in the discharged effluent over the five year period exceeded the health standard of 30 pg TCDD/l. In addition, the TEQ concentration of the total 12 dioxin-like PCB congeners was 1.65 pg TEQ/l (intermittent) and 0.261 pg TEQ/l (continuous), respectively.

Table 2.2. Five-year summary of PCB homolog discharges from intermittent and continuous effluents.

Intermittent effluent										
Year	mono-CB (g)	di-CB (g)	tri-CB (g)	tetra-CB (g)	penta-CB (g)	hexa-CB (g)	hepta-CB (g)	octa-CB (g)	nona-CB (g)	deca-CB (g)
2011	0.28 (± 0.01)	1.58 (± 0.03)	3.89 (± 0.08)	7.26 (± 0.14)	12.44 (± 0.24)	13.14 (± 0.28)	5.66 (± 0.11)	1.43 (± 0.03)	0.39 (± 0.01)	0.27 (± 0.01)
2012	0.13 (± 0.005)	1.78 (± 0.06)	5.48 (± 0.19)	9.07 (± 0.27)	15.48 (± 0.46)	22.74 (± 0.76)	15.67 (± 0.56)	3.08 (± 0.11)	0.24 (± 0.01)	0.10 (± 0.002)
2013	0.18 (± 0.003)	1.05 (± 0.02)	2.94 (± 0.06)	6.78 (± 0.15)	12.77 (± 0.30)	11.94 (± 0.27)	4.46 (± 0.09)	1.16 (± 0.02)	0.26 (± 0.01)	0.94 (± 0.03)
2014	0.26 (± 0.01)	4.11 (± 0.2)	9.07 (± 0.22)	13.35 (± 0.27)	19.93 (± 0.39)	19.43 (± 0.38)	10.79 (± 0.21)	2.09 (± 0.04)	0.38 (± 0.01)	0.09 (± 0.002)
2015	0.06 (± 0.001)	0.66 (± 0.01)	1.62 (± 0.03)	2.78 (± 0.05)	4.70 (± 0.09)	5.00 (± 0.10)	2.75 (± 0.06)	0.53 (± 0.01)	0.09 (± 0.002)	0.03 (± 0.001)
Continuous effluent										
Year	mono-CB (g)	di-CB (g)	tri-CB (g)	tetra-CB (g)	penta-CB (g)	hexa-CB (g)	hepta-CB (g)	octa-CB (g)	nona-CB (g)	deca-CB (g)
2011	5.68 (± 0.02)	58.99 (± 0.13)	87.09 (± 0.14)	105.06 (± 0.16)	98.99 (± 0.10)	51.62 (± 0.036)	17.22 (± 0.005)	2.01 (± 0.001)	0.10 (± 0.001)	0.09 (± 0.001)
2012	6.71 (± 0.01)	22.10 (± 0.04)	85.82 (± 0.13)	125.93 (± 0.19)	121.67 (± 0.29)	45.70 (± 0.09)	11.43 (± 0.03)	1.64 (± 0.01)	0.18 (± 0.001)	7.01 (± 0.09)
2013	4.82 (± 0.01)	34.97 (± 0.02)	60.31 (± 0.04)	74.52 (± 0.05)	72.22 (± 0.08)	41.90 (± 0.06)	15.79 (± 0.03)	2.53 (± 0.01)	0.04 (± 0.003)	0.20 (± 0.001)
2014	2.07 (± 0.01)	31.81 (± 0.02)	66.85 (± 0.04)	89.30 (± 0.08)	79.86 (± 0.09)	44.16 (± 0.06)	15.04 (± 0.02)	2.08 (± 0.01)	0.08 (± 0.001)	0.35 (± 0.001)
2015	2.95 (± 0.004)	36.50 (± 0.03)	68.51 (± 0.06)	91.30 (± 0.09)	76.21 (± 0.07)	36.32 (± 0.04)	13.28 (± 0.02)	2.15 (± 0.005)	0.06 (± 0.0004)	0.17 (± 0.001)

Table 2.3. Average TEQs of 12 dioxin-like PCBs for the intermittent effluent (33 observations) and for the continuous effluent (56 observations).

Intermittent effluent													
	TEQ PCB-77 (pg-TEQ/l)	TEQ PCB-81 (pg-TEQ/l)	TEQ PCB-105 (pg-TEQ/l)	TEQ PCB-114 (pg-TEQ/l)	TEQ PCB-118 (pg-TEQ/l)	TEQ PCB-123 (pg-TEQ/l)	TEQ PCB-126 (pg-TEQ/l)	TEQ PCB-156 (pg-TEQ/l)	TEQ PCB-157 (pg-TEQ/l)	TEQ PCB-167 (pg-TEQ/l)	TEQ PCB-169 (pg-TEQ/l)	TEQ PCB-189 (pg-TEQ/l)	Total TEQs (pg-TEQ/l)
Mean	4.96×10^{-3}	9.67×10^{-3}	2.02×10^{-2}	5.10×10^{-4}	1.39×10^{-2}	2.72×10^{-3}	1.09	2.87×10^{-3}	4.18×10^{-4}	1.40×10^{-3}	5.07×10^{-1}	7.54×10^{-4}	1.65
STD	1.68×10^{-2}	4.30×10^{-2}	2.12×10^{-2}	1.17×10^{-3}	1.37×10^{-2}	9.88×10^{-3}	4.06	3.39×10^{-3}	1.88×10^{-3}	2.33×10^{-3}	1.87	1.81×10^{-3}	4.61
Max	9.63×10^{-2}	2.41×10^{-1}	8.57×10^{-2}	6.63×10^{-3}	5.70×10^{-2}	4.74×10^{-2}	23.1	1.45×10^{-2}	1.07×10^{-2}	1.22×10^{-2}	10.2	8.67×10^{-3}	23.5
Min	1.93×10^{-4}	3.11×10^{-4}	3.40×10^{-4}	2.84×10^{-5}	4.86×10^{-5}	1.92×10^{-5}	1.50×10^{-1}	4.22×10^{-5}	4.22×10^{-5}	2.88×10^{-5}	6.50×10^{-2}	3.78×10^{-5}	2.28×10^{-1}
Continuous effluent													
	TEQ PCB-77 (pg-TEQ/l)	TEQ PCB-81 (pg-TEQ/l)	TEQ PCB-105 (pg-TEQ/l)	TEQ PCB-114 (pg-TEQ/l)	TEQ PCB-118 (pg-TEQ/l)	TEQ PCB-123 (pg-TEQ/l)	TEQ PCB-126 (pg-TEQ/l)	TEQ PCB-156 (pg-TEQ/l)	TEQ PCB-157 (pg-TEQ/l)	TEQ PCB-167 (pg-TEQ/l)	TEQ PCB-169 (pg-TEQ/l)	TEQ PCB-189 (pg-TEQ/l)	Total TEQs (pg-TEQ/l)
Mean	2.03×10^{-4}	3.19×10^{-4}	1.19×10^{-3}	3.92×10^{-5}	8.23×10^{-4}	2.34×10^{-5}	1.93×10^{-1}	1.29×10^{-4}	4.53×10^{-5}	4.42×10^{-5}	6.57×10^{-2}	4.13×10^{-5}	2.61×10^{-1}
STD	2.18×10^{-4}	6.05×10^{-5}	2.49×10^{-3}	5.10×10^{-5}	1.73×10^{-3}	3.06×10^{-5}	2.24×10^{-16}	3.20×10^{-4}	2.22×10^{-5}	9.47×10^{-5}	4.40×10^{-3}	2.86×10^{-5}	6.42×10^{-3}
Max	1.77×10^{-3}	7.59×10^{-4}	1.88×10^{-2}	3.21×10^{-4}	1.31×10^{-2}	2.46×10^{-4}	1.93×10^{-1}	2.29×10^{-3}	2.06×10^{-4}	7.23×10^{-4}	9.63×10^{-2}	2.49×10^{-4}	2.95×10^{-1}
Min	6.22×10^{-5}	3.11×10^{-4}	3.75×10^{-4}	1.38×10^{-5}	1.43×10^{-4}	1.81×10^{-5}	1.93×10^{-1}	3.13×10^{-5}	3.00×10^{-5}	8.04×10^{-6}	6.50×10^{-2}	2.27×10^{-5}	2.59×10^{-1}

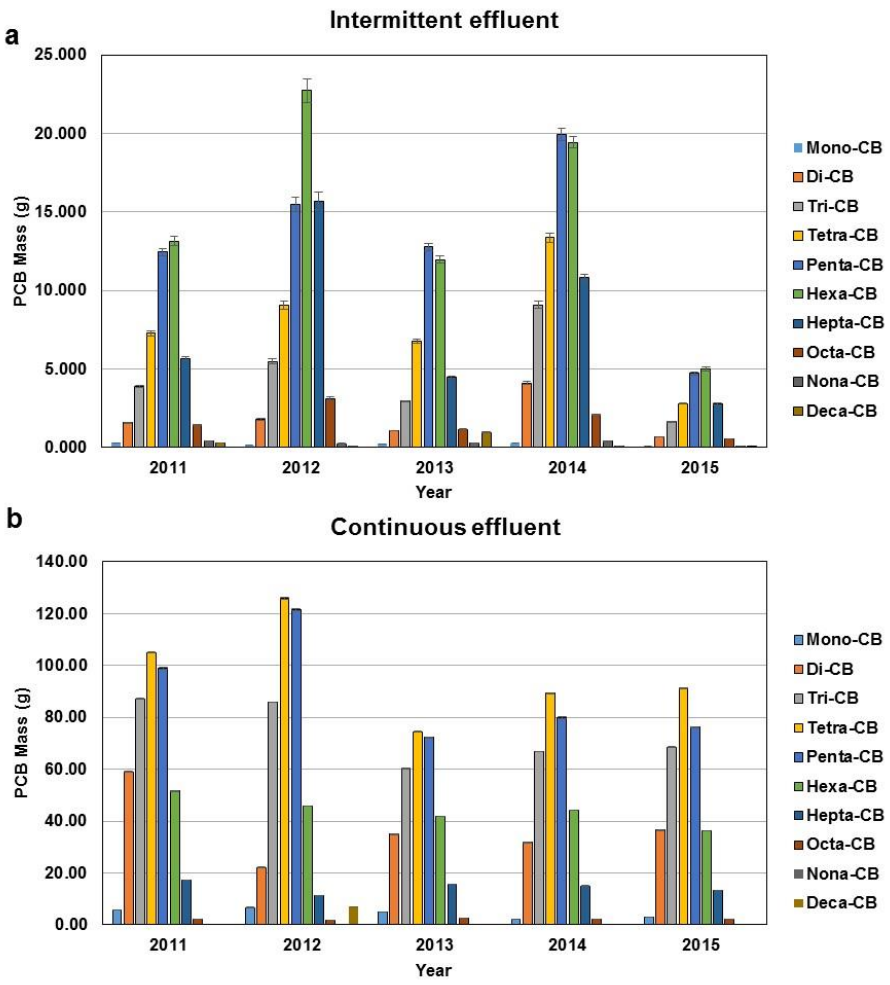

Figure 2.3 PCB homolog distribution from A) Intermittent effluent and B) continuous effluent.

2.3.3 PCB organohalide respiration patterns for discharged effluents

The average number of chlorine/biphenyl for 209 PCB congeners from the continuous effluent was 3.83-4.03 over a five-year study period (Table 2.4), which was 19% lower than that of the intermittent effluent (4.80 to 4.93). This difference of an average number of chlorine/biphenyl indicated that a PCB organohalide respiration process might occur during normal wastewater treatment. This was mainly attributed by the mass of tetra- and penta- chlorinated congeners that accounted for 47.8-57.8% of the total annual PCBs over five years for the continuous effluent (Table 2.2). In contrast, penta- and hexa-

chlorinated congeners had an increased abundance in the intermittent effluent with 49.5-58.2% of total annual PCBs from 2011 to 2015. Higher chlorinated PCB congeners can often be biotransformed to lower chlorinated PCBs by anaerobic organohalide respiration processes thus explaining the difference in chlorine per biphenyl values.

Table 2.4. The average number of chlorine/biphenyl for total PCBs from intermittent and continuous effluents.

	Intermittent effluent	Continuous effluent
Year	Cl/Biphenyl	Cl/Biphenyl
2011	4.88 (± 0.14)	3.83 (± 0.30)
2012	4.88 (±0.54)	4.02 (±0.39)
2013	4.93 (±0.18)	3.94 (±0.25)
2014	4.87 (±0.22)	4.03 (±0.15)
2015	4.80 (±0.37)	3.91 (±0.15)
Average	4.87 (±0.30)	3.94 (±0.28)

2.3.4 Aerobic and anaerobic biodegradation potential

Aerobic degradation of PCBs can occur for congeners with four or less chlorines per biphenyl. More specifically, the total annual PCB mass discharging to the river from the intermittent effluent only 27.8% can theoretically be aerobically biodegraded (Figure 2.4a), where tri- (4.60 g) and tetra- (7.85 g) chlorinated congeners had the highest abundance. In contrast, the potential for aerobic biodegradation for the continuous effluent was more pronounced and 58.3% (Figure 2.4b) could potentially be mineralized compared to the intermittent effluent. Similarly, tri- (73.7 g) and tetra- (97.2 g) chlorinated congeners contributed the most towards the total mass. On the other hand, intermittent effluent has high aerobically biodegraded PCBs accounting for 72.2% of the total annual PCB discharges over the five years period. For the intermittent effluent, penta- (13.1 g), hexa- (14.5 g), and hepta- (7.9 g) chlorinated congeners contributed the most with a total of 94.1% of the total PCB mass towards the anaerobic biodegradation potential. In comparison, the continuous effluent

was dominated by penta- (89.8 g), hexa- (43.9 g) and hepta- (14.6 g) chlorinated congeners that altogether made up 97.5% of the anaerobic biodegradation potential. More specifically, both the intermittent and continuous effluents had high anaerobic PCB degradation potentials (Ranaerobic = 98.7% for intermittent effluent, Ranaerobic = 93.7% for continuous effluent) considering all the potential PCB congeners that would be anaerobically degraded to *ortho*-PCB congeners (Figure 2S).

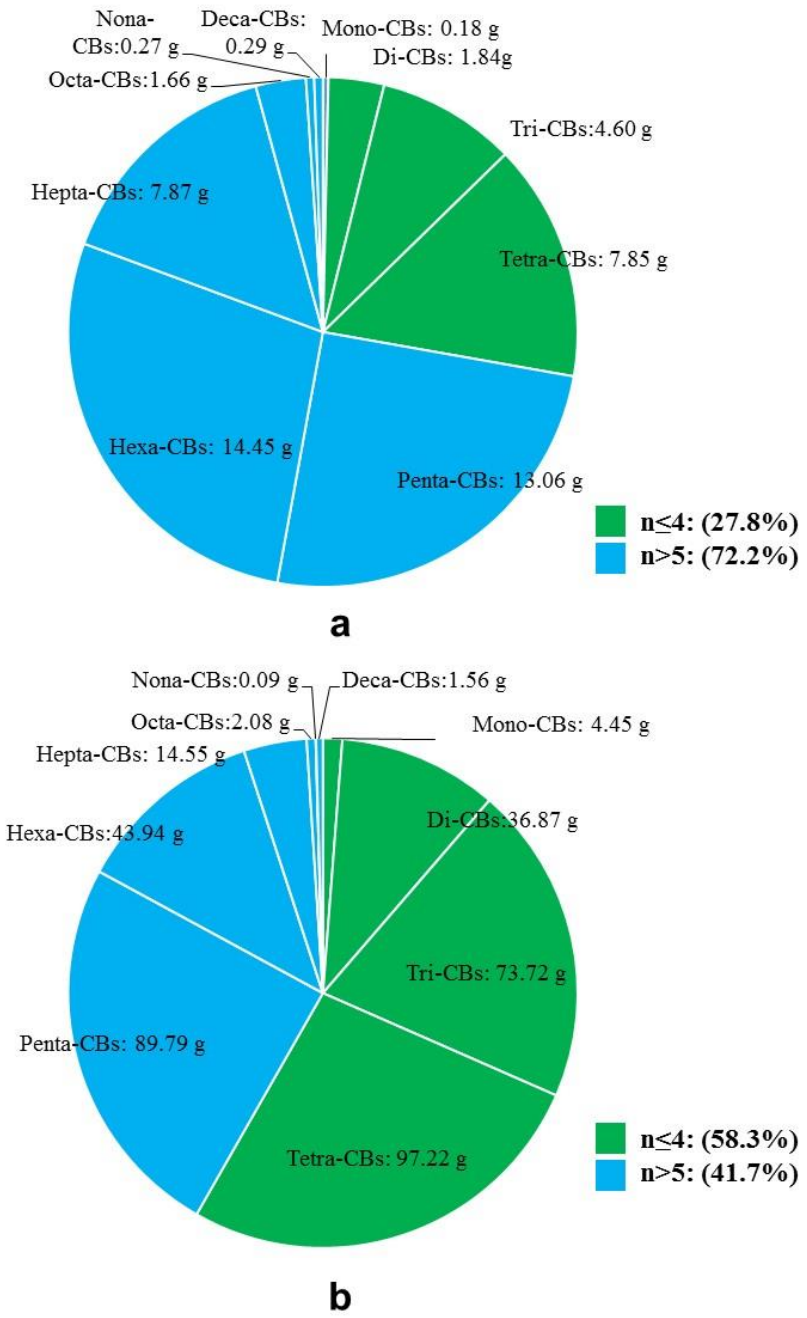

Figure 2.4 The potential for anaerobic organohalide respiration (n > 5) and aerobic degradation (n ≤ 4) for PCBs A) Intermittent effluent and B) continuous effluent.

2.4 Discussion

The evaluation of the PCB abundance and homolog analysis in the two effluents from the large municipal WWTP showed the presence of high levels of PCBs. In the continuous effluent, the average number of chlorines per biphenyl over the five year period was 3.94 ± 0.28 chlorines per biphenyl compared to 4.87 ± 0.30 chlorines per biphenyl for intermittent effluent containing the stormwater overflow. This difference indicated that organohalide respiration of PCBs likely occurred during the wastewater treatment processes. The rate and extent of PCB respiration depend on the number and positions of chlorines assuming the reductive conditions are present in the anaerobic environment (Grimm et al., 2015).

The amount of PCBs measured in the effluent might originate from historical PCB contamination sites in the area. PCBs have been considered as legacy pollutants thus potential sources could enter stormwater and sewage system (in case of combined sanitary and stormwater systems) thereby continuously release PCBs into the sewers, the WWTP and the receiving river. For instance, activities at a nearby Navy facility from 1995 to 1997 resulted in a potential PCB source releasing into the aquatic environment (Rosenfeld and Feng, 2011). Some sites housed PCB-containing portable generators and leaking generators were thought to have contaminated surrounding soil (Francingues et al., 2008). The high level of PCBs (Aroclor 1260) in the sediments in front of the Navy facility indicated potential PCB sources from ship bilge. The highest level of Aroclor 1260 (12,000 g/kg) was detected in sediment that was located at the end of a storm sewer line that drained a large transformer vault (Albright, 2013). It was also reported that a research laboratory in the area stored 148,800 gallons of PCB oil that were used to refill leaking or new transformers (Albright, 2013) thus risks for leaks were present. Furthermore, a nearby power plant was another historical PCB contamination source in the area (Hopf et al., 2014). Several studies reported that spills including PCB laden transformer fluids and fuel oils were released into the soil environment

between 1985 and 2003 (Haynes, 2013; Hwang and Foster, 2006, 2008a). As a result, infiltration into the stormwater system could be a current source of PCBs (Hwang and Foster, 2008b). A long-term plan for upstream monitoring of PCBs in the sewer system and thus PCB source control would be a potential solution to overcome the reduction in PCB discharge required according to the TMDL. Such a long-term plan could also include alternatives to reduce the possible PCB sources in the WWTP influent such as in-situ bioremediation at historical PCB contaminated sites and promotion of organohalide respiration of PCBs in the sewer pipe system/network. (Rodenburg et al., 2012) has demonstrated that organohalide respiration of PCBs occurs in many older sewer systems due to the accumulation of anaerobic material in the sewer lines.

The results from this study showed that there was a potential for aerobic and anaerobic biodegradation of PCBs present in the effluent. In the intermittent effluent from stormwater overflow 27.8% of total PCB was estimated to be susceptible to aerobic biodegradation, while 58.3% could undergo aerobic degradation in the continuous effluent. This difference could be explained by the processes that the PCBs in each of the effluents experience. For the continuous effluent, the PCBs pass through all the processes that are in place for removal of nutrients (carbon, nitrogen and phosphorous) including a long hydraulic time and several anaerobic processes that could allow for organohalide respiration to take place. The present organohalide respiration bacteria, therefore, can respire with the high chlorinated PCBs entering the WWTP (>4 chlorines/biphenyl) (De et al., 2006; Field and Sierra-Alvarez, 2008b) thereby transforming the higher chlorinated PCBs to lower chlorinated PCBs. However, even if the PCBs were biodegraded according to their estimated potential, the TMDL would still be exceeded by approximately 130 g of PCBs thus more extensive measures would have to be established to comply with the TMDL, which the WWTP is currently in the process of.

As a result, the PCB distribution pattern in the continuous effluent had increased potential for aerobic PCB degradation as compared to that in the intermittent effluent, since this effluent by-passed all the biological processes in the WWTP. The result of aerobic biodegradation of the lower chlorinated congeners is complete mineralization to carbon dioxide via the intermediate products cis-dyhydriol and 2,3-dihydroxy intermediate with low toxicity (Tu et al., 2011a). This process is performed by aerobic bacteria that possess the genes that encode for biphenyl-2,3-dioxygenase (bphA gene) and a dehydrogenase (bphB) (Tu et al., 2011a).

On the other hand, both effluents had high PCB anaerobic degradation potential (organohalide respiration) if all potential PCB congeners that would be anaerobically degraded to the *ortho*-PCB congeners are considered. This was mainly attributed that tetra- through hexa- chlorinated congeners that partially dechlorinated during the wastewater treatment processes that occur under normal flow conditions. Therefore the mass of tri- though penta- chlorinated congeners was higher in continuous effluent. Furthermore, the TEQ concentration of the dioxin-like PCBs from this effluent was reduced by 1-2 orders of magnitude compared to the intermittent effluent. This change in toxicity indicated a reduction of dioxin-like PCBs in the WWTP with stabilized anaerobic processes such as denitrification. Some studies have also shown that PCBs organohalide respiration occurred in urban wastewater treatment systems (Levén et al., 2012; Rodenburg et al., 2012; Rosińska, 2014; Rosińska and Karwowska, 2017). Rosinska (2014) demonstrated that approximately 98% of the PCB toxicity in wastewater sludge was reduced during thermophilic digestion. Rosińska and Karwowska (2017) suggested that PCB 169 was dominant in the wastewater sludge with concentrations ranging from 8.2 to 23.4 µg/kg. However, a significant degradation (77.8 to 80.5%) was observed for this dioxin-like PCB congener after the organohalide respiration process during anaerobic digestion. The PCB organohalide respiration signal from the

continuous effluent was also supported by results from molecular analysis of nine sampling locations from the metropolitan WWTP (Table S1).

Anaerobic organohalide respiration and aerobic degradation of PCBs are two complementary biological processes that allow for potential mineralization and thus removal of commonly used PCB mixtures (Abramowicz, 1990). In a wastewater treatment system, the PCBs could be aerobically biodegraded during the multi-stage aerobic treatment processes such as aeration for organic matter oxidation and the subsequent nitrification process. On the other hand, specific types of bacteria such as *Chloroflexi* could be enriched, when the microorganisms are present in an anoxic/anaerobic zone thus anaerobically dechlorinate PCBs in these parts of the plant (Bedard, 2008). Therefore, it is important to identify the proportion of PCBs that can be anaerobically and aerobically biodegraded based on the annual PCB discharge. Aerobic bacteria can attack the benzene ring and open the molecule so it becomes linear. PCB congeners with four or less chlorine atoms can be degraded by aerobic bacteria (e.g., Alcaligenes sp. JB1) growing with biphenyl as a carbon source (1996; Correa et al., 2010; Passatore et al., 2014). On the other hand, anaerobic organohalide respiring bacteria can attack highly chlorinated PCB congeners through the removal of *meta*- and *para*-chlorines (Lombard et al., 2014). As a result, highly chlorinated PCB congeners can be transformed to five different PCB congeners solely having *ortho* chlorine atoms (i.e., 2-Monochlorobiphenyl, 2,6-Dichlorobiphenyl, 2,2'-Dichlorobiphenyl, 2,2',6'-Trichlorobiphenyl, 2,2',6,6'-tetrachlorobiphenyl). Many studies have shown that highly chlorinated PCB congeners were reductively dechlorinated to lower chlorinated PCB congeners in a wastewater and sediment samples (Berkaw et al., 1996; Cutter et al., 1998; Fagervold et al., 2007; Kaya et al., 2013b; Wu et al., 2000). Many studies have indicated that the organohalide respiration predominantly attacked flanked *meta*-chlorines (Berkaw et al., 1996; Cutter et al., 1998; Fagervold et al., 2007; Fagervold et al., 2011; Wu et al., 2000), which has been

reported by other studies. Kaya et al. (2013a) investigated the effect of anaerobic digestion for different doses of PCB-118. A maximum of 7.95 ppm PCB-118 (26.5% removal efficiency) was dechlorinated to PCB-70 and PCB-67. This indicated that anaerobic digesters in WWTP could be a potential solution for PCBs organohalide respiration under suitable conditions (Kaya et al., 2013a).

