College of Engineering
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Feasibility of Natural Attenuation for the Remediation of Soil Contaminants at the Santa Susana Field Laboratory

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Executive Summary

Area IV of the Santa Susana Field Laboratory (SSFL) was used for energy development research by the U.S. Department of Energy (DOE) from the mid-1950s until approximately 2000. These activities resulted in soil contamination by petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated dioxins, and metals such as mercury and silver. An evaluation of possible soil treatment technologies conducted by Sandia National Labs in 2011 identified six technologies that could be evaluated using Area IV soil and conditions. DOE, based on a recommendation made by the community, decided to have local universities conduct five of the proposed six treatability studies (DOE elected not to conduct the thermal treatment study at this time). The five in-depth treatability studies selected for evaluation include bioremediation, phytoremediation, soil partitioning, an evaluation of mercury contamination, and natural attenuation.

The purpose of the natural attenuation treatability study is to determine if natural processes occurring at Area IV can reduce certain soil contaminant concentrations and to ascertain what rates of biodegradation could be expected in the field under natural attenuation conditions. This study is being conducted in two phases. The first phase (reported here) is a literature review to determine which soil contaminants in Area IV are amenable to biodegradation and other weathering processes, what contaminant biodegradation and weathering pathways are known, and what rates of biodegradation and weathering of the contaminants have been observed in published field and laboratory studies.

Estimates were made of the time periods required to reduce soil contaminant concentrations to acceptable levels using natural attenuation alone based on what was known of the site conditions at Area IV. The second phase of the natural attenuation study will use the findings of companion studies on bioremediation and phytoremediation of Area IV soils to make better site-specific predictions of natural attenuation rates at the site. These companion studies include microcosm experiments to measure biodegradation rates of the contaminants in Area IV soils under natural attenuation conditions. The bioremediation study also includes an investigation of the microbial communities present in Area IV soils, and the results of that investigation will provide an indication of whether or not bacteria and/or fungi are present in the soils which are known degraders of the contaminants.

The literature review suggests that all of the contaminants in Area IV soils are amenable to natural attenuation processes, but that the rates of natural attenuation may be slow for some of the contaminants. These processes include abiotic weathering by volatilization, leaching, and photo-oxidation, as well as biodegradation by bacteria and fungi, and phytoremediation by plants. Abiotic processes are expected to have limited effects because most of the remaining contaminants have low volatility and the contaminants are highly weathered which would have likely already led to volatilization of the lighter components, leaving the less volatile components in the soil. Bacterial and fungal biodegradation appear to be the most likely processes to contribute to reductions in concentrations of the treatability study chemicals of interest (COIs). The study also included an assessment of mercury and other metals, which do not biodegrade, but may be addressed through phytoremediation. Biodegradation processes have been researched for each of the COIs, and lists of microorganisms capable of mediating

biodegradation are provided for each COI. In addition, tables of published biodegradation rates under natural attenuation conditions are provided in this report for each COI. The potential for these processes to contribute to natural attenuation of each COI are described below.

Petroleum hydrocarbons: Biodegradation of non-aromatic petroleum hydrocarbons in soils is well documented and hydrocarbon-degrading microorganisms are nearly ubiquitous in the environment. However, some hydrocarbon compounds are more difficult to biodegrade than others, such as longer-chain hydrocarbons. The half-lives for biodegradation of petroleum hydrocarbons in soil range have been reported to range from days to several years. Based on published or calculated first-order rate constants, the time to reduce SSFL hydrocarbons to the DTSC-specified background concentration of 5.7 ppm is 0.42 to 69 years. This wide range is due to both the range of published rates and the range of hydrocarbon concentrations at different locations in Area IV. Since hydrocarbon contaminants at SSFL are highly weathered, the most rapid rates would not be expected for natural attenuation at SSFL.

Polyaromatic hydrocarbons (PAHs): Numerous aerobic PAH-degrading bacteria and fungi have been reported in the literature. Their ability to biodegrade PAHs is dependent on the number of aromatic rings, with the slowest rates for PAHs with the greatest number of aromatic rings, such as benzo-a-pyrene. Half-lives of 60 days to 3 years have been reported for PAH mixtures in soil. The time estimated to reach the background levels specified for PAHs at the site (2.5 - 5.6 ppb) range from 5 to 15 years based on comparison to relevant published field studies. However, weathering of soil contaminants at SSFL may have greatly reduced their bioavailability, and this could increase the time required. Biodegradation rates could likely be accelerated by amending soils with surfactants to increase the bioavailability of the sequestered PAHs. Phytoremediation has also been successful for PAHs, and data from one study suggests that the PAHs in Area IV soils could be remediated in 1.5 to 2.7 years with active phytoremediation.

Polychlorinated biphenyls (PCBs): PCB biodegradation is more complex than hydrocarbon biodegradation, often requiring a combination of anaerobic and aerobic conditions. Bacterially mediated PCB degradation typically involves anaerobic dechlorination followed by aerobic biodegradation. Only a few species of bacteria have been identified with the ability to reductively dechlorinate PCBs, and these are found mostly in aquatic sediments. Reported rates of PCB biodegradation are extremely low, even under ideal conditions. In fact, a half-life of 40 years was reported for Aroclor 1260, which is the predominant PCB contaminant found in Area IV. If anaerobic conditions do not exist in SSFL soils, then bacterial dechlorination is unlikely. Fungal biodegradation of PCBs may be more promising at SSFL than bacterial biodegradation, because fungi do not require anaerobic conditions. Phytoremediation of PCBs is also a possibility for soils at SSFL.

Dioxins: Like PCBs, bacterial biodegradation of chlorinated dioxins requires a combination of anaerobic and aerobic processes, so if anaerobic conditions are not found in the SSFL soils, then significant dioxin biodegradation by bacteria would not be expected. Based on the published literature, biodegradation of the dioxins in SSFL soils could take 1 to 50 years under natural attenuation conditions. As noted for PCBs, fungal biodegradation of dioxins may be more promising at SSFL if the soils are not anaerobic. Bioaugmentation with fungi could improve biodegradation rates, and laboratory experiments are currently underway to assess this strategy. Limited research has been done on phytoremediation of dioxins, but some researchers suggest that its effectiveness for dioxins might be similar to that for PCBs.

Perchlorate: Leaching into the underlying groundwater is likely to be an important mechanism of soil perchlorate natural attenuation because of its high solubility in water. Biodegradation of perchlorate requires anaerobic conditions, which may not be present at SSFL. Fungal biodegradation of perchlorate has not been reported. Phytoremediation may enhance perchlorate remediation in SSFL soils based on one published study, but this study was done with saturated soil, and thus may not be applicable to SSFL.

Mercury: Volatilization of elemental mercury and/or methyl mercury is a possible natural attenuation mechanism for mercury removal from SSFL soils, but this process is likely to be very slow, and it could create air pollution issues. Phytoremediation of mercury is a potential method of removing mercury from the soil, although this would not be a natural attenuation method since it would involve active removal of plants from the site. It is unlikely that plants at SSFL will take up mercury into their roots unless the mercury is first chelated. Greenhouse experiments are underway to test the use of a chelating agent to facilitate mercury uptake by plants from SSFL in a companion study.

General conclusions:

Estimates of times predicted to reach proposed clean-up levels via natural attenuation varied widely due to a lack of site-specific information. These predictions can be narrowed and more reliable after the companion studies are completed. Also, predictions can be improved once more site characterization work is completed, particularly for the determination of redox conditions in the soil and soil temperature profiles. Detailed chemical analyses could also be used to help determine the extent of current biodegradation at the site.

Natural attenuation at SSFL is expected to be slow – on the order of decades - based on the history of soil contamination at the site. Since the soil contaminants have been in the soil for decades, natural attenuation processes have already been acting on the soils for a long time. It is highly unlikely that natural attenuation rates would accelerate at the site without active intervention. Natural attenuation processes often follow first-order kinetics, which means that the rates of natural attenuation would decrease over time as the contaminant concentrations decrease. In addition, biodegradation typically slows down even more than expected from first-order kinetics over time as contaminants become sequestered in the soil and the most easily biodegraded components of the contaminants have biodegraded early in the weathering process, leaving the more recalcitrant fractions in weathered soils.

In some cases, long remediation times are predicted because the clean-up goal for this site requires reaching very low background levels of the surrounding natural environment. Much shorter remediation times would be expected if clean-up goals were set similar to those set for typical industrial sites.

Natural attenuation should be considered on a case-by-case basis for the different sub-areas in Area IV. Soils with very high contaminant concentrations will likely need to be excavated and hauled off site, but natural attenuation should be considered for soils with lower contaminant concentrations. This could greatly reduce the quantity of soil that needs to be excavated and the many associated environmental impacts of such excavation. Although the focus of this investigation was on natural attenuation, the findings suggest that more active bioremediation methods could be successfully employed at SSFL, and such methods should be further explored.

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1.0 Introduction

The Santa Susana Field Laboratory (SSFL) was the site of numerous rocket engine tests and energy-related research projects for decades, which included the construction and testing of small nuclear reactors (U.S. DOE, 2014). A variety of chemicals were used for this research within Area IV at the Energy Technology Engineering Center (ETEC) site. These chemicals included polychlorinated biphenyls (PCBs) in electrical components and hydraulic fluids, fuels to run auxiliary generators and heat water for steam, solvents to clean components, metals such as mercury for energy transfer applications, and silver for photograph development. Burning of wastes onsite produced dioxins, and releases of PCBs, metals, fuels, lubricants, and solvents from transformers, storage tanks, drums in storage areas, and at leach fields contaminated soil within Area IV. Extensive soil sampling investigations have identified the locations and soil concentrations of these contaminants of interest (COIs[1]) in Area IV.

The Sandia National Laboratories conducted a preliminary soil treatability study in 2011 in which several potential methods were identified for on-site remediation of the COIs in the soils at Area IV. One of the strategies recommended for further evaluation is natural attenuation, which the US EPA defines as the "use of natural processes to contain the spread of contamination from chemical spills and reduce the concentration and amount of pollutants at contaminated sites (US EPA, 1999)." Natural attenuation processes include biological degradation by on-site bacteria, plants and fungi, as well as abiotic processes such as volatilization, dispersion, dilution, radioactive decay, and sorption of contaminants onto organic matter and clay minerals in the soil. Models can be made from bench scale experiments and literature reviews which can be used to estimate how long it will take for natural attenuation to reduce the concentrations and bioavailability of contaminants in the soil. Site monitoring during natural attenuation is essential to ensure there is no risk to the environment or public health.

Early use of natural attenuation was primarily for remediation of benzene, toluene, ethylbenzene and xylenes (BTEX) (Yadav & Reddy, 1993), but more recently it has been applied to chlorinated hydrocarbons as well (Weber et al., 2008). In theory it could be applied to any contaminant as long as the timeline is long enough (Mulligan & Yong, 2004). If measured natural attenuation rates are high enough without more active remediation methods, and the remediation timeline is long enough, then natural attenuation can be a cost effective option (Alvarez & Illman, 2005). However, it is important to assess the potential for natural attenuation of the particular contaminants and conditions at each particular site. Natural attenuation rates can be highly influenced by the microbial community, climate, and soil characteristics, as well as the chemistry of the particular COI.

The purpose of the research described in this report is to estimate the ability of natural attenuation to reduce soil contaminant levels to low enough values that the soil can be left on site and not hauled off-site. The goal is to provide the information necessary to guide a sustainable approach to remediation that minimizes environmental impacts of the remediation

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[1] Contaminants of interest for the soil treatability study, other chemicals detected in Area IV, such as pesticides and herbicides, are not part of the soil treatability study.

efforts. While it is desirable to reduce soil contaminant concentrations to the lowest levels possible, this desire must be weighed against the environmental impacts involved in transporting contaminated soil. Excavation of soil will result in the release to the environment of contaminants in the soil by volatilization and airborne dust transport, and also PAH emissions from the diesel excavation equipment. Similarly, trucking of the soil will result in PAH and other air pollutant emissions, as well as causing noise and road safety issues in the local area. In fact, a study of a remediation site on the Central Coast of California showed that 90% of PAH exposure during remediation was associated with diesel exhaust (Ozaki, 2000). Therefore, it would clearly be advantageous to minimize the volume of soil which needs to be trucked off-site. Natural attenuation may have the potential to reduce some contaminant concentrations to acceptable levels, thus reducing the need for hauling some of the contaminated soil at SSFL.

This natural attenuation study is being conducted in two phases. The first phase (reported here) is a literature review determining which COIs are amenable to biodegradation and other weathering processes, what biodegradation and weathering pathways are known, and what rates of biodegradation and weathering of the COIs have been observed in field and laboratory studies. This first phase relies on literature reports on natural biodegradation and other processes that might reduce the concentrations of these contaminants. An attempt was made to consider the published literature in terms of the site conditions at SSFL. This evaluation is used to estimate the time required to reduce COI concentrations to desired clean-up levels via natural attenuation alone. The COIs investigated in this study include petroleum hydrocarbons, PAHs, PCBs, dioxins, and metals (mercury in particular). This Year-1 report summarizes the literature review and the potential for natural attenuation of these COIs based on currently available site data.

The second phase of this study will use bioremediation and phytoremediation field and laboratory study results to make more site-specific predictions of natural attenuation rates at the SSFL site. Companion studies are underway to separately assess the feasibility of using bioremediation and phytoremediation as active methods of remediation at SSFL. Both of these studies include microcosm experiments to measure biodegradation rates of the COIs in soils under natural attenuation conditions. The bioremediation study also includes an investigation of the microbial communities present in Area IV soils, and the results of this investigation will provide an indication of whether or not bacteria and/or fungi are present in the soils which are known degraders of the COIs. Field studies will also provide better site characterization in terms of soil temperature, moisture and redox conditions, which can be used to further ascertain the potential rates of natural attenuation at the site. The second-phase natural attenuation study will also include an analysis of historical soil data, combined with recent sampling for COIs at the site to see if concentrations are decreasing in the field. The soil sampling associated with the other treatability studies will also be designed to identify potential intermediates or biodegradation end-products from the COIs to provide evidence that natural biodegradation is occurring at the site. Thus, the Year-2 natural attenuation report will provide more accurate estimates of natural attenuation rates based on the new information that will be made available by these additional studies.

This study is being conducted in compliance with the Administrative Order on Consent (AOC) that DOE signed with the California Environmental Protection Agency Department of Toxic

Substances Control (DTSC) in 2010. The AOC specifies the processes for completing site characterization and remedy identification for Area IV. Included within the AOC is a requirement for DOE to conduct soil treatability studies to remove contaminants found in soil in Area IV. This treatability study plan addresses the AOC requirement to conduct soil treatability studies. DTSC has the regulatory authority for approving and accepting the results of all Area IV treatability studies.

1.1. SSFL Site Background

The Santa Susana Field Laboratory (SSFL) was the site of numerous liquid-propulsion rocket engine tests performed by the U.S. Department of Defense (DOD), the National Aeronautics and Space Administration (NASA), the United States Air Force (USAF), and several commercial companies, including Boeing. North American Aviation (NAA) originally established SSFL in 1947. The site was used for government and commercial research and development of both nuclear technology and large static-fire rocket engines. A 290-acre section of SSFL known as "Area IV" was designated for energy research in 1954. The Energy Technology Engineering Center (ETEC) was established on a 90-acre portion of Area IV which was leased by the Atomic Energy Commission (AEC) to DOE. Area IV was used for nuclear energy research as well as other research projects. The ETEC was also the site of DOE's Liquid Metals Center of Excellence. During the ETEC's operations, 10 small nuclear reactors were built and tested. Sodium and potassium were tested as coolants in these reactors as alternatives to water or gas. The majority of these tests took place between 1956 and 1970, with the last reactor being shut down in 1974. In 1959, the sodium-cooled reactor experienced excess heating of fuel elements resulting in a partial meltdown, releasing radioactive contamination (CEC, 2014). Research and handling of nuclear materials in Area IV were completed in 1988.

During the 1970s demolition and removal of the nuclear research facilities began. This process continued through the 90s. The last non-nuclear research in Area IV was halted in 2001 with the closure of the Sodium Pump Test Facility. Since then all nuclear materials have been removed from ETEC. Only a few shells of reactor buildings persist. The Radioactive Materials Handling Facility (RMHF) is the only remaining active facility and will assist in the final building demolition and soil cleanup operations in the event that radioactive material is found during the cleanup process of SSFL.

During the various research projects and tests conducted in Area IV and in other areas of SSFL, a variety of chemicals were used. PCBs were used in electrical components such as transformers. Various hydraulic fluids and fuels were used to run generators, heat water for steam, and other applications. Solvents were used to clean parts during and after tests. Mercury and sodium were used in energy transfer applications, and silver was used in photograph development. Waste was burned on the site, which produced dioxins and released the PCBs, metals, fuels and lubricants, and solvents from transformers, storage tanks, and drums in storage areas and at leach fields. All these factors led to the contamination of the soil in Area IV with petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), dioxins, PCBs, perchlorate and various metals, including mercury and silver.

In 2005 the Topanga Wildfire burned almost all of the brush on the SSFL site and the surrounding area. The fire burned 24,000 acres, including 2,000 acres of SSFL (roughly 80%).

Some buildings had substantial fire damage, about 10 out of the 200 on SFFL at the time. During the fire roughly 150 pounds of Freon were lost from air conditioning units (ETEC, 2005). The effects of the fire on the contaminants are largely unknown. However, wildfires are known to produce dioxins (Persson and Simonson, 2005), and because of the large release of Freon (a chlorinated compound) during the fire, it is possible that more dioxins were produced (ETEC 2005).

Another contaminant of interest is perchlorate, which is an anion commonly used for solid rocket fuels or explosives (Coates & Achenbach, 2004). The extent of perchlorate contamination in Area IV is limited to the site of the Former Sodium Burn Pit where contaminated soils have been removed; this report addresses the natural attenuation of perchlorate in case this becomes of interest. Perchlorate is highly soluble in water, but also very non-reactive which causes it to accumulate in groundwater. Perchlorate was not recognized as a significant water contaminant in the U.S. until 1996 (Coates & Achenbach, 2004).

The DOE remains responsible for addressing soil and groundwater contamination that resulted from the research activities in the parts of Area IV leased by DOE. Contamination on the site has become a large public issue for the nearby growing communities. Soil sampling and chemical analysis have determined the areas and extent of the contamination. The ranges of the contaminants found in the soil of Area IV are shown in **Table 1.1**. The locations of the areas with the highest contamination are shown in **Figures 1.1 and 1.2**. The soil treatability studies will be used to determine the best way to reduce the contamination in these areas.

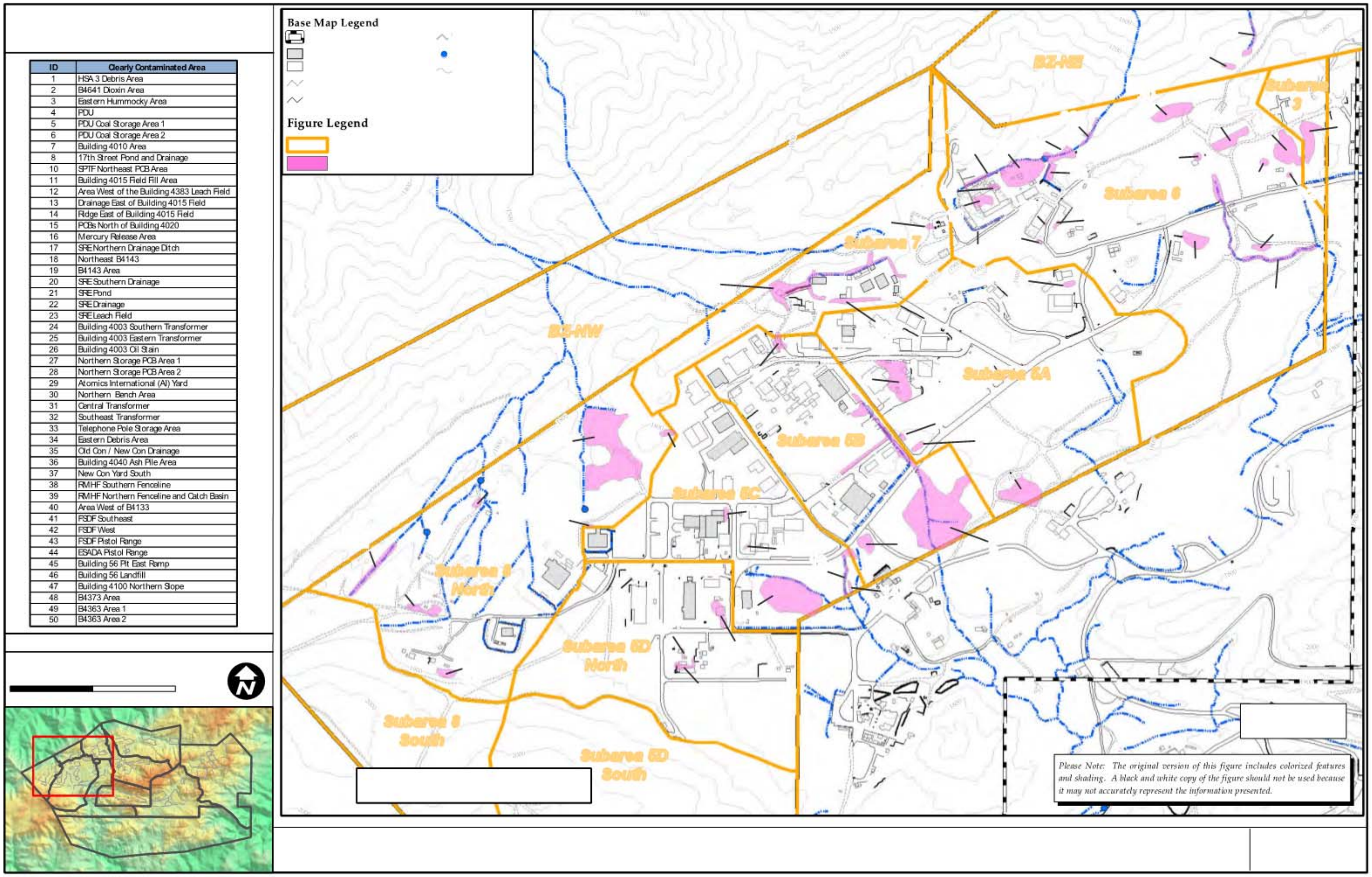
1.2. SSFL Soil Treatability Studies

In May 2011 the DOE contracted Sandia National Laboratories (Sandia) to initiate the treatability study process. Sandia evaluated the options for soil treatability and made recommendations for the best technology options applicable to Area IV. During this process DOE engaged the community through a local community working group called the Soil Treatability Investigation Group (STIG). STIG members attended the meetings led by Sandia during the process of Sandia's evaluation of treatability study options. STIG provided it's input to Sandia during these meetings.

Sandia recommended natural attenuation, bioremediation, phytoremediation, and soil partitioning be considered as potential technologies to use at Area IV. The DOE has commissioned five treatability studies to address these contaminants and determine the best way to restore the site to reasonable levels for health and safety. These studies will examine the most promising methods of reducing the volume of contaminated soil that will need to be trucked from SSFL and disposed of using traditional means. Collectively the studies will investigate the feasibility of natural attenuation, bioremediation, phytoremediation, and soil partitioning. The fifth study will examine the chemical state of the mercury on the site. This report will cover the Natural Attenuation Study, performed by California Polytechnic State University.

Table 1.1. High and Low Concentrations of the Major Contaminants found in Subarea 5B of Area IV (CDM Smith, 2012).

Contaminant	Class	Lowest Measured Concentration	Highest Measured Concentration	Units
2,3,7,8-TCDD	Dioxin	<1	5.27	ppt
Aluminum	Metal	2770	38500	ppm
Antimony	Metal	<1	11.8	ppm
Arsenic	Metal	1.51	70.5	ppm
Barium	Metal	22.5	399	ppm
Beryllium	Metal	<1	1.84	ppm
Boron	Metal	<1	16.4	ppm
Cadmium	Metal	<1	1.12	ppm
Chromium	Metal	5.33	62.1	ppm
Chromium (hex)	Metal	0.3	4.8	ppm
Cobalt	Metal	1.95	20.7	ppm
Copper	Metal	2.68	37	ppm
Lead	Metal	1.12	549	ppm
Lithium	Metal	2.8	67.2	ppm
Manganese	Metal	85.8	1490	ppm
Mercury	Metal	<1	23.6	ppm
Nickel	Metal	3.28	48.8	ppm
Selenium	Metal	<1	2.61	ppm
Silver	Metal	<1	63.8	ppm
Strontium	Metal	8.87	270	ppm
Vanadium	Metal	11.8	93.5	ppm
Zinc	Metal	11.8	929	ppm
1-Methylnaphthalene	PAH	<1	550	ppb
2-Methylnaphthalene	PAH	<1	700	ppb
Anthracene	PAH	<1	380	ppb
Benzo(a)anthracene	PAH	<1	1500	ppb
Benzo(a)pyrene	PAH	<1	17000	ppb
Benzo(b)fluoranthene	PAH	<1	20000	ppb
Benzo(g,h,i)perylene	PAH	<1	90000	ppb
Benzo(k)fluoranthene	PAH	<1	4900	ppb
Chrysene	PAH	<1	3700	ppb
Dibenzo(a,h)anthracene	PAH	<1	1600	ppb
Fluoranthene	PAH	<1	6800	ppb
Ideno(1,2,3-cd)pyrene	PAH	<1	49000	ppb
Naphthalene	PAH	<1	690	ppb
Phenanthrene	PAH	<1	2900	ppb
Pyrene	PAH	<1	9300	ppb
Aroclor 1248	PCB	<1	18000	ppb
Aroclor 1254	PCB	<1	2900	ppb
Aroclor 1260	PCB	<1	5300	ppb
TPH- C5-C12	TPH	<1	<1	ppm
TPH-C12-C20	TPH	<1	14	ppm
TPH-C21-C30	TPH	<1	420	ppm
TPH-C30-C40	TPH	<1	1100	ppm



ID	Clearly Contaminated Area
1	HSA 3 Debris Area
2	B4641 Dioxin Area
3	Eastern Hummocky Area
4	PDU
5	PDU Coal Storage Area 1
6	PDU Coal Storage Area 2
7	Building 4010 Area
8	17th Street Pond and Drainage
10	SPTF Northeast PCB Area
11	Building 4015 Field Fill Area
12	Area West of the Building 4383 Leach Field
13	Drainage East of Building 4015 Field
14	Ridge East of Building 4015 Field
15	PCBs North of Building 4020
16	Mercury Release Area
17	SRE Northern Drainage Ditch
18	Northeast B4143
19	B4143 Area
20	SRE Southern Drainage
21	SRE Pond
22	SRE Drainage
23	SRE Leach Field
24	Building 4003 Southern Transformer
25	Building 4003 Eastern Transformer
26	Building 4003 Oil Stain
27	Northern Storage PCB Area 1
28	Northern Storage PCB Area 2
29	Atomics International (AI) Yard
30	Northern Bench Area
31	Central Transformer
32	Southeast Transformer
33	Telephone Pole Storage Area
34	Eastern Debris Area
35	Old Con / New Con Drainage
36	Building 4040 Ash Pile Area
37	New Con Yard South
38	RMHF Southern Fenceline
39	RMHF Northern Fenceline and Catch Basin
40	Area West of B4133
41	FSDF Southeast
42	FSDF West
43	FSDF Pistol Range
44	ESADA Pistol Range
45	Building 56 Pit East Ramp
46	Building 56 Landfill
47	Building 4100 Northern Slope
48	B4373 Area
49	B4363 Area 1
50	B4363 Area 2

Please Note: The original version of this figure includes colorized features and shading. A black and white copy of the figure should not be used because it may not accurately represent the information presented.

Figure 1.1. Map of SSFL Area IV with the areas of highest contaminant concentrations in pink (CDM Smith, 2012).

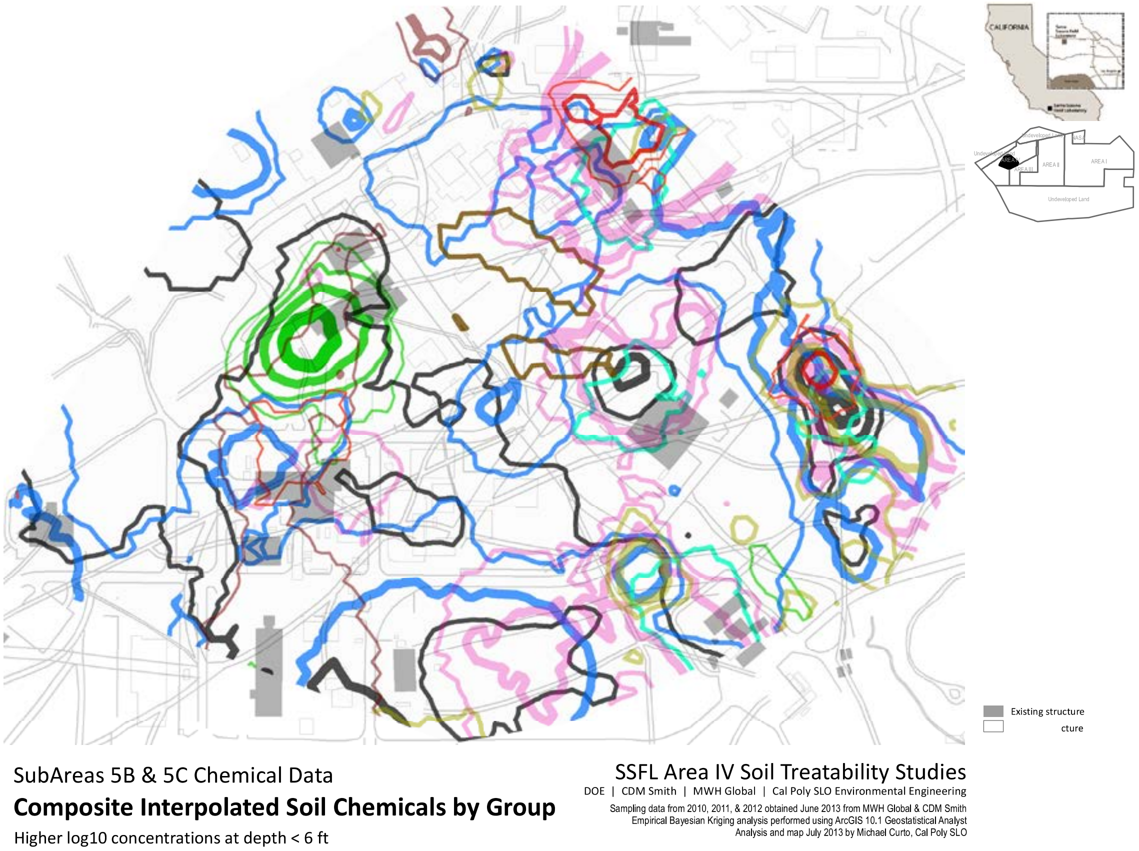


Figure 1.2. Contour Map of Area IV Subareas 5B and 5C showing the concentrations of the major contaminants (Cal Poly 2013).

1.3. Scope and Overview of Literature Review

The purpose of this natural attenuation study is to determine if the soil contaminants in Area IV of SSFL can be reduced to acceptable levels using natural attenuation. The first phase of the natural attenuation study used an in-depth literature review to identify the possible natural attenuation processes that may be occurring. Rates reported for the natural processes were tabulated and used to provide an estimate of the possible range of natural attenuation rates that could be expected at the SSFL site.

The Phase-1 literature review covers natural attenuation processes affecting:

- Petroleum hydrocarbons
- Polyaromatic hydrocarbons (PAHs)
- Polychlorinated biphenyls (PCBs)
- Polychlorinated dibenzo-*p*-dioxins (PCDD) and dibenzofurans (PCDF) (dioxins)
- Perchlorate
- Mercury and other metals

Each contaminant is considered separately - synergistic effects between the various contaminants are not considered in this analysis. Radionuclides are not considered in this study.