2.5 Conclusions

Annual PCB discharges were determined from intermittent and continuous effluent in a WWTP by using five years of data from 2011 to 2015. The continuous effluent contributed a significantly larger mass of PCBs with a total mass (1821.4 g) for five years, which showed that this effluent contributed the majority of PCBs into the nearby river. A 19% difference in the average number of chlorine/biphenyl for the 209 PCB congeners for intermittent versus continuous effluent indicated a potential for organohalide respiration of PCBs during normal wastewater treatment. Also, a high mass distribution of tri-, tetra- and penta- chlorinated PCBs accounted for approximately 75% of the anaerobically biodegraded PCBs further indicated the possibility of PCB organohalide respiration occurrence for the continuous effluent. Based on the results from this study, a long-term monitoring program and PCB source control program needs to be established to prevent the exceedance of the TMDL. This could be done by detecting PCBs in the influent and establish appropriate action for further transformation of PCBs during wastewater treatment operation. Enhanced biodegradation of PCBs via bioaugmentation could be an alternative for removal PCBs. A combination of anaerobic organohalide respiration followed by aerobic biodegradation would be the best solution to achieve the complete removal of PCBs by final biomineralization. Further studies need to be conducted to establish a PCB mass balance for both the wastewater and biosolid streams in the WWTP to identify the behavior and fate of PCBs in wastewater. This study is

contributing to a continued evaluation of the PCB annual discharge and biodegradation potential in the wastewater effluents thus more recommendations will be gained from subsequent studies.

Chapter 3: Predicting the potential for organohalide respiration in wastewater: Comparison of intestinal and wastewater microbiomes

Ran Jing [a] and Birthe V. Kjellerup [a,*]

[a]University of Maryland at College Park, Department of Civil and Environmental Engineering, 1147 Glenn L. Martin Hall, College Park, MD 20742, USA;

Contact Information:

Ran Jing: rjingratmond88@gmail.com; Tel: 301-851-1728.

Birthe V. Kjellerup: bvk@umd.edu; Tel: 301-314-1535. *Corresponding author.

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ABSTRACT

Halogenated compounds such as polychlorinated biphenyl (PCBs) and polybrominated diphenyl ethers (PBDEs) enter wastewater treatment plants (WWTPs) via the sewage system. These organic contaminants partition between the aqueous and the biosolid phase, where the former is discharged as wastewater effluent. Biosolids from a WWTP provide a hydrophobic surface for adsorption and thus the presence and potential growth of organohalide respiring (OHR) bacteria. In this study, the aim was to assess the potential organohalide respiration capacity in wastewater biosolids by investigating actively organohalide respiring bacteria with a focus on organohalide respiration of PCBs and PCE. The results of the biosolids

analysis showed increased amounts of products from PCB respiration. Simultaneously, experiments with organohalide respiration of PCE in biosolids samples showed significant decreases PCE concentration after 46 days (28-92%). Subsequently, it was evaluated if the OHR microbial populations in biosolids were similar to those present in intestinal human biofilms by applying a bioinformatic approach. The OHR populations of the communities were analyzed from existing American and Chinese human intestinal microbiomes. The overall groups *Proteobacteria*, *Bacteroides*, *Actinobacteria*, and *Firmicutes* phyla dominated the microbiomes in all datasets. The OHR groups in biosolids and intestinal biofilms included *Dehalogenimonas*, *Dehalobacter*, *Desulfitibacter*, *Desulfovibrio*, *Sulfurospirillum*, *Clostridium*, and *Comamonas*. The results of this study showed that several OHR phyla were present in all samples independent of origin. Wastewater and intestinal microbiomes also contained OHR phyla. Overall, the results points towards using bacterial communities in biosolids as indicators of organohalide respiration in wastewater and intestinal microbiomes, which is related to ingestion or halogenated compounds.

Keywords: Biosolids; Intestinal biofilm; Organohalide respiring bacteria; Wastewater; halogenated compounds.

3.1 Introduction

Natural halogenated compounds that are present in seafood such as red algae, cod, oysters, shrimp, and herring are commonly a part of mammalian diets and thus being ingested by humans (Atashgahi et al., 2018b; Cardozo et al., 2007; Fielman et al., 1999). Therefore, the human gut microbiome comprises microorganisms that can break down these halogenated compounds (Atashgahi et al., 2018c; Claus et al., 2016). Within the past century, halogenated

compounds (both chlorinated and brominated) have also been chemically synthesized thus causing an increase of anthropogenic halogenated compounds present in the environment (Carvalho, 2017; Schafer and Kegley, 2002). Many of these halogenated compounds are persistent organic pollutants that bioaccumulate in the food web due to their high affinity for adsorption to hydrophobic organic materials (PCBs: $\log K_{ow}$ = 4.46-8.18, PBDEs: $\log K_{ow}$ = 5.74-10.33) and they subsequently biomagnify (Burreau et al., 2006; Kelly et al., 2007). Since the halogenated compounds have been a part of the human diet for thousands of years, it is likely that the intestinal biofilms in the human gut can provide an environment for the presence and potential growth of OHR bacteria (Yim et al., 2008). This would mainly be attributed to the fact that OHR bacterial cells are located in the biofilm in close proximity to the surface of intestinal villi thereby interacting with the hydrophobic organohalide compounds via adsorption and organohalide respiration. In this process, halogenated compounds act as terminal electron acceptors for anaerobic respiration with organic molecules as the electron donors (Atashgahi et al., 2016).

Studies of sewer systems (Hoai et al., 2010; Rodenburg et al., 2012) and wastewater treatment plants (Rodenburg et al., 2010; Vogelsang et al., 2006) have shown that organohalide compounds such as polychlorinated biphenyl (PCBs) and polybrominated diphenyl ethers (PBDEs) can enter wastewater treatment plants (WWTP) via the sewer system. In addition, 17 polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) congeners have been detected from 14 WWTPs in the Pilica River catchment at central Poland (Urbaniak et al., 2017). The concentrations of PCDD and PCDF in the effluents are ranged from 2.99 to 177.19 pg/l and 6.05 to 51.30 pg/l, respectively. Moreover, triclocarban (TCC) and triclosan (TCS) have also been detected in the biosolids samples from a WWTP in the Mid-Atlantic region of the US (Andrade et al., 2015). The results of their study indicated that the initial concentrations of TCC and TCS were 22900

and 25800 μg/kg dry activity sludge. Hexachlorocyclohexanes, dichlorodiphenyltrichloroethane (DDT) and its metabolic products (i.e., dichlorodiphenyldichloroethylene (DDE) and Dichlorodiphenyldichloroethane (DDD)), and dieldrin were also found in sewage treatment plants (Katsoyiannis and Samara, 2004; Kenny et al., 2017; Lian et al., 2019). In the WWTP, removal of the majority of halogenated organic compounds will take place due to adsorption to biosolids, whereas biodegradation during aerobic and anaerobic treatment processes can partially biodegrade halogenated compounds (Luo et al., 2014). However, despite the hydrophobicity of the halogenated compounds, a partition to the aqueous phase will occur, resulting in low concentrations being discharged via wastewater effluents (Jing et al., 2019a; Lee et al., 2014; Xiang et al., 2013). The biosolids in a WWTP system can provide a hydrophobic surface for adsorption and subsequent biodegradation of halogenated compounds thus enabling activity and potential growth of OHR bacteria in the system. Studies have identified OHR bacteria such as *Dehalococcoides sp*. and *Dehalobacter sp*. in biosolids and municipal wastewater treatment processes such as anaerobic digestion (Krumins et al., 2018; Smith et al., 2015). Other results showed that bacterial communities from biosolids samples were similar to those found in biofilm samples in the human gut (Cai et al., 2014b). The similarity was mainly attributed to the fact that human feces are the main source of content discharging into the sewage system. The results showed that 12-15% of the 16S rRNA gene sequences from biosolids and wastewater samples were also identified in intestinal biofilm samples (Shanks et al., 2013), while 97% of bacterial taxa from the intestinal biofilm samples were found in sewage samples (McLellan et al., 2010; Newton et al., 2015). McLellan et al. (2013) investigated the microbial communities of WWTP influents from 12 American cities by using massively parallel sequencing to examine if *Lachnospiraceae sp*. could be used to differentiate between human and non-human fecal pollution sources (McLellan et al., 2013). The results showed that

Lachnospiraceae sp. was the most abundant species in WWTP influents that could also be identified in human fecal microbiomes, since *Lachnospiraceae sp*. was detected in 46 of 48 human fecal samples (McLellan et al., 2013). Since this finding, Newton et al. (2015) compared the microbial community patterns in 137 human fecal samples and 200 sewage influent samples from 71 American cities. The results showed that the wastewater samples captured 97% of human fecal oligotypes based on sequencing. The most commonly identified bacterial families (*Bacteroidaceae*, *Ruminococcaceae*, *Lachnospiraceae*) that were found in the fecal samples were similar to those in sewage samples (Newton et al., 2015). These similar taxonomical patterns indicated that WWTP influents can represent the human fecal microbial community and capture the characteristics of the human intestinal microbiome (Price et al., 2018). Therefore, the presence of the specific taxa such as OHR bacteria in WWTPs and whether they are present in the human intestinal microbiome could be conducted on the basis of the general analyses of the microbiomes from the human gut and from WWTPs that show large similarities.

This current study focus on the presence of OHR bacteria in WWTP and whether these bacteria can also be found in human intestinal biofilms. Based on the analyses of the microbiomes from the human intestinal microbiomes and from WWTP, the question was developed whether OHR bacteria were present in the human intestinal microbiome due to the presence of halogenated compounds in food as well as the anthropogenic sources of halogenated compounds that can contaminate the food chain. The objectives of this study were to 1) identify potential organohalide respiring activity in WWTP biosolids; 2) evaluate the presence of OHR bacteria in WWTP biosolids; 3) Identify OHR bacteria in gut microbial communities from selected American and Chinese human populations through already existing microbiome datasets.

3.2 Materials and methodology

3.2.1 Wastewater and biosolids collection

The WWTP involved in this study daily treats approximately 380 million gallons of wastewater (Jing et al., 2019a). As shown in Figure 3.1, the untreated wastewater influent enters the WWTP, then large particles are removed by screens and in gravity settling basins. After that, organic matter is removed through aerobic biological treatment. Ammonia is simultaneously converted to nitrate and nitrite during nitrification and subsequently converted to nitrogen gas during denitrification. Finally, the wastewater passes through a filtration unit and is disinfected by sodium hypochlorite before it is discharged to a river. Wastewater samples were collected from 12 locations at the WWTP (Figure 3.1): 1) East Primary Influent, 2) West Primary Influent, 3) East Primary Effluent, 4) West Primary Effluent, 5) East Secondary Effluent, 6) West Secondary Effluent, 7) Nitrification/Denitrification Effluent, 8) Belt Filter Centrate, 9) Belt Filter Washing Water, 10) Centrifuge Centrate, 11) Dissolved Air Flotation Centrate, and 12) Gravity Thickening Centrate. In addition, biosolid samples were collected at seven other locations (Figure 3.1): 1) Primary Sludge, 2) East Secondary Sludge, 3) West Secondary Sludge, 4) Thermal Hydrolysis Processes, 5) Post Anaerobic Digester, 6) Dried Biosolids, and 7) Belt Filtration Cake. The collected samples were stored in 800 ml glass wide mouth jars and kept at 4 °C during transportation to the laboratory, where they were further stored at 4 °C until the start of the experiment the next day.

3.2 Wastewater and biosolids sampling and PCBs congener analysis

In this study, 10 PCB homologs and 209 PCB congeners were evaluated in different locations during the treatment processes (Figure 3.1). The wastewater samples were collected

as composite samples by using a Sigma 900Max #006 composite sampler (HACH Company, Loveland, Colorado), see Jing et al, (2019) for details on autosampler setup and sampling methods (Jing et al., 2019a). In addition, approximately 500 g of biosolids were collected as grab samples. The 209 PCB congeners were analyzed at a certified laboratory by using EPA method 1668B as described in Jing et al. 2019. The anaerobic dechlorination of PCBs can result in a reduction of chlorine numbers from the PCB molecular. Therefore, a lower mol% distribution of PCB homologs could indicate that a PCB organohalide respiration could potentially occur during the sewage collection system and wastewater treatment process. Evaluation of organohalide respiration with PCBs was performed by comparing the mol% distribution of PCBs and the chlorine per biphenyl ratio of the wastewater samples.

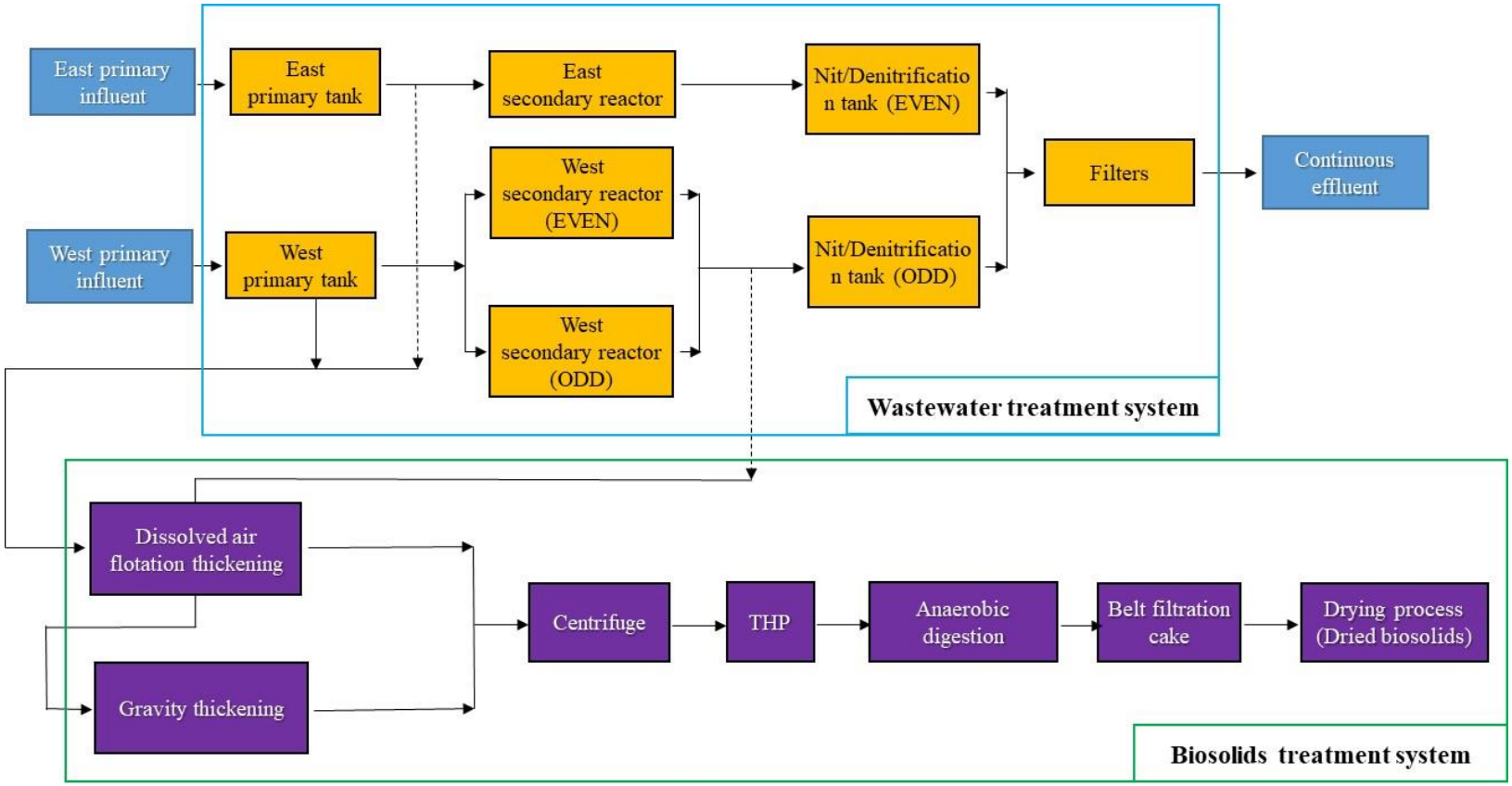

Figure 3.1 Flowchart of the major wastewater treatment processes for the WWTP described in this study.

3.2.3 Evaluation of organohalide respiration activity in biosolids

Five biosolid mesocosms (west secondary, east secondary, THP, primary, and post-anaerobic digester) were set up and spiked with tetrachloroethylene (PCE) to evaluate the potential for organohalide respiration. All mesocosms were set up in triplicates. In each, 10 g of biosolid was mixed with 75 ml of anaerobic E-Cl medium (Miller et al., 2005) and spiked with 3 ul PCE (Lombard et al., 2014) to a final concentration of 1.5×10^{-3} mg/ml (39 ppm). Stock solutions of acetate, propionate, and butyrate were prepared in 100 ml of boiling water to an individual final concentration of 1 mM in a fume hood under N_2 flow as a mix of carbon sources. After that, the mixed carbon source stock solution was added to the mesocosms at a final concentration of 2.5 mM (of each fatty acid) (Wu et al., 2000). Finally, the mesocosms were inoculated in an incubator without shaking at 30℃ for 30 days.

A gas chromatograph (Agilent 6890N) with a Flame Ionization Detector (GC-FID) (Agilent Technologies, Inc, Santa Clara, California) was applied in this study to analyze the headspace for the chlorinated compounds PCE, TCE, DCE, VC, and ethene. The presence of TCE, DCE, VC or ethane would indicate that organohalide respiration was occurring due to the detecting of organohalide respiration products. Headspace samples were collected under nitrogen pressure by using a clean syringe. A blank sample of nitrogen was run first followed by headspace samples of the mesocosms. The samples were injected into the port on the top of the FID inlet. The GC program used in this study was: Carrier gas (N_2) flow of 3 ml/min1, column temperature of 180 °C, a temperature program starting at 40 °C, increasing to 75 °C at 20°C/min, holding for 0.75 min, then increasing to 150°C at 45 C /min and holding for 0.35 min, using an injector temperature of 200 °C. After that, the potential organohalide respiration products from the headspace samples was captured by the FID and the concentration were calculated based on a standard curve with five concentrations (in triplicate) for each potential degradation product.

3.2.4 DNA extraction and polymerase chain reaction (PCR) of biosolid samples from a WWTP

DNA was extracted from samples collected at 11 different locations at the WWTP (Figure 3.1) by bead beating with a Fastprep Cell Disruptor (Qbiogene, Inc., Carlsbad, California) and extraction with MoBio Power Soil DNA Extraction Kit (Qiagen Sciences Inc., Germantown, Maryland). 1 ml of wastewater, 0.25 g of biosolid or mesocosm sample were extracted following the manufacturer's instructions (Qiagen Sciences Inc., Germantown, Maryland). The DNA concentration and quality were assessed using a spectrophotometer (NanoDrop ND-1000; NanoDrop Technologies LIc., Concord Plaza, Wilmington, North Carolina).

PCR was performed to evaluate the presence of OHR bacteria in the collected samples. PCR conditions and primers specific for the 16SS rDNA of OHR bacteria were. In this study, five different PCR primer sets TceA 1270F/1336R, Dhb447F/647R, Dhc1200F/1271R, and Dehcl348F/884R were applied to identify potential OHR bacteria (Fagervold et al., 2005; Payne et al., 2013; Sinclair et al., 2015). Tests of the assay with DNA extracted from the commercial SDC-9 bioaugmentation culture containing *Dehalococcoides mccartyi*, *Dehalogenimonas spp*., *Desulfovibrio spp*., *Desulfitobacterium spp*., Methanogenic bacteria, and Sulfate-Reducing bacteria was used as a positive control. After amplification, gel electrophoresis of PCR products were conducted for verification of formed fragments of correct size an ethidium bromide-stained agarose gel.

3.2.5 Metagenomic analysis of potential OHR bacteria from mesocosms

The bioinformatics data of mesocosms was based on the sequencing of the universal V3-V4 region. The mesocosm data were compared to the human intestinal microbiomes based on the V4 region (Shendure and Ji, 2008). The forward primer sequence was: was 5'-TCG-TCG-GCA-GCG-TCA-GAT-GTG-TAT-AAG-AGA-CAG-CCT-ACG-GGN-GGC-WGC-AG-3', while the reverse primer sequence was: 5'- GTC-TCG-TGG-GCT-CGG-AGA-TGT-GTA-TAA-GAG-ACA-GGA-CTA-CHV-GGG-TAT-CTA-ATC-3' (Integrated DNA Technologies, Inc. Coralville, Iowa). The human intestinal microbiome datasets used in this study were downloaded from The National Center for Biotechnology Information (NCBI) as the Sequence Read Archive (SRA) data. The wget and fastq-dump programs were applied to transfer SRA data to fastq files. After that, substitution and chimeras errors were removed prior to bioinformatics analysis.

The sequencing data for both biosolid samples and human fecal microbiota were analyzed by following protocol of the DADA2 Pipeline Tutorial v1.8 (https://benjjneb.github.io/dada2/tutorial.html). The sequencing fastq data of biosolid samples were filtered with the standard filtering parameters: (fnFs, filtFs, fnRs, filtRs, truncLen = c(290, 256), maxN = 0, maxEE = c(2, 2), truncQ = 2, rm.phix = TRUE, compress = TRUE, multithread=TRUE, trimLeft = 33). For the American intestinal microbiome dataset, the filtering parameters were sete up as: fnFs, filtFs, fnRs, filtRs, maxN = 0, truncLen = c(240, 140), maxEE = c(2, 2), truncQ = 2, trimLeft = c(35, 21). For the Chinese intestinal microbiome dataset, the filtering parameters were set up as: (fnFs, filtFs, fnRs, filtRs, maxN = 0, truncLen = c(275, 250), maxEE = c(2, 2), truncQ = 2, trimLeft = c(19, 20), rm.phix = TRUE, compress = TRUE, multithread = FALSE). The outputs of the DADA2 pipeline were assigned with microbial taxonomies through a modified SILVA database with a systematic clean-up of the noisy sequences which was updated with the exciting OHR bacterial collected

from the literature. The final product was an OTU amplicon sequence variant (ASV) table that records the number of times each exact amplicon sequence variant was observed in each sample.

3.2.6 Human fecal microbiota dataset

The 16S rRNA dataset included two parts. One was V4 16S-rRNA region (BioProject Accession PRJNA386260) from the National Center for Biotechnology Information (NCBI). The American human intestinal microbiomes used in this study were V4 16S-rRNA region with primer set 515F and 806R containing 5089759 reads. This dataset was sequenced using Illumina technology of intestinal biofilm samples collected from 211 healthy American human individuals from Michigan and it was used as the health control in a previous study of *Clostridium difficile* infection (Daquigan et al., 2017). The second was from the BioProject (Accession PRJNA383300). This 16S rRNA dataset contained intestinal biofilm samples collected from 94 Chinese humans. It was also sequenced using Illumina technology using V3-V4 16S rRNA region. The detailed information about the American and Chinese human intestinal microbial datasets was summarized into Table S2.

3.3 Results

3.3.1 Presence of organohalide respiring bacteria in wastewater and biosolids

The distribution of PCB homologs in wastewater samples showed that di- (4-20%), tri- (9-22%), tetra- (18-27%), penta- (18-32%), and hexa- (8-24%) chlorinated PCBs were most abundant (Table S3). Similar results were observed for the seven biosolid samples. Here, the distribution of these five homologs comprised 4-14%, 9-25%, 19-32%, 17-32%,

and 8-21%, respectively, of the total PCBs (Table S4). For the belt filter washing water, centrifuge centrate, and dissolved air flotation centrate, the most abundant homologs were also di- (8-11%), tri- (11-14%), tetra- (17-24%), penta- (29-32%), and hexa- (15-23%) (Table S5). For the belt filter centrate, however, the most abundant homologs were tri- (8%), tetra- (21%), penta- (33%), hexa- (20%), and hepta-chlorinated PCBs (8%). Similar results were also observed for the gravity thickening centrate with the distribution of these five homologs being 8.2%, 27.9%, 32.3%, 6.5%, and 20.9% of the total PCBs. More specifically, the number of chlorines per biphenyl for the intermittent and continuous effluents from the same WWTP in the previous study could also support the PCB organohalide respiration during the biological treatment process. The average number of Cl/biphenyl for 209 PCB congeners from the continuous effluent was ranged from 3.83±0.30 to 4.03±0.15 over a five-year study (Jing et al., 2019a). It is 19% lower than the Cl/biphenyl of the intermittent effluent (4.80±0.37 to 4.93±0.18). These could indicate that the PCB organohalide respiration occurred in the treatment process such as the anaerobic digester. The anaerobic digester can provide an appropriate environment that can result in the OHR bacteria growing on the surface of the biosolid particles. In addition, a relatively long residential time (22 days) of the anaerobic digester process could further provide allow the OHR bacteria interacting with the PCB molecules (Jing et al., 2019).

3.3.2 Organohalide respiration potential in wastewater and biosolid samples

Evaluation of the presence of organohalide respiting microorganisms in wastewater and biosolid samples (Table 3.1) was performed with molecular methods applying already published primer sets for 16S rRNA and functional genes (Da Silva and Alvarez, 2008; Kjellerup et al., 2008; Payne et al., 2011). The samples from east primary effluent, east secondary effluent, west secondary effluent, nit/denitrification effluent, west secondary

sludge and THP showed presence of organohalide respiring bacteria with the primer set for the primer set TceA 1270F/TceA 1336R (Integrated DNA Technologies, Inc. Coralville, Iowa) indicating the potential for biodegradation of TCE. The remaining samples showed the absence of this group of bacteria or presence below the detection limit (Smith et al., 2015). In addition, east primary effluent, west primary effluent, west secondary effluent, west secondary sludge, post anaerobic digester and belt filtration cake showed presence of one or more of the organohalide-respiring group, when the samples were evaluated with the primer set Dhb 447F/Dhb 647R (Integrated DNA Technologies, Inc. Coralville, Iowa). Moreover, most of the wastewater and biosolid samples showed the presence of OHR bacteria when tested with the primer set Dhc 1200F/Dhc 1271R (Integrated DNA Technologies, Inc. Coralville, Iowa) except samples from primary sedimentation and pre-THP. Furthermore, the presence of OHR bacteria was detected with the primer set Dehcl 348F/Dehcl 884R (Integrated DNA Technologies, Inc. Coralville, Iowa). Altogether, the results showed the potential for organohalide respiration in many locations in both the wastewater and biosolids processing steps.

Table 3.1. Presence and absence of organohalide respiring bacteria in the wastewater and biosolids samples as a result of PCR evaluation.