Each section of this report covers the chemical structures of the contaminant, production and sources, chemical species and concentrations found in Area IV, health concerns including toxicity mechanisms, and biodegradation mechanisms. Tables are provided with published biodegradation rates in both field and laboratory studies, as well as microbial species known to biodegrade the COI. Degradation mechanisms are presented for bacterial and fungal biodegradation, as well as abiotic weathering processes. Rates of natural attenuation reported under conditions as closely matching those at the SSFL site as possible were sought in terms of climate, contaminants, and soil type. Phytoremediation studies of each contaminant are also described. Finally, an analysis of biodegradation rates is made to provide an estimate of the time that would be required to reduce COI concentrations to background levels.

1.4. Roles and responsibilities

The natural attenuation study team consists of five entities:

California Polytechnic State University, San Luis Obispo is jointly responsible for preparing the study plan and study report with CDM, conducting the study with CDM, and presenting the study plan and study report to the STIG. The Cal Poly team consists of Yarrow Nelson (Principal Investigator) and graduate students Kenneth Croyle, Mackenzie Billings, and Matthew Poltorak. Undergraduate students Adam Caughy, Adam Donald and Nicole Johnson also contributed significantly to this study.

CDM provided overall project management and contracting, and was jointly responsible for preparing this study report with the university, performing the study with the university, and working with DTSC to gain regulatory acceptance of the study plan and study report.

DOE is a responsible party at the site and is providing funding for this study.

DTSC is the regulatory agency over Area IV of SSFL and retains ultimate approval authority of use of soil treatment technologies as a remedial measure.

The STIG participated in the progress of the study and will be updated on progress and results.

2.0 Study Approach

2.1. Phase 1 Literature Review Methods

The literature review addresses the following questions:

- Are the Area IV COIs investigated in this study amenable to natural attenuation based on published studies and data sets from other field sites?
- What geochemical, biological, or other weathering natural attenuation processes are possibly occurring at Area IV?
- What are the mechanisms of PCB and dioxin dechlorination, and how have these been determined in other studies?
- What are the intermediate and end products of biodegradation of the Area IV COIs, and what is the toxicity of these degradation intermediate and end products?
- What range of rates of natural attenuation could be expected at Area IV?
- How have natural attenuation rates for the Area IV COIs been calculated at other sites?
- How would natural attenuation at Area IV be effectively monitored?
- What is known about mercury valence state changes in soils similar to those found at Area IV?

The literature review was conducted according to the following standard operating procedure (SOP):

1. The databases and journals used to research natural attenuation of the Area IV COIs included those listed below (accessed through the Cal Poly Library System):
   a. Science Citation Index (Web of Knowledge/Web of Science)
   b. Science Direct
   c. BIOSIS
   d. Google Scholar
2. Journals with specific relevance were searched, including, but not limited to:
   a. Environmental Science and Technology
   b. Bioremediation Journal
   c. Biodegradation
   d. Applied Environmental Microbiology
   e. Chemosphere
   f. Biotechnology and Bioengineering

3. Papers and abstracts from conference proceedings were also used, such as:
    a. Battelle Conference on Chlorinated and Recalcitrant Compounds
    b. Symposium on Bioremediation and Sustainable Environmental Technologies
    c. American Chemical Society Division of Environmental Chemistry
4. Review articles (articles providing critical evaluation of previously published studies) were used to help identify the most important studies and provide a broad perspective and identify important prior publications.
5. All information cited was obtained from the original papers in which information was published (not as cited by subsequent publications).
6. All publications were indexed into a database using Mendeley
7. Colleagues and professionals with experience in the field of biodegradation of the Area IV COIs were contacted via email and telephone for guidance in:
    a. Identifying other current researchers in this area
    b. Identifying field sites with similar Area IV COIs
8. An attempt was made to identify field sites analogous to Area IV for which past research could be applied to estimating natural attenuation potential at this site. Such "analogous" sites ideally met the following criteria:
    a. Soils are of similar type to those found in Area IV (sandy loam)
    b. Concentrations of Area IV COIs are within the range of levels measured at Area IV
    c. Climate (temperatures, rainfall) is similar to Area IV
    d. Vegetation type and cover is similar to what is found at Area IV
9. Results of the literature review are presented in tables of published findings.
10. Interpretation is provided based on published data and what is currently known of site conditions at SSFL.

2.2. Phase 2: Use of Site-Specific Data for Estimating Natural Attenuation Rates

The second phase of this study (results not presented in this report) will make use of results of the concurrent treatability studies on bioremediation and phytoremediation of soils at the SSFL site to more specifically determine the feasibility and potential rates of natural attenuation that could be expected at the site. Questions to be addressed by the Phase-2 study making use of results of concurrent studies include:

- Are known bacterial and fungal degraders of the contaminants of concern present in the soils at SSFL?
- Are biodegradation intermediates present at the site or in the lab experiments?
- What biodegradation rates could be expected under existing conditions at the SSFL site without biostimulation or bioaugmentation?
- What phytoremediation rates could be expected at the SSFL site without changing the plant community?
- Can existing natural attenuation rates be enhanced and, if so, how?

3.0 Petroleum Hydrocarbons (not including PAHs)

3.1. Physical Properties and Toxicity of Petroleum Hydrocarbons

Petroleum hydrocarbons include mixtures of hydrocarbons found in crude oil and refined fuels. This includes polycyclic aromatic hydrocarbons (PAHs), but these are considered separately in the next section of this report. There are four main classes of the thousands of organic compounds found in oil: saturated hydrocarbons, aromatic hydrocarbons, asphaltenes (phenols, fatty acids, ketones, esters, and porphyrins), and resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) (Marshall & Rodgers, 2008). The COIs at SSFL include both aliphatic and aromatic hydrocarbons. Aromatics will primarily be addressed in the discussion of PAHs. Because the petroleum hydrocarbons at the site are extensively weathered, benzene, toluene, ethylbenzene, and xylene (BTEX) are likely to have evaporated and/or biodegraded and thus are not be discussed in this report.

Total petroleum hydrocarbon (TPH) is a term used for the collective quantification of petroleum hydrocarbons. TPH can be determined in ranges of equivalent carbon atoms per molecule. For example, TPH C12-C14 is a measurement of hydrocarbons with the equivalent of 12 to 14 carbons in terms of when they elute in a gas chromatogram. Hydrocarbons are hydrophobic, and the longer the carbon chain, the more hydrophobic the compound. Most of the constituents in petroleum hydrocarbon mixtures have relatively high vapor pressures and low solubilities in water (see **Table 3.1**).

Table 3.1. Physical properties of petroleum hydrocarbons for specific fractions (Leaking Underground Storage Tank Program, Division of Environmental Response and Remediation, 2012)**.**

Fraction	Molecular Weight (g/mol)	Aqueous Solubility at 20-25°C (pure compound) (mg/L)	Vapor pressure (mm Hg)	Henry's Law Constant (L H_2O/L air, unitless)	K_{oc} (mL/g)
ALIPHATICS					
C7 – C8	100	3.60 E+01	4.80 E+01	7.70 E+01	3.16 E+03
C9 – C10	130	5.40 E+00	5.00 E+00	1.60 E+02	3.16 E+04
C11 – C12	160	4.30 E-01	4.80 E-01	1.60 E+02	3.16 E+05
C13 – C16	200	3.40 E-02	3.60 E-02	1.60 E+02	5.00 E+06
C17 – C21	270	7.60 E-04	8.40 E-04	1.10 E+02	4.00 E+08
C22 – C35	280	2.50 E-06	8.40 E-04	1.10 E+02	4.00 E+08
AROMATICS					
C10 (Naphthalene)	128	3.10 E+01	2.76 E-01	1.74 E-02	8.44 E+02
C9 – C10 (alkyl benzenes)	120 – 176	1.10 E+02	5.00	4.20 E-01	1.26 E+03
C11 – C13 (total alkyl naphthalenes)	142 – 176	1.45 E+03	5.00 E-02	2.30 E-02	7.06 E+03
C12 – C22 (poly aromatic hydrocarbons)	152 – 278	4.86 E+01	2.70 E-03	4.12 E-01	6.29 E+04

Soil contamination by petroleum hydrocarbons is common due to human activity and accidents such as fuel and oil spills (Brooijmans, Pastink, & Siezen, 2009). TPH has been measured in Area IV of SSFL at concentrations up to 82,000 ppm, but most of the soils in the clearly contaminated areas have TPH concentrations between 100 and 1,000 ppm.

Petroleum compounds have a range of toxic effects, including developmental, hematological, hepatic, immunological, and renal disturbances ("ATSDR - Toxic Substances - Total Petroleum Hydrocarbons (TPH),"). Concern about petroleum hydrocarbon exposure is primarily related to BTEX and PAHs. PAH toxicity is discussed in Section 4 below. There are known effects and established minimal risk levels for acute, intermediate, and chronic exposure to hydrocarbons. Toxicity from hydrocarbon ingestion most often affects the lungs (Levine, 2013). Neurological, respiratory, reproductive, and renal effects are associated with exposure to aliphatic hydrocarbons with 5-8 carbons; those with 8-35 carbons are associated with hepatic, adaptive, and metabolic effects (U.S. Department of Health and Human Services, 1999). Exposure to aromatic hydrocarbons has similar health effects. BTEX exposure has been shown to affect the immunological/lymphoreticular, neurological, renal, hepatic, and developmental systems (U.S. Department of Health and Human Services, 1999). Exposure to aromatic hydrocarbons with 9-16 carbons (i.e. naphthalene, isopropylbenzene, acenaphthylene, etc.) may cause renal, endocrine, hepatic, and respiratory effects (U.S. Department of Health and Human Services, 1999). Exposure to aromatic hydrocarbons with 16-35 carbons may cause hepatic and renal effects (U.S. Department of Health and Human Services, 1999). According to the U.S. EPA Integrated Risk Information System (IRIS) database, there is inadequate information to assess the carcinogenic potential of aliphatic hydrocarbons such as hexane (U.S. Department of Health and Human Services, 1999). The concern about cancer from hydrocarbons comes overwhelmingly from aromatic hydrocarbons and chlorinated hydrocarbons (MACTEC Engineering and Consulting, 2010).

3.2. Petroleum Hydrocarbon Weathering

Petroleum compounds typically "weather" in the environment, meaning some components of the petroleum hydrocarbon mixture are either removed or transformed over time. Weathering processes include abiotic processes such as volatilization, chemical or photochemical oxidation, and adsorption into the pore structure of the soil, and biological processes such as biodegradation. Volatilization may decrease the amounts of smaller hydrocarbons that have a higher vapor pressure. This primarily affects gasoline- and kerosene-range hydrocarbons in the C10-C16 range (Nishiwaki et al., 2011). However, one study on diesel-contaminated soil showed that volatilization accounted for only 2% of initial TPH removal (Namkoong et al., 2002). Preferential biodegradation of the most biodegradable hydrocarbon substrates results in a change in composition, with weathered petroleum spills typically depleted of straight-chain alkanes due to biodegradation (Whittaker & Pollard, 1997).

In many cases, weathering processes can hinder bioremediation through sequestration of contaminants in the soil. Hydrocarbons are slowly absorbed into the organic phase of the soil, which can significantly reduce their bioavailability, resulting in lower biodegradation rates (Gallego et al., 2010).

3.3. Bacterial Biodegradation of Petroleum Hydrocarbons

Petroleum hydrocarbon biodegradation by naturally occurring microflora is very well documented (Atlas, 1981; Bento et al., 2005; Sarkar et al., 2005; and Gieg et al., 1999). Aerobic bacteria are reported to perform the vast majority of biodegradation, but yeast and fungi also biodegrade hydrocarbons (K. S. M. Rahman et al., 2003 and Brooijmans et al., 2009). Common genera of hydrocarbon-degrading bacteria include *Pseudomonas, Acinetobacter, Burkholderia, Mycobacterium, Haemophilus, Rhodococcus, Paenibacillus*, and *Ralstonia* and numerous other genera (Tyagi et al., 2011; Margesin et al., 2003; and Das & Chandran, 2011).

Petroleum hydrocarbons have a wide range of chemical properties and thus exhibit a wide range of biodegradation rates. The New Zealand Ministry for the Environment has cited benchmark biodegradation rates for different classes of hydrocarbons (**Table 3.2**), and these rates can serve as a general guide for biodegradability of the different fractions. More specific published rates are presented below in Section 3.5. Clearly, short-chain aliphatic hydrocarbons biodegrade faster than longer-chain hydrocarbons and aromatic compounds. This means that weathered petroleum contamination usually consists of longer chain and aromatic compounds, which are more difficult to biodegrade.

Table 3.2. Benchmark biodegradation rates adopted by the New Zealand Ministry for the Environment
(Ministry for the Environment, 1996).

Hydrocarbon Fraction	Biodegradation time
C7-C9	2 years
C10-C14	5 years
C15-C36	10 years
Benzene	5 years
Toluene	5 years
Ethyl benzene	5 years
Xylene	5 years
Naphthalene	5 years
Pyrene	10 years
Benzo(a)pyrene	10 years

The mechanism of aerobic hydrocarbon biodegradation is oxidation mediated by enzymes such as monooxygenase, dioxygenase and peroxidase, as well as cytochrome p450 systems (e.g. Wiedemeier et al, 1999). In aerobic biodegradation of petroleum hydrocarbons, monooxygenase enzymes typically attack alkanes, while dioxygenase enzymes attack aromatic compounds, both mechanisms using oxygen as an oxidizing agent (**Figure 3.1**) (Das & Chandran, 2011 and Wiedemeier et al., 1999). Oxidation of alkanes and many other compounds produces alcohols, aldehydes, epoxides and carboxylic acids. These compounds are then completely broken down and the carbon is either respired as carbon dioxide or incorporated into cell biomass (**Figure 3.2**).

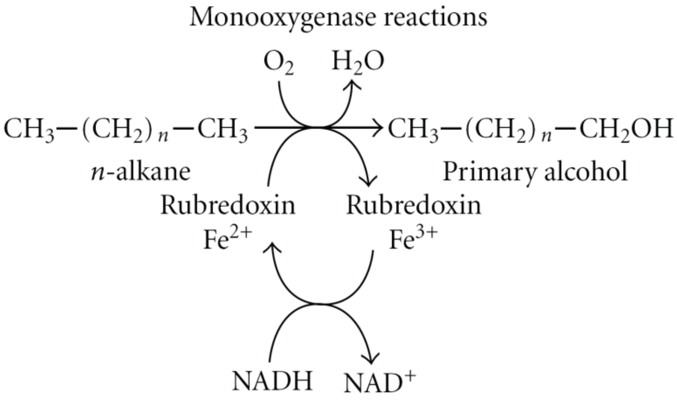


Figure 3.1. Reaction of monooxygenase on linear hydrocarbons (Das & Chandran, 2011)**.**

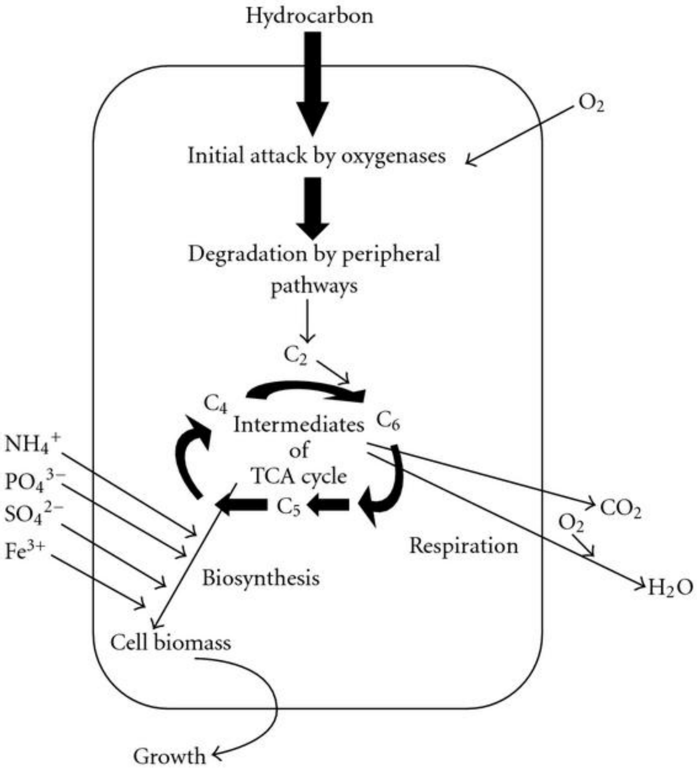


Figure 3.2. General pathway of aerobic degradation of small alkanes and other hydrocarbons (Das & Chandran, 2011)**.**

There are several organisms that express enzymes related to monooxygenases that have a very narrow substrate range (for example, methane monooxygenase metabolizes methane). For the most part, these enzymes are responsible for oxidizing C1 through C4 hydrocarbons (Van Beilen & Funhoff, 2005). Microbes with these enzymes are fairly specialized and will not likely play a large role in the natural attenuation of the larger hydrocarbons at SSFL. A mechanism more likely to occur in SSFL soils is carried out by particulate alkane hydrolases like those expressed in *P. putida* GPo1, which preferentially oxidize alkanes longer than C10.

Two classes of alkane-hydroxylating p450 systems have been identified. Class 1 p450s consist of a three-component system comprised of cytochrome p450, ferredoxin, and ferredoxin reductase subunits (Van Beilen & Funhoff, 2007). Class 2 p450s have a microsomal 2-component system comprised of a membrane-bound p450 and a reductase. These are found in various soil yeast strains and oxidize n-alkanes to yield fatty acids and carboxylic acids. The most active of the p450 enzymes is p450BM-3. The Alk B gene, which is required for p450 enzymatic activity, is present in *M. tuberculosis, Prauserella rugosa, Rhodococcus erythropolis, Burkholderia cepacia, Pseudomonas aeruginosa, Acinetobacter sp.* and *Alcanivorax borkumensis*, organisms that are prevalent in soil (Van Beilen & Funhoff, 2005). These enzyme systems have a wide range of substrates from C5 – C12, and others can oxidize C10 – C16 alkanes.

Anaerobic petroleum hydrocarbon biodegradation has been studied far less than aerobic biodegradation (Wiedemeier et al., 1999). In order for anaerobic degradation to occur, both alternative electron acceptors and microorganisms that are able to use them must be present (Ulrich & Suflita, 2001). Hydrocarbon constituents have been shown to biodegrade under Fe(III)-reducing, denitrifying, and sulfate-reducing conditions, and manganese oxides, soil humic acids, and fumarate have also been implicated in anaerobic hydrocarbon biodegradation (Van Hamme et al., 2003 and Townsend et al., 2003). Both facultative anaerobes (nitrate-, iron-, and manganese-reducing microorganisms) and strict anaerobes (e.g. sulfate-reducers) can biodegrade hydrocarbons anaerobically (Grishchenkov et al., 2000). However, compared to aerobic biodegradation, anaerobic biodegradation lends itself to fewer hydrocarbon substrates at much lower rates and to a lesser extent than aerobic biodegradation (Grishchenkov et al., 2000). A study by one of the authors of this report (Nelson) concluded that anaerobic degradation of petroleum compounds in groundwater at the former Guadalupe Oil Field was extremely slow compared to aerobic biodegradation (Chell et al., 2007).

3.4. Fungal Biodegradation of Petroleum Hydrocarbons

Fungi are also common degraders of hydrocarbons. White-rot fungi *(Phanerochaete sp.)* have been shown to effectively biodegrade a wide variety of hydrocarbon compounds (Pointing, 2001). Ligninolytic enzymes are thought to be primary contributors in fungal breakdown of petroleum hydrocarbons. Most of the research on fungi such as white-rot fungi has been done for more recalcitrant compounds than alkanes, such as PAHs and PCBs (Pointing, 2001).

In a study conducted by Yateem et al. (1998), the fungi species *Phanerochaete chrysosporium, Pleurotus ostreatus,* and *Coriolus versicolor* were tested for their ability to degrade petroleum hydrocarbons in soil microcosms. The results indicated that *Coriolus versicolor* was the most active degrader. After 12 months, 78.1% of TPH was biodegraded under nitrogen-rich conditions. *P. chrysosporium* removed 77.1% under nitrogen-limiting conditions.

3.5. Reported Rates of Natural Attenuation of Petroleum Hydrocarbons

Reported biodegradation rates of petroleum hydrocarbons span a wide range and tend to decrease in the following order: saturated aliphatic hydrocarbons > light aromatics > high-molecular-weight aromatics > polar compounds (Leahy & Colwell, 1990). These rates can be affected by multiple biological, physical, and chemical factors (**Table 3.3**). Based on a thorough literature review, un-amended first-order biodegradation rate constants range from approximately $3.8x10^{-4}$ to $3.3x10^{-2}$ day^{-1} in field studies and $8.1x10^{-4}$ to 0.27 in lab studies (**Table 3.4**). Since PHC biodegradation rates have been reported in over 100 publications, the rates reported in Table 3.4 are those reported only in the most cited papers.

Table 3.3. Factors affecting bioremediation rates (Boopathy, 2000)**.**

Microbial
• Growth until critical biomass is reached (until the minimum amount of biomass is reached, microbial populations cannot participate in some processes) • Mutation and horizontal gene transfer • Enzyme induction • Enrichment of the capable microbial populations • Production of toxic metabolites
Environmental
• Depletion of preferential substrates • Lack of nutrients • Inhibitory environmental conditions
Substrate
• Too low concentration of contaminants • Chemical structure of contaminants • Toxicity of contaminants • Solubility of contaminants
Biological aerobic vs anaerobic process
• Oxidation/reduction potential • Availability of electron acceptors • Microbial population present at the site
Growth substrate vs. cometabolism
• Type of contaminant • Concentration • Alternate carbon source present • Microbial interaction (competition, succession, and predation)
Physico-chemical bioavailability of pollutants
• Equilibriums sorption • Irreversible sorption • Incorporation into humic matters
Mass transfer limitations
• Oxygen diffusion and solubility • Diffusion of nutrients • Solubility in water

Table 3.4. Biodegradation rates of petroleum hydrocarbons reported in the literature (most cited field and laboratory studies).

Contaminant	Matrix	Lab/Field	Soil TPH Concentration (mg/kg)		Length of Study (days)	Biodegradation Metric		Reference
			Initial	Final		Percent Degraded	Rate of Degradation (mg/kg/day)	
Diesel	Soil	Lab	10,000	3,550	30	65%	215	Namkoong et al., 2002
Octane	Soil	Lab	700	691.6	15	1.2%	0.56	Moldes et al., 2011
Octane	Soil	Lab	700,000	532,000	15	24%	1,100	Moldes et al., 2011
PHC	Soil	Lab	2,815	1,439	60	48%	22.9	Llado et al., 2013
PHC	Soil	Lab	2,985	788	160	74%	13.7	Li et al., 2012
PHC	Soil	Lab	21,100	8,229	210	61%	61.3	Tang et al., 2012
PHC	Soil	Lab	11,533	7,496	270	35%	15	Couto et al., 2010
PHC	Soil	Lab	8,378	1,608	112	81%	60	Baek et al., 2007
PHC	Sludge	Lab	48,800	20,984	365	57%	76	Hutchinson et al., 2001
PHC	Soil	Lab	4,000	624	7	84.4%	482	Sarkar et al., 2005
PHC	Soil	Lab	99.2	82.5	120	16.8%	0.14	Mishra et al., 2001
PHC	Soil	Lab	11,975	5361	270	55%	25	Sabaté et al., 2004
TPH (C10-C32)	Soil	Field	2,440	952	168	61%	8.9	Kaplan and Kitts, 2004
TPH (C12-C23)	Soil	Lab	2,800	1,436.40	84	48.7%	16	Bento et al., 2005
TPH (C12-C23)	Soil	Lab	3,300	2,531	84	23.3%	9.2	Bento et al., 2005
TPH (C23-C40)	Soil	Lab	9,450	5,131	84	45.7%	51	Bento et al., 2005
TPH (C23-C40)	Soil	Lab	7,450	6,891	84	7.5%	6.65	Bento et al., 2005
PHC	Soil	Field	14,000	12,200	365	12.8%	4.9	Balba et al., 1998
PHC	Soil	Field	100,000	33,000	210	77%	320	Rhykerd et al., 1999
PHC	Soil	Field	9,500	3,750	630	60%	9.2	Nedunuri et al., 2000
PHC	Soil	Field	72,000	42,000	390	42%	77	Euliss et al., 2008
TPH (C10-C40)	Soil	Field	9,000	7,164	7	20.4%	260	Lai et al., 2009
TPH (C10-C40)	Soil	Field	3,000	2,838	7	5.4%	23	Lai et al., 2009
Crude oil	Soil	Field	9,500	8,265	28	13%	44	Schaefer and Juliane, 2007
Crude oil	Soil	Field	5,000	4,625	28	7.5%	13	Schaefer and Juliane, 2007
PHC	Soil	Lab	60,600	57,570	35	5%	87	Mancera-López et al., 2008
PHC	Soil	Field	7,000	7,448	730	6.4%	-0.61	Phillips et al. 2009
PHC	Soil	Field	99,300	22,900	180	77%	424	Rojas-Avelizapa et al., 2007

Rates observed in laboratory studies are often higher than rates observed in the field for a number of reasons. Compared to some lab studies which spike fresh contaminants into soil, contaminants in the field are more weathered, leaving the more recalcitrant compounds. Contaminants in the field may also be sequestered in the soil matrix and less bioavailable. Also, in the lab, better aeration is often provided compared to the field.

An important consideration is that petroleum hydrocarbons are comprised of thousands of different compounds, each with its own biodegradation kinetics. Biodegradation of some compounds is more complete than others, and some compounds are more recalcitrant than others (Leahy & Colwell, 1990). For this reason, biodegradation may follow first-order kinetics during initial biodegradation, followed by much slower biodegradation of the more recalcitrant, sequestered compounds. This hindered kinetics is sometimes referred to as "hockey stick kinetics" because of the modified shape of the concentration vs. time curve. Thus, hydrocarbon-contaminated sites are often left with some residual contamination, which is recalcitrant and has low bioavailability.

3.6. Biostimulation of Petroleum Hydrocarbon Biodegradation

The term biostimulation umbrellas several remedial technologies used to enhance biodegradation in the field by supplementing soils with growth substrates and/or co-substrates, and several of these technologies have been used to accelerate petroleum hydrocarbon degradation. Popular biostimulation agents include aeration, use of surfactants, nutrient supplementation and bulking with organic materials. **Table 3.5** below outlines the results of several studies assessing various biostimulation methods for improving biodegradation rates of petroleum hydrocarbons.

Table 3.5. Reported results of biostimulation and bioaugmentation studies for petroleum hydrocarbon biodegradation.

Additive	**Percent Degradation (%)**	**Study Duration**	**Reference**
Rhamnolipid biosurfactant + bioaugmentation	***92***	100 days	(Lin et al., 2011)
Bioaugmentation	86		
Biosurfactant	78		
Landfarming	68		
Combined bacterial consortium + poultry litter + coir pith + rhamnolipid biosurfactant (Pseudomonas sp. DS10-129)	***78***	90 days	(Rahman et al, 2002)
Bioaugmentation + biostimulation	***90.2***	120 days	(Mishra et al., 2001)
Surfactant	***76***	30 days	(Moldes et al., 2011)
Unamended	24	15 days	
Rice hulls at 10% w/w and nutrients	***91***	4 months	(Rastegarzadeh et al., 2006)
Unamended	24		
Bioaugmentation	***75.2***	6 weeks	(Bento et al, 2005)
Natural attenuation	48.7		
Nutrient addition	45.8		

Table 3.5. Reported results of biostimulation and bioaugmentation studies for petroleum hydrocarbon biodegradation.

Additive	Percent Degradation (%)	Study Duration	Reference
Nutrient addition + bioaugmentation + nonionic surfactant	***50***	9 months	(Couto et al., 2010)
Natural attenuation	30		
Manual aeration			
Nutrient addition			
Nutrient addition + nonionic surfactant			
Nutrient addition + bioaugmentation			
Bioagumentation	***90.2***	120 days	(Mishra et al., 2001)
Natural attenuation	16.8		
Biostimulation	***75***	10 weeks	(Abdulsalam et al., 2011)
Bioaugmentation	66		
Natural attenuation	50		

Aeration is one of the most important biostimulation methods for hydrocarbon biodegradation because aerobic biodegradation rates are so much greater than anaerobic rates. Numerous approaches have been used to deliver oxygen to the subsurface, including biosparging, ozonation, peroxide addition and the use of oxygen release compounds (Wiedemeier et al., 1999). For soils, addition of bulking agents may also work to increase soil aeration (Rastegarzadeh, Nelson, & Ririe, 2006).

Nutrient supplementation is very useful when conditions at a given site are deemed nutrient-limited. Nitrogen, phosphorus, and potassium are required to sustain microbial growth. To ensure that nutrient limitation is not a factor impeding biodegradation, nutrients are often added to soil during remediation activities. Fertilizers are applied in various forms, including water-soluble, slow-release, and oleophilic fertilizers.

Surfactants help to release compounds sequestered in soil and increase their bioavailability (Inakollu, Hung, & Shreve, 2004). Many studies indicate that surfactants accelerate degradation of the contaminants by increasing their bioavailability ((Chrzanowski et al., 2012; Fava & Di Gioia, 2001; Fava et al, 2004); (Gorna et al, 2011; Harkness et al., 1993a; Inakollu et al., 2004; (Lawniczak et al, 2013; Mukherjee & Das, 2010; (Mulligan et al, 2001; Tiehm et al 1997; Viisimaa et al 2013 and Whang et al, 2009). Surfactant molecules increase the bioavailability of hydrophobic and/or recalcitrant compounds that are embedded in the soil matrix to microorganisms by increasing their solubility in the aqueous phase (Lawniczak et al., 2013; Inakollu et al., 2004 and Whang et al., 2009). They may also change cell membrane properties and increase microbial adherence, increasing the likelihood of direct substrate uptake when two immiscible phases are present (Neu, 1996 and Franzetti et al., 2009). Both synthetic (petrochemical) and natural (oleochemical) surfactants sources have been used for biostimulation of hydrocarbon biodegradation.

Since hydrocarbon contaminants at SSFL are highly weathered, use of surfactants may be necessary to increase the bioavailability of these contaminants. Laboratory soil microcosm experiments should be conducted to assess surfactants' ability to improve biodegradation rates of SSFL petroleum hydrocarbons.

3.7. Bioaugmentation of Petroleum Hydrocarbon Biodegradation

Bioaugmentation involves the addition of microorganisms known to biodegrade contaminants at a site. Bioaugmentation has produced mixed results for petroleum hydrocarbon remediation. It improves biodegradation rates only if microorganisms capable of degrading the contaminants are not present at the site (Tyagi et al., 2011). If microorganisms capable of degrading the COIs are present at the site, they do not need to be added; in fact, the best degraders are often those adapted to site conditions (Couto et al., 2010). In many locations, petroleum hydrocarbons have been around for a century or more and therefore there are often many microorganisms adapted to metabolizing them.

One study showed that enrichment, isolation, and re-inoculation of indigenous organisms at an oil-contaminated sandy beach resulted in 88% removal of hydrocarbons, a significant improvement over the natural attenuation rate of only 15% at this site over the same time period (Rosenberg et al., 1992). A similar study found that re-injecting enriched indigenous organisms obtained from a site increased the number of contaminant-degrading organisms in the soil (Bento et al., 2005).

Many researchers have used a combination of biostimulation and bioaugmentation using nutrients, microbes, and bulking agents (Krumins et al., 2009; Lee et al., 2011; Lin et al., 2011; Lladó et al., 2013; Rahman & Gakpe, 2008; Rastegarzadeh et al., 2006; Richardson et al., 2012; and Yong-lei et al., 2011). Rahman et al (2003) reported that combining bioaugmentation with nutrient and biosurfactant amendments to the soil significantly improved biodegradation of n-alkanes in the 12-40 equivalent carbon range.

Bioaugmentation can also be an effective strategy when using metal-resistant bacteria in the presence of toxic co-contaminants (in this case heavy metals) that inhibit biodegradation rates. For example, one study showed increased respiration rates and a 75% reduction of total hydrocarbons when bioaugmentation with a metal-tolerant bacteria was compared to unaugmented controls (Alisi et al., 2009).