	TceA 1270F/1336R	Dhb 447F/647R	Dhc 1200F/1271R	Dehcl 348F/884R
East primary effluent	+	+	+	+
West primary effluent	-	+	+	+
East secondary effluent	+	-	+	+
West secondary effluent	+	+	+	+
Ni/denitrification effluent	+	-	+	+
Primary sludge	-	-	-	-
East secondary sludge	-	-	+	+
West secondary sludge	+	+	+	+
Pre-THP	+	-	-	+
Post anaerobic digester	-	+	+	+
Final cake	-	+	+	-

3.3.3 Organohalide respiration of PCE in mesocosms

A previous study of the large municipal wastewater treatment plant showed that organohalide respiration with PCBs occurred (Jing et al., 2019a). In this current study, biosolid samples from the same WWTP but from different locations were set up as mesocosms to assess whether organohalide respiration with PCE can occur. After 36 days, DCE was detected in two mesocosms (east secondary sludge and post-anaerobic digester) thus showing organohalide respiration (Table 3.2). For the east secondary mesocosm, the organohalide respiration was observed after 36 days (the average mass percent of PCE decreased from 99 to 7%). Similar results were observed for the post-anaerobic digester mesocosm after 46 days (PCE from 99 to 71%) with simultaneous detection of respirations products.

Table 3.2. Organohalide respiration of PCE in activity assays for selected samples from the wastewater treatment plant.

		Day 1				Day 36				Day 46			
	Mesocosm	PCE	TCE	DCE	VC	PCE	TCE	DCE	VC	PCE	TCE	DCE	VC
East secondary sludge	1	99.0%	0.0%	1.0%	0.0%	83.4%	16.6%	0.0%	0.0%	19.8%	67.2%	13.0%	0.0%
	2	100.0%	0.0%	0.0%	0.0%	16.1%	76.3%	7.6%	0.0%	0.0%	15.3%	36.3%	48.3%
	3	98.6%	0.0%	1.4%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	0.2%	98.6%	1.2%
Post-anaerobic digester	1	96.6%	0.0%	3.4%	0.0%	88.7%	11.3%	0.0%	0.0%	100.0%	0.0%	0.0%	0.0%
	2	100.0%	0.0%	0.0%	0.0%	94.5%	0.0%	5.5%	0.0%	68.1%	22.2%	9.7%	0.0%
	3	100.0%	0.0%	0.0%	0.0%	82.5%	12.6%	4.9%	0.0%	73.5%	22.1%	4.4%	0.0%

Table 3.3. The abundance of identified organohalide respiring bacterial genera from the biosolid samples.

	Genus	Primary sludge (OTU)	East secondary sludge (OTU)	West secondary sludge (OTU)	THP sludge (OTU)	Post-anaerobic digester (OTU)
Obligate OHR bacteria	*Dehalogenimonas*	0	0	0	0	36
Versatile OHR bacteria	*Dehalobacter*	22	0	0	0	27
	Desulfitibacter	0	0	17	0	7
	Desulfovibrio	0	29	0	54	0
	Sulfurospirillum	0	17	0	0	121
	Clostridium	0	85	0	17	0
	Comamonas	0	113	0	67	1436

3.3.4 Microbial community structure and presence of OHR-bacterial genera in biosolid samples

The microbial populations in the five biosolid samples used for mesocosms were assessed (Figure 3.2) and showed that the main phyla were similar in east secondary sludge, west secondary sludge, post-anaerobic digester sludge, and primary sludge. *Firmicutes* were the most dominant phylum (up to 32% in primary sludge, 44% in west secondary sludge, and 39% in post-anaerobic digester sludge). *Bacteroidetes* (with relative abundances between 26 and 34% of the classified reads) were the second most abundant phylum. The bacterial community composition in biosolids collected post-THP was notably different compared to other biosolid samples. Here, *Proteobacteria* was the most dominant phylum (64%) (Figure 3.2).

Evaluation of OHR-bacterial genera showed that obligate OHR bacteria were found in biosolids from east secondary, post-anaerobic digester sludge, and primary sedimentation locations (Table 3.3). According to Atashgahi et al. (2016), known OHR bacteria can be classified as facultative and obligate groups based on their energy-gaining metabolism (Figure S2 and Figure S3). Both obligate and versatile OHR bacteria were found in five biosolid samples. The post-anaerobic digester sample contained the most diverse OHR population (six bacterial genera) and also the largest relative abundance (Table 3.3).

The most abundant OHR bacterial detected genus was *Comamonas* accounting for 5.3% of the total microbial community. One of the species in this genus is a marine bacterium *Comamonas sp*. 7D-2 that can respire with organohalide substrates (Atashgahi et al., 2018a). Another OHR bacterium in the genus was detected in an anaerobic digester, *Sulfurospirillum* (relative abundance of 0.4%). *Dehalogenimonas* was the only obligate OHR bacterial group detected in the five biosolid samples in this study based on the bioinformatic approach. The presence of *Dehalogenimonas spp*. was also demonstrated by PCR with the primer set Dhb

447F/Dhb 647R. *Dehalogenimonas spp*. Can respire with several organohalides including PCBs and dibenzo-p-dioxins, which have been found in WWTP solids (Liu et al., 2014; Wang and He, 2013). In addition, biosolids from east secondary and post-THP also had a diverse OHR bacterial population. For the east secondary sludge, four OHR bacterial genera were identified including *Desulfovibrio* (0.11%), *Sulfurospirillum* (0.07%), *Clostridium* (0.3%), and *Comamonas* (0.4%). For the post-THP sample, the OHR bacterial genera included *Desulfovibrio* (0.2%), Clostridium (0.07%), *Comamonas* (0.3%), and Enterobacter (0.4%), which in total accounted for 2.8% of the total relative microbial abundance.

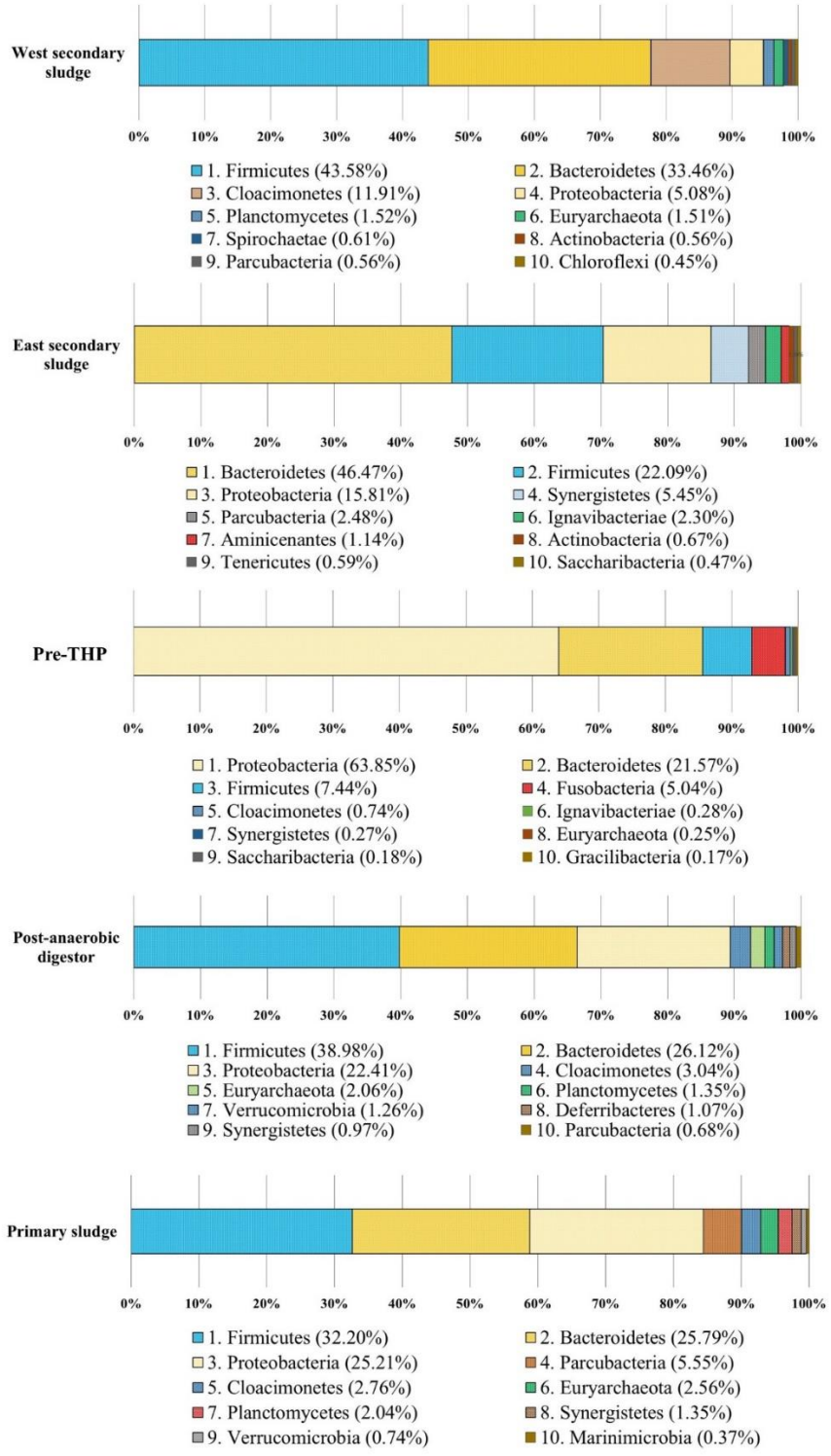

Figure 3.2 Relative abundance of phyla within the bacterial community of five biosolid samples from different locations at the WWTP

3.3.5 OHR bacteria in American and Chinese intestinal microbiomes

The microbiomes from 211 healthy American human intestinal samples contained 12 phyla, 23 classes, 34 orders, 61 families and 203 genera after the analysis of the V4 region of the 16S rRNA gene (Figure 3.3). In total, the 12 phyla covered 95.7% known microorganisms and among these the following four phyla were dominant: *Firmicutes* (60.8%), *Bacteroidetes* (24.4%), *Actinobacteria* (6.5%), and *Proteobacteria* (4.0%). For the 94 Chinese intestinal microbiomes, 13 phyla were identified and the relative abundance was similar to the American samples with *Firmicutes* (61.1%), *Bacteroidetes* (25.7%), Actinobacteria (11.6%), and Proteobacteria (1.5%). These results were consistent with other studies (Arumugam et al., 2011; Bäckhed et al., 2015; Huttenhower et al., 2012; Thursby and Juge, 2017) and indicated that the gut microbiome is primarily composed of the *Bacteroidetes* and *Firmicutes*.

The American and Chinese microbiomes exhibited similar compositions of OHR bacterial genera (Table 3.4). Four OHR bacterial genera were detected in the American dataset: *Desulfovibrio* (0.2%), *Clostridium* (0.6%), *Comamonas* (0.001%), and *Enterobacter* (0.2%), whereas five OHR genera were detected in the Chinese dataset. Four were the same as those observed in the American dataset and the fifth was *Enterobacter* (0.01). Three OHR bacterial genera dominated: *Dehalobacter* (0.15%), *Desulfovibrio* (0.06%) and Clostridium (0.12%). The relative abundances of the remaining two genera *Comamonas* and Enterobacter were insignificant compared to the other OHR-bacterial genera.

Table 3.4. Abundance of identified OHR bacterial genera from the human fecal microbiomes (total OTU from 211 American and 94 Chinese individuals, respectively).

	Genus	American fecal microbiome (OTU)	Chinese fecal microbiome (OTU)
Obligate OHR bacteria	*Dehalogenimonas*	0	0
Versatile OHR bacteria	*Dehalobacter*	0	0
	Desulfitibacter	0	0
	Desulfovibrio	8786	1587
	Sulfurospirillum	0	0
	Clostridium	21941	3349
	Comamonas	26	3

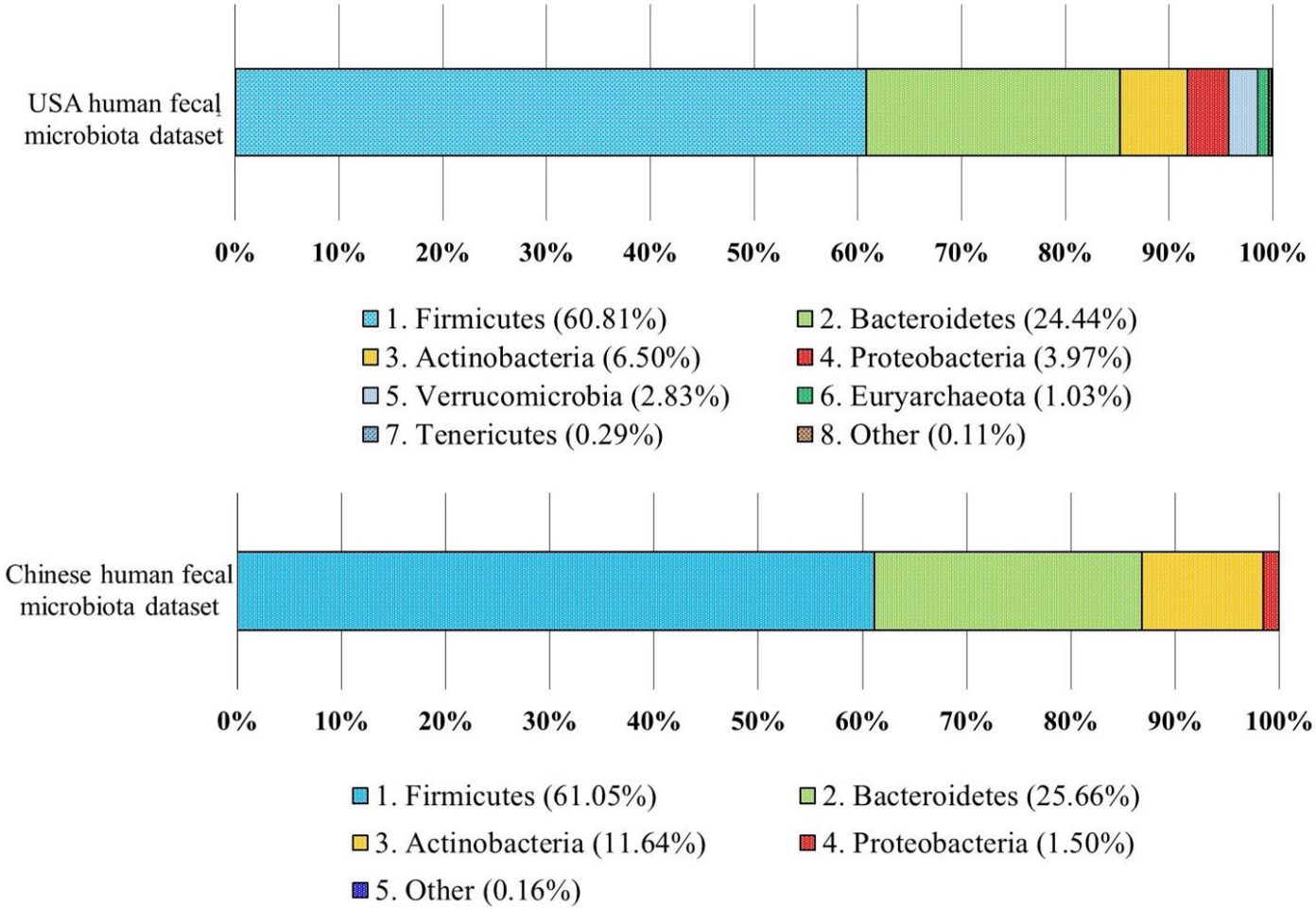

Figure 3.3 Relative abundance of phyla in bacterial communities from American and Chinese human fecal microbiomes.

3.4 Discussion

The distribution of PCB homologs indicated that PCB organohalide respiration occurred in the biosolid treatment system of the WWTP, with a higher degree during belt filtration and cake processing. In the wastewater system, the amount of lowly chlorinated PCB homologs (Cl < 4) increased across the east primary process to the nitrification/denitrification process. The post-digester biosolids were compressed through the belt filtration process, which can potentially result in an increased number of bacteria due to water loss and OHR bacteria can be physically forced to close proximity of the surface of the biosolid particles. As a result, lowly chlorinated PCBs might be accumulated due to organohalide respiration of PCBs (Payne et al., 2011). In addition, a large volume of the final cake during the final processing could further provide an anaerobically sealed zone thereby potentially allowing the OHR bacteria interacting with the PCB molecules (Praveckova et al., 2016).

An accumulation of *ortho*-PCBs (i.e., PCB-1, PCB-4, PCB-10, PCB-19, PCB-54) could further support the occurrence of PCB organohalide respiration in both wastewater and biosolid samples (Figure 3.4). *Ortho*-PCBs are mainly generated by OHR bacteria that remove chlorines from *meta*- and *para*-positions during respiration (Sowers and May, 2013b). Only a few studies have shown reductive dechlorination of *ortho*-PCBs in the environment (Fagervold et al., 2011; May et al., 2006; Sowers and May, 2013b) thus the accumulation of ortho-PCBs is a sign of organohalide respiration.

An accumulation of *ortho*-PCBs was also observed in the biosolid samples accross the biosolid treatment processes. This could be attributed to the fact that the biosolid treatment facility had a relatively long biosolid retention time in for instance the anaerobic digestion process (22 days) thus providing an environment, which support sulfidogenesis and methanogenesis.

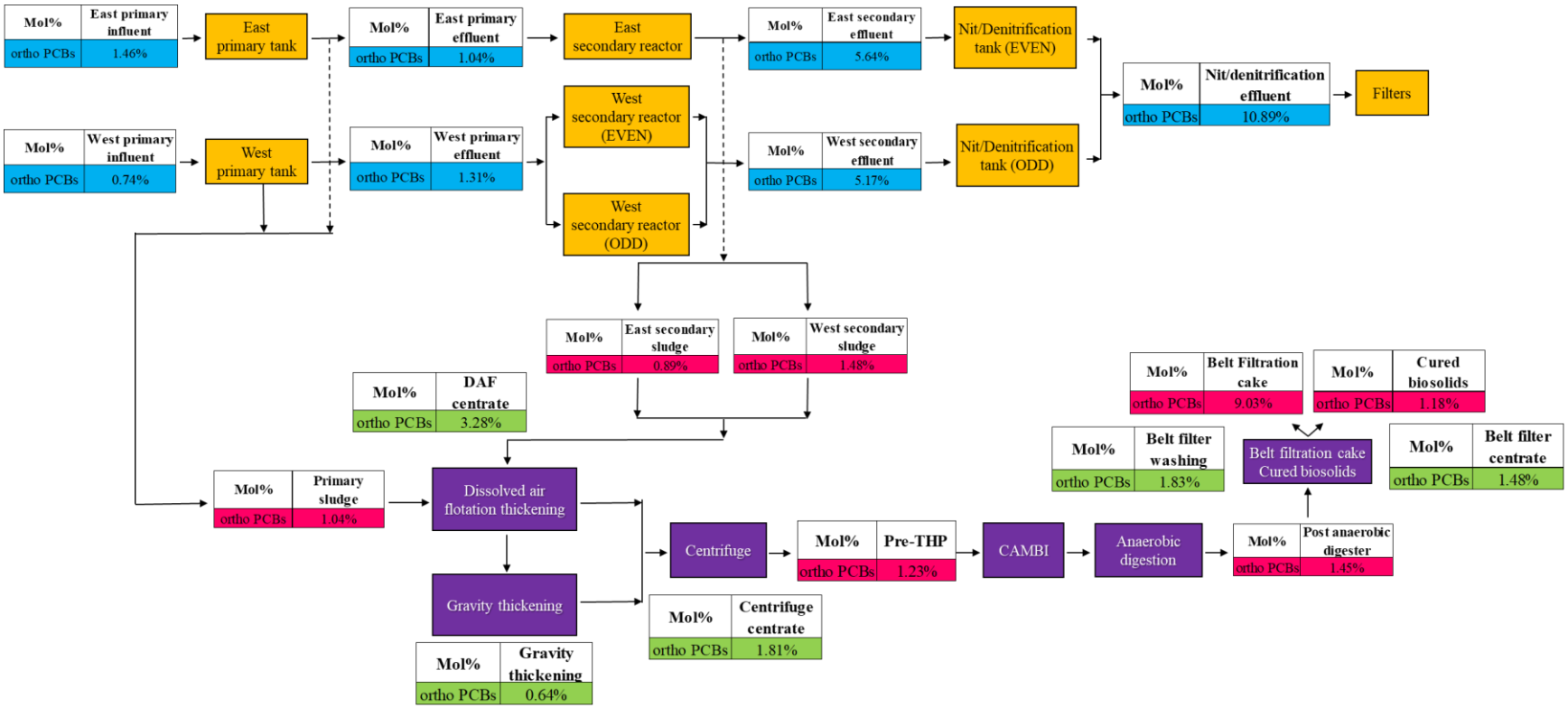

Figure 3.4 Assessment of organohalide respiration of PCBs shown as the presence of non-flanked *ortho* chlorinated PCBs (PCB 1, 4, 10, 19, 54) in wastewater and biosolid samples. The numbers are shown as mol% *ortho* chlorination. A high level of non-flanked *ortho* chlorinated PCB congeners shows that organohalide respiration of PCBs has occurred.

In this study, three out of seven identified OHR bacterial genera (*Desulfovibrio*, *Clostridium*, and *Comamonas*) from the American and Chinese human intestinal microbiomes were also identified in wastewater and biosolid samples. These three genera have been implicated in organohalide respiriration (Chang et al., 2000; Hou and Dutta, 2000; Mayer-Blackwell et al., 2016). In addition, the OHR bacteria at the genus level were similar among the east secondary, post THP, American and Chinese human intestinal microbiomes, although differences in relative abundances were detected. The post-anaerobic digester biosolids sample contained six OHR bacterial genera dominated by *Comamonas* and *Sulfurospirillum* (Figure 3.5). Based on these results it is possible that the potential for organohalide respiration in the WWTP system could be attributed to the OHR bacterial communities originating from the human intestinal microbiomes.

As discussed previously, three facultative OHR genera (*Desulfovibrio*, *Clostridium*, and *Comamonas*) from the American and Chinese human intestinal microbiomes were also identified in wastewater and biosolid samples. This observation suggests that the potential of organohalide respiration from the WWTP system could be attributed to the OHR bacterial communities originating from the human intestinal microbiomes. The organohalide compounds can reach into the food chain and bioaccumulate in the human body thereby inducing the gut microbiota composition such as the OHR bacterial communities (Atashgahi et al., 2018c; Vrieze et al., 2014). Ingestion of contaminated food such as micro-concentration of residues in vegetables and fruits is one of the main routes of exposure to the organohalide compounds for the general population (Atashgahi et al., 2018c). The organohalogen antibiotics added to animal feed can reach into the human body through the food chain, once the animal's stools were used for fertilizers. In addition, a subconscious food lifestyle in America and China can also impact the structure of the human gut microbiome (David et al., 2014) particularly for the microbial community of the OHR bacteria. American

people ate large amount of seafood with the average American consumed 16.0 pounds of fish and shellfish in 2017 (Morson et al., 2017). Marine food webs are the main route for the bioaccumulation of the organohalide compounds such as PCBs and polychlorinated dibenzo-p-dioxins (PCDDs) (Zanaroli et al., 2015b). A recent study highlighted the bioaccumulation of organohalide compounds that a regular monitoring of trout from the Great Lakes has identified more than 60 organohalogens including PCBs, polybrominated diphenyl ethers (PBDEs), and Dichlorodiphenyltrichloroethane (DDTs) (Fernando et al., 2018). Moreover, Chinese people have traditions to use plants containing natural halogens such as *Foeniculum vuLgare* and *Angelica dahurica* to cook food (Gao et al., 2014; Wang et al., 2016). After ingestion of these foods, the anaerobic environment of the human gut is appropriate for their reductive metabolism. The generated by-products are transported and further metabolized in the liver by oxidative and conjugative enzymes (Atashgahi et al., 2018c).

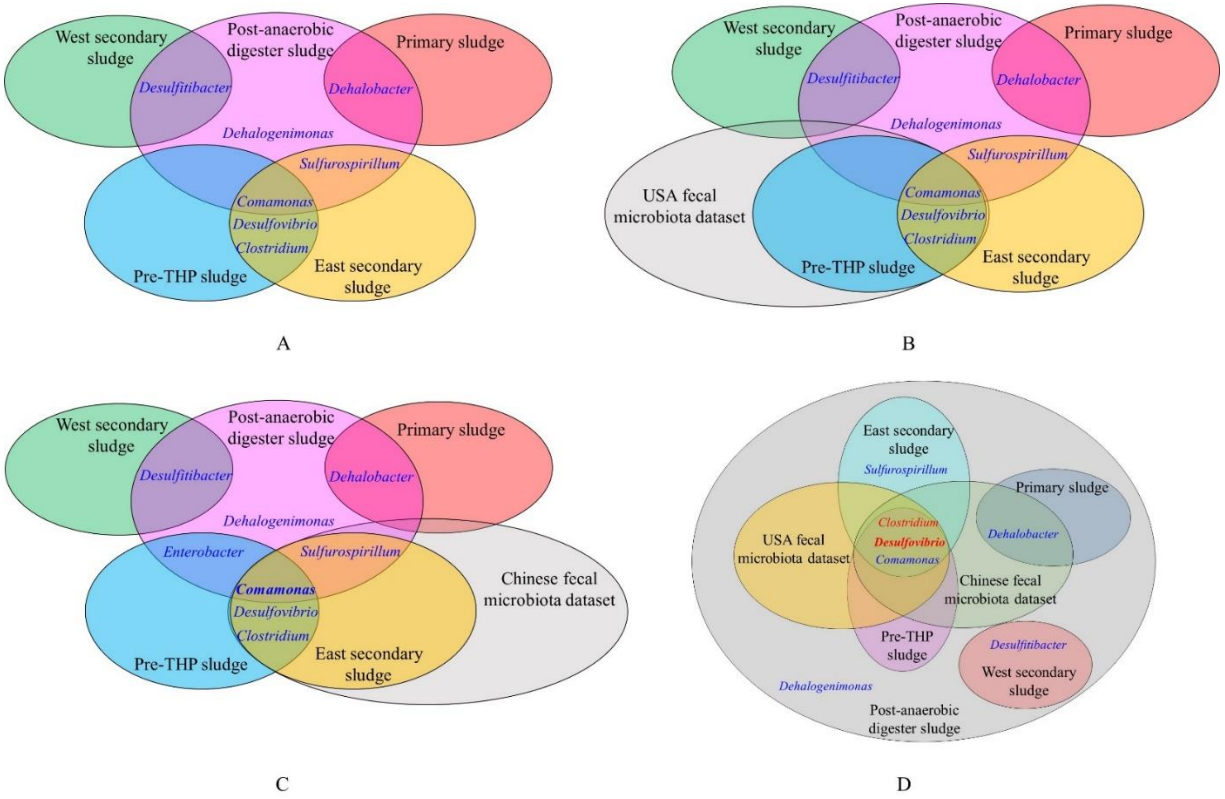

Figure 3.5 Relationship of OHR bacterial genera among the five biosolids samples and the American and Chinese human fecal microbiomes. A) All the samples contained OHR bacteria that can potentially use PCE in organohalide respiration; B) American human fecal microbiomes had an overlap containing three genera of OHR bacteria in the biosolids from the thermophilic hydrolysis process (THP); C) Chinese human fecal microbiomes had an overlap containing four genera of OHR bacteria with the east secondary sludge; D) post-anaerobic digester sludge covered the major genera of OHR bacteria from the human fecal microbiomes except for *Clostridium* and *Desulfovibrio* (highlighted by red color).