Bioaugmentation has not always produced successful results (Silva & Alvarez, 2010 and Mariano et al., 2009). Failure of bioaugmentation to increase biodegradation rates can be due to several causes (Boon et al., 2010). The inoculated microbes may be subjected to predation by other organisms on site, reducing its viable population. In addition, added organisms often will not remove a contaminant over other preferable substrates (Mackay et al., 1992). Furthermore, chemicals that inhibit microorganism growth may also be present on-site. Finally, added organisms may not be able to physically contact a contaminant if the contaminant is sequestered in the soil matrix. This is why researchers have often used a combination of bioaugmentation and surfactant addition.

In some cases, bioaugmentation has actually been shown to interfere with diesel biodegradation (e.g. Demque et al., 1997). Another study showed that biostimulation (nutrients) and combined biostimulation (nutrients) and bioaugmentation (with native microorganisms) accelerated biodegradation rates in sand, but there was no significant difference between the two (Venosa et al., 1996). Similarly, research by one of the authors of this report suggested that bioaugmentation was ineffective for improving biodegradation of hydrocarbons in groundwater at the Guadalupe Oil Field (Waudby & Nelson, 2004). This site had a long history of petroleum contamination, and this exposure likely resulted in a well-adapted microbial community better able to biodegrade the local contaminants than the microbial consortium used for bioaugmentation.

At the SSFL site, the hydrocarbon contaminants are highly weathered, and it is likely that hydrocarbon-degrading indigenous microorganisms are already present at the site because of the long-term presence of contamination. In a companion study, research has been proposed to identify hydrocarbon-degrading microorganisms at the SSFL site using a combination of classical culturing techniques and DNA-based genetic analysis of soil samples. This site characterization will be useful to determine if bioaugmentation is likely to improve biodegradation rates. Soil microcosm experiments are also proposed to test the efficacy of bioaugmentation with white-rot fungi – an organism shown to biodegrade many of the contaminants at SSFL.

3.8. Phytoremediation of petroleum hydrocarbons

Phytoremediation is another potential means of improving biodegradation rates of petroleum hydrocarbons. A phytoremediation strategy called 'rhizoremediation' is particularly suited for remediating petroleum hydrocarbons (PHCs) in the soil (Gerhardt et al., 2009). Rhizoremediation is the enhancement of biodegradation by stimulating bacteria in the soil-root zone, known as the rhizosphere. This root-zone environment often contains many microorganisms that are capable of degrading petroleum hydrocarbons (Vangronsveld et al., 2009). Plant root exudates can enhance the degradation of pollutants by stimulating the survival and action of these microbes present in the rhizosphere (Kuiper et al., 2004; Salt et al., 1998). Plants can also supply oxygen to the rhizosphere to stimulate fungal and bacterial aerobic reactions (Schnoor et al., 1995). Grasses are known to stimulate the rhizosphere and much of the phytoremediation research for removal of organic compounds such as PHCs focuses on *Poaceae* species which are grasses (Hall et al., 2011).

Phytodegradation, in which contaminants are biodegraded in the plant itself, can also be used to remediate hydrocarbons. However, for this mechanism the compounds must first be phytoextracted by the plant (Vangronsveld et al., 2009). In general, moderately hydrophobic contaminants are more readily taken up into plants because compounds with a log $K_{ow} < 0.5$ cannot readily pass through plant membranes, and organics with a log $K_{ow} > 3.0$ often get stuck in membranes and cell walls on the outside of the plant and do not enter the fluids of the cell (Pilon-Smits, 2005; Salt, 1998; Schnoor et al., 1995; Vangronsveld et al., 2009). This can limit the effectiveness of phytoremediation of some hydrocarbons because hydrocarbon compounds have K_{ow} values that range from 0.37 to 6.57 (Heath et al., 1993).

Based on published data, phytoremediation of PHCs could be effective. One study showed over 50% degradation of approximately 7000 ppm of TPH in one year (Phillips et al., 2009b). Another study showed over 63% degradation of 5000 ppm of TPH in only 127 days (Peng et al., 2009). Slower rates have been reported by Banks et al. (2003), with 50% reduction of 3000 ppm of TPH observed in 870 days. Based on these studies, the time to remediate 5000 ppm of TPH down to the SSFL background level of 5.7 ppm could take between 1.3 and 23 years (assuming first order kinetics).

3.9. Potential for Natural Attenuation of Petroleum Hydrocarbons at the SSFL Site

A wide range of hydrocarbon biodegradation rates has been reported in the literature, making it difficult to precisely predict hydrocarbon remediation times required for soils at SSFL via natural attenuation. As described above (Table 3.4), first-order rate constants calculated from published data range from approximately 3.8×10^{-4} to 3.3×10^{-2} day^{-1} in field studies. The median first-order rate constant was 5.0×10^{-3}. To make a conservative calculation, only field rates were used to estimate remediation times for SSFL soils, not the higher rates reported for lab studies. Also, only rate constants from highly cited papers were used. Using these rate constants and the range of TPH concentrations measured in SSFL soils, the time required to reach background levels was calculated. A background level of 5.7 mg/kg is currently the clean-up goal specified in the DTSC "look-up tables," and this value was used for the current estimation. This calculation was performed for a range of TPH concentrations, from 860 to 82,000 mg/kg for illustration purposes. A sample calculation is shown in **Table 3.6**.

The first-order calculations based on published biodegradation rates suggest that the target TPH concentration of 5.7 mg/kg could be reached in anywhere from less than a year to almost 70 years through natural attenuation, assuming weathering does not impede contaminant degradation (**Table 3.7**). Clearly the wide range of TPH biodegradation rates reported for different sites results in a large uncertainty of the prediction of natural attenuation rates at SSFL. It would be helpful to run microcosm experiments under conditions mimicking those at SSFL to get a better idea of potential biodegradation rates at SSFL.

An important assumption in the above calculations was that the same first-order rate constant would be valid throughout the remediation period. As stated above, there are a couple of reasons this may not be a valid assumption: 1) The more easily biodegraded fractions of the hydrocarbon mixture will biodegrade first, leaving the more recalcitrant compounds towards the end, and 2) some fraction of the hydrocarbons will likely remain sequestered in the soil matrix and unavailable for biodegradation. For these reasons, longer remediation times than those calculated in Table 3.7 may be required at SSFL.

Site testing and analyses should be done at SSFL to better ascertain the feasibility of bioremediation of petroleum hydrocarbons at SSFL. Some useful tests include nutrient availability, oxygen levels, soil moisture and soil temperature. These characteristics all play a role in controlling site-specific microbial activity.

Although preliminary calculations indicate that natural attenuation may take decades to biodegrade TPH present at SSFL, biostimulation and/or bioaugmentation may help accelerate contaminant degradation. Popular methods of biostimulation/augmentation, such as fertilizer, bulking agents, surfactants, and microbe addition described above, show promise for petroleum hydrocarbons. Microcosm experiments are being conducted in a companion study to ascertain the effectiveness of these bioremediation strategies for soils at SSFL.

Table 3.6. Sample calculation of time required to reach background TPH concentration.

Assumptions:

1. First-order rate of decay is assumed for all cited studies. Based on initial and final concentrations, a first-order rate constant was derived in order to calculate a time to reach background TPH concentration of 5.7 mg/kg.
2. No rate-limiting factors inhibit petroleum hydrocarbon degradation (e.g. sufficient nutrients are available, including oxygen, and weathering does not impede contaminant degradation).

Calculations:

For first-order biodegradation, concentration (C) decreases exponentially:

$$C = C_0 e^{-kt}$$

where:

C_0 = initial concentration at t = 0 (sample: 12,000 mg/kg)
k = first-order decay rate constant (sample: k = 7.06 x 10^{-3} day^{-1})
C_f = background TPH level as specified by DTSC = 5.7 mg/kg
t = time required to reach C_f, days

Taking the natural logarithm and rearranging, solve for t:

$$t = \frac{ln\left(\frac{C}{C_0}\right)}{-k} = \frac{\ln\left(\frac{5.7}{12{,}000}\right)}{-7.06\ x\ 10^{-3}\ day^{-1}} * \frac{year}{365\ days} = 2.97\ years$$

Table 3.7. Estimated time required to reach TPH background level (5.7 mg/kg) assuming first-order kinetics using minimum and maximum reported rate constants.

Initial TPH Concentration (mg/kg)	Time to reach background (years)		
	Minimum[1]	Maximum[2]	Median[3]
860	0.42	36	2.8
1650	0.47	41	3.1
6450	0.58	51	3.9
16600	0.66	58	4.4
23000	0.69	60	4.6
82000	0.79	69	5.3

[1] based on maximum reported rate constant
[2] based on minimum reported rate constant
[3] based on median reported/calculated first-order rate constant

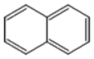
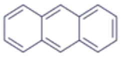
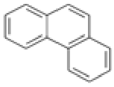
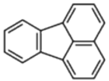
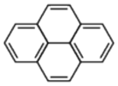
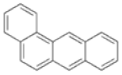
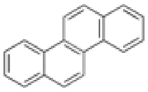
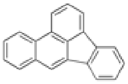
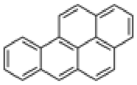
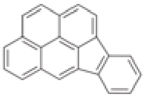
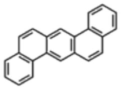
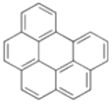
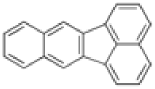
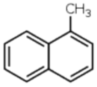
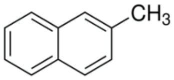
4.0 Polyaromatic Hydrocarbons (PAHs)

4.1. Physical properties and toxicity of PAHs

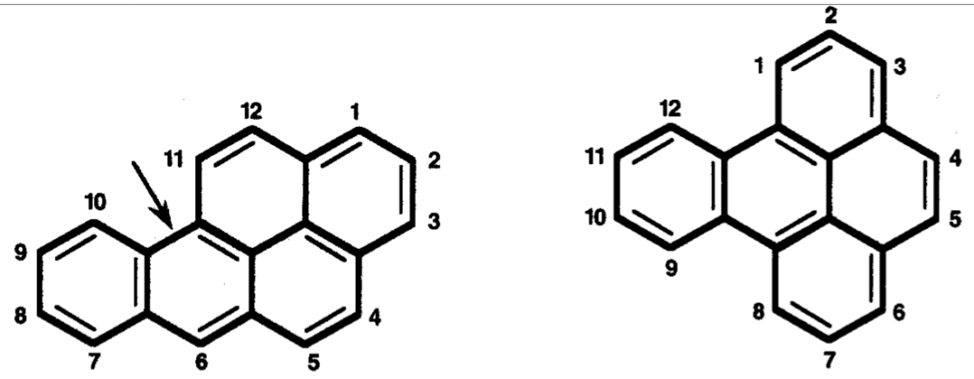
Polyaromatic hydrocarbons, also known as polynuclear aromatic hydrocarbons, are hydrocarbons with multiple aromatic rings (usually between 2 and 10) which do not contain heteroatoms or substituents. They have low solubilities in water, which decrease as molecular weight increases (see **Table 4.1**). They typically have high melting and boiling points and low vapor pressures. Melting and boiling points increase at higher molecular weights, while vapor pressure decreases (Haritash & Kaushik, 2009). PAHs are common airborne pollutants produced from burning fuel. They also occur naturally in oil, coal, and tar deposits. Their close link to fossil fuel processing and combustion makes them one of the most common organic pollutants (Lindsey et al., 1989). Many PAHs are known carcinogens, teratogens, and/or mutagens, and are therefore important to monitor (Srivastava et al., 2010). Since they are largely insoluble in water, air pollution is the primary concern for this group of hydrocarbons. Larger PAHs are less volatile, and are primarily found in sediment and oil-contaminated soil. Soil particles with bound PAHs are also a concern for particulates in the air. Information about molecular weight, formula, structure, solubility, vapor pressure, log K_{ow}, carcinogenicity, and number of rings of each PAH found in Area IV is shown in Table 4.1.

PAHs are formed naturally during the formation of coal and oil by reactions that convert biological molecules such as steroids to PAHs. This also occurs during the heating or incomplete burning (500–800°C) of fossil fuels, tar, and cooking oils (Haritash & Kaushik, 2009). For example, studies have found that consumption of repeatedly heated coconut oil can cause genotoxic changes in the liver (Srivastava et al., 2010). PAHs are also produced from the burning of wood, diesel, fat, tobacco, and incense. The relative amounts of each PAH species in air emissions varies based on the type of combustion. For example, gasoline combusted in a car engine will make a different mixture of PAHs than the burning of trees in a forest fire (Vergnoux et al., 2011). These differences can be used to determine the source of certain pollutants. PAHs have also been linked to oil spills, particularly in the recent Deepwater Horizon spill (Ortmann et al., 2012). Studies done on the ecosystem after this spill show possible bioaccumulation in the marine life of the Gulf of Mexico (Ortmann et al., 2012).

Table 4.1. Physical properties of PAHs found in Area IV ("ChemSpider," 2013; N. K. Nagpal, 1993). NC= non-carcinogenic; WC=weakly carcinogenic; C=carcinogenic; SC=strongly carcinogenic; U=Not yet determined; Kow=Octanol/water partition coefficient; Koc= partitioning coefficient for organic carbon

PAH	***Mol. Wt.(g /mol)***	***Molecular Formula***	***Structure***	***Solubility at 25 °C (µg/L)***	***Vap. Pressure at 25 °C (mm Hg)***	***Log Kow***	***Carcinogenicity***	***Benzene (and total) rings***
Naphthalene	128.2	$C_{10}H_8$		3.10E+04	8.50E-02	3.3	NC	2
Anthracene	178.2	$C_{14}H_{10}$		1.29E+03	6.60E-06	4.5	NC	3
Phenanthrene	178.2	$C_{14}H_{11}$		1.15E+03	1.20E-04	4.46	NC	3
Fluoranthene	202.3	$C_{16}H_{10}$		2.30E+02	9.20E-06	5.16	NC	3 (4)
Pyrene	202.1	$C_{16}H_{10}$		1.33E+02	4.50E-06	4.88	NC	4
Benzo[a]anthracene	228.3	$C_{18}H_{12}$		1.10E+01	5.00E-09	5.79	C	4
Chrysene	228.3	$C_{18}H_{12}$		1.90E+00	6.20E-09	5.63 (5.30)	WC	4
Benzo[b]fluoranthene	252.3	$C_{20}H_{12}$		1.50E+00	5.00E-07	6.6	C	4 (5)
Benzo[a]pyrene	252.3	$C_{20}H_{12}$		1.60E+00	5.50E-09	6.13	SC	5
Indeno(1,2,3-cd)pyrene	276.3	$C_{22}H_{12}$		6.20E+01	6.60E-06	6.58 (6.20)	C	5(6)
Dibenzo[a,h]anthracene	278.3	$C_{22}H_{14}$		4.00E-01	1.30E-10	6.7	C	5
Benzo[ghi]perylene	276.4	$C_{22}H_{12}$		3.00E-01	1.00E-10	6.63	NC	6
Benzo(k)flouroanthene	252.3	$C_{20}H_{12}$		8.00E-01	7.90E-10	6.84	C	4 (5)
1-Methylnaphthalene	142.2	$C_{11}H_{10}$		2.58E+04	6.70E-02	3.87	U	2
2-Methylnaphthalene	142.2	$C_{11}H_{10}$		2.46E+04	5.50E-02	3.86	U	2

Thirteen of the 15 PAHs found in Area IV are on the EPA's list of 127 Priority Pollutants (EPA, 2013). The toxicity of different PAHs is largely dependent on their chemical structure. Two PAHs with the same number of carbons and rings may exhibit different toxicities. Often PAH toxicity is caused by its reactive metabolites. The main concern of PAHs is their carcinogenic properties. The carcinogenic properties of PAHs are believed to be due to the binding of reactive PAH metabolites directly to DNA (ATSDR, 2009). An example of a reactive metabolite derived from a PAH is a diol epoxide. These compounds are known mutagens that disrupt cell replication and bind to DNA, forming adducts (ATSDR, 2009). Additionally, the location of the epoxide group formed on the PAH may be key to the toxicity of the reactive metabolite. The "bay theory" predicts that PAH intermediates and metabolites will have higher mutagenic and reactive properties if the epoxide is in a "bay region" (see **Figure 4.1**). A "bay region" is an area on the PAH that resembles a cis-butene, with the ends facing away from the main body of the molecule (Jerina et al., 1976 and Weis et al., 1998).



Benzo[*a*]pyrene

Benzo[*e*]pyrene

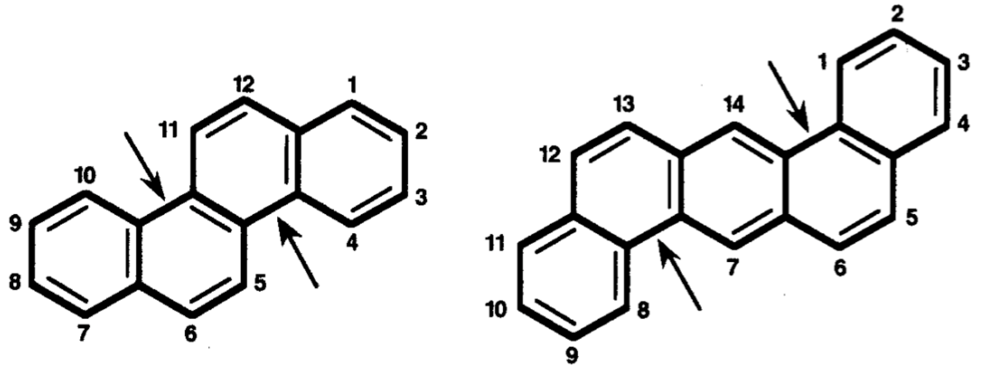


Figure 4.1. Bay regions (see arrows) on some common PAHs (ATSDR, 2009).

Six possible fates of PAHs in the environment were reported by Wild & Jones (1995):

- volatilization
- adsorption on soil particles
- photo-oxidation
- chemical oxidation
- leaching
- microbial degradation

These abiotic and biotic processes are discussed in the sections below, and an assessment is made of their potential effect on the natural attenuation of PAHs at the SSFL site.

4.2. Abiotic Weathering Processes Affecting PAHs in Soil

Volatilization: Volatilization of PAHs from soil is likely only for PAHs with higher vapor pressures, such as naphthalene and methyl-naphthalenes (see Table 4.1). Experiments by Park, et al., (1990) showed that naphthalene and 1-methyl naphthalene accounted for 30 and 20% of the reductions, respectively. Volatilization of all other PAHs in the study was negligible because of their low vapor pressure (Park et al., 1990).

The wildfire at SSFL in 2005 may have aided in the volatilization of some PAHs in the top few inches of the soil. This reduces the potential for future volatilization of PAHs from the soil at the site.

Adsorption to soil matrix: Weathering of PAHs for long periods of time (5-10 years or more) causes PAHs to become absorbed into the organic phase of soil. This reduces the total toxicity of the contaminants in the soil, which relieves stress on the microbial community. At the same time it greatly reduces the bioavailability of these compounds, reducing the potential for biodegradation (Alexander, 1995).

Photo-oxidation: Photo-oxidation of PAHs can be significant in aquatic environments, but is not thought to be significant in terrestrial environments (Vilanova et al., 2001). Photo-oxidation requires direct exposure to sunlight, which is likely only in the top few millimeters of soil, and thus it is unlikely to be a significant mechanism for SSFL soils.

Chemical oxidation: Abiotic chemical oxidation can degrade PAHs significantly, depending on the size of the PAH molecule. For PAHs with 3 or fewer rings chemical oxidation can account for 2-20% of the total reduction. However, for PAHs that have more than 3 rings chemical oxidation is not a significant reduction mechanism (Park et al., 1990). Chemical oxidation can be stimulated by adding oxidants such as ozone and hydrogen peroxide, either *in situ* or *ex situ* (Lundstedt, 2003).

Leaching: Leaching of PAHs from soil into groundwater or surface water is limited by the low solubility of PAHs, particularly for higher molecular weight PAHs. In one study naphthalene and phenanthrene were reported to dissolve into water and be leached out of soil, while larger, more hydrophobic PAHs became bound to colloids, which could also be leached through the soil (Bergendahl, 2005). This study found first-order desorption constants of 7.75×10^{-3} and 5.87×10^{-5} hr-1 for naphthalene and phenanthrene, respectively. Thus, the smaller PAH (naphthalene) was leached about 100 times faster than the larger phenanthrene. Another study quantified leaching of PAHs from sewage-farm soils with soil PAH concentrations between 1100 and 1400 ng/g, and found leachate PAH concentrations of only 2.23 and 0.90 ng/g (Reemtsma & Mehrtens, 1997). Only 0.2 and 0.06 % of the original PAH in the soil was leached out. Additionally, 80-90% of the PAHs in the leachate had 3 rings or less, while only constituting 18-25% of the PAH content in the soil. This further shows that smaller PAHs are preferentially leached from soils.

Biological weathering: Biodegradation can reduce the concentrations of PAHs in soil over time (see Sections 4.3 and 4.4 below). During this biological weathering most of the lower molecular-weight PAHs are biodegraded early, leaving higher molecular weight fractions in the soil. This process has undoubtedly occurred at the SSFL, and needs to be accounted for in the plan for remediation of the site.

4.3. Bacterial Biodegradation of PAHs

PAHs biodegrade slowly under natural conditions, with the larger PAHs (large number of rings) degrading particularly slowly (Haritash & Kaushik, 2009). Along with the chemical structure of the PAH, environmental conditions such as pH, temperature, and oxygen availability have a large impact on biodegradation rates. These conditions are often interrelated and their effects are difficult to predict (Haritash & Kaushik, 2009). In biological degradation, the bacterial and/or fungal species present and their population size is key. Other factors include microbial acclimation, nutrient accessibility, cellular transport, and chemical partitioning (Haritash & Kaushik, 2009). Numerous PAH-degrading bacterial and fungal species have been isolated from PAH-contaminated soil (Jacques et al., 2009). Mechanisms of bacterial PAH biodegradation are described in this section, while fungal biodegradation is described in Section 4.4.

The rate of PAH degradation by bacteria can be increased by several methods. One way is to increase the bioavailability of the PAHs using light oil or biosurfactant-producing bacteria. Another way is to add nutrients or compost to the soil. These methods of biostimulation are described below in Section 4.5.

Pathways of bacterial biodegradation of lower molecular weight PAHs (2-3 rings) have been studied extensively (Haritash & Kaushik, 2009), but biodegradation pathways of higher molecular weight PAHs (4 or more rings), are not as well supported by research (Haritash & Kaushik, 2009). There are far fewer organisms known that can use these larger molecules as carbon or energy sources.

The generalized pathways of bacterial and fungal PAH biodegradation are shown in **Figure 4.2**. In these catabolic pathways oxygen must be present to initiate a reaction with the PAH ring (Gibson et al., 1968). The hydroxylation of benzoid aromatics (aromatics containing benzene rings, which are especially stable) involves the integration of molecular oxygen (Gibson, 1984). Bacteria use dioxygenase enzymes to incorporate both oxygen atoms of molecular oxygen to form cis-dihydrodiols (Gibson et al., 1990). These compounds are selectively dehydrogenated by cis-dihydrodiol dehydrogenases (Patel & Gibson, 1974). This process rearomatizes the benzene nucleus to form dihydroxylated intermediates. This is followed by either an ortho or a meta fission by dioxygenases, with respect to the connected aromatic ring. This step is largely dependent on which dioxygenase is produced by the bacteria. For this reaction to occur, the benzene ring must have two hydroxyl groups ortho or para to each other. If this requirement is met, the benzene ring can be cleaved either between (intradiol fission) or adjacent to (extradiol fission) the hydroxyl groups (see **Figure 4.3**) (Cerniglia, 1992). The enzymes that perform this step are highly region- and stero-selective (Gibson, 1984).

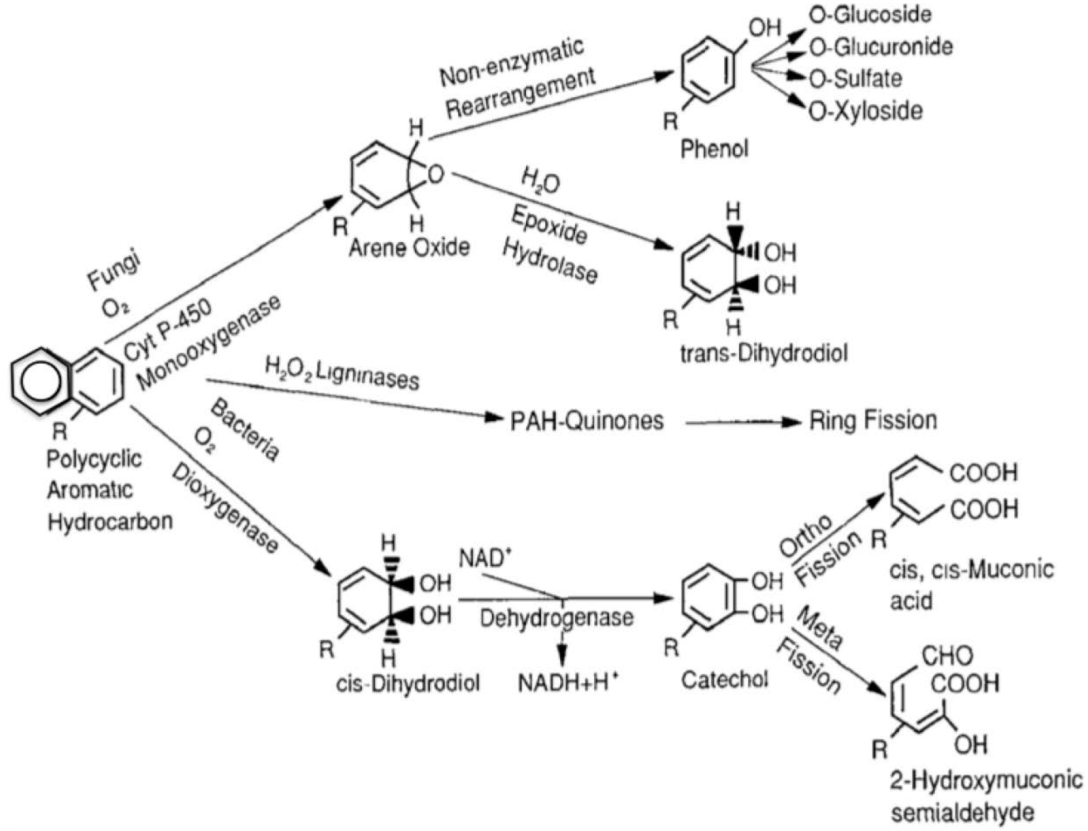


Figure 4.2. A summary of microbial and fungal catabolism of polycyclic aromatic hydrocarbons (Gibson et al., 1990)**. Many of the end products shown here are easily biodegraded further to CO_2.**

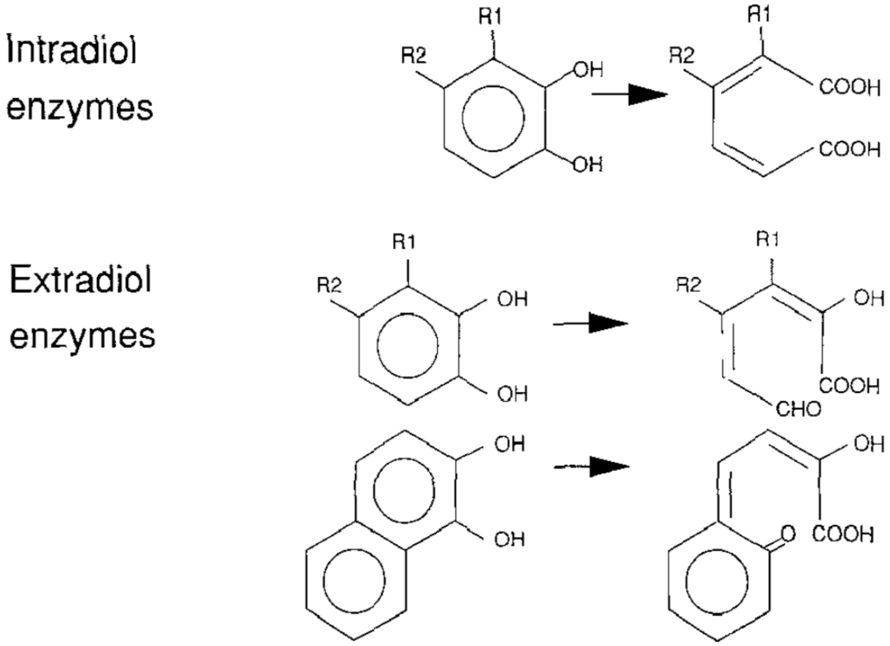


Figure 4.3. Ring cleavage by extradiol (meta fission) and intradiol (ortho fission) enzymes (Cerniglia, 1992)**.**

These processes can be repeated to breakdown large PAHs into smaller and smaller ones, yielding smaller, less-toxic molecules that are easier to break down by the normal soil microflora. However, the more rings in a PAH the less likely this process is to be initiated. Four and five ring PAHs remain particularly resistant to biodegradation, due to their high resonance energy and low solubility in water (Cerniglia, 1992). It should also be noted that there has been relatively little research done on complex mixtures of PAHs, and most studies focus on single PAHs in order to minimize variables. However, PAHs often exist in complex mixtures in the environment, and this is certainly true in Area IV at SSFL.

4.4. Fungal Biodegradation of PAHs

A number of fungi species, both lignolytic and non-lignolytic, have been identified as being capable of PAH biodegradation (see Appendix A for a list of fungi species and reported rates of degradation). Some fungi that produce lignolytic enzymes have been shown to degrade PAHs. These enzymes include lignin peroxidase, laccase, and manganese peroxidase. They function by oxidizing carbon polymers common in natural lignins, and these same enzymes can oxidize PAHs. Other enzymes involved include oxygenase and dehydrogenase, which are common catabolic enzymes. Fungi secrete these enzymes, and others, and digest molecules outside their cells and then absorb the products of the enzymatic reactions for nutrients. These reactions oxidize molecules to destabilize bonds, catalyzing radical formation. These enzymes not only degrade lignin but also catalyze one-electron oxidations of PAHs to quinones (see **Figure 4.4**). Lignin peroxidases are known to oxidize PAHs that have less than a 7.6 eV ionization energy (Haemmerli et al., 1986). Those PAHs would include pyrene, anthracene, coronene and others (Kuroda, 1964).