3.5 Conclusion

In this study, the potential OHR bacteria in a WWTP were identified. The bioinformatic profiles of the biosolid samples were compared with that of the gut microbial community from the selected American and Chinese human intestinal datasets. The distribution of PCB homologs and the number of chlorines per biphenyl of the wastewater samples showed that the organohalide respiration occurred in both wastewater and biosolids samples. The bacterial community composition showed the presence of OHR bacteria including *Dehalogenimonas*, *Dehalobacter*, *Desulfitibacter*, *Desulfovibrio*, *Sulfurospirillum*, *Clostridium*, and *Comamonas* thus supporting organohalide respiring capacities in wastewater and biosolids. In addition, the analyzed microbiomes of the WWTPs shared similarities with the human fecal microbiome and contained potential OHR bacteria. The possibility of organohalide-respiring in a WWTP system has been identified by analyzing the microbial communities from the biosolid samples. According to this study, an important factor that impacts the microbial community of the OHR bacteria in human gut could be the food contaminated with organohalide pollutants or the food containing natural halogenated compounds. Moreover, the bacterial communities of the biosolid samples from a WWTP system could be good indicators of a city's estimated level of organohalide compounds ingestion due to their similarities with the human fecal microbiome and contained potential OHR bacteria.

Chapter 4: Remediation of Polychlorinated Biphenyls (PCBs) in Contaminated Soils and Sediment: State of Knowledge and Perspectives

Ran Jing [a] Soliver Fusi[a], and Birthe V. Kjellerup [a,*]

[a]University of Maryland at College Park, Department of Civil and Environmental Engineering, 1147 Glenn L. Martin Hall, College Park, MD 20742, USA;

Contact Information:

Ran Jing: rjingratmond88@gmail.com; Tel: 301-851-1728.

Birthe V. Kjellerup: bvk@umd.edu; Tel: 301-314-1535. *Corresponding author.

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ABSTRACT

Polychlorinated biphenyls (PCBs) are one of the persistent organic pollutants (POPs) used worldwide between the 1930s and 1980s. Many PCBs can still be found in the environment such as in soils and sediments, even though their use has been heavily restricted. This review summarizes the most frequent remediation solutions including, phytoremediation, microbial degradation, dehalogenation by chemical reagent, and PCBs removal by activated carbon. New insights that emerged from recent studies of PCBs remediation including supercritical water oxidation, ultrasonic radiation, bimetallic systems, nanoscale zero-valent iron based

reductive dehalogenation and biofilm covered activated carbon, electrokinetic remediation, and nZVI particles in combination with a second metal are overviewed. Some of these methods are still in the initial development stage thereby requiring further research attention. In addition, the advantages and disadvantages of each general treatment strategy and promising technology for PCBs remediation are discussed and compared. There is no well-developed single technology, although various possible technologies have been suggested. Therefore, the possibility of using combined technologies for PCB remediation is also here investigated. It is hoped that this present paper can provide a basic framework and a more profound prospect to select successful PCB remediation strategies or combined technologies.

Keywords: Bioremediation, Dehalogenation, Ex-situ, In-situ, Polychlorinated biphenyls (PCBs), Remediation technologies

4.1 Introduction

Polychlorinated biphenyls (PCBs) have been used for industrial purposes since 1929 (Alcock et al., 1994). The physical and chemical properties of PCBs allow them for a wide range of industrial applications. Their electrical insulating properties allow for their use with electrical equipment, such as in cooling, for instance (Merkel et al., 1999). PCBs are classified as persistent organic pollutants (POPs) with high toxicity, and have undesirable effects on the environment and on humans (Lallas, 2001a). Once released into the environment PCBs they could bioaccumulate within the food chain, due to their high affinity for organic materials. They have been found in human's tissues, blood, and breast milk and are introduced via the consumption of meat, fish, and dairy products (Van den Berg et al.,

2006). Consequentially, they have been linked to chronic effects in humans including immune system damage, decreased pulmonary function, bronchitis and interferences with hormones leading to cancer (Schecter et al., 2006). Additionally, studies indicated that children will show serious developmental problems such as low birth-weight, behavioral disorders, and hearing loss at relatively high exposure to PCBs (more than 10 pg/kg body weight per day) (Urban et al., 2014). Literature also provides evidence of the effects of PCBs exposure to animals (e.g., rats) such as: liver damage, immune system suppression, abnormalities in fetal development, enzyme induction, sarcomas, non-Hodgkin lymphomas, and serum lipids (Goncharov et al., 2008; Kogevinas, 2001).

The PCB molecule consists of two connected benzene rings and chlorine atoms that can attach to any or all of 10 different positions allowing for 209 different congeners and 10 different homologs (Alder et al., 1993; McFarland and Clarke, 1989). High chlorinated PCB congeners typically have relatively high octanol-water partition coefficients (Kow) thus are often found in organic matter such as soils and sediments. Due to a low water solubility and vapor pressure, PCBs partition between the aquatic and solid phase thus exist in multiple compartments resulting in widespread contamination (Tanabe, 1988).

PCBs can be released into the atmosphere by incineration of PCB-containing waste, leaking from landfills with PCB containing products, and disposed industrial waste, among others (Van Gerven et al., 2004). In US, the most common remediation technology for PCB-contaminated soil or sediments are incineration or disposal in landfills (Gomes et al., 2013). Other strategies such as biological, chemical, physical and thermal methods are also widely applied in PCB remediation. However, most of these solutions are disruptive, unsustainable and transfer PCBs to different sections of the environment, rather than ridding them (Agarwal et al., 2007). Therefore, current research is being conducted to find sustainable, alternative remediation technologies for persistent organic pollutants. This review paper aims to: 1)

critically evaluate the state of these promising technologies for PCB contaminated soils, sediments, and waters; 2) discuss the possibility of improving PCB remediation by simultaneously combing multiple treatment strategies; 3) explore future research prospects regarding PCB remediation on the basis of a comprehensive review of the current PCB remediation technologies discussed in this paper.

4.2 Traditional PCB Remediation Technologies

4.2.1 Phytoremediation of PCBs

Phytoremediation has been recognized as an ecologically responsible alternative for removing organic pollutants in soils, water and sediments (Meagher, 2000). The most precise of phytoremediation techniques is plant-mediated bioremediation (Macek et al., 2000). Phytoremediation processes can be summarized as two steps: the biodegradation of organic pollutants in soil or groundwater and uptake into plant tissues through their roots followed by transformation by plant enzymes or direct volatilization into the atmosphere (Harvey et al., 2002). Plant species used for PCB phytoremediation including *Medicago sativa* (alfalfa), *Lespedeza cuneate* (Chinese bushclover), *Lathyrus sylvestris* (everlasting pea), *Phalaris arundinacea* (reed canary grass), *Cucurbitaceae* (cucurbits), *Sparganium* (bur-reed), *Salix alaxensis* (Alaska willow) and *Picea glauc*a (white spruce) as well as 27 different weeds (Chekol et al., 2004); Slater et al. (2011) Ficko et al. (2010). Tu et al. (2011b) showed a decrease in PCB soil concentrations by 31.4% and 78.4% after the first and second years of field scale phytoremediation by *M. sativa* via situ phytoremediation. *Sparganium* has been shown to promote the oxidation of the low-chlorinated PCBs via *rhizodegradation*, while *panicum virgatum* (switchgrass) and other popularly used plants have shown degradation of a high and low congener mix (Meggo et al., 2013). Wyrwicka et al. (2014) found that

Cucurbitaceae could be used to reduce PCBs concentration in sludge and sediment by 38.63% and 27.38% respectively.

Microorganisms play a significant role in PCB biodegradation during rhizoremediation process due to a relatively large K_{ow}. Several studies demonstrated that a distributed network of plant roots can activate microbial processes i.e., "rhizodegradation" or "rhizoremediation", which are capable of degradation of PCBs in contaminated soils (Passatore et al., 2014). For example, plant roots can release microbial growth factors and extracellular enzymes or organic acids which can be used as electron donors for anaerobic dehalogenation processes or facilitate microbial metabolism (Gerhardt et al., 2009).

Phytoremediation is a green (environmentally friendly) and low-tech remediation method with low environmental impacts. An ideal plant for phytoremediation should include the following characteristics: high biomass production, broad root distribution, and an ability to tolerate and accumulate contaminants (Gomes et al., 2013). Phytoremediation is a low cost method due to the absence of energy-consuming equipment (Pivetz, 2001). In addition, phytoremediation has little to no destructive impact on soil fertility and structure, while the introduction of plants can improve the overall condition of the soil due to the plant and mirobes introducing minerals and nutrients (Gerhardt et al., 2009). It should be noted that PCBs intaking by plants mainly attributed by uptake and translocation. However, plants are not only capable of PCB attenuation in soil and sediments, but also the capability of plants to metabolize PCBs. Some studies indicated that dichloro-, trichloro-, and tetrachlorobiphenyl congeners can be metabolized by plant cell cultures of *Rosa spp* (Lee and Fletcher, 1992). More persistent 2,2′,5,5′-tetrachlorobiphenyl could be oxidized to 3,4-dihydroxy-2,2′,5,5′-tetrachlorobiphenyl by plant cell cultures of Rosa spp. (Harms et al., 2003). Other studies have indicated that the PCB concentration increased in the stems and leaves of pumpkins (Cucurbita sp.) in Aroclor contaminated soil (Åslund et al., 2008). However, the PCB

concentration in the pumpkin roots was unchanged. These observations support that the mechanism of PCB transport in plants can mainly be attributed to uptake and translocation compared to volatilization and deposition (Aken et al., 2009). PCBs have high octanol-water partition coefficients (K_{ow}) from $10^{4.10}$ (20°C) for mono-chlorobiphenyl to $10^{7.93}$ for deca-chlorobiphenyl influencing the mobility of PCBs in the environment (Zhang et al., 2013). Therefore, the higher-chlorinated PCB congeners with a high K_{ow} (log K_{ow} > 6) tend to be present in soils and sediments compared to lower-chlorinated PCB congeners. As a result, soils often contain a higher proportion of highly chlorinated PCB congeners. More importantly, most of the studies suggested that the metabolism of PCBs by plants is only limited to tetrachlorinated and lower congeners (Aken et al., 2009). Due to the high hydrophobicity of higher-chlorinated PCB congeners, these are less often taken up and transported inside of the plants thereby experiencing limited metabolism by plant tissues. Due to these mechanisms. High-chlorinated PCB congeners are usually more resistant to the metabolism process than the lower chlorinated congeners in most of the cases. As a result, the high-chlorinated PCB congeners accumulate in biomass and tend to release to the environment, when the process of plant decomposition occurs after the plants are dead. However, phytoremediation also offers several limitations. One of the drawbacks is that PCB remediation generally implies long-term monitoring. One estimate was that an industrial site contaminated with a PCB mixture (trichlorobiphenyl and tetrachlorobiphenyl) requires 20 years to be remediated (Kaštánek et al., 1999). More importantly, plants lack the biochemical pathway to achieve mineralization of pollutants. Another significant disadvantage is that phytoremediation systems may lose their effectiveness, when plant growth slows or stops due to extreme weather and other such factors (Pivetz, 2001). Furthermore, the effectiveness of phytoremediation systems can be restricted by plant depth due to the generally shallow distribution of plant roots or by the transfer of contaminants from plants to other ecosystems

(Pivetz, 2001). Additionally, the introduction of noxious or invasive vegetation can bring negative impacts to animals and other plants in the ecosystem (Pivetz, 2001).

4.2.2 Microbial Degradation of PCBs

Microbial degradation or bioremediation is defined as a natural biological process that relies on microorganisms (e.g. bacteria, fungi) to degrade, break down, transform, and remove contaminants or hazardous materials (Vidali, 2001). Evidence for the microbial degradation of PCBs in natural environments such as soil, sediment, and surface water has been well reported in various studies (Anyasi and Atagana, 2011; Bedard, 2008). Microbial degradation of PCBs encompasses two possible pathways: anaerobic dehalogenation and aerobic degradation.

Microbial degradation of highly chlorinated PCB congeners is generally achieved by organohalide respiration under anaerobic conditions (Field and Sierra-Alvarez, 2008a). Organohalide respiration of PCBs is a biological process that potentially decreases the toxicity of PCBs through the removal of chlorines (Häggblom et al., 2003). During this process the chlorine substituent is replaced with hydrogen. The PCB congeners serves as the terminal electron acceptor with three potential chlorine substituent positions; *para*, *meta* and *ortho*. (Brown et al., 1987). Substitution of a chlorine with a hydrogen atom preferentially occurs at the first two sites. The potential pathway for anaerobic dehalogenation of a highly chlorinated PCB congener is illustrated in Figure 4.1, using 2,3,4,5,6-Pentachlorobiphenyl (PCB-116) as an illustrated example (Van Dort et al., 1997). Here, the highly chlorinated PCB is transformed to a lighter chlorinated congener (Abramowicz and Olson, 1995). The first case study of anaerobic dehalogenation of PCBs was reported by Brown et al (1987). Since then, studies have validated the potential of anaerobic organohalide respiration in marine sediments (Quensen III et al., 1988).

Over the past decade in-situ bioremediation technologies which can be applied to treatment of PCB impacted soils and sediments in the field have been conducted (Sowers and May, 2013a; Tyagi et al., 2011). Two major types of bioremediation techniques include biostimulation and bioaugmentation. Some studies indicated that biostimulation by halopriming with halogenated aromatic compounds can increase the dehalogenating microbial catalysts of indigenous PCB dechlorinating bacteria and induce genes required for dehalogenation (Sowers and May, 2013a). Biostimulation is a process to add the nutrients or substrates to a contamination site to stimulate the activities of autochthonous microorganisms (Azubuike et al., 2016). For the cases of anaerobic degradation of PCBs, biostimulation of PCB indigenous dechlorinating bacteria can be achieved by halopriming with halogenated aromatic compounds such as halogenated benzoates (Sowers and May, 2013). The chlorine atoms of PCB congener are replaced with hydrogen during this process. Over the past decade the biostimulation of anaerobic PCB degradation was widely applied to treatment of PCB impacted soils and sediments, however, the precise mechanism is still unclear. On the other hand, biostimulation can also apply to aerobic bioremediation that the microorganisms can use oxygen to breakdown the low-chlorine content PCBs. Biphenyl is the primary substrate that can support co-metabolism of PCBs (Sharma et al., 2017). During the aerobic degradation, the benzene ring with less chlorines of the PCB molecular is destructed. This process involves several genes which are mainly bph gene clusters i.e. bphA (dioxygenation of the biphenyl ring), bphB (dehydrogenase), bphC (ring cleavage dioxygenase). The bphA gene is involved in dioxygenation of the biphenyl ring with the formation of dihydrodiol. The bphB gene is involved the oxidation of biphenyl-2,3-dihydrodiol to 2,3-dihydroxybiphenyl. The bphC gene is responsible for biphenyl ring cleavage to generate phenylcatechol. An earlier example is the stimulation of Aroclor 1260 in sediment slurries which demonstrated that the addition of bromated biphenyl congeners (PBBs) achieve more effective stimulation

leading to complete dehalogenation. Other haloprimers such as tetrachlorobenzene (TeCB), pentachloronitrobenzene (PCNB), tetrabromobisphenol A (TBBPA) have resulted in an increase of native PCB dechlorinating bacteria such as *Dehalococcoides sp, Ochrobactrum sp, Parasegetibacter sp, Thermithiobacillus sp, Phenylobacterium sp*, and *Sphingomonas sp* (Krumins et al., 2009; Li et al., 2016; Park et al., 2011). Biostimulation has also been achieved by using electrochemical techniques to treat the PCB contaminated groundwater or sediment (Chun et al., 2013; Yu et al., 2016). Voltage was applied to the contaminated sediments from a superfund site (Fox River) to stimulate the oxidative and reductive transformation of Aroclor 1242 with an overall concentration reduction of 40-60% (Chun et al., 2013). In Guangdong, China, an application of bioanode stimulation resulted in dehalogenation of 2,3,4,5-tetrachlorobiphenyl in an electronic waste recycling site by 42% after 110 days of incubation (Yu et al., 2016).

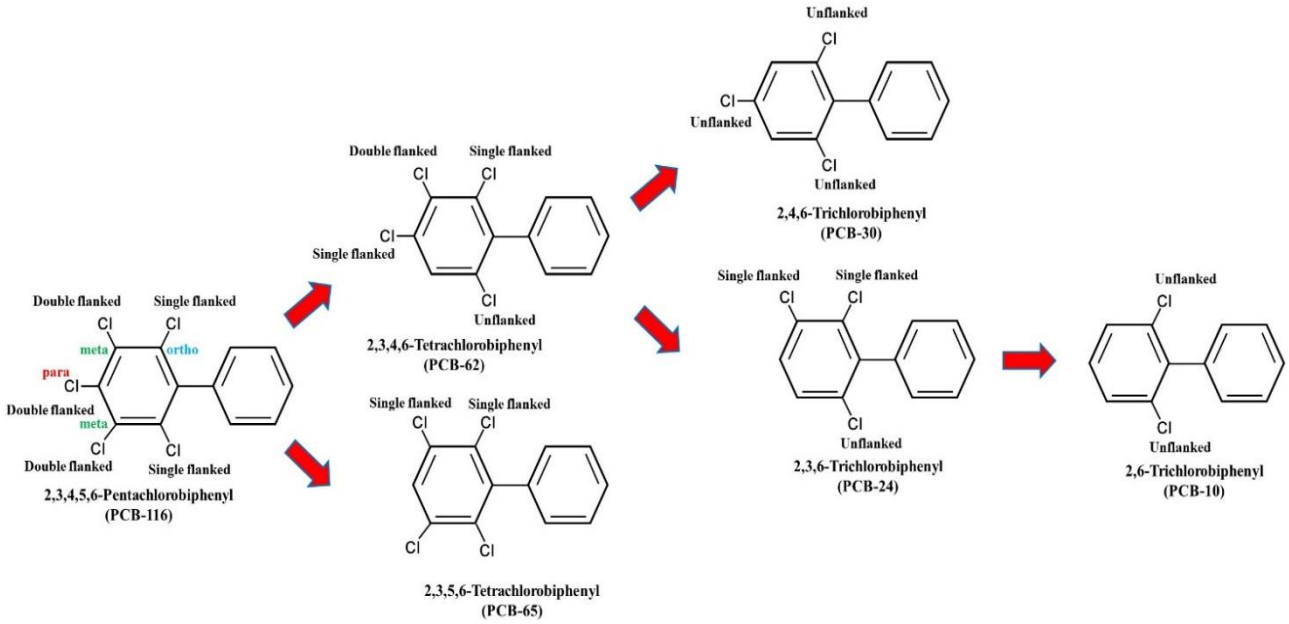

Figure 4.1 Possible dechlorination pathway of 2,3,4,5,6-Pentachlorobiphenyl and the distribution of products (Van Dort et al., 1997).

Bioaugmentation, a feasible, in-situ, PCB transformation pathway, is defined as the addition of bacterial cultures to accelerate the degradation rate of a contaminant (Tyagi et al., 2011). May et al (2008) reported that the isolated ultramicrobacterium from sediment is capable of dechlorinating Aroclor 1260, containing double-flanked chlorines, when they were added to the contaminated soil (May et al., 2008). Similarly, Payne et al (2011) found that *D. chlorocoercia* DF1 could enhance the dehalogenation of weathered Aroclor 1260, containing double and single flanked chlorines (Payne et al., 2011). The total concentration of pentapolychlorinated biphenyls in mesocosm experiments decreased by 56% after bioaugmentation with DF1 for 120 days. More recently, biofilm covered activated carbon particles as a delivery system for bioaugmentation to enhance PCB dehalogenation have been studied. For example, Kjellerup et al. (2013) showed that the dehalogenation of Aroclor 1248 was enhanced in a mesocosm study with sediment (Kjellerup and Edwards, 2013). These studies provide convincing evidence that biofilm covered activated carbon as an in-situ treatment of weathered PCBs exhibits a number of advantages. Therefore, this is will be discussed in more detail as an independent technology for PCB remediation in the following section. The major environmental condition that could affect the efficiency of bioaugmentation technique is different phases. When PCBs release into the environments, the PCB distribution among different phases such as solid phase and aqueous phase is driven by their partition coefficient. The transport and partitioning behaviors of the 10 different PCB homologs vary with their structural differences, which represent by different octanol-water partition coefficients (K_{ow}) indicating the fugacity of PCBs in the environment, from $10^{4.10}$ (20 °C) for monochlorobiphenyl to $10^{7.93}$ (20 °C) for decachlorobiphenyl (Zhang et al., 2013). As a result, the concentration of PCBs in different phases could significant vary. The average air concentrations of PCBs may range from 120 to 170 pg/m3, however, the average

concentration of PCBs in background soil could be from 100 to 1000 pg/g (Batterman et al., 2009). One example could be monochlorobiphenyl which is more likely to accumulate to air phase based on its aqueous solubility, vapor pressure and log K_{ow}. Bioaugmentation is mainly *applied* in sediment, soil, and solid phases. Therefore, it is expect that the use of bioaugmentation approaches may considerably limited by the environmental fate of monochlorobiphenyl. In addition, the distribution of high-chlorine content PCB congeners in different phases also highly depend on their partitioning coefficients. Some highly chlorinated PCB congeners sharing similar chemical properties such as the similar vapor pressure for both PCB-77 and PCB-153 (4.4×10^{-7} mm Hg at 25 ℃ and 3.80×10^{-7} mm Hg at 25 ℃) (Erickson, 1997; Mackay et al., 1992). However, it does not mean they have the similar chemical fate. The logK_{ow} of the PCB-153 (8.35 at 25 ℃) is two orders of magnitude higher than that of PCB-77 (6.04-6.63 at 25 ℃) resulting a significantly different distribution in soil or water phase (Dunnivant et al., 1992). As a result, the bioavailability of PCB-153 is significantly higher than that of the PCB-77 in soil phase resulting in a high removal efficiency of bioaugmentation.

Lightly chlorinated PCB congeners, with three or less chlorine atoms per molecule, could be biodegraded by aerobic bacteria (Borja et al., 2005). Two different clusters of genes are involved during this aerobic, oxidative, destruction process: one is responsible for converting the PCB congeners to chlorobenzoic acid, the other for the degradation of the chlorobenzoic acid (bphA) through cometabolic aerobic oxidation (Borja et al., 2005). Many bacterial strains are capable of oxidative degradation of PCBs such as *Pseudomonas*, *Burkholderia*, *Comamonas*, *Rhodococcus* and *Bacilus* (Anyasi and Atagana, 2011). The common by-product of biphenyl degradation is chlorobenzoic acid through 1,2-dioxygenative ring cleavage. Monochlorobiphenyl, for example, can serve as substrates for bacteria growth, because the biphenyl can be used as a carbon and an energy source when PCB congeners

degrade by a cometabolic process (Anyasi & Atagana, 2011). Some biphenyl-utilizing bacteria such as *Burkholderia sp*. LB400 can convert PCBs into chlorobenzoic acids using biphenyl dioxygenase (bphA) through cometabolic aerobic oxidation (Furukawa et al., 2004). Other bacterial species can further breakdown chlorobenzoic acid into less toxic compounds (Bianucci et al., 2004). This has been observed in many studies regarding *Pseudomonas sp.* and *Micrococcus sp* (Bevinakatti and Ninnekar, 1922; Boyle et al., 1992).

The aerobic and anaerobic bacterial biodegradation of PCBs has been well studied and successfully used. Microbial degradation has relatively low technical barriers as compared with the promising remediation technologies for PCBs. As mentioned previously, the residues for microbial degradation of PCBs are generally less-toxic products and cell biomass. Highly chlorinated molecules can be degraded through the pathway of reductive dehalogenation resulting in 2 to 3 chlorine congeners as the major metabolites. In addition, microbial degradation of PCBs does not disrupt normal activities. This eliminates the waste transported off site thereby reducing the potential threats to human health and the environment. It is worth noting that microbial degradation has a smaller environmental footprint than incineration of PCB contaminated soils. The results of a life cycle assessment indicated that the impact on global warming was nine times less than that of incineration producing 6.5×10^5 kg CO_2-eq and 7.2×10^4 kg CO_2-eq, respectively (Busset et al., 2012). However, microbial degradation of PCBs is also considered problematic, even though it is used worldwide (Kumar et al., 2011). The anaerobic dechloronation of PCBs is a long-term, labor-intensive process with efficiencies specific and limited to target PCB and the bacterial species. Not all PCB congeners can be rapidly or even completely degraded (Kumar et al., 2011). Many aquatic environments are not suitable for the growth of aerobic microorganisms, because only the top layer of sediments is aerobic.