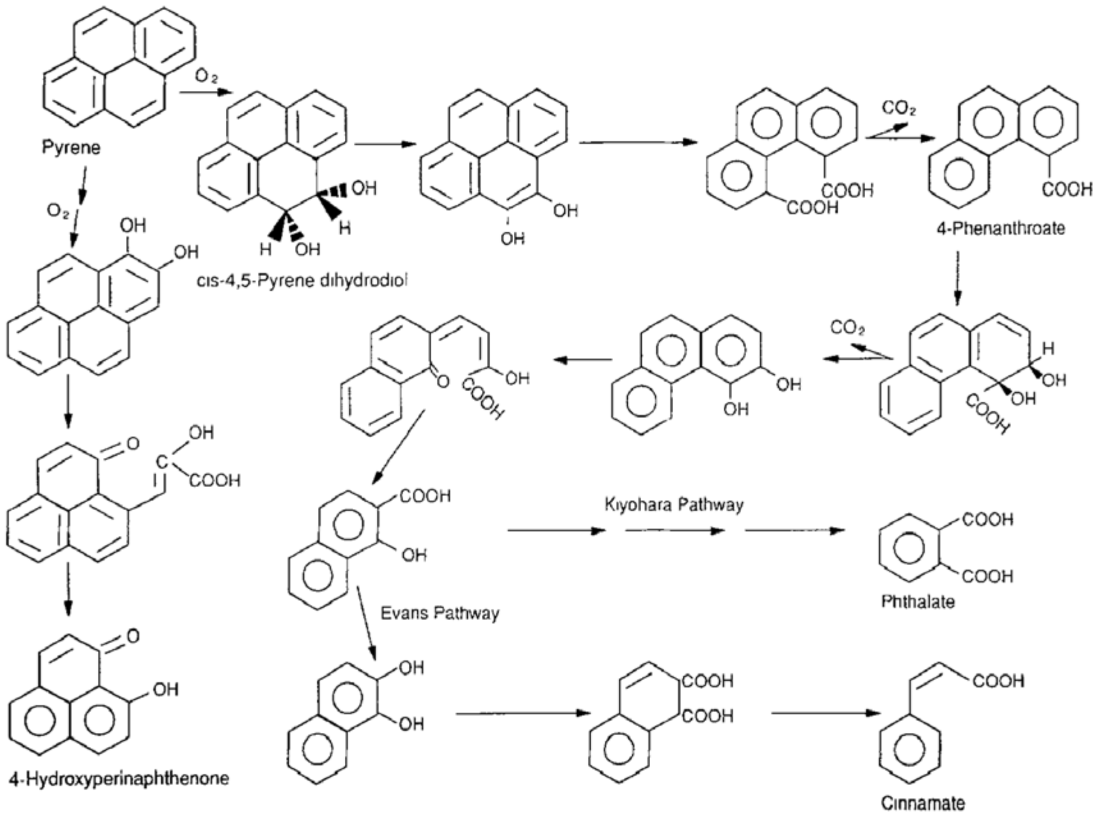


Figure 4.4. Proposed pathway for the metabolism of pyrene by *Mycobacterium* sp. PYR-1 (Cerniglia, 1992)**.**

The best studied non-lignolytic fungus, *Cunninghamella elegans,* uses cytochrome P-450 moonoxygenase to break down aromatic rings. The enzyme integrates 1 of the 2 oxygen atoms from molecular oxygen into the aromatic nucleus of a benzene ring (see Figures 4.2 and 4.4 for pathways). The remaining oxygen atom is used to form water (Laskin, 1984). The resulting structure is an arene oxide intermediate, which can undergo one of two changes (Figure 4.2). The first is further degradation by an epoxide hydrolase, forming a trans-dihydrodiol. The other option is a rearrangement to phenol, which is not catalyzed by an enzyme. Phenol can be conjugated by sulfate, glucose, or glucuronic acid. These processes have been shown to work on a number of PAHs and the methyl, nitro, and fluoro-substituted derivatives and produce trans-dihydrodiols, phenols, quinones, tetralones and conjugates of these primary metabolites. For a list of metabolites produced from PAHs by fungi, see (Cerniglia, 1992).

4.5. Natural Attenuation Rates of PAHs

The kinetics of the biodegradation of mixtures of PAHs in soil is very complex because multiple microorganisms using multiple pathways are operating on multiple PAH substrates. Among other things, these microbial processes are competing for nutrients and electron acceptors. Guha et al. (1999) suggested a multi-substrate Monod model to describe the kinetics of PAH biodegradation based on the biomass of degrading bacteria present in the soil (Guha, Peters, & Jaffé, 1999). This model is based on the assumption that all substrates are being degraded simultaneously by the same system of enzymes, but this model has not been tested on highly complex mixtures of PAHs (Guha et al., 1999). Some studies have shown that mixtures of PAHs can cause substrate interactions which are difficult to account for in rate estimations (Guha et al., 1999).

Several studies have correlated biodegradation rates inversely to the molecular weight of PAHs (Heitkamp & Cerniglia, 1988). Some of the higher molecular weight PAHs biodegrade extremely slowly. These larger PAHs (4 to 5 rings) are considered recalcitrant, and are often observed to either not biodegrade completely or not biodegrade at all (Fernández-Luqueño et al., 2011; Lladó et al., 2009).

Other factors affecting biodegradation rates are degree of sequestration by the soil and the population of PAH-degrading microbes present. The degree of adsorption onto the soil matrix will affect bioavailability of PAHs, which in turn affects biodegradation rates. And of course, it is important to determine whether or not there are bacteria present in the soil which are able to degrade PAHs, particularly the larger, more recalcitrant PAHs. If the microbial population in the soil at the site does not contain bacteria to degrade some of these larger PAHs, bioaugmentation with known-degraders may be necessary.

A wide range of PAH biodegradation rates have been reported in the literature, based almost exclusively on soil laboratory microcosm experiments. These rates are summarized in **Table 4.2**. The length of the reported microcosm studies ranged from 7 days to 96 months. In one study, no PAH biodegradation was observed over 500 days (Richardson et al., 2012), while in others greater than 50% degradation was observed in as little as 100 days (eg Espinoza & Dendooven, 2007 and Alvarez-Bernal et al., 2006). Half lives of up to 96 months were reported for mixtures of PAHs (Ouvrard et al., 2013). The average first order degradation rate constant for PAHs with 3 or less rings was 0.84 mg/kg/day, while PAHs with 4 or more rings have an average rate of 0.92 mg/kg/day. The reason for this is likely to be 2 fold. First, if degradation rates are not measurable for a PAH (i.e. zero, or NSD) it is less likely that the research will be published. Since larger PAHs have slower degradation rates, fewer papers that report the rates of biodegradation for these PAHs were published. This artificially inflates the rate for PAHs with 4 or more rings. The second reason is that there are far fewer rates based on long term studies (1 year or longer) compared to short term studies. This is probably due to time constraints of researchers and projects. However, short study durations combined with low biodegradation rates of PAHs with 4 or more rings results in very low difference in initial and final concentrations. This will increase the chance for error in measuring the difference in these concentrations, which could cause error in rate calculations.

Table 4.2. Reported Natural Attenuation Biodegradation Rates of PAHs in Soil.

Compound	No. Rings	Matrix	Lab/ Field/ Unknown (L/F/U)	PAH Concentrations (mg/kg)		Length of study (days)	Biodegradation Kinetics		Reference
				Initial	Final		Percent Degraded	Degradation Rate (mg/kg/day)	
2-Ethenylnaphthalene	2	Soil	L	50	43	150	14	O.047	Fava et al., 2004
2-Methylnaphthalene	2	Soil	L	65	35.8	150	45	0.195	Fava et al., 2004
Biphenyl	2	Soil	L	1	0.7	21	30	0.015	Aronstein et al, 1991
Dimethylnaphthalene	2	Soil	L	141	57.8	150	59	0.56	Fava et al., 2004
Naphthalene	2	Soil	L	42	19.7	150	53	0.15	Fava et al., 2004
Naphthalene	2	Soil	L	9.5	8.7	534	8	0.0015	Richardson et al., 2012[1]
Naphthalene	2	Soil	L	871	565	53	35	5.77	Tiehm et al., 1997
Acenaphthene	3	Soil	L	11.9	5.7	534	53	0.012	Richardson et al., 2012
Acenaphthene	3	Soil	L	97	62.9	53	35	0.64	Tiehm et al., 1997
Acenaphthene	3	Soil	L	294	191	53	35	1.95	Tiehm et al., 1997
Anthracene	3	Soil	L	350	59.5	100	83	2.91	Alvarez-Bernal et al., 2006
Anthracene	3	Soil	L	4002	3162	150	21	5.6	Fava et al., 2004
Anthracene	3	Soil	L	10.5	3.1	534	70	0.014	Richardson et al., 2012
Anthracene	3	Soil	L	75	25	100	67	0.50	Rivera-Espinoza & Dendooven, 2007[2]
Dibenzofuran	3	Soil	L	355	198.8	150	44	1.04	Tiehm et al., 1997
Fluorene	3	Soil	L	970	776	150	20	1.29	Fava et al., 2004
Fluorene	3	Soil	L	83	30	60	64	0.88	Llado et al., 2013
Fluorene	3	Soil	L	9.5	3.6	534	62	0.011	Richardson et al., 2012
Fluorene	3	--	--	308	200	53	35	2.04	Richardson et al., 2012
Phenanthrene	3	Soil	L	500	15	100	97	4.85	Alvarez-Bernal et al., 2006
Phenanthrene	3	Soil	L	1	0.952	21	5	0.0023	Aronstein et al., 1991
Phenanthrene	3	Slurry	L	50	36.5	35	27	0.39	Providenti, Flemming, Lee, & Trevors, 1995
Phenanthrene	3	Slurry	L	50	38.5	35	23	0.33	Providenti et al., 1995
Phenanthrene	3	Slurry	L	114	31.5	27	72	3.06	Tiehm et al., 1997[3]
Phenanthrene	3	Soil	L	129	37.2	534	71	0.17	Richardson et al., 2012
Phenanthrene	3	Soil	L	325	163	100	50	1.62	Richardson et al., 2012

Table 4.2. Reported Natural Attenuation Biodegradation Rates of PAHs in Soil.

Compound	No. Rings	Matrix	Lab/ Field/ Unknown (L/F/U)	PAH Concentrations (mg/kg)		Length of study (days)	Biodegradation Kinetics		Reference
				Initial	Final		Percent Degraded	Degradation Rate (mg/kg/day)	
11H-Benzo[b]fluorene	4	Soil	L	210	210	150	0	0.00	Tiehm et al., 1997
Benz[a]anthracene	4	Soil	L	13.8	7.1	534	49	0.0125	Richardson et al., 2012
Benz[a]anthracene	4	--	--	254	165	53	35	1.68	Tiehm et al., 1997
Benzo[a]anthracene	4	Soil	L	37	21	62	43	0.26	Llado et al., 2013
Chrysene	4	Soil	L	68	40	63	41	0.44	Llado et al., 2013
Chrysene	4	Soil	L	14	6.7	534	52	0.137	Richardson et al., 2012
Chrysene	4	--	--	173	112	53	35	1.15	Tiehm et al., 1997
Fluoranthene	4	Soil	L	2065	1652	150	20	2.75	Fava et al., 2004
Fluoranthene	4	Soil	L	25.2	11.3	534	55	0.026	Richardson et al., 2012
Fluoranthene	4	--	--	681	442	53	35	4.51	Tiehm et al., 1997
Pyrene	4	Soil	L	84	35	61	58	0.80	Llado et al., 2013
Pyrene	4	Soil	L	0.00688	0.00688	95	0	0.0	Cheung & Kinkle, 2001
Pyrene	4	Soil	L	0.00688	0.00647	95	6	$4.26\text{x}10^{-6}$	Cheung & Kinkle, 2001
Pyrene	4	Soil	L	0.00688	0.00673	95	2	$1.49\text{x}10^{-6}$	Cheung & Kinkle, 2001
Pyrene	4	Soil	L	0.00688	0.00659	95	4	$2.98\text{x}10^{-6}$	Cheung & Kinkle, 2001
Pyrene	4	Soil	L	1215	1215	150	0	0.0	Fava et al., 2004
Pyrene	4	Soil	L	100	35	32	65	2.03	S. Hwang & Cutright, 2002
Pyrene	4	Soil	L	100	21.7	32	78	2.45	S. Hwang & Cutright, 2002
Pyrene	4	Soil	L	100	18.2	32	82	2.56	S. Hwang & Cutright, 2002
Pyrene	4	Soil	L	40.9	18.4	534	55	0.042	Richardson et al., 2012
Pyrene	4	Soil	L	448	291	53	35	2.97	Tiehm et al., 1997
Triphenylene	4	Soil	L	302	302	150	0	0.0	Fava et al., 2004
Benzo[a]pyrene	5	Soil	L	22	17	66	23	0.76	Llado et al., 2013
Benzo[a]pyrene	5	Soil	L	150	39	7	74	0.16	Armenante, Pal, & Lewandowski, 1994
Benzo[a]pyrene	5	Soil	L	13.5	10.4	534	23	0.0058	Richardson et al., 2012
Benzo[a]pyrene	5	Unknown	U	169	110	53	35	1.12	Tiehm et al., 1997

Table 4.2. Reported Natural Attenuation Biodegradation Rates of PAHs in Soil.

Compound	No. Rings	Matrix	Lab/ Field/ Unknown (L/F/U)	PAH Concentrations (mg/kg)		Length of study (days)	Biodegradation Kinetics		Reference
				Initial	Final		Percent Degraded	Degradation Rate (mg/kg/day)	
Benzo[a]pyrene	5	Soil	L	95	66	100	31	0.29	Rivera-Espinoza & Dendooven, 2007
Benzo[k]fluoranthene	5	Soil	L	38	24	65	37	0.215	Llado et al., 2013
Benzo[k]fluoranthene	5	Soil	L	4.2	3.2	534	24	0.0019	Richardson et al., 2012
Benzo[k]fluoranthene	5	Soil	L	158	103	53	35	1.05	Tiehm et al., 1997
Benzo[g,h,i]perylene	6	Soil	L	5.3	5.3	534	0	0.0	Richardson et al., 2012
Acenaphthene	3	Soil	L	11.9	5.7	534	52	0.0116	Cheung & Kinkle, 2001
Acenaphthene	3	Unknown	U	97	--	53	--	--	Cheung & Kinkle, 2001
Acenaphthene	3	Unknown	U	294	--	53	--	--	Cheung & Kinkle, 2001
Benzo[b]fluoranthene	5	Soil	L	57	39	64	32	0.28	Llado et al., 2013
Benzo[b]fluoranthene	5	Soil	L	6.9	5.2	534	25	0.0032	Richardson et al., 2012
Benzo[b]fluoranthene	5	Soil	L	125	81	53	35	0.83	Tiehm et al., 1997
PAH mixture	varied	Soil	L	293	NSD	1140	NSD	--	Ouvrard et al., 2013
PAH mixture	varied	Soil	L	1371	NSD	2490	NSD	--	Ouvrard et al., 2013
PAH mixture	varied	Soil	L	446	NSD	2880	NSD	--	Ouvrard et al., 2013
PAH mixture	varied	Soil	L	555	383	2880	31	0.060	Ouvrard et al., 2013

Notes

[1] Nitrogen-limited

[2] Nitrogen-sufficient

[3] Percent degradation is contributable to biodegradation only (dissolution and sampling contributed to percent PAH removed in this study). Final concentration calculated using only attenuation due to biodegradation.

[4] Percent degradation is total PAH degradation, not individual constituent.

[5] Percent degradation is total PAH degradation.

[6] Concentration read from graph. Total sum of initial anthracene, phenanthrene, and benzo[a]pyrene concentrations was provided (988 mg/kg); percent degradation of each individual PAH constituent was also provided.

[7] Concentration estimated from graph provided in article

4.6. Biostimulation of PAH Biodegradation

PAH biodegradation rates can be stimulated beyond natural attenuation rates using a variety of biostimulation approaches. Bulking agents can be added to improve the aeration of soils, surfactants can be added to help solubilize PAH compounds sequestered in the soil structure, nutrients such as oxygen, nitrogen and phosphate can be added to improve growth of biodegrading microbes, and cometabolites can be added which provide an additional substrate to sustain the growth of microbes. Each of these biostimulation approaches is reviewed below for its applicability to PAH bioremediation.

4.6.1. Bulking Agents for Attempted Improvement of Biodegradation of PAHs

Bulking agents are materials added to soil to increase the volume of the soil and create air pockets. Examples include wood chips, sawdust, straw and rice hulls. Such bulking agents have been shown to improve biodegradation rates of compounds such as petroleum compounds which degrade aerobically because of the improved aeration (Rastegarzadeh, Nelson, & Ririe, 2006). However, benefits of bulking agents for PAH biodegradation have not been clearly shown by all experiments reported in the literature. Straube et al (2003) observed increased PAH degradation rates with the addition of ground rice hulls and blood meal, but these researchers did not examine rice hulls separately. Oleszczuk et al. found that the addition of bulking agents did not increase PAH degradation; in fact, degradation rates were slightly lower in supplemented soils (Oleszczuk, 2006). Similarly, the addition of straw had no effect on PAH degradation in a creosote-contaminated soil compared to the control (Hultgren et al., 2009). It is possible that the bulking agents add organic matter to the soil which may serve as a PAH sink and reduce the bioavailability of the PAHs (Doick & Semple, 2004; Ouvrard et al., 2013; and Totsche et al., 1997).