4.2.3 Dehalogenation of PCBs by Chemical Reagent

The objective of dehalogenation using chemical reagents is converting PCBs to low level toxic compounds through progressive replacement of chlorines (Kulkarni et al., 2008a). In order to destroy PCBs, chemical reagents additionally need high temperatures and pressures. The chemical reagents most commonly used are Mg and Zn/acidic or basic solution, Fenton's reagent (FR), and low-valent metals (e.g., alkali metal in alcohol) (Kulkarni et al., 2008a). The first application of dehalogenation using chemical reagents can be traced back 90 years when commercial phenols were first used to dehalogenate dioxins and furans. Likewise, chemical reagents can also be used to dehalogenate PCBs, converting them to non-hazardous or less toxic compounds during the treatment. PCB congeners can be dehalogenated with a relatively high efficiency, in short to medium time periods compared to biological treatments (Huling and Pivetz, 2006). Mitoma et al. (2004) conducted their study for detoxification of PCBs using metallic calcium in ethanol for 24 hours under atmospheric pressure and room temperature, resulting in 98% reduction. Ryoo et al. (2007) developed a practical disposal of PCBs using polyethylene glycol 600, potassium hydroxide and aluminum. The average removal efficiency of PCBs was about 78% at 100 °C with 2 hours, which increased to 99% at 150°C and 4 hours, particularly for PCB-77, PCB-105, PCB-118, PCB-123 and PCB-169. Nah et al. (2008) used a fine metal powder, glycol and alkali to remove PCBs from waste insulating oil resulting in a removal efficiency of 99.9% for total PCB concentration.

In addition, some studies reported that a combination of chemical solutions and catalysts i.e., catalytic hydrodehalogenation, can result in a higher dechlorination performance (Xu and Bhattacharyya, 2007). It is usually performed with transition metals such as Ni or Pd as heterogeneous catalysts in organic solvents. Extensive research has been conducted on catalytic hydrodehalogenation of PCBs. Gomes et al. (2013) summarized the

studies on reductive dehalogenation of PCB in their review paper (Gomes et al., 2013). Such a combination of chemical solution and catalysts could allow PCB dehalogenation in short times under mild conditions (e.g., ambient temperature) with low energy requirement (Ehsan et al., 2003). Nonetheless, this technology is still difficult to apply to the actual presence of PCB contaminated sites because of necessary consideration of multiple uncertainties. For example, when this technology is used to treat contaminants at high concentrations, excessive amounts of reagents are necessary (Gomes et al., 2013). Furthermore, this technology is more impactful on soils due to high temperatures and strong acid and alkali conditions (Gomes et al., 2013).

4.2.4 Removal of PCBs by Activated Carbon

Activated carbon are widely applied for removal of hazardous organic and inorganic compounds due to the highly porous structure of carbonaceous materials that increase the surface area (500-2500 m^2/g) for adsorption or chemical reactions. Adsorption is a physical process where adsorbates are attracted onto the surface structure of materials such as activated carbon. Activated carbon are generally made from natural, renewable and low cost materials (e.g., coconut shell, pall fiber, hardwood, bamboo, lignite, bark husk, peanut hull, coir pith, maize cob, sawdust, and rice husk) (Das et al., 2015). Activated carbon can be classified into three categories depending on the pore size: micropores (diameter < 2 nm); mesopores (2 nm < diameter < 50 nm); and macropores (diameter > 50 nm) (Kim et al., 2015). Activated carbon applied for removal of PCBs can also be classified on the basis of their morphologies: granular activated carbon (GAC), powdered activated carbon (PAC), bead activated carbon (BAC), activated carbon fibers (ACFs), and carbon nanotubes (CNTs). Physical properties of these selected activated carbon involved in removal of PCBs from

literature are summarized in Table 4.1 (Hung et al., 2011; Ji et al., 2014; K.Vasilyeva et al., 2010; Kim et al., 2007; Kim et al., 2008; Li et al., 2003; Mangun et al., 2001).

The particle size and pore size of activated carbon result in relative larger contact surface area between adsorbents and adsorbates, which provide conditions for PCB adsorption to occur. Activated carbon has been widely applied for PCB-contaminated soils. Activated carbon is a sensible choice for PCB removal from contaminated soil and it is recognized as one of the most efficient fundamental approaches (Foo and Hameed, 2010). Cho et al. (2014) used activated carbon to enhance PCB immobilization (Choi et al., 2014). They also developed a pilot study in Hunters Point Shipyard at San Francisco in which there was a 73% decrease of PCBs transferred from sediments into the aquatic environment during a 60-month span, when activated carbon (3.7% dry wt.) was added to the sediment (Choi et al., 2009). Vasilyeva et al. (2010) evaluated the removal performances of PAC and GAC in soils contaminated with PCBs (i.e. trichlorobiphenyl, tetrachlorobiphenyl and pentachlorinated congeners). The results of this study indicated that reduction of PCBs in AC-amended soil is mainly attributed to a decrease in trichlorobiphenyl and tetrachlorobiphenyl congeners. Kjellerup et al. (2013) used granular activated carbon to reduce the concentration of PCB contaminated sediments by sequestration. This study showed that the homolog distribution of PCB dechloronation products significantly changed after 500 days. The final dehalogenation products of penta- through hepta-chlorobiphenyl congeners were shifted from tri- through penta-chlorobiphenyls to mono- through tri-chlorobiphenyls. More specifically, Denyes et al. (2012) showed an 89% reduction in the bioavailability of PCBs in historically contaminated soils using biochar (Denyes et al., 2012). Comprehensive reviews of the possibility of biochar for PCB-contaminated soils and sediments can be found in (Beesley et al., 2011; Gomes et al., 2013; Hilber and Bucheli, 2010; Rakowska et al., 2012).

Table 4.1 Physical properties of the selected activated carbon

Materials	Particle Size (mm)	Pore Size (nm)	BET Surface Area (m^2/g)	Total Pore Volume (cm^3/g)	References
PAC	0.001-0.1	<2	1000	0.78	(Vasilyeva et al., 2010)
PAC	0.006-0.16	15.8	1380	0.513	(Kim et al., 2007)
PAC	0.004-0.33	17.4	2031	0.771	(Kim et al., 2007)
PAC	0.005-0.23	13.1	1221	0.435	(Kim et al., 2007)
GAC	N/D[a]	2.25	374.9	0.21	(Ji et al., 2014)
GAC	N/D	3.50	715.8	0.63	(Ji et al., 2014)
GAC	0.4-1.5	<2	880	0.86	(Vasilyeva et al., 2010)
BAC	230	53	193.8	0.26	(Kim et al., 2008)
ACFs	N/D	8.1	730	N/D	(Mangun et al., 2001)
ACFs	N/D	12.2	1585	N/D	(Mangun et al., 2001)
ACFs	N/D	13.4	1890	N/D	(Mangun et al., 2001)
CNTs	N/D	N/D	122	0.28	(Li et al., 2003)

[a]N/D stands for “No Data”.

Moreover, activated carbon can easily be integrated with other auxiliary technologies (e.g., microwave decomposition) or catalysts and this combination of different technologies has been used in recent studies (Liu et al., 2007). Some examples of this combination of multi-technology are; the application of microwave and granular activated carbon for the treatment of soil contaminated by 2,4,5-trichlorobiphenyl (PCB-29) (Liu and Yu, 2006), synthesis of reactive nano-fe/pd bimetallic system-impregnated activated carbon for the simultaneous adsorption and dehalogenation of PCBs (Choi et al., 2008), substituted chlorines of high-chlorinated PCB congeners by activated carbon impregnated with Fe coupled with Pd (Choi et al., 2009), removal of dioxin-like PCBs from fish oil by countercurrent supercritical CO_2 extraction and activated carbon (Kawashima et al., 2009), and nano-zerovalent iron contained porous carbon for the adsorption and dehalogenation of PCBs (Liu and Zhang, 2010). However, limited field application has been developed for the most of these studies, though they all show promising lab-scale results. It should be noted that considerable studies on PCB remediation by a combination of anaerobic bacteria (biofilm) and activated carbon as a microbial inoculum delivery system have been conducted and remarkable progress has been made in recent decades (Das et al., 2017). Therefore, it will be independently discussed in the next section.

4.3 Advanced PCB Remediation Technologies

4.3.1 Supercritical Water Oxidation

Supercritical water oxidation (SCWO) is a clean technology which occurs in water at temperatures and pressures above the critical point of water (647 K and 22.064 MPa) (Marulanda and Bolaños, 2010). Under these supercritical conditions, water loses its hydrogen bonds and starts to transition from a polar solvent to a nonpolar solvent. As a result,

the solubility of PCBs increases in the supercritical water. PCBs are then degraded at these high temperatures and the final products are carbon dioxide, water and mineral acids (Rahuman et al., 2000). Typical operating conditions for commercial SCWO systems are 550-650 °C, 250 bar and this technology has been shown to be very efficient, achieving over 99% PCB destruction (Marrone et al., 2004). Additionally, SCWO systems in high temperature environments can rapidly complete the oxidation of PCBs to CO_2 and H_2O without toxic byproducts.

Studies showed that SCWO is an effective technique for destroying PCBs. Hatakeda et al. (1999) determined the efficiencies of hydrogen peroxide and oxygen for destruction of 3-chlorobiphenyl at different temperatures and oxidant concentrations. In this study, over 99% of 3-chlorobiphenyl was decomposed. Weber et al. (2002) assessed the PCBs destruction in supercritical water under alkaline conditions and over 99% of PCBs were destroyed. Fang et al.'s study showed that SCWO can destroy 93% of decachlorobiphenyl with excess O_2 (Fang et al., 2004). Marulanda and Bolaños. (2010) used a large scale mineral transformer for PCB-contaminated oil and found that 99.6% of the mixture of PCBs and hydrocarbons was destroyed with 350% oxygen excess at 539 °C.

SCWO, however, introduces a few complicating aspects that should be considered. For example, chlorine atoms from the biphenyl ring can produce hydrochloric acid during SCWO, which can corrode the system. Additionally, due to the low dielectric constant of supercritical water, both sticky salts and non-sticky solids are completely precipitated during SCWO (Fang, 2014). These salts deposit on equipment surfaces causing fouling, plugging, and erosion. Researchers have analyzed SCWO and conclude that the salts can accumulate on the surface of equipment requiring high cost maintenance and other operational maintenance procedures (Marrone et al., 2004).

4.3.2 Ultrasonic Radiation

Ultrasonic radiation is a promising method for PCB degradation. It is generally agreed upon that the possible mechanism of ultrasonic radiation is acoustic cavitation (Gedanken, 2004). Cavitation is a process in which mechanical activation destroys the attractive forces of molecules in the liquid phase thereby allowing bubble growth through the diffusion of solute vapor (Yeow and Peng, 2012). The energy inside of the bubbles will release and lead to high temperatures and pressures in microscopic regions resulting in chemical excitation that breaks chemical bonds. As a result, degradation of PCBs can be carried out with high effectiveness and simple handling conditions such as low temperatures and fast reaction times. Ultrasonic radiation is capable of high PCB removal efficiencies (more than 90%) (Lu et al., 2009). Rodríguez and Lafuente (2008) examined the dehalogenation of a PCB mixture using an ultrasonic radiation system at 40 °C with a hydrazine hydrochloride/palladium (HZ/Pd) catalyst. Gas chromatography results indicated that the concentration of PCBs in this mixture sample decreased from 2768 ppm to 25 ppm after 15 minutes and the PCBs were completely dechlorinated after 30 minutes. It is important to note that these concentrations are high thus the removal efficiencies may be large due to this factor. Okuno et al. (2000) observed 80%-90% degradation of 2-chlorobiphenyl, 4-chlorobiphenyl and 2,2'-dichlorobiphenyl in aqueous solutions in 30-60 min with a 200 kHz ultrasound. Lu and Weavers (2002) explored the laboratory scale application of ultrasound for the treatment of 4-chlorobiphenyl contaminated sediments. Over 90% of 4-chlorobiphenyl in the aqueous, homogeneous solution was destroyed at 20 kHz with a power density of 460 W/L after 20 min. It is to be noted that this sample contained only one congener thus the high efficiencies may be a result of this. A recent variation of PCB remediation by ultrasonic radiation is the ultrasound-assisted chemical process (UACP). Chen et al. (2013) developed a combination of ultrasonic irradiation and radical generations

using di-tert-butyl peroxide as a radical initiator to dechlorinate Aroclor 1260. The results demonstrated that UACP has a PCBs removal efficiency of 97 % within 3 hours.

Even through ultrasonic radiation has many advantages such as high remediation efficiency, no byproducts production, and lowered environmental impact, it also has several drawbacks when compared to other conventional methods (e.g., thermal treatment). For example, ultrasonic radiation is costly due to catalyst loadings and operations; therefore, it cannot easily be operated for large-scale, industrial and commercial purposes. In addition, commercial ultrasonic radiation generally has high energy requirements (Khoddami et al., 2013; Nur Ismayuslini, 2010).

4.3.3 Catalytic Hydrodehalgenation of PCBs by Bimetallic Systems

A bimetallic system consists of two metals sharing an interface or boundary in separate phases such as core-shell bimetallics (Lens et al., 2013). Both phases play an active role in bimetallic systems and the alloyed shell is the catalytically active phase. Bimetallic systems are usually carried out with two different metals: one zero-valent form with a negative reduction potential and the other a transition metals with a high reduction potential as the reducing catalyst (Gomes et al., 2013; Patel and Suresh, 2007). In this process of hydrodehalogenation, the corrosion of the zero-valent metal with water will firstly generate hydrogen at room temperature and pressure. Following that, the hydrogen is absorbed onto the surface of the catalyst to form a metal hydride as the target substrate of dehalogenation (Patel & Suresh, 2007).

Core-shell bimetallics have been successfully utilized to dechlorinate PCBs and other chlorinated organics (Patel and Suresh, 2007; Wu et al., 2012). Studies have been conducted to investigate bimetallic formulations to improve catalyst activity for PCB degradation (Hennebel et al., 2013). Hydrogen gas is generated through the corrosion of Mg by the

reduction of water, when the Mg/Pd bimetal system is applied to catalytic hydrodehalogenation of 2-chlorobiphenyl (Wu et al., 2012). The generated hydrogen then adsorbs to the Pd surface, relying on hydrogen bonds (Yang et al., 2011). This yields a stable adsorbed system of PdH2 while dehalogenating the PCB. Other general bimetallic systems for hydrodehalogenation of PCBs include iron/palladium (Fe/Pd), iron/nickel (Fe/Ni), and aluminum/palladium (Al/Pd). Table 4.2 summarizes experimental conditions and main findings in literature regarding hydrodehalogenation of PCBs by bimetallic systems (Agarwal et al., 2009; DeVor et al., 2008; He et al., 2010; Venkatachalam et al., 2008).

Bimetallic systems have several potential benefits. They have induced physicochemical properties, such as catalytic performance (Kang et al., 2011). Magnetic metals in combination with noble metals form bimetallic systems which exhibit irreplaceable advantages in pollutions remediation, since noble metals (e.g., silver (Ag), gold (Au), platinum (Pt)) have more recondite electron structures based on surface plasmon resonance (Duan and Wang, 2013). Additionally, two active metals of a bimetallic system can enhance catalytic activity thus treat PCBs and other chlorinated organic compounds in short times with no specialized laboratory equipment (Sakrittichai, 2001). Bimetallic systems also have distinct drawbacks. Some magnetic metals such as cobalt (Co), Ni, and noble metals such as Ag, Au, Pt, are too costly to be used in large commercial operations. Moreover, the reaction rate will significantly reduce, when the passivation of metals carry on during the remediation process (Doong and Wu, 1992). More generally, the zero-valent metals involved in hydrodehalogenation of PCBs by bimetallic systems such as iron, zinc or magnesium can generate partially dehalogenated products that may be more toxic than the previous target compounds (transformation of target compounds to phenol) (Grittini et al., 1995).

Table 4.2 Summary of experimental conditions and main findings of studies on hydrodechlorination of PCBs by bimetallic systems.

Bimetallic Systems	Compounds	Main Operating Conditions	Main Findings	References
Al/Pd	2-chlorobiphenyl	The 50 ml solution of 2-PCB was added into a 100 ml serum bottle with 5.0 g/L of Al/Pd loading and fixed on a horizontal shaker (180 rpm) at ambient temperature.	2-PCB was completely dechlorinated into BP within 60 min by the 1.43 wt.% of Al/Pd. Al/Pd presented high stability and reactivity to dechlorinate 2-PCB in acid aqueous solution.	(Yang et al., 2011)
Mg/Pd	Mix of PCB congeners	The contaminated substrate was mixed with Mg/Pd and contacted intimately by tumbling at 20 rpm. PCB extracts (0.5 ml) were spiked with 10 µL of 200 ppm D-8 naphthalene in DCM and analyzed in a GC/MS.	The PCBs changed from a higher chlorinated mixture to lower congenersat the end of 26 h. The distribution of PCB congeners towards lower chlorinated PCBs after 8 h and at least until 26 h.	(Agarwal et al., 2009)
Fe/Pd	2,3,2′,5′-tetrachlorobiphenyl (TeCB)	PCBs (<1 µg/g) combined 5 g of Pd/Fe were placed in a vial with 500 ml of ethanol and isopropanol solution and shaken for 16 h for batch tests. The PCB-laden solvent was repetitively recycled and used to column testing to verify the results from the batch studies.	2,3,2′,5′- tetrachlorobiphenyl was completely transformed to biphenyl in 9 h. The dominant Chlorinated byproducts was 2,3,2′-trichlorobiphenyl followed by 2,5,2′-trichlorobiphenyl.	(Korte et al., 2002)
Fe/Pd	Aroclor 1254	The degradation was initiated by injecting 25 µL of Aroclor 1254 (100 mg L-1) into 1 ml of solution per vial containing 1 g/L as Fe of a certain type of nanoparticles. PCBs (2.5 ppm) were added into vials and placed on a rotary shaker (40 rpm) at 22 °C in an incubator.	The degradation was clearly enhanced when 0.1% (w/w) of Pd was coated on the Fe particles, which resulted in a 24% reduction of PCBs within 100 h. The starch-stabilized bimetallic Fe/Pd particles were able to transform over 80% PCBs.	(He & Zhao, 2010)
Fe/Pd	3,3′,4,4′-tetrachlorobiphenyl (PCB77)	The polypyrrole film (16.2 cm2) that contained the Pd nanoparticles was added to a 20-ml solution of PCB 77. The dechlorination efficiency of the Fe/Pd nanoparticles was evaluated using a 15.6 mg/L PCB 77 in 65% (v/v) ethanol in water with Fe/Pd (Pd: 2.3 wt%).	85% of PCB77 was dechlorinated within 2 h with 0.82 g/L of palladium nanoparticle loading. PCB77 was completely dechlorinated by Fe/Pd in the PAA/PVDF membrane within 2 h and biphenyl was formed as the main dechlorination product.	(Venkatachalam et al., 2008)
Mg/Pd	2-Monochlorobiphenyl 3-Monochlorobiphenyl 4-Monochlorobiphenyl	0.25 g of Mg/Pd and 10 ml of PCB solution were added into 20 ml vials and were shaken for 2 min. Analysis of the extracted samples were performed on a Shimadzu GC-2014 and a Thermo Finnigan Trace GC/DSQ.	The rate of dechlorination for monochlorinated congeners in water was PCB-003>PCB-002>PCB-001. There is no significant degradation of biphenyl apparent with pure methanol as the solvent in more than 30 d.	(DeVor et al., 2008)

4.3.4 Nanoscale Zero-Valent Iron (nZVI) based Reductive Dehalogenation

The diameter of nZVI particles is less than 100 nm and they usually have a core-shell structure. The outside ion of nZVI particles can react with water and oxygen to form an outer (hydr)oxide layer in aqueous environments (Nurmi et al., 2005; O'carroll et al., 2013). As a result, this outer oxide layer allows electron transfer from the metal through the oxide conduction band or localized band. Furthermore, the outer oxide layer could serve as an adsorbent for PCBs. In 1994, Schreier and Reinhard (1994) reported dehalogenation of alkyl halides (RX) by Fe powder in oxygen-free and buffered water, previously a method with difficult to predict outcomes. Since then, a three step mechanisms was proposed to explain the dehalogenation process of alkyl halides (Matheson and Tratnyek, 1994). Regarding to PCBs, the electrons can directly transfer from Fe^0 to the PCB moleculars under an acid environment and the Fe^{2+} will be generated as an product (Figure 4.2) (Gomes et al., 2016). On the basis of the proposed mechanism, nZVI with a large surface area has been successfully used to achieve the dehalogenation process in water or PCB-contaminated soil in the presence of catalysts. Current research is exploring the ability of nZVI particles to dechlorinate highly chlorinated PCBs. A study conducted by Gardner et al. (2004) indicated that 3% of nZVI (w/w) can rapidly and extensively dechlorinate PCB-contaminated sediments in the New Bedford Harbor and the Housatonic River (Gardner et al., 2004). The nZVI particles removed 84% of PCBs from the Housatonic River sediments which had an initial concentration of approximately 50 ppm. In 2007, it was found that more than 95% of 10 different PCB congener remediation, with concentrations ranging up to 1 mg/kg soil or filter cake, can be catalyzed by iron oxide and V_2O_5/TiO_2 at 300 °C, when the iron nano-particles were applied to remediate PCB-contaminated soil (Varanasi et al., 2007). Long et al. (2014) found that dehalogenation of Aroclor 1260 in soil can be enhanced by anaerobic composting with nZVI. The nZVI can provide an appropriate pH (8-9) and reduce volatile

fatty acid inhibition thereby stimulating the growth of microorganisms and by generating hydrogen gas via corrosion of nZVI, thereby enhance dehalogenation. The results showed that dehalogenation performance was enhanced by 34% after adding 10 mg g-1 of nZVI to soil containing 1mg/kg for 140 days. Liu et al. (2014) used thermal desorption combined with nZVI to remediate PCB-contaminated soil at different temperature conditions (300°C - 600°C). They found that 97.40 % of trichlorobiphenyl (TrCB) and tetrachlorobiphenyl (TeCB) was removed without nZVI at 600 °C. In contrast, 98.35% of TrCB and TeCB was eliminated with the use of 100 mg of nZVI at the same temperature.

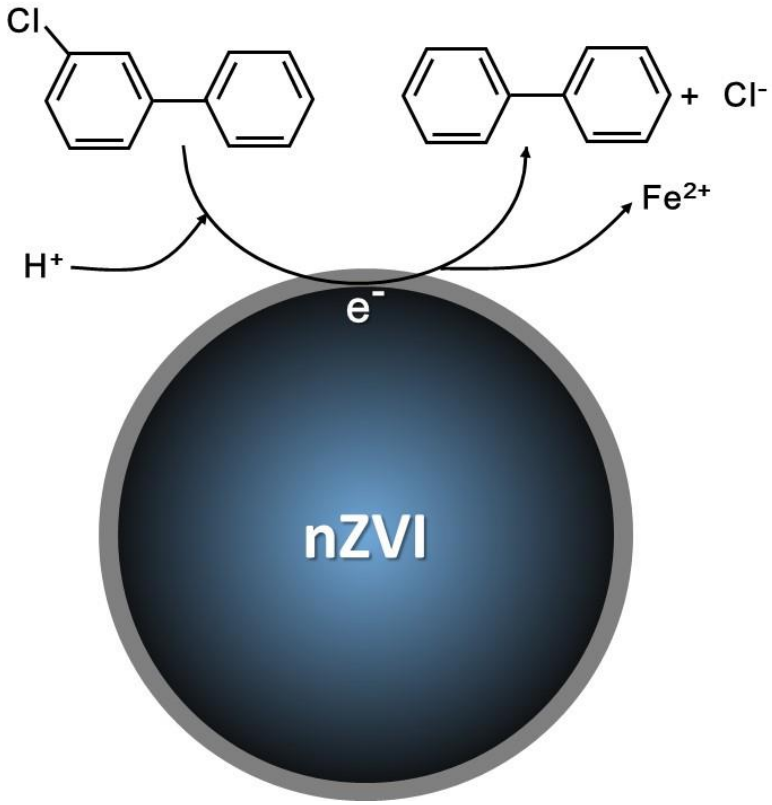

Figure 4.2 Proposed mechanisms for abiotic dechlorination of PCBs: Electron transfers from Fe^0 to monochlorobiphenyl (Gomes et al., 2016).

NZVI-remediation could offer advantages as compared to other in situ remediation technologies. For example, nZVI has been used to remediate PCBs as an advanced technology in the environment due to its catalytic properties and high degradation performance (Ahmadi et al., 2011). A large surface area of nZVI particles can increase reactivity and, therefore, results in high speed contaminant degradation (Cook, 2009; Nurmi et al., 2005). Additionally, nZVI can be used in recalcitrant contaminated water such as deep contaminated ground water since nZVI can suspend in water for a longer time due to its small size (Singh et al., 2012). However, the application of nZVI is still controversial. To date, the nZVI technologies in practice are mostly the application of chlorinated solvents in aquifers and most cases of nZVI applications are lab scale and have taken place in North America in comparison to the few field applications in Europe (e.g., Czech Republic, Germany and Italy) (Bardos et al., 2015). In broad terms the surface of nZVI particles in an aquifer will be oxidized into iron (II) and (III) oxides/hydroxides. As a consequence, the particle surface reactivity will be limited (Lee and Jou, 2012). In addition, the nZVI particles will undesirably agglomerate and convert to large particles because the most reactive particles agglomerate more readily (Bardos et al., 2015). The toxicological effect of nZVI particles are usually related to their nano-scale size. The nZVI used in remediation are typically 10-100 nm as aforementioned and the unique toxicity of nZVI has been only observed in nanoparticles smaller than 30 nm (Bardos et al., 2014). Some studies reported the toxicity of nZVI is specific to bacteria and biota such as *collembolan* (srpingtails) and *lumbricina* (earthworms) (Bardos et al., 2015).

4.4 Possibility of PCB Remediation Using Multi-Technology

4.4.1 PCBs Removal by Biofilm Covered Activated Carbon

In the early 1970s, it was found that biofilm covered activated carbon particles can efficiently remove organic pollutants (Dussert and Tramposch, 1997). Since then, the combination of bacterial biofilm and activated carbon has been widely used as a practiced treatment method for wastewater treatment, post-treatment, potable water purification, and organic contaminants removal (Tammaro et al., 2014). Kolb and Wilderer (1997) used an integrated activated carbon adsorption and biodegradation system to reduce the concentration of critical components (e.g., Benzene and 2-chlorophenol) from industrial process wastewaters, thereby mitigating the inhibitory effects on the bacteria. Islam et al. (2016) recently reported that the biofilm treatment process with activated carbon adsorption can effectively treat naphthenic acids (NAs) in oil sand process affected waters (OSPW). After 28 days, the NAs removal efficiencies of OSPW for biodegradation and adsorption were 14% and 63% respectively. Such successful attempts have led to the recognition of the combination of bacterial biofilm and activated carbon as a viable remediation technology for PCB-contaminated sediment as suggested by the USEPA. Some preliminary research of PCB-contaminated sediments treated with granular activated carbon (GAC) have been conducted. A bench-scale study on bioaugmentation of PCBs in aqueous wastes conducted by Ghosh et al. demonstrated that 62% of PCBs were removed by a granular activated carbon column Ghosh et al. (2011) In addition, a field study indicated that the bioaccumulation of PCBs were reduced in clams, worms, and amphipods after the was sediment treated with 1-5% (w/w) of GAC. Sediment treated with 3.4% of coke activated carbon achieved 85% and 92% reductions in aqueous equilibrium PCB concentrations during one-month and six-month experimental spans, respectively (Zimmerman et al., 2004). These studies showed that the indigenous bacteria found in sediment were capable of PCB dehalogenation. However,

microbial degradation of PCBs are limited in performance due to the relatively low abundance of PCB-dechlorinating microorganisms and low bioavailability of PCBs, especially for in-situ microbial degradation (Kjellerup and Edwards, 2013). There was an insignificant decrease in the overall concentration of parent PCB congeners. Tri-, tetra- and penta-chlorinated congener concentrations were still relatively high, while mono- and di-chlorinated congeners were dominant in the presence of activated carbon (Kjellerup et al., 2014). Therefore, a combination of adsorbent sequestration and bioaugmentation through biofilm covered activated carbon systems is proposed to enhance the biodegradation of low concentration PCBs in sediment. An adsorbent (e.g., activated carbon and biochars) could adsorb PCBs from aquatic sediments and concentrate PCB dechlorinating microorganisms onto its surface thereby applying the microbial communities to the PCB-contaminated sediment.

Such a combination of PCB-dechlorinating microbial communities in the form of biofilms on surfaces of activated carbon provide several advantages. The biofilm covered activated carbon system usually has a removal efficiency of over 60% due to simultaneous adsorption and biodegradation (Kjellerup and Edwards, 2013). A compact space between biofilms with large cell density and activated carbon surface could allow microorganisms to utilize PCBs as an electron acceptor thereby enabling subsequent degradation. In addition, the microorganisms embedded within an adherent biofilm attain a high resistance to toxic pollutants (Köhler et al., 2006). Furthermore, a biofilm covered activated carbon systems can maintain long solid retention times thereby biodegrading persistent organics at a low growth rate (Aktaş and Eçen, 2007). One drawback of biofilm covered activated carbon system is that some slow growing PCB dechlorinating microorganisms might be bloom-growing thus affected by other easily-degradable organic compounds or pollutants (Abromaitis et al., 2016). For example, some researchers pointed out that the concentration of persistent organic

compounds (e.g., PCBs) in a municipal wastewater treatment plant effluent is very low (0.019 to 1.7 μg/L), though the biochemical oxygen demand of secondary effluent is generally between 1.7 and 7.7 mg/L (Abromaitis et al., 2016; Deblonde et al., 2011b).

4.4.2 PCBs Remediation Technologies Coupled to Electrokinetic Remediation

Soil and aquatic environments (e.g., lake, river, and groundwater) contaminated with PCBs through direct or secondary exposure have resulted in detrimental environmental impacts (Beesley et al., 2011). More advanced alternatives to unsustainable PCB remediation techniques have been sought. Electrokinetic remediation uses low-level direct current as a ‘‘cleaning agent’’ to remove organic pollutants from the soil or other environments (Acar and Alshawabkeh, 1993). Electrokinetic remediation can be conducted in-situ. This generally includes an external, direct current source, and an anode and cathode immersed in the electrolytic solution. The organic pollutants driven by ionic migration and electrophoresis will migrate to the electrodes when the direct current is applied. Other technologies (e.g., nZVI dehalogenation) coupled to electrokinetic remediation could lead to new advancements in PCB remediation (Gomes et al., 2012). Fan et al. (2013) used nano Pd/Fe particles coupled with electrokinetic technology to remediate PCB contaminated soil. The results indicated that high electroosmotic flow can facilitate nano Pd/Fe transport, but the degradation was low without the solubilization of PCBs strongly adsorbed in soils. Gomes et al. (2014) proposed a cost-effective solution for PCB contaminated soil remediation by using electrodialytic remediation combined with nZVI particles. Electrodialytic remediation combines electrodialysis with the electrokinetic movement of ions to remove heavy metals (Ottosen et al., 1997). Two surfactants (saponin and Tween 80) were involved in this study to enhance PCB desorption and removal from polluted soil. The results show that the removal efficiencies of highly chlorinated PCB congeners (penta-, hexa-, hepta-, and octa-

chlorobiphenyl) were between 9% and 96%. Chun et al. (2013) developed an innovative approach to significant dehalogenation of PCBs in sediment using electrical stimulation which provide electron donors and acceptors to PCB dechlorinating microorganisms. The results of their study indicated that the concentration of weathered PCBs decreased by 40% to 60%, from original concentrations of about 20 mg/kg dry sediments, in microcosms treated with electric current as compared to that of PCBs observed in control reactors.

4.4.3 nZVI Particles in Combination with a Second Metal as a Promising Technology for PCBs Remediation

The effectiveness of nZVI based reductive dehalogenation described in this paper are quite specific. One major concern in nZVI based reductive dehalogenation processes is the reduction of surface reactivity due to iron oxide formation on the surface of the nanoparticles. As aforementioned, combining two or several different remediation technologies as a potentially viable alternative for PCBs remediation can overcome the deficiencies associated with individual treatment methods (Naddeo et al., 2011). Furthermore, optimization of PCBs remediation efficiency can be achieved within appropriate costs. In recent years, the effectiveness of nZVI has been proven in the treatment of a wide variety of contaminants such as chlorinated methanes, brominated methanes, trihalomethanes, chlorinated ethenes, chlorinated benzenes, and other polychlorinated hydrocarbons (Nowack, 2008). Regarding nZVI particles coupled with electrokinetic remediation discussed above, some studies indicated that nZVI particles in combination with a second metal (e.g., Pt, Ag) are more effective in treating chlorine-containing organic pollutants with high reaction rates (Cao et al., 2011; Liou et al., 2005). Metal-covered nZVI particles can effectively reduce the activation energy of the pollutants and increase the reaction rate of dechlorinaton reactions (Liou et al., 2005). Additionally, metal-covered nZVI particles can rapidly achieve

dehalogenation through increasing the particle surface area and surface activity (Clark et al., 2003). Zhuang et al. (2011) have proven that 2,3,4-trichlorobiphenyl (PCB-21) can be rapidly dechlorinated by palladized, nanosized ZVI (Pd/nFe) (Zhuang et al., 2011). Their results suggested that the degradation rate of PCB-21 (normalized rate constant of 10-1) by using Pd/nFe was three orders of magnitude faster than that of PCB-21 when using unpalladized ZVI (normalized rate constant of 10-4). Le et al. (2015) developed an integrated remediation system for dehalogenation of Aroclor 1248 using bimetallic nanoparticles Pd/nFe and biodegradation via *burkholderia xenovorann* LB400. The dehalogenation efficiencies of tri-, tetra-, penta-, and hexachlorinated biphenyls were 99%, 92%, 84%, and 28%, respectively. After nano-bio treatment, the toxicity of the residual PCBs in terms of toxic equivalent values decreased from 33.8×10^{-5} μg/g to 9.5×10^{-5} μg/g.

4.4.4 Assessment of PCB Remediation Technologies

Four criteria including the cost, removal efficiency, time duration, and environmental burdens were provided to determine a comprehensive framework for PCB remediation strategies and the combinations of these technologies. As shown in Table 4.3, chemical reagent, supercritical water oxidation, ultrasonic radiation, bimetallic systems, nZVI, and nZVI combination with a second metal have a high remediation efficiency (78%-99%) with a rapid reaction time. More importantly, most of these technologies does not generate toxic byproducts except the supercritical water oxidation and bimetallic systems. The generated byproducts from these technologies are the major drawbacks that could be released to the environment with negative impacts. However, these technologies usually require excessive costs. Therefore, any efforts relative to the implementation of these technologies to the contaminated filed sites or large commercial operations will encounter with challenges. Phytoremediation and microbial degradation have a limited remediation efficiency (40%-

60%). They usually cannot immediately apply to the PCB sources due to requiring a long remediation and monitoring time as compared to the technologies mentioned above. Even though phytoremediation and microbial degradation have a limited remediation efficiency with a long-term remediation time, the major advantage is the relatively low implementation costs. Therefore, they could be applied to an in-situ contamination site as a full-scale application. Activated carbon and the biofilm covered activated carbon obtained the highest scores according to this table. A low cost and relatively high remediation efficiency allow them to be applied to either in-situ or ex-situ PCBs remediation. More specifically, the biofilm covered activated carbon system has a considerable removal efficiency which is usually over 60% due to simultaneous adsorption and biodegradation. A compact space between biofilms and activated carbon surface could allow bacteria to efficiently attack PCB molecular as an electron acceptor thereby. However, it should be noted that some studies on PCBs remediation by a combination of anaerobic biofilm and activated carbon involved in this paper were conducted under a strictly experimental condition (e.g., mesocosms with extremely anaerobic environments), even though a remarkable progress has been made in recent decades. A successful treatment of PCBs not only depends on its remediation efficiency, it is also need to consider the cost, time duration, and environmental burdens. On the basis of the discussion of this paper, therefore, the future vision of PCBs remediation could be a comprehensive treatment based on the biofilm covered activated carbon particles as a microbial inoculum delivery system.

PCBs are very toxic chemical compounds. Thus a comprehensive assessment of the environmental impacts of PCB destruction is an important criterion for selection of PCB remediation technologies. However, there is little evidence/information that supports this sort of assessment for most of the proposed PCBs destruction technologies involved in this paper (Weber, 2007). The comprehensive assessment of PCB formation and destruction are only

available for the incineration process (Buekens and Huang, 1998 ; Weber, 2007). In addition, assessments of PCB removal stability is another significant parameter for PCB destruction technologies. For instance, the destruction efficiency of a wastewater treatment facility could be difficult to evaluate due to low concentration levels in the effluent streams. This would require continuous PCB sampling and long-term data monitoring and collection such as time series analysis on the basis of PCB emission. Therefore, the assessment of the PCB destruction technologies should include two essential parts: 1) assessment of destruction efficiencies for in-situ and ex-situ PCB remediation technologies and the environmental impacts or risks associated with the implementation and operation of the respective technology; 2) continuous PCB sampling and long-term data monitoring and collection in order to assess the reliability of the proposed PCB remediation technology.

Table 4.3 Matrix of criteria for different PCB remediation technologies.

	Cost	Removal efficiency	Time duration	Toxic byproducts	Overall Score
Phytoremediation	Low cost (+)	Effectiveness restriction by shallow distribution of plant roots	Long remediation and monitoring time	NA	+
Microbial degradation	Low cost (+)	Low (40%-60%) (-)	A long-term period for bioremediation (-)	Less toxic byproducts (+)	NA
Chemical reagent	Additional expenses requirement for high concentrations (-)	High (78%-99%) (+)	Short or medium time periods (+)	NA	+
Activated carbon	Low cost materials (+)	High (73%-89%) (+)	NA	None (+)	+++
Supercritical water oxidation	High (-)	High (93%-99%) (+)	NA	Sticky salts and non-sticky solids causing fouling, and erosion (-)	-
Ultrasonic radiation	Too costly due to catalyst loadings and operations (-)	High (80%-90%) (+)	Short times required (+)	No byproducts production (+)	++
Catalytic hydrodehalgenation by bimetallic systems	Too costly to be used in large commercial operations (-)	High (+)	Short times required (+)	Partially dehalogenated products (-)	NA
Nanoscale zero-valent iron	High (-)	High (84%-98.3%) (+)	Short times required (+)	nZVI is toxic to bacteria and biota (-)	NA
Biofilm covered activated carbon	Low cost materials (+)	High (60%-92%) (+)	Relatively short times required (+)	None (+)	+++
Electrokinetic remediation	NA	High (40%-96%) (+)	NA	None (+)	++
nZVI particles combination with a second metal	High (-)	High (84%-99%) (+)	Short times required (+)	NA	+

4.5 Conclusions

This paper reviewed the treatment strategies for PCBs remediation. Chemical reagent, supercritical water oxidation, ultrasonic radiation, bimetallic systems, nZVI, and nZVI combination with a second metal have a high remediation efficiency (78%-99%) with a rapid reaction time. However, these technologies usually require excessive costs. Phytoremediation and microbial degradation have a limited remediation efficiency (40%-60%). Even though phytoremediation and microbial degradation have a limited remediation efficiency with a long-term remediation time, the major advantage is the relatively low implementation costs. Therefore, they are not appropriate for an in-situ contamination site as a full-scale application. Activated carbon and the biofilm covered activated carbon approaches obtained the highest scores according to this table as compared to the technologies mentioned above. A low cost and relatively high remediation efficiency (more than 60%) allow them to be applied to either in-situ or ex-situ PCBs remediation. PCBs are complex chemicals, so knowledge of their chemical and physical properties is important to better understand their transport and fate thereby for selecting appropriate remediation approaches. The possibility of PCB remediation by using multiple technologies discussed in this paper need more data and pilot scale experiments in order to evaluate the effectiveness. The future vision of PCB remediation could be a comprehensive treatment solution. Because successful treatment of PCBs not only depends on the appreciated selection of the most effective remediation technology, it is also needs to consider the public acceptance and environmental and human health impacts of the remediation technology, neither of which have been achieved.

Chapter 5: Application of Biofilm-based Inoculum Delivery System for Organohalide Respiration of Polychlorinated Biphenyls (PCBs) in Sediments

Ran Jing[a] and Birthe V. Kjellerup[a],*

[a]University of Maryland at College Park, Department of Civil and Environmental Engineering, 1147 Glenn L. Martin Hall, College Park, MD 20742, USA

Contact Information:

Ran Jing: rjingratmond88@gmail.com; Tel: 301-851-1728.

Birthe V. Kjellerup: bvk@umd.edu; Tel: 301-314-1535. *Corresponding author.

ABSTRACT

This study aims to utilize organohalide respiring bacteria as a biofilm on surfaces of pinewood biochar as an enhanced bioaugmentation approach. Polychlorinated biphenyl (PCB) degrading biofilm on surfaces of adsorbents has the potential to enhance the PCB degradation capacity. The formation of the biofilm can provide a higher cell density as compared to that of a traditional free-floating delivery system in addition to simultaneously sequestering PCBs from the sediment matrix. High sorption capacity of pinewood biochar can adsorb PCB molecules, so they are in close proximity to the biofilm resulting in increased interaction between the organohalide biofilm and the hydrophobic and adsorbed PCBs. In this study, biofilms made up by the organohalide-respiring bacteria *D. chlorocoercia* DF-1 were formed on the surface of pinewood biochar. The biochar-biofilms were subsequently applied

to PCB contaminated sediment from the Grass River in Michigan, USA. The goal was to evaluate the organohalide respiration of the PCB contaminated sediment in the absence/presence of the biofilm and free-floating inoculum. This approach can provide an efficient method for utilizing organohalide respiring bacteria for PCB bioaugmentation thereby increasing the potential for long-term and sustained organohalide respiration.

5.1 Introduction

Polychlorinated biphenyls (PCBs) as one of the persistent organic pollutants (POPs) is becoming a serious environmental concern (Cheng and Hu, 2010; Jing et al., 2019b). They are bioaccumulating in the food chain due to their lipophilicity in the environment for a long period when they are released into the environment. Over the past decade, PCB remediation technologies such as bioremediation, phytoremediation, dehalogenation by a chemical reagent, thermal methods, and PCBs removal by pinewood biochar are widely studied (Jing et al., 2018; Passatore et al., 2014; Wu et al., 2012). However, some of these remediation technologies are disruptive and unsustainable. The current effort is to find a sustainable alternative remediation technology for PCBs. An enclosed mesocosm system such as mesocosms is an important experimental tool for the lab-scale organohalide respiration of PCBs from contaminated soils or sediments (Zhang et al., 2010). However, a normal mesocosm system of a weathered PCBs remediation study usually has a limited performance due to their low concentration and low bioactivities of natural organohalide-respiring microorganisms (Jing et al., 2018). To overcome these challenges, this study intends to concentrate one of the organohalide-respiring bacteria i.e., *D. chlorocoercia* DF-1 onto surfaces of the absorbent materials (e.g., pinewood biochar). Such a PCB-degrading biofilm on surfaces of adsorbent could enhance their PCB degradation capacity (Edwards and Kjellerup, 2013; Kjellerup et al., 2014). The formation of biofilm can provide a higher cell

density as compared to that of a free-floating system (Jefferson, 2004). High sorption capacity of pinewood biochar particles can adsorb PCB molecular in close proximity to the adsorbent resulting interaction between organohalide-respiring microorganisms and hydrophobic PCBs (Liu et al., 2015; McDonough et al., 2008).

Studies indicated that *D. chlorocoercia* DF-1 (originating from Charleston Harbor, SC) can survive in contaminated soil was also capable to dechlorinate the high-chlorinated PCBs (May and Sowers, 2016). More specifically, PCB organohalide respiration of a DF-1 biofilm inoculum has been shown in the recent bioaugmentation studies (Nuzzo et al., 2017). Payne et al. (2011) implemented a bioaugmentation study in 2-L mesocosms with the *D. chlorocoercia* DF-1 and 1.3 ppm of weathered Aroclor 1260 contaminated sediments which were collected from Baltimore Harbor, MD (Payne et al., 2011). The total penta-chlorinated PCBs decreased by 56% in the bioaugmented mesocosms after 120 days. The results of their study indicated that bioaugmentation with *D. chlorocoercia* DF-1 can enhance the dechlorination of doubly flanked chlorines of weathered PCBs. In another study, Kjellerup et al., (2012) evaluated the effects of anaerobic bioaugmentation of soil samples (Mechanicsburg, PA) contaminated with 4.6 to 265 ppm of Aroclor 1260 by using *D. chlorocoercia* DF-1 (Kjellerup et al., 2012a). A changing of PCB homolog distributions indicated that the dechlorination of weathered PCBs has been observed. The mole percent of the hepta- and octa-chlorinated congeners were decreased by 14% and 5%, respectively.

In addition, biofilms have a complex architecture in which microorganisms can exist in, for instance, discrete pillar or mushroom-shaped structures (Stoodley et al., 2002). Such a complex channel network can provide efficient access to nutrients thereby resisting dramatic changes in environmental conditions such as pH and temperature changes (Flemming and Wingender, 2010). In addition, a highly organic porous surface the pinewood biochar particles have high affinities for the simultaneous attraction of biofilm-forming by

organohalide-respiring microorganisms and adsorption of PCB molecular (McDonough et al., 2008). Both the biofilm and the absorbent with a highly porous surface are essential components for the implementation bioaugmentation of weathered PCBs in sediments (Capozzi et al., 2019). One of the objectives in this study is to inoculate the biofilm formation of the existing organohalide-respiring microorganisms i.e., *D. chlorocoercia* DF-1 on the surface of pinewood biochar. The organohalide performance of the DF-1 biofilm covered pinewood biochar on the PCB contaminated sediments is also evaluated and compared with that of the liquid *D. chlorocoercia* DF-1 inoculums. This microbial inoculum delivery system could be essential for the future application to the PCB contaminated sites with low-concentration PCBs. In addition, this approach can provide an efficient method for inoculating microorganisms for PCB bioaugmentation thereby increasing the potentials for long-term bioaugmentation.

5.2 Materials and methodology

5.2.1 Inoculation of D. chlorocoercia DF-1 culture and biofilm

The *D. chlorocoercia* DF-1 culture was inoculated with 75 ml of E-Cl medium in a 160 ml of serum bottle spiking with 2 ppm PCE. The protocol of the E-Cl medium was prepared as described in the previous study (Miller et al., 2005). In this study, PCE was used to inoculate the *D. chlorocoercia* DF-1 biofilm as an alternative electron acceptor instead of PCBs. This is mainly attributed that the *D. chlorocoercia* DF-1 can rapidly grow by utilizing PCE as terminal electron acceptors by converting PCE to cis-DCE (Wang et al., 2003). Studies reported that OHR bacteria such as *D. mccartyi* can rapidly grow with PCE (Sung et al., 2003; Wang et al., 2014). Their cell density was achieved to 1.2×10^8 to 1.3×10^8 cells per ml inoculating with PCE after 30 days (Wang et al., 2014). However, the cell density of

the *Dehalococcoides mccartyi* culture inoculated with PCBs was only 5.9×10^6 to 10.4×10^6 cells per ml after 150 days. In addition, the residual PCE and its biodegradation products (e.g., TCE, DCE) of the DF-1 culture and DF-1 biofilm mesocosms in this study could be easily removed from the headspace of the serum bottles after inoculation. As a result, the competition of the organohalide respiration between the residual PCE and the weathered PCBs could be avoided when the DF-1 biofilm covered pinewood biochar particles was added to the sediments. Moreover, not all PCB congeners can be rapidly or even completely degraded such as the *ortho*-PCBs (Kumar et al., 2011) if they are used as the electron acceptors to inoculate DF-1 culture and biofilm in this study. Therefore, the PCBs attached to the pinewood biochar surface after inoculation will release and future contaminates to the environments when the DF-1 biofilm covered pinewood biochar implemented to the PCB contaminated sites. Propionate stock solution was prepared in 100 ml of boiling water to a final concentration of 1 mM in a fume hood under N_2 flow as a mix of carbon sources. 1 ml of propionate (1 M) is then added to the culture as the carbon source at a final concentration of 2.5 mM (Wu et al., 2000). All the DF-1 cultures were inoculated in an incubator without shaking at 30 ℃ for 30 days. After 30 days inoculation, the headspace of DF-1 cultures was flashed under a N_2 gas in a flume hood to remove residual PCE and the degradation products. After identifying the bioactivity of DF-1 culture, 20 ml of DF-1 culture was then transferred to 80 ml of fresh E-Cl medium with 3 g of pinewood biochar particles and 55.87 ppm PCE (liquid phase concentration). Finally, the E-Cl medium inoculated with DF-1 cultures and pinewood biochar particles were inoculated in an incubator without shaking at 30 ℃.

5.2.2 Assessment of biofilm growth on pinewood biochar through the headspace measurements using gas chromatography-flame ionization detector (GC-FID)

In this study, a GC-FID (Agilent Technologies, Inc, Santa Clara, California) was used to analyze the potential PCE degradation products (i.e., trichloroethylene (TCE), 1,2-dichloroethene (DCE), and vinyl chloride (VC)) from the headspace of the DF-1 culture by utilizing a hydrogen flame to oxidize organic molecules and produce ions. To measure the gas components, 5 ml of gas from the headspace of the inoculation bottles was extracted by using a 1 ml gas syringe (Valco Instruments Company Inc., Houston, Texas). The GC program used in this study was shown as the following: a carrier gas (N_2) flow of 3 ml/min1, column temperature of 180 °C, an injection volume of 60.0 μL with a splitless mode; a temperature program starting at 40 °C, increasing to 75 °C at 20 °C/min, holding for 0.75 min, then increasing to 150 °C at 45 °C/min and holding for 0.35 min, and an injector temperature of 200 °C. A blank sample of nitrogen was also taken and measured through the GC system.

5.2.3 Mesocosms setup

Mesocosms were conducted with DF-1 biofilm-based inoculum with sediments collected from the Grass River (Antrim County, Michigan). For each mesocosm, 170 -180 g of sediment samples with 150 ml of E-CL medium and DF-1 biofilm inoculum are added into the 250 ml of serum bottles (Thomas Scientific inc., Swedesboro, New Jersey) in triplicates. The bacterial cells for both DF-1 biofilm and liquid inoculum were enumerated by using qPCR assays with 348f/884r primers specific for the 16S rDNA of organohalide-respiring bacteria. The DF-1 biofilm and liquid inoculum were then added into the mesocosm sediments to maintain a ratio of 1.46 to 1.56×10^7 gene copies/g sediments. The PCE

accumulated in the biofilm covered pinewood biochar were purged out by using N_2 gas in a flume hood before adding them to the mesocosms to avoid the interference of the organohalide respiration of the weathered PCBs by PCE as the electron acceptors. The bottles are sealed with Teflon septa and secured with aluminum crimp caps and incubated in the dark at 30 ℃. Negative control was also set up in triplicates to determine the organohalide respiration of the natural species in the sediments. In addition, abiotic control experiments with and without pinewood biochar were also set up under the same conditions but with autoclave killed-sediments. To avoid exposure of the cultures to air, all serum bottles are set up in an anaerobic chamber. The mesocosm experiments of biofilm inoculum and liquid inoculum are setup as Table 1. Finally, a gas chromatography (GC) analysis for the individual mesocosms was established to assess whether a statistically significant transformation of the weathered PCBs occurred.

Table 5.1 Mesocosm experiments with DF-1 biofilm and liquid inoculum.

Mesocosms	Experimental setup
Negative control	Grass River sediment×3
Abiotic control	Grass River sediment with autoclaved×3
Biochar adsorption group	(Grass River sediment + pinewood biochar)×3
Bioaugmentation group 1	(Grass River sediment + liquid DF-1 culture)×3
Bioaugmentation group 2	(Grass River sediment + DF-1 biofilm)×3

5.2.4 DNA extraction

For each mesocosm, DNA was extracted in triplicate by using the MoBIO PowerSoil DNA Extraction Kit (Qiagen Sciences Inc., Germantown, Maryland). 0.25 g of sediment samples in triplicate were collected and added into the Zirconia/Silica Beads tubes. After that, all samples in the tubes were horizontally shaking on a beat beating at speed "4.5" using a FastPrep120 (Q-Biogene, Inc., California) for 10 min. The rest of the extraction steps

involving proprietary reagents and spin filters were performed according to the protocol provided by the manufacturer. The final extracts were eluted in 100 μL of solution C6 and stored at -80°C. Finally, the extracted DNA samples were analyzed on the nano-drop (Fisher Scientific, Hampton, New Hampshire) to measure the concentration of the DNA and the purity. Each DNA should have an A260/280 ratio of ≥1.6 and an A260/230 ratio of ≥2.0 (Payne et al., 2017).

5.2.5 Identification and enumeration of D. chlorocoercia DF-1

Polymerase chain reaction (PCR) is available for the evaluation of *D. chlorocoercia* DF-1 biofilm samples. In this study, the DF-1 biofilm samples with the pinewood biochar particles were centrifuged at 10000 g for 3 minutes. The pellets at the bottom were then collected into a 1.5 ml tube and sonicated at 1000 Hz for 60 minutes to separate the biofilm and pinewood biochar particles. The DNA for each mesocosm samples was extracted performed in triplicate as described above. After that, 25 ul PCR reactions were conducted with 2 ul of the extracted DNA, 1 ul of forward primer, 1 ul of reverse primer, 12.5 ul of DreamTaq Green PCR Master Mix (Fisher Scientific, Hampton, New Hampshire), and 8.5 ul of DNA free water. The PCR program was conducted as the following: initial denaturation at 95 °C for 2 min, 95 °C for 45 sec, 58 °C for 45 sec, 40 cycles at 72 °C for 60 sec, 72 °C for 30 sec. A PCR primer set (348F/884R) was designed to select 16s-rRNA gene amplicons of all putative PCB dechlorinating *Chloroflexi*. After that, the specificity of the PCR products was confirmed to analyze the reaction quality and yield by performing the electrophoresis at a voltage of 100 V for 40 minutes on an ethidium-bromide-stained agarose gel. The electrophoresis can reveal the size of the product band, which is compared with the predicted result. In this study, the predicted size of the product band should be 536 bp.

In this study, real-time polymerase chain reaction (qPCR) assays specific for the 16S rDNA of DF-1 were used to assess the biofilm inoculum coated pinewood biochar particles. Enumeration was performed by using iQ SYBR Green Supermix (Bio-Rad Laboratories Inc., Hercules, California) and primers specific for the 16s-rRNA gene of *Chloroflexi* community (348F/884R) as described previously. The qPCR program was conducted as the following: initial denaturation at 95 °C for 5 min, followed by 35 cycles of 95 °C for 45 s, 55 °C for 25 s, and 72 °C for 25 s. The detection limit of all qPCR reaction was 3×10^2 gene copies/g biosolids/wastewater (weight) (Payne et al., 2013). The final gene copy numbers for each sample was determined by standard curves based on 5 dilutions of DNA samples with known microorganisms such as SDC-9 using the same qPCR procedure as for the biofilm inoculums.

5.2.6 Scanning Electron Microscopy coupled with Energy Dispersive X-ray (SEM/EDX) Spectroscopy

A Zeiss Supra 55 Field emission scanning electron microscope (Carl Zeiss AG, Oberkochen, Ostalbkreis) was used for SEM analysis of DF-1 biofilm samples after 45-day inoculation and an abiotic control (Pinewood biochar) without biofilm inoculation. EDX analysis was conducted with Scanning Auger Electron Nanoprobe-Physical Electronics 710 (Physical Electronics, Inc., Chanhassen, Minnesota). Firstly, the pinewood particles covered DF-1 biofilm samples and the abiotic control was put on a double-sided sticky carbon tape and dried in air. The samples were then coated with a thin film of gold on the surface of the sample to prevent charging and put into a vacuum chamber for analysis. SEM imaging analysis has been conducted with 1 keV primary electrons with the focus to detect the presence of biofilms. Imaging was performed at x3000, x10000, x15000, x30000, and x65000 magnifications at a working distance of 5-8 mm. EDX analysis was conducted using Integrated Auger Nanoprobewhich. Four to five different regions were selected for each

sample and the elemental distribution of each region was mapped for both DF-1 biofilm covered pinewood particles and the abiotic control. In addition, EDX spectra from the selected locations on each region were obtained to determine the elemental composition of that location.

5.2.7 PCB sampling, extraction, and analysis

In this study, the organohalide respiration of the weathered PCBs from each mesocosm was investigated by identifying their pattern transformation using a gas chromatography with an electron capture detector (ECD). Therefore, PCBs extraction was conducted for these potential organohalide respiration products. In addition, PCBs extraction is also evaluated in the abiotic control mesocosms without any bacterial inoculum and background organohalide respiring in the presence of weathered PCBs in the sediments. The PCB results analyzed by a GC from mesocosms are based on the surrogate recovery that can reflect the composition of weathered PCBs due to the organohalide respiration.

Microwave-assisted extraction was used to extract PCBs from the sediment samples. 5 grams of air-dried sediment samples were carefully transferred to the extraction vessels. 3 grams of clean sea sand (Merck) was used as a blank control and 18 ml hexane-acetone (1:1) is added into the vessels. Prior to extraction, 20 µL of the mixed solution of surrogates (0.5 µg ml-1 tetrachloro-m-xylene (TCMX), 2,4,6-trichlorobiphenyl (PCB-30) and 2,2’,3,4,4’,5,6,6’-octachlorobiphenyl (PCB-204)) were added into each sample. After that, extractions were performed at 115 °C for 10 min at 1000 W. After extraction, the vessels were allowed to cool to room temperature before they are opened. Then the extracts are settled and the supernatants were collected using by a Pasteur pipette. The residues were washed with hexane, hexane-acetone (1:1), acetone respectively. The supernatants are

collected and then were concentrated to less than 200 µl by using nitrogen blowdown. Finally, hexane was added to dissolve the extracts again and then stored at -20 °C.

The protocol of PCB extracts cleanup was conducted as following: copper was washed by sulfuric acid and put into a desiccator. Pasteur pipettes packed with glass wool covered with a layer of sodium sulfate then Cu/fluorosil (1:4 copper/fluorosil) and finally a layer of sodium sulfate. The volume and mass are not so important. The columns were washed with pure hexane prior to extraction. Then both the extract and the rinse were transferred into the pipette and the effluent was collected. The collected effluent was reduced in volume and spiked with 20 µl of the mixed solution of internal standards (50 ng ml-1 4-bromobiphenyl and 2,2',4,5,5'-pentabromobiphenyl) for further analysis. Finally, samples were analyzed by gas chromatography (Agilent 7890B) with an electron capture detector (GC-ECD) (Agilent Technologies, Inc, Santa Clara, California).

5.3 Results

5.3.1 Characterization of D. chlorocoercia DF-1 culture growing and their bioactivity of PCE dechlorination

All the DF-1 liquid culture of six inoculation bottles indicated the degradation of PCE after 10 days (Figure 5.1). The results of the headspace measurement indicated the PCE decreased from 94%±1.19% to 32%±29.50% after 10 days of incubation. Bottle 6 exhibited the most of the degradation in which 85% of PCE was observed. As a result, 69% of TCE, 12% of DCE, and 10% of VC were generated. After 20 days inoculation, approximately 100% of PCE were removed. Correspondingly, 14%±4.30% TCE and 81%±4.66% DCE were generated. After 30 days of incubation, all the PCE eventually transferred to 15%±2.66% TCE and 81%±10.59% DCE. Correspondingly, an average concentration of 5.53×10^{8} gene

copies/mg for each culture was eventually achieved over the 30 days. Similar growth was also observed in the inoculation of PCB-respiring *Dehalococcoides mccartyi* strains with PCE after 30 days (1.2×10^8 to 1.3×10^8 cells/ml) reported by Wang et al. (2014). In contrast, only 5.9×10^6 to 10.4×10^6 cells/ml were obtained after inoculation with Aroclor 1260 for 150 days.

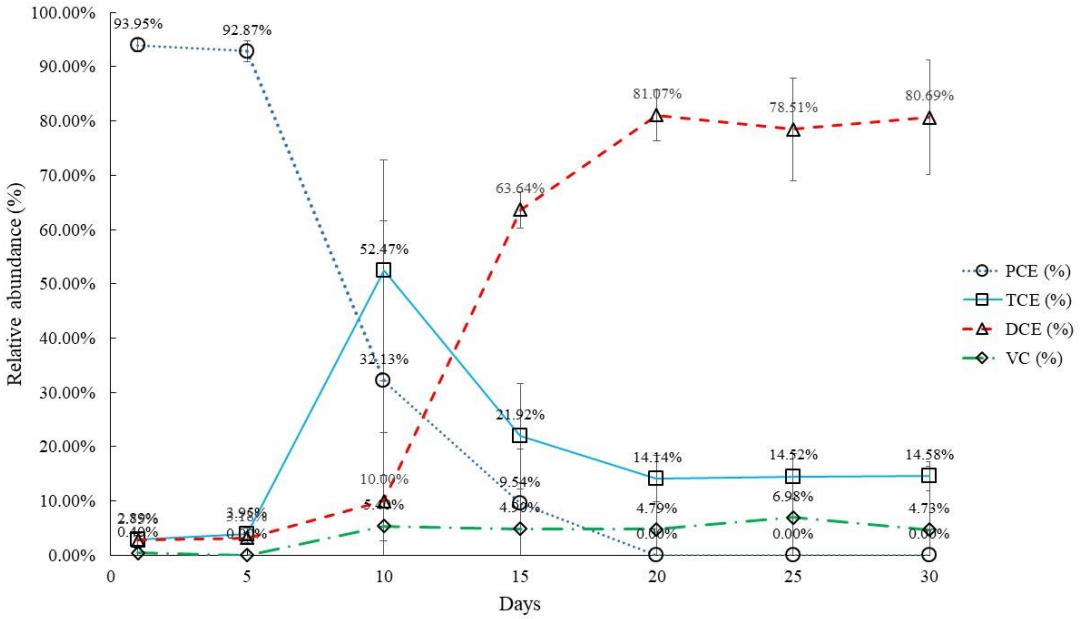

Figure 5.1 PCE dechlorination of DF-1 culture.

5.3.2 Characterization of D. chlorocoercia DF-1 biofilm growing and their bioactivity of PCE dechlorination

In this study, the *D. chlorocoercia* DF-1 was initially inoculated with 5-10 ppm of PCE. However, the inoculation was unsuccessful. The biodegradation products (i.e., TCE, and DCE) were detected in the headspace, when the DF-1 culture was inoculated with 55.87 ppm PCE in mesocosms. After 21 days inoculation, one out of ten mesocosms didn't show the dechlorination activity. According to Figure 5.2, the mole percent of the total PCE in the

headspace decreased from 100% to 70.4%±17.6% for the rest of nine mesocosms which suggested that the *D. chlorocoercia* DF-1 biofilm converted PCE to TCE. After another 5 days, approximately 41.2% of PCE were removed. The mole percent of TCE from PCE increased slightly from 29.6 %±17.4% to 37.6%±15.4%. Moreover, DCE (3.6%±5.9%) was firstly detected during the inoculations. No VC was ever detected in the headspace of the mesocosms during the inoculations. It is possible that the DCE was not converted to by *D. chlorocoercia* DF-1 biofilm. It is also attributed that the generated VC was observed into the pinewood biochar particles due to their high sorption capacity.

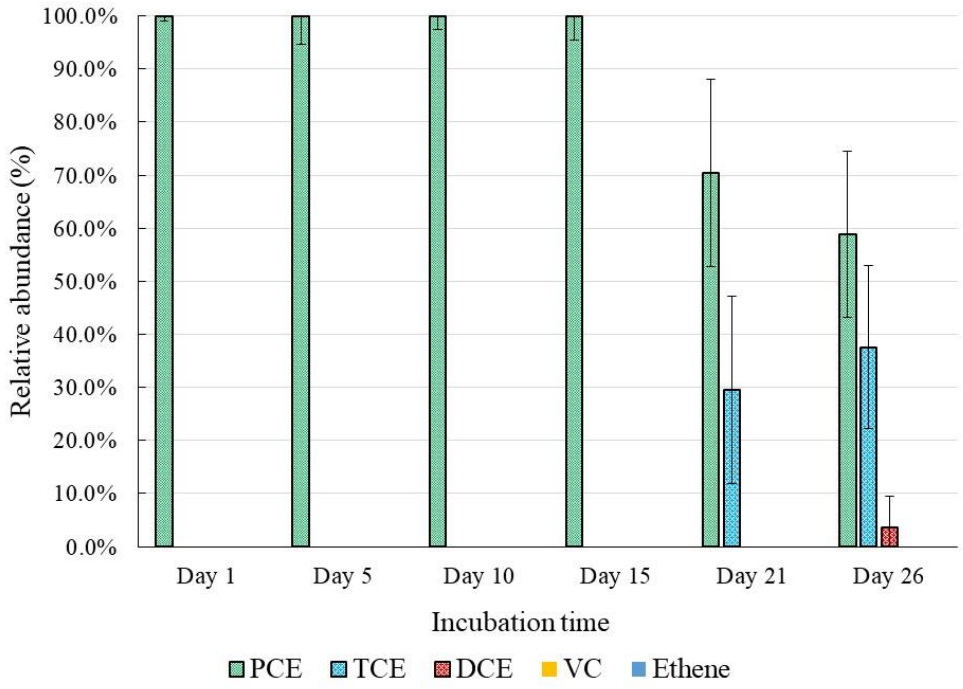

Figure 5.2 PCE dechlorination of DF-1 biofilm in mesocosms. 29.6% of the PCE was transferred to TCE in nine mesocosms after 21 days inoculation. After 26 days, 3.6% of DCE in the headspace of the mesocosms was detected.

5.3.3 Molecular characterization and enumeration of D. chlorocoercia DF-1 biofilm

Detecting the growth of DF-1 biofilm was difficult due to their low growth rate (May et al., 2008). This difficulty of the DF-1 stain growth has also been reported by other studies (May et al., 2008; Sowers and May, 2013b). The extracted DNA was tested by using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). All samples had an A260/280 ratio of ≥ 1.8 and an A260/230 ratio of ≥ 2.0 (Payne et al., 2017a). An A260/280 ratio of 1.6 to 1.8 is considered as pure for DNA (Kim et al., 2014). The value of ratio absorbance at 260 nm and 230 nm is commonly expected in the range of 2.0-2.2. If the ratio is lower than 1.6 to 1.8 or greater than 2.0-2.2, it indicates the presence of contaminants in the extracted DNA samples. The growth of DF-1 biofilm was successfully monitored by using PCR assay 348F/884R primer sets in this study. According to Figure 5.3, all the PCR fragment sizes of the DF-1 biofilm on pinewood biochar were comparable to the expected amplified sizes (536 bp) of the assay with DNA extracted from SDC-9 bioaugmentation culture as the positive control.

Enumeration of DF-1 biofilm was performed by using iQ SYBR Green Supermix (Bio-Rad Laboratories, California, USA) and primers specific for the 16s-rRNA gene on the basis of a standard curve with 6 dilutions of DNA sample with SDC-9 using the same qPCR procedure as for the biofilm inoculums. Amplification efficiencies of standards and samples were 92±8.0% with $R^2 = 0.98$. After 35 days inoculation, the DF-1 biofilm grown with PCE showed an increase of approximate 2 orders of magnitude in the copies of 16S rRNA genes as compared to the gene copies of DF-1 culture (10^6 gene copies/ml culture). Table 5.2 indicated that the gene copy numbers of nine mesocosms which are ranging from 1.95×10^8 to 8.30×10^8 gene copies/g pinewood biochar. The results of the qPCR assay used in this study indicated that the *D. chlorocoercia* DF-1 biofilm was rapidly grown with PCE and formed on the surface of a solid medium (i.e., pinewood biochar particles) during 35 days inoculation.

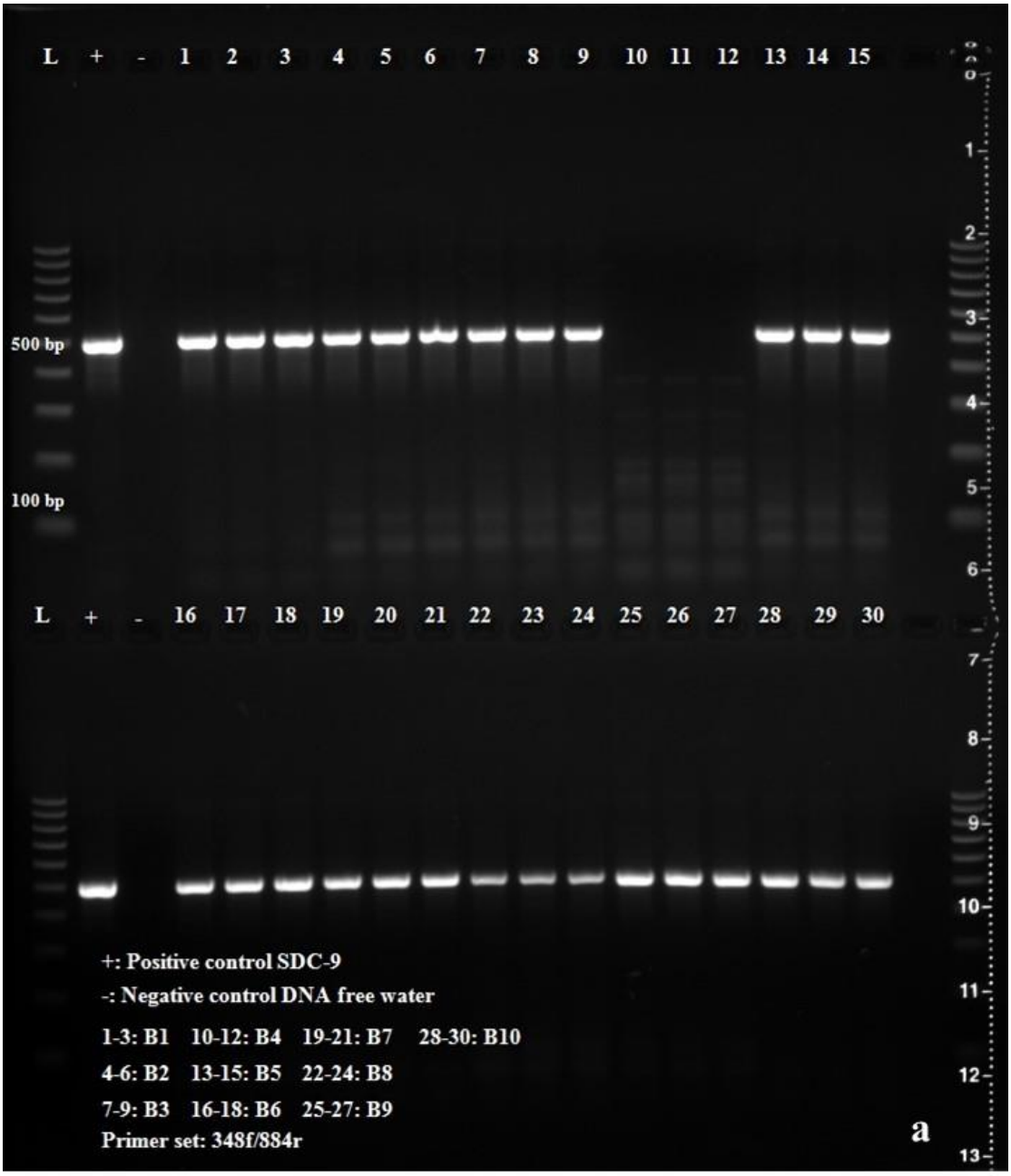

Figure 5.3 PCR of DF-1 biofilm with the 348f/884r primer set. The PCR products of the DF-1 biofilm on pinewood biochar were positive as compared to the expected amplified sizes (536 bp). The results of the qPCR assay indicated that the DF-1 biofilm were successfully inoculated on the surface of pinewood biochar particles during 35 days inoculation.

Table 5.2 results of Q-PCR for DF-1 biofilm with the 348f/884r primer set.

	Gene copies of DF-1 biofilm/g pine wood biochar	Mean	Stdev
B1	5.94×10^{8} 6.61×10^{8} 5.61×10^{8}	6.05×10^{8}	5.11×10^{7}
B2	5.88×10^{8} 5.70×10^{8} 5.80×10^{8}	5.79×10^{8}	8.89×10^{6}
B3	6.95×10^{8} 7.09×10^{8} 8.18×10^{8}	7.41×10^{8}	6.72×10^{7}
B5	6.75×10^{8} 7.08×10^{8} 6.83×10^{8}	6.89×10^{8}	1.70×10^{7}
B6	1.74×10^{8} 2.07×10^{8} 2.02×10^{8}	1.95×10^{8}	1.76×10^{7}
B7	2.21×10^{8} 2.19×10^{8} 2.25×10^{8}	2.22×10^{8}	3.12×10^{6}
B8	6.79×10^{8} 8.09×10^{8} 7.30×10^{8}	7.39×10^{8}	6.52×10^{7}
B9	6.39×10^{8} 7.03×10^{8} 7.03×10^{8}	6.82×10^{8}	3.72×10^{7}

5.3.4 Scanning electron microscopy and energy-dispersive X-ray spectrometry mapping of D. chlorocoercia DF-1 biofilm

This is the first report of the micromorphology of the *D. chlorocoercia* DF-1 biofilm based on an SEM/EDX analysis. The results of micromorphology for pinewood biochar particles (abiotic control) and *D. chlorocoercia* DF-1 biofilm were presented in Figures 5.4(a1-a3) and 4(b1-b3), respectively. Figure 5.4a indicated the micromorphology of the surface of pinewood biochar particles. A large number of densely packed hive-structures can be seen on the surface (Figure 5.4 (a1)). The micromorphology of the outer surface of the

hive-structures showed a striated cuticle layer (Figure 5.4 (a2)). In addition, Figure 5.4 (a3) suggested that the hive-structures have a similar size with microporous structures located on the inside of each hive-structure. According to chemical composition analysis, the major elements of the pinewood biochar surface include the carbon, oxygen, magnesium, and calcium. According to Figure 5.4 (b1-b3), an extracellular matrix deposition of the surface of pinewood biochar. It is possibly related to extracellular polymers production and matrix formation due to the *D. chlorocoercia* DF-1 cells attach to and grow on the surface. Moreover, another evidence of extracellular polymers formation on the surface of pinewood biochar is a chemical composition analysis indicating the deposition of phosphorus and sodium. The phosphorus and sodium are usually considered as the essential elements for the cell membrane (Kaiser et al., 2007).

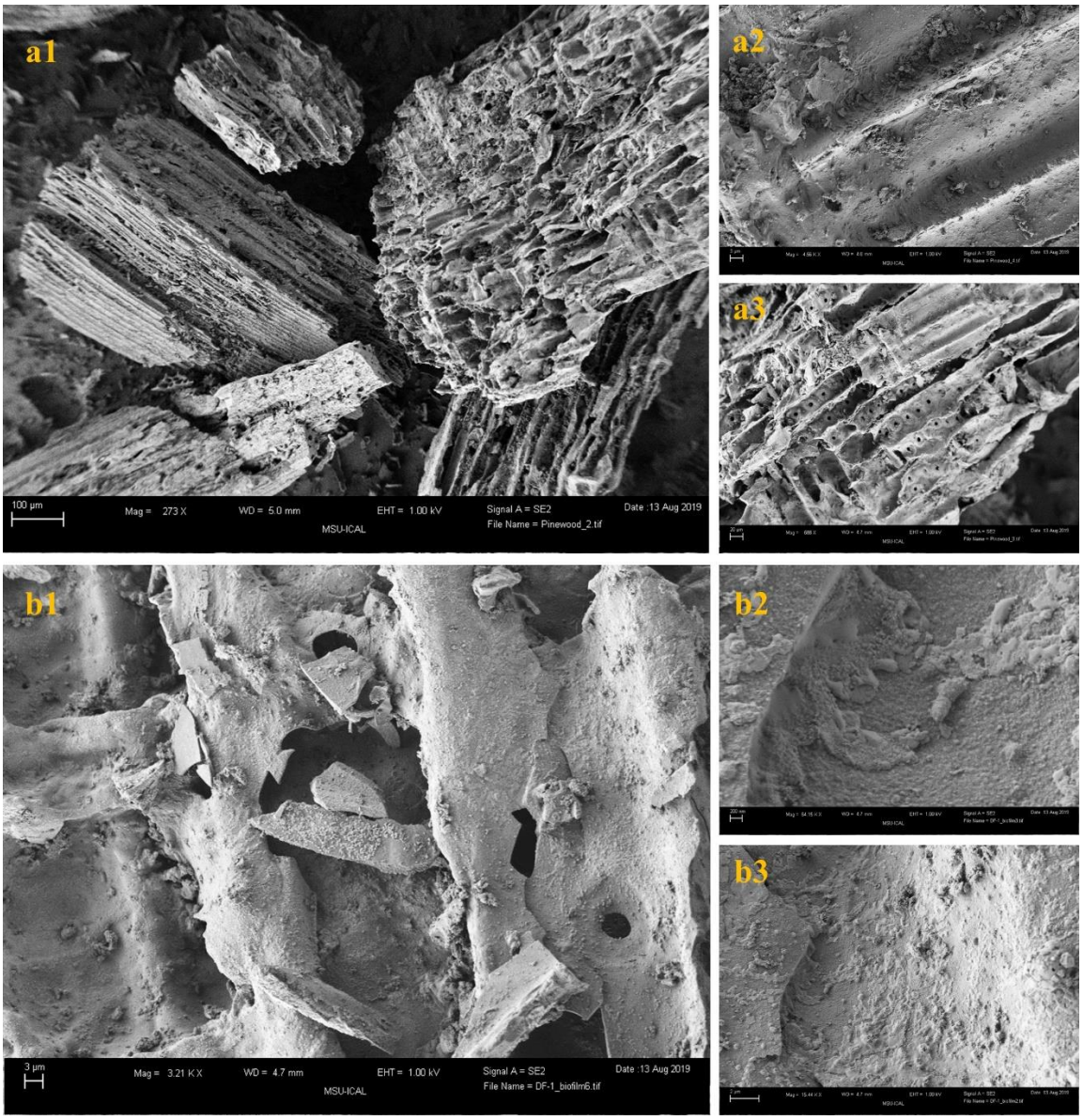

Figure 5.4 Scanning electron microscopy and energy-dispersive X-ray spectrometry mapping of pinewood biochar particles (a1-a3) and *D. chlorocoercia* DF-1 biofilm (b1-b3). Figure 4a1-a3: a large number of hive-structures with the more complex microporous structures can be seen on the surface of pinewood biochar particles; Figure 4b1-b3: the extracellular matrix deposition of the surface of pinewood biochar has been observed.

5.4 Discussion

The *D. chlorocoercia* DF-1 biofilm was initially inoculated with 5-20 ppm of PCE. However, the biofilm inoculation was not successful. The biodegradation products (i.e., TCE, DCE, and VC) were not detected in the headspace of the mesocosms. In addition, the DF-1 biofilm was not significantly growing based on the data of qPCR analysis. The gene copies of the DF-1 biofilm per grams of pine wood biochar was only 10^6-10^7 gene copies/g pine wood biochar. This could be attributed that the PCE absorbed by pinewood biochar particles thereby resulting in unevenly distributed in the surface of pinewood biochar under a relatively low concentration (5-20 ppm) that the *D. chlorocoercia* DF-1 cells cannot utilize as the terminal electron acceptors. Moreover, the DNA extraction recovery of the DF-1 biofilm was low. The DNA concentration of the extracted biofilm ranged from 5 to 15 ng/ul which is comparable to that of the *D. chlorocoercia* DF-1 liquid culture. Therefore, an additional experiment was implemented to separate the biofilm from the surface of pinewood biochar particles. According to the results, the DF-1 biofilm can be successfully separated from the pinewood biochar particles after sonicated at 1000 Hz for 60 minutes. After that, the DNA concentration of the extracted biofilm was significantly increased which ranged from 25 to 45 ng/ul. Due to the reasons that mentioned above, the bioaugmentation experiments of DF-1 biofilm were delayed.

Chapter 6. Conclusions and future works

6.1 Conclusions

The second Chapter evaluated the potential for bioremediation of PCBs in the intermittent and continuous effluent from a WWTP on the basis of five years of data from 2011 to 2015. According to the results, the first hypothesis was proven that the characteristics of PCBs present in by-pass and regularly treated effluents are different in mass and composition due to a high organohalide respiring potential in the regular treatment process. A 19% difference in the average number of chlorine/biphenyl for the 209 PCB congeners for intermittent versus continuous effluent indicated a potential for organohalide respiration of PCBs during normal wastewater treatment. Also, a high mass distribution of tri-, tetra-, and penta-PCBs accounting for 20.65%, 29.34%, and 24.83% of the anaerobically biodegraded PCBs further demonstrated the possibility of PCB organohalide respiration occurrence for Outfall 2 wastewater stream. The results can help to understand the biodegradation potential in the wastewater effluents thus more recommendations will be gained from subsequent studies.

In Chapter 3, to answer the question that if the organohalide respiring (OHR) bacteria present in the wastewater and biosolid from the WWTP, the potential OHR bacteria in the wastewater and biosolid samples from the WWTP were investigated. The mol % distribution of PCB homologs and numbers of chlorine/biphenyl of the wastewater samples showed that the organohalide respiration could occur in both wastewater and biosolid samples in varying degrees. In addition, two mesocosms

(east secondary sludge and post-anaerobic digester) inoculated with PCE future indicated the potential of organohalide respiration in wastewater and biosolid samples from the WWTP. The biodegradation products of PCE were generated from the east secondary sludge and post-anaerobic digester mesocosms. The total mole percent of PCE decreased from 99.2%±0.7% to 6.6% ±11.4% and 98.9%±1.9% to 70.8%±3.8% after 46 days. More interestingly, the bioinformatics profiles of the biosolid samples indicated that potential upstream sources of the OHR bacteria from the WWTP are related to the human gut microbial community. According to the 16S rRNA gene amplicon sequencing and analyzing by Data 2 pipeline, OHR bacterial genera including *Dehalogenimonas*, *Dehalobacter*, *Desulfitibacter*, *Desulfovibrio*, *Sulfurospirillum*, *Clostridium*, and *Comamonas* were found from the biosolid samples. Three out of these seven OHR genera (i.e., *Desulfovibrio, Clostridium,* and *Comamonas*) were also found in both American and Chinese human fecal microbiota datasets. These similarities of the OHR bacteria from the WWTP and the human fecal microbiome potentially revealed upstream sources of the OHR bacteria of the wastewater and biosolid samples could be from the human gut.

Chapter 4 has reviewed the most frequent remediation solutions including, phytoremediation, microbial degradation, dehalogenation by the chemical reagents, and PCBs removal by activated carbon. In addition, new insights that emerged from recent studies of PCBs remediation including supercritical water oxidation, ultrasonic radiation, bimetallic systems, nanoscale zero-valent iron-based reductive dehalogenation and biofilm covered activated carbon, electrokinetic remediation, and nZVI particles in combination with a second metal were overviewed. The advantages

and disadvantages of each general treatment strategy and promising technology for PCBs remediation are discussed and compared. nZVI combination with a second metal has a high remediation efficiency (78%-99%) with a shorter time. However, these technologies usually require excessive costs. Phytoremediation and microbial degradation have limited remediation efficiency (40%-60%). Even though phytoremediation and microbial degradation have a limited remediation efficiency with a long-term remediation time, the major advantage is the relatively low implementation costs. Therefore, they are not appropriate for an in-situ contamination site as a full-scale application. The conclusion of this chapter is a biofilm covered activated carbon system could be the most appropriate remediation approach with a low cost and relatively high remediation efficiency (more than 60%) that can be applied to either in-situ PCBs remediation.

Based on the conclusion of Chapter 4, Chapter 5 aims to inoculate the biofilm formation of the existing organohalide-respiring microorganisms i.e., *D. chlorocoercia* DF-1 on the surface of pinewood biochar particles. After that, the organohalide performance of the DF-1 biofilm covered pinewood biochar on the PCB contaminated sediments is going to be evaluated and compared with that of the liquid *D. chlorocoercia* DF-1 inoculums. The preliminary results indicated that DF-1 has been successfully inoculated with 2 ppm of PCE. Six bottles of DF-1 inoculum exhibited the degradation of PCE in which approximately 100% of PCE were removed after 20 days inoculation. Correspondingly, an average concentration of 5.53×10^{8} gene copies/mg for each culture was eventually achieved over the 30 days. Moreover, biofilm formation on the surface of pinewood biochar particles has been

detected based on the qPCR assay and an SEM/EDX analysis. The results of the qPCR assay indicated that the *D. chlorocoercia* DF-1 biofilm was rapidly grown with PCE and formed on the surface of pinewood biochar particles during 35 days inoculation. Furthermore, the deposition of the extracellular matrix on the surface of pinewood biochar has also been observed on the basis of SEM/EDX analysis. This is the first report of the micromorphology of the *D. chlorocoercia* DF-1 biofilm based on an SEM/EDX analysis.

6.2 Recommendations for further research

Organohalide respiration of PCBs in a WWTP system has been shown in this study. The second chapter focused on evaluating the annual amount of PCBs discharged to the nearby river from effluents originating from an intermittent and an continuous wastewater effluent. A 19% difference in the average number of chlorine/biphenyl for the 209 PCB congeners for intermittent versus continuous effluent indicated a potential for organohalide respiration of PCBs during normal wastewater treatment.

Moreover, another line of results suggested this PCB anaerobic organohalide respiration is that similar results of mol% of *ortho*-PCBs in the west and east primary influents (0.90% and 1.98%) as compared to that of intermittent effluent were also observed. This potentially indicates that specific types of bacteria such as putative PCB-dechlorinating *Chloroflexi* could be enriched, when the microorganisms are present in an anoxic/anaerobic zone thus anaerobically dechlorinate PCBs in these parts of the plant. However, two important research questions were still unsolved: 1)

which location of the WWTP has the most bioactive of PCB organohalide respiration and 2) Can the organohalide-respiring bacteria can be enriched using mesocosms for subsequent PCB bioremediation in sediments? To assist in answering the research questions, biosolid samples need to be future collected from the wastewater operational units to enrich the potential organohalide-respiring bacteria by setting up mesocosms.

In Chapter 5, *D. chlorocoercia* DF-1 was inoculated as biofilm growing on the surface of pinewood biochar to evaluate its organohalide performance on the PCB contaminated sediments. The methods of PCBs extract cleanup and GC analysis were established in this chapter. However, the analytic outputs of PCB analysis in the GC chromatography has large numbers of the interference peaks. Therefore, more efforts on the cleanup of PCBs extracts need to be conducted. In addition, the microbial inoculum delivery system could be essential for the future application to downstream sites of a WWTP contaminated with low-concentration PCBs. In addition, this approach can provide an efficient method for inoculating microorganisms for PCB bioaugmentation thereby increasing the potentials for long-term bioaugmentation. However, several research questions need to be answered.

1. What are the implications of the native *organohalide-respiring bacteria* from the contaminated sediments for the PCB bioremediations of DF-1 biofilm covered pinewood biochar?
2. What is the PCB bioremediation performance of the DF-1 biofilm covered pinewood biochar under in-situ conditions in the environment at a PCB contaminated

site?

3. Does the DF-1 biofilm covered pinewood biochar can withstand the extreme environmental condition such as *high-velocity flow* and natural aerobic conditions?

Appendices

Equations

$$M_i = C_i * Q_j \quad (S1)$$

$$M_{PCB} = \sum_{j=1}^{365} \cdot \sum_{i=1}^{10} . C_i * Q_j \quad (S2)$$

Where i (i = 1, 2, 3, ……10) denotes the types of 10 different PCB homologs; j (j = 1, 2, 3, ……365) denotes the operation days for the WWTP; Ci denotes the mass concentration (g/l) of each PCB homolog from both the intermittent and the continuous effluent for each day; Q_i is the flow rate for both outfall 1 and outfall 2 effluent (l/day) for each day; M_i denotes the mass (g) of each PCB homolog of effluents to the nearby river each day; MPCB is the total PCB mass (g) loading to the river of each operation year.

$$n_{Cl/Biphenyl} = \frac{\sum_{i=1}^{10} . \frac{M_i}{m_{wi}} * n_i}{\sum_{i=1}^{10} . \frac{M_i}{m_{wi}}} \quad (S3)$$

$$^{year}n_{j,Cl/Biphenyl} = \frac{\sum_{j=1}^{365} . n_{j\ Cl/Biphenyl}}{365} \quad (S4)$$

Where i (i = 1, 2, 3, ……10) denotes the types of 10 different PCB homologs, j (j = 1, 2, 3, ……365) denotes the operation days for WWTP; M_i denotes the calculated total mass (g) of each PCB homolog from both the intermittent and continuous effluents for each day; m_{wi} is the molecular weight (g/mol) for each PCB homolog; n_i denotes the number of chlorine for each PCB homolog; $n_{Cl/Biphenyl}$ is the number of chlorine per biphenyl ring for each day; $year_{n,Cl/Biphenyl}$ denotes the average number of Chlorine/Biphenyl for each year.

$$R_{aerobic} = \frac{\sum_{j=1}^{365} \cdot \sum_{k=1}^{4} . C_k * Q_j}{\sum_{j=1}^{365} \cdot \sum_{i=1}^{10} . C_i * Q_j} \quad (S5)$$

$$R_{anaerobic} = \frac{\sum_{j=1}^{365}\cdot\sum_{i=1}^{10}.C_i*Q_j - \sum_{j=1}^{365}\cdot\sum_{h=1}^{5}.C_{h,\ ortho}*Q_j}{\sum_{j=1}^{365}\cdot\sum_{i=1}^{10}.C_i*Q_j} \quad (S6)$$

Where i (i = 1, 2, 3, ……10) denotes the types of 10 different PCB homologs; k (k = 1, 2, 3, 4) denotes the types of the PCB homologs with four or less chlorine atoms (i.e., Monochlorobiphenyls, Dichlorobiphenyls, Trichlorobiphenyls, Tetrachlorobiphenyls); j (j = 1, 2, 3, ……365) denotes the operation days for the WWTP; h denotes the types of 5 different PCB congeners with *ortho* chlorine atoms; C_i is the mass concentration (g/l) of each PCB homolog from both the intermittent and continuous effluent for each day; $C_{h,ortho}$ represents the mass concentration (g/l) of 5 types of the PCB congeners with *ortho* chlorine atoms for each day; Q_i is the flow rate for both effluents (l/day) for each day.

Table S1 Concentration of DNA extracted from different locations of the WWTP.

	Sample locations	Total Solids (g/ml)	DNA content (ug DNA/ul DNA extract)	DNA content (mg DNA/g Dry solid)
1	Primary sedimentation tank	0.006	3.0	0.536
2	Nitri/denitrification Reactor	0.050	4.3	0.087
3	Primary effluent	0.008	3.6	0.468
4	Anaerobic digestion reactor (Digested biosolids)	0.984	9.4	0.009
5	Final cake	0.605	5.7	0.009
6	Nitri/denitrification sedimentation	0.006	3.2	0.533
7	Secondary reactor	0.093	13.1	0.141
8	Secondary sedimentation tank	0.007	2.7	0.391
9	Centrifuge pre-dewatering (Liquid and biosolid)	0.040	3.2	0.079

Table S2 Information on American and Chinese human intestinal microbial datasets in this study.

	American intestinal microbiomes	Chinese intestinal microbiomes
BioProject ID	PRJNA386260	PRJNA383300
Primers	515F and 806R	338F and 806R
Sequencing area	V4 16S-rRNA	V3-V4 region of 16S-rRNA
Data source	University of Michigan	China Agricultural University
Sample size	211	94
Total reads	5089759	4302225
Sequence Technologies	Illumina MiSeq	Illumina MiSeq

Table S3 Presence of PCB homologs in wastewater samples collected for this study.

Homolog	East primary influent	West primary influent	East primary effluent	West primary effluent	East secondary effluent	West secondary effluent	Nit/denitrification effluent
Monochlorobiphenyl	1.1%	0.5%	0.7%	0.9%	1.0%	1.1%	2.3%
Dichlorobiphenyl	9.2%	4.1%	6.1%	6.8%	15.0%	15.0%	19. 9%
Trichlorobiphenyl	14.1%	9.4%	10.6%	12.5%	19.0%	17.8%	21.6%
Tetrachlorobiphenyl	21.9%	18.3%	19.2%	25.8%	23.7%	25.0%	27.3%
Pentachlorobiphenyl	31.9%	30.7%	31.8%	24.3%	25.4%	26.2%	17.8%
Hexachlorobiphenyl	21.8%	23.6%	20.6%	9.3%	12.0%	11.3%	8.3%
Heptachlorobiphenyl	0.1%	9.9%	8.1%	19.7%	3.1%	3.1%	2.3%
Octachlorobiphenyl	0.0%	2.9%	2.3%	0.7%	0. 7%	0.5%	0.5%
Nonachlorobiphenyl	0.0%	0.5%	0.5%	0.00%	0.1%	0.1%	0.1%
Decachlorobiphenyl	0.0%	0.1%	0.2%	0.00%	0.0%	0.0%	0.0%

Table S4 Presence of PCB homologs in biosolid samples collected for this study.

Homolog	Primary sludge	East secondary sludge	West secondary sludge	Pre-CAMBI	Post anaerobic digester	Cured biosolids	Belt Filtration cake
Monochlorobiphenyl	0.6%	0.1%	1.0%	1.0%	1.0%	4.3%	1.2%
Dichlorobiphenyl	6.0%	5.0%	6.5%	8.7%	6.6%	4.4%	13.9%
Trichlorobiphenyl	10.6%	10.8%	12.7%	11.3%	8.9%	14.4%	25.2%
Tetrachlorobiphenyl	19.2%	28.5%	23.2%	25.5%	21.7%	28.4%	31.8%
Pentachlorobiphenyl	31.8%	25.6%	31.6%	24.7%	32.4%	19.4%	17.3%
Hexachlorobiphenyl	20.6%	9.8%	18.2%	9.6%	18.6%	13.6%	7.3%
Heptachlorobiphenyl	8.1%	19.4%	5.3%	15.1%	8.3%	12.0%	3.0%
Octachlorobiphenyl	2.3%	0.7%	1.2%	4.1%	2.2%	3.5%	0.4%
Nonachlorobiphenyl	0.6%	0.0%	0.2%	0.0%	0.4%	0.0%	0.0%
Decachlorobiphenyl	0.2%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%

Table S5 Presence of PCB homologs in biosolid centrate samples (mol%).

Homolog	Belt filter centrate	Belt filter washing water	Centrifuge centrate	DAF centrate	Gravity thickening centrate
Monochlorobiphenyl	1.1%	1.2%	0.8%	1.1%	0.5%
Dichlorobiphenyl	6.3%	8.1%	8.7%	10.6%	3.2%
Trichlorobiphenyl	8.0%	11.3%	13.9%	13.5%	8.2%
Tetrachlorobiphenyl	20.7%	17.0%	24.3%	23.7%	27.9%
Pentachlorobiphenyl	33.0%	29.2%	31.9%	29.5%	32.3%
Hexachlorobiphenyl	19.5%	23.0%	14.9%	15.6%	6.5%
Heptachlorobiphenyl	8.3%	7.7%	4.3%	4.7%	20.9%
Octachlorobiphenyl	2.5%	2.2%	1.1%	1.2%	0.6%
Nonachlorobiphenyl	0.5%	0.4%	0.2%	0.2%	0.0%
Decachlorobiphenyl	0.2%	0.0%	0.0%	0.0%	0.0%

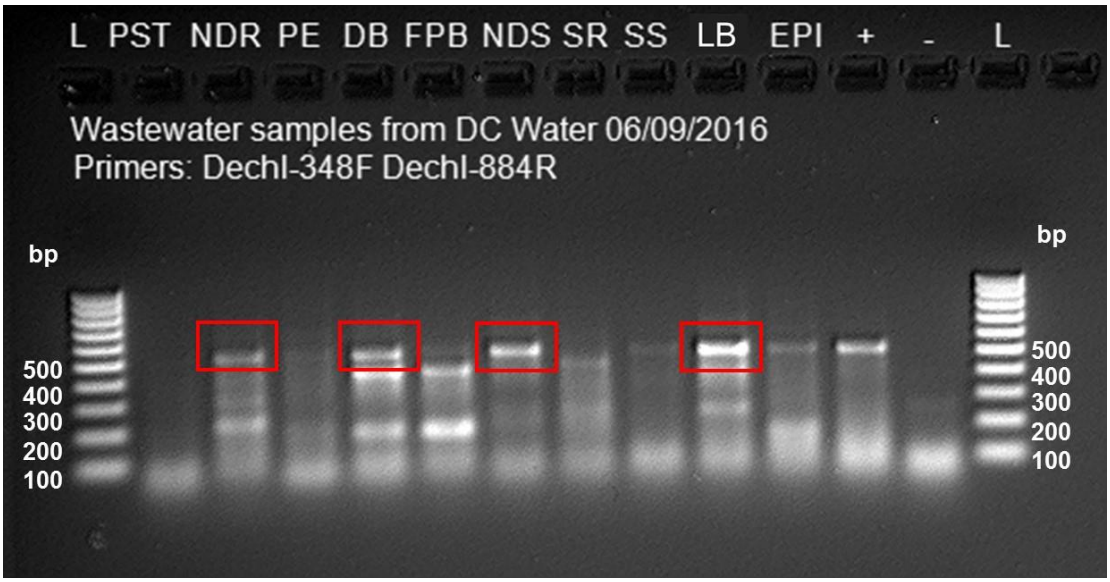

Figure S1 Agarose gel electrophoresis analysis of DNA samples from different locations of the WWTP; NDR: Nitri/denitrification reactor; DB: Anaerobic digestion reactor (Digested biosolids); NDS: Nitri/denitrification sedimentation; LB: Centrifuge pre-dewatering (Liquid and biosolid) L: DNA ladders; +: Positive control, DNA extracted from DHC microbial consortium (SDC-9) by using Dechl-348F/Dechl-884 primer set.

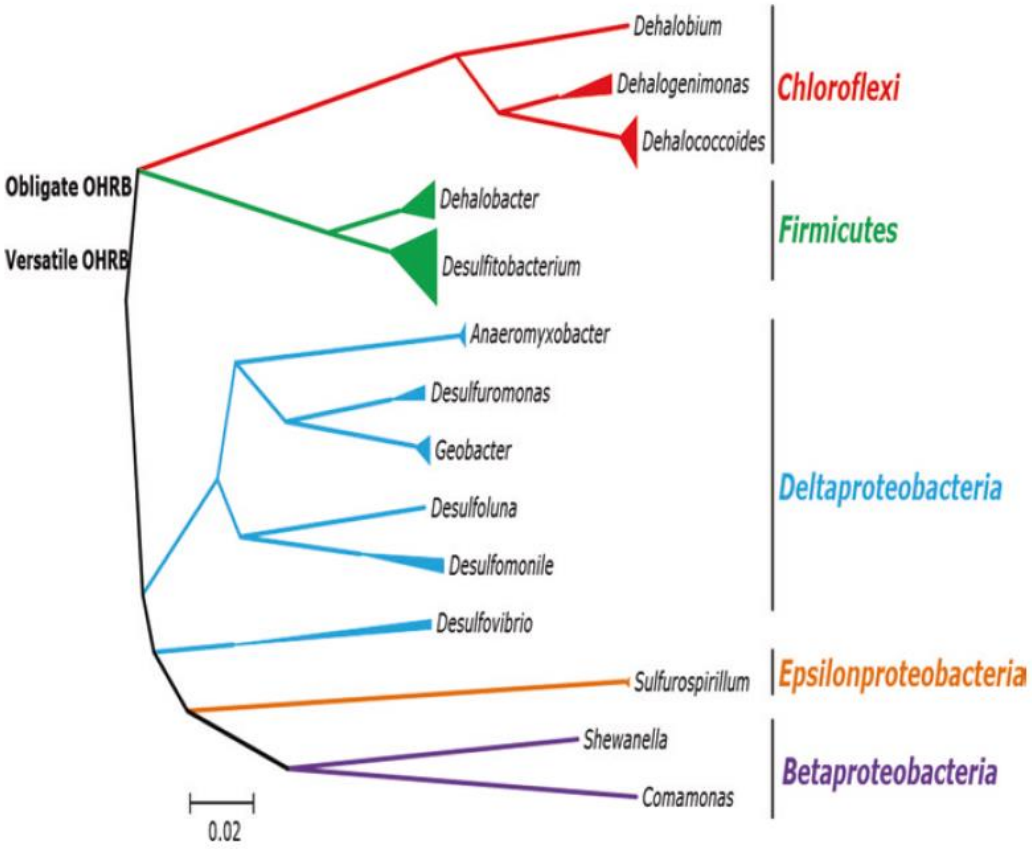

Figure S2 Phylogenetic tree of known OHR bacteria based on 16S rRNA gene sequences. Alignment and phylogenetic analysis were performed with MEGA and the tree was constructed using the neighbor-joining (NJ) method. The *reference bar at the bottom* indicates the branch length that represents 2% sequence divergence. Color Key: *Chloroflexi* (red), *Firmicutes* (green), *Deltaproteobacteria* (blue), *Betaproteobacteria* (violet), *Epsilonproteobacteria* (brown).

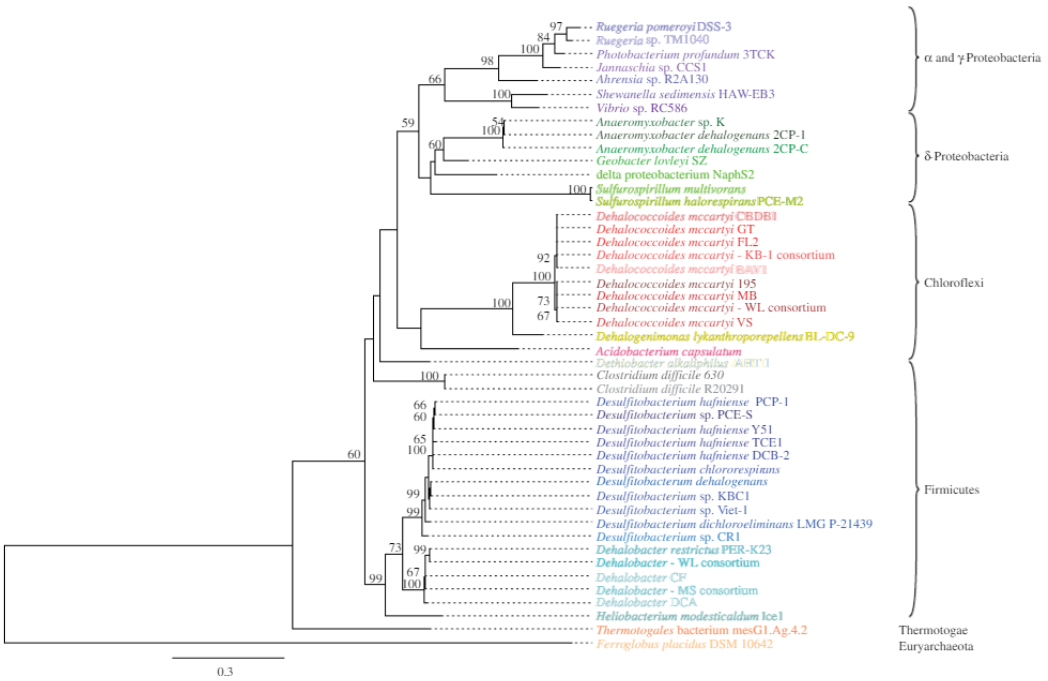

Figure S3 Maximum-likelihood (ML) tree of 16S rRNA gene sequences from known organisms containing a reductive dehalogenase homologous (rdhA) gene.

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