4.6.2. Surfactants for Improvement of PAH Biodegradation

It has been well established that PAHs become bound to the organic fraction of soils, sequestering some fraction of contaminants and reducing their bioavailability to microbes degrading these compounds (Hatzinger & Alexander, 1995). This effect reduces biodegradation rates of PAHs over time as the contaminants are aged in the soils (Hatzinger & Alexander, 1995). Surfactants can be used to release sorbed PAHs and increase their bioavailability. Surfactants have been confirmed to enhance mobilization and biodegradation of PAHs in soils (Tiehm et al., 1997). Nonionic surfactants (e.g. Alfonic 810-60 and Novel II 1412-56) have been shown to increase desorption of phenanthrene from soils (Aronstein et al. 1991). Natural surfactants, or biosurfactants, are known to rival their synthetic counterparts' efficiency while being more biodegradable and less toxic to contaminant-degrading microorganisms (Lawniczak et al., 2013). One natural anionic surfactant, soya lecithin, has been shown to elevate PAH degradation rates (Soeder et al., 1996). Important considerations when applying biosurfactants for bioremediation of contaminants include bio-compatibility between the pollutants, microorganisms, and biosurfactants. Native microflora may also impact *in-situ* biosurfactant treatment. Rhamnolipids used as biosurfactants can sometimes be biodegraded preferentially over contaminants (Chrzanowski et al., 2012). One way to avoid preferential degradation of surfactants over that of the contaminant is to apply microorganisms that do not preferentially degrade biosurfactants, a trait commonly observed in biosurfactant producers (Providenti et al., 1995). Before surfactants are applied in the field, though, several factors must be considered: Cost, efficacy at concentrations not exceeding 3%, low toxicity, low adsorption to soil, low soil dispersion, and low surface tension should all be considered prior to surfactant selection and use (Mulligan et al. 2001).

4.6.3. Nutrient Supplementation to Improve Biodegradation

The literature indicates that nutrient supplementation can enhance biodegradation of PAHs in some cases (Lee et al., 2003 and Richardson et al., 2012). However, one study indicated that nutrient amendment did not significantly reduce PAH concentrations in soil (Hwang and Cutright 2002). In fact, excess nitrogen can inhibit the lignin-degrading system of white-rot fungi (Higson 1991). This indicates that SSFL soils should be assessed to determine both whether or not PAH degradation is nutrient-limited and if nitrogen-stimulation accelerates PAH degradation.

4.7. Cometabolic Methods of PAH Biodegradation

Cometabolism may aid in biodegradation of PAHs by providing an additional substrate to sustain the growth of biodegrading microbes. For example, enhancement of native microbiota by means of lignocellulosic substrate amendment (adding material that contains lignin and cellulose) has been shown to promote higher biodegradation rates of high molecular-weight-PAHs, even of those with five aromatic rings or more (Llado et al. 2013). Similarly, biodegradation of high molecular weight PAHs has been improved by the addition of lower molecular weight PAHs. For example, cometabolic bioremediation of pyrene has been demonstrated when phenanthrene is present as a primary substrate (Hwang & Cutright, 2003). Also, fluorene has been shown to be cometabolically degraded by *Mycobacterium* sp. strain BB-1 (Boldrin et al., 1993). One study assessing degradation of pure PAHs found that naphthalene was toxic to all organisms not isolated on the pure compound (Bouchez et al., 1995). However, another study emphasized the importance of conducting PAH biodegradability studies in the presence of PAH mixtures to consider the more realistic field scenario in which PAH mixtures are more likely to be present in soil (van Herwijnen et al., 2003). The effect of mixtures of PAHs on biodegradation rates can be positive or negative, and are difficult to predict. In most cases the biodegradation rates for higher molecular weight PAHs increase while the biodegradation rates of lower molecular weight PAHs decrease, in comparison to observed rates for individual compounds (Guha et al., 1999). Clearly, SSFL's site-specific PAH contamination profile should be considered when determining whether or not cometabolism can serve to aid remediation of PAHs in SSFL soils.

4.8. Bioaugmentation of PAH Biodegradation

Some sites contaminated with PAHs are blessed with microbes which biodegrade high molecular weight PAHs, while other sites may benefit from bioaugmentation with microorganisms known to degrade PAHs (Juhasz & Naidu, 2000). Particularly for high molecular weight PAHs, such as benzo-a-pyrene, fungi may be more promising candidates for bioaugmentation than bacteria (Juhasz & Naidu, 2000). The most commonly used group of fungi for bioaugmentation in the bioremediation of PAH contaminated soil is white-rot fungi.

These fungi use both lignin and manganese peroxidase to break down complex lignins in rotting wood, and these same enzyme systems can metabolize PAHs. Fungi have been shown to degrade a broad range of PAHs under low-oxygen conditions. Low-molecular-weight PAHs (2-3 rings) are biodegraded most extensively by *Aspergillus sp.*, *Trichocladium canadense*, and *Fusarium oxysporum*. Higher molecular weight PAHs are more recalcitrant (Juhasz & Naidu, 2000), but biodegradation of high molecular-weight PAHs (4–7 rings) has been observed by *T. canadense*, *Aspergillus sp.*, *Verticillium sp.*, and *Achremonium sp* (Silva et al., 2009). The bacterium *Pseudomonas aeruginosa* may also be a promising microorganism that has been shown to degrade PAHs and can produce its own rhamnolipid biosurfactants, thereby potentially increasing PAH bioavailability (Hwang and Cutright 2002).

An important question to answer before expending effort for bioaugmentation is if the native fungi and bacteria at the particular site are better suited to conditions at the site than microorganisms that may be introduced to the site for bioaugmentation. Consider for example that at least one study showed that fungi used in bioaugmentation, *Trametes versicolor* and *Lentinus tigrinus*, were clearly outcompeted by native fungi (Llado et al. 2013). Soil microcosm experiments can be used for screening to test the efficacy of any proposed bioaugmentation strategy.

4.9. Phytoremediation of PAHs

Rhizoremediation, as described above for aliphatic hydrocarbons, is also well suited for remediation of PAHs (Pilon-Smits, 2005). However, PAHs are often more recalcitrant to rhizoremediation due to high chemical stability and high log K_{ow} values which causes them to resist degradation and bind tightly to soil (Haritash & Kaushik, 2009). For example, benzo*[a]*pyrene has a log K_{ow} of 6.2, and anthracene has a log K_{ow} of 4.44 (Heath et al., 1993). Even so, studies have shown remediation of PAHs through rhizosphere stimulation and even some phytoextraction into the tissue of several different plant species, although phytoextraction was limited (Gao & Zhu, 2004; Hall et al., 2011). For instance, a study by Gao and Zhu (2004) showed that phytoextraction alone contributed to only 0.01% (phenanthrene) and 0.24% (pyrene) of the total PAH reduction by plants.

The phytoremediation rates for PAHs can be very rapid as shown in one study that removed 67.5% of pyrene from soil in 28 days (Liste & Alexander, 2000). The rates of removal vary depending on the specific PAH, the plant species, and concentration of contaminant. Based on the published studies, phytoremediation of PAHs could reduce soil PAH concentrations at SSFL from 50 ppm (typical value at SSFL) to 0.00447 ppm (background level) on a time scale of 1.5 to 2.7 years. This estimate is based on first order rate constants for various PAHs calculated in a study by Robinson et al. (2003). However, slower degradation rates (9.1% reduction in a year) have been observed with weathered soil that could indicate a longer remediation time of around 98 years at SSFL based on first order kinetics (Parrish et al., 2004). Clearly it would be helpful to conduct site-specific phytoremediation experiments with contaminated soil from SSFL to get a more precise idea of the potential phytoremediation rate of PAHs at SSFL.

4.10. Potential for Natural Attenuation of PAHs at SSFL

Based on published studies (Juhasz & Naidu, 2000), it is likely that the low molecular weight PAHs will biodegrade under natural conditions at SSFL over time, but this process will be slow, and some non-bioavailable fraction of these PAHs is likely to remain sequestered in the soil indefinitely. Biodegradation of the more recalcitrant high molecular weight PAHs would be even slower and less complete under natural conditions. Because of the long aging time of the soil contamination at SSFL, the more biodegradable fraction of the PAHs may have already biodegraded, leaving the more recalcitrant and more sequestered PAHs in the soil at present.

Predicting natural PAH biodegradation rates at the SSFL site is difficult because there are many different PAHs present as well as various other contaminants. It is likely that a consortium of bacteria and/or fungi are biodegrading PAHs at the site, which makes modeling of biodegradation even more complicated. Further, some contaminants may be toxic to the degraders of other contaminants. Bioavailability of PAHs at SSFL may be low because of their high hydrophobicity and long time period in the soils at the site. As the PAHs bind to organic colloids in soil, they become difficult to access for bacteria (Lladó et al., 2013). Other factors that affect biodegradation rates of PAHs include inorganic salt concentrations, pH, other carbon sources, nitrate concentrations, phosphate concentrations, soil type, climate, other constituents present, biomass of degraders, and species of degraders (Guha et al., 1999; Haritash & Kaushik, 2009; Manilal & Alexander, 1991). Because of all these complex factors, the best ways to estimate biodegradation rates for PAHs in the soils at this site are 1) comparison to field data from similar sites, if possible and 2) empirically, using microcosm experiments.

Experiments conducted by Ouvrard et al. (2013) may be good indicators of the rates to be expected at SSFL because they used mixtures of PAHs in soil. However, Ouvard et al. used PAH concentrations of 380 to 2077 ppm, which are much higher than those at SSFL (1-8 ppm). They found that the half-life of their PAH mixture with a starting concentration of 293 ppm was 36 months (Ouvrard et al., 2013). Another study conducted by Llado et al. (2013) is relevant to this study because it focused on weathered and recalcitrant PAHs in soil. This study found the half-life of flourene and chrysene to be 60 and 63 days in unamended soil, respectively (Llado et al., 2013). The same study also showed pyrene had a half life of 63 days with a starting concentration of 84 ppm. The soil from this experiment was collected from a location near Barcelona, Spain, which has a similar subtropical Mediterranean climate to SSFL (*Meteorological Service of Catalonia*, 2012).

Based on the published laboratory studies of PAH biodegradation by Ouvard et al. and Llado et al. described above, and those presented in Table 4.2, it is unlikely that biodegradation alone under natural attenuation conditions will reduce PAH concentrations to the background levels for SSFL specified in the DTSC "look-up tables" (DTSC, 2013) in a reasonable amount of time. The DTSC specified target background concentrations for PAHs ranging from 2.5 ppb for anthracene to 5.6 ppb for pyrene (**Table 4.3** and DTSC, 2013). For a best-case scenario, consider the biodegradation of one of the more lightly contaminated soils at SSFL with a PAH concentration of 100 ppb. Using the short half-life of 60 days as reported by Llado et al. (2013), and assuming uninhibited exponential decay, it would take 4 to 5 years for the concentration to be reduced to 5 ppb. Using the more conservative half-life published by Ouvard et al.

(36 months), it would take about 15 years to reach background levels. However, these outcomes are very unlikely to be observed because as time goes on the remaining PAHs are the more recalcitrant and the more sequestered compounds. Additionally, there are many different PAHs in the soils at SSFL that are known to be recalcitrant, and will not likely degrade via natural attenuation. Further, it should be noted that the lowest final PAH concentration reported for any of the lab studies reviewed in Table 4.2 is about 3 ppm, which is about three orders of magnitude higher than the background levels specified for SSFL by the DTSC.

Table 4.3. Background levels of PAHs and other semi-volatile compounds as specified by the DTSC (2013).

Chemical Constituent	**Units**	**Look-Up Table Value**	**Basis**
Semi-Volatiles (SVOCs)/PAHs - EPA Method 8270C(SIM)			
Acenaphthylene	µg/kg	2.5	BG MRL
Anthracene	µg/kg	2.5	BG MRL
Benzo(a)anthracene	µg/kg	see note [2]	---
Benzo(a)pyrene	µg/kg	see note [2]	---
Benzo(b)fluoranthene	µg/kg	see note [2]	---
Benzo(g,h,i)perylene	µg/kg	2.5	BG MRL
Benzo(k)fluoranthene	µg/kg	see note [2]	---
Bis(2-Ethylhexyl)phthalate	µg/kg	61	BTV
Butylbenzylphthalate	µg/kg	100	BTV
Chrysene	µg/kg	see note [2]	---
Dibenz(a,h)anthracene	µg/kg	see note [2]	---
Diethyl phthalate	µg/kg	27	BG MRL
Dimethyl phthalate	µg/kg	27	BG MRL
Di-n-butylphthalate	µg/kg	27	BG MRL
Di-n-octylphthalate	µg/kg	27	BG MRL
Fluoranthene	µg/kg	5.2	BTV
Fluorene	µg/kg	3.8	BTV
Indeno(1,2,3-cd)pyrene	µg/kg	see note [2]	---
Naphthalene	µg/kg	3.6	BTV
Phenanthrene	µg/kg	3.9	BTV
Pyrene	µg/kg	5.6	BTV
1-Methyl naphthalene	µg/kg	2.5	BG MRL
2-Methylnaphthalene	µg/kg	2.5	BG MRL
Acenaphthene	µg/kg	2.5	BG MRL
Benzo(a)pyrene Equivalent			
Benzo(a)pyrene TEQ[2]	µg/kg	4.47 (see note[2])	BTV-TEQ

Note: Benzo(a)pyrene Toxicity Equivalency Quotient applied to these PAHs.

Although natural attenuation alone does not appear to be a viable method of reducing PAH concentrations down to background levels, enhanced bioremediation through biostimulation or bioaugmentation may improve biodegradation rates significantly. Biostimulation through addition of nutrients and/or bulking agents may increase biodegradation rates as well as bioagmentation by addition of lignolytic fungi. Since the sequestration of PAHs in the soil is likely to be a major impediment to biodegradation, the use of biosurfactants could also be explored. The best way to test the efficacy of these active bioremediation approaches would be to conduct controlled laboratory soil microcosm experiments.

Additional natural attenuation of PAHs may be possible through abiotic processes such as volatilization and photooxidation. However, volatilization is likely to have already occurred over the decades the contaminants have been in the soil, and thus further reduction of PAH concentrations by volatilization are not likely. Photooxidation is also unlikely to be significant because light cannot penetrate the soil to the depths where the PAH contaminants are located. Leaching is unlikely to be a major factor in removal of PAHs from Area IV soils because the high molecular weight PAHs in these soils are expected to have very low desorption rates.

5.0 Polychlorinated biphenyls (PCBs)

5.1. Description and Toxicity of PCBs

PCBs are chlorinated biphenyl compounds (**Figure 5.1**). Although the name is "polychlorinated", monochlorinated biphenyls are generally included under the name "PCBs". There can be between 1 and 10 chlorine atoms bonded to the biphenyl in 10 different positions, making 209 possible unique congeners. PCB congeners are often classified by the number of chlorine atoms they contain. PCBs with the same number of chlorines are called homologs. Another more specific way of naming PCBs is by stating the location(s) of the chlorines on the biphenyl groups. Each of these congeners are also given a number, which are often used to describe specific congeners (ASTDR, 2010). The most common way of naming PCBs was created by Monsanto Corp., who produced PCBs commercially. This method names mixtures of PCBs with a 4-digit number, with the first 2 digits indicating the number of carbons (12) and the second 2 digits indicating the chlorine content by weight percent. These mixtures were sold by Monsanto under the trade name Aroclor. For example, Aroclor 1254 is a mixture of PCBs that includes mono-through heptachloroinated homologs with a 54% chlorine content by weight (ASTDR, 2010).

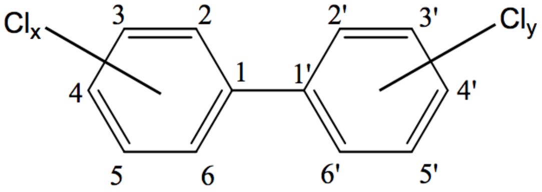


Figure 5.1. Generic Structure of PCBs (ASTDR, 2010)**. Positions 2,2',6, and 6' are ortho positions, positions 3,3',5 and 5' are meta positions and positions 4 and 4' are para positions.**

PCBs were produced worldwide for their wide range of applications. About 1.5 million tons were produced between 1930 and 1977, 600,000 of which were produced in the US (Breivik et al., 2002). PCBs are versatile compounds because they have different physical-chemical properties depending on their degree of chlorination. These properties include low electrical conductivity and low flammability. PCBs were used in fluid-filled electrical components such as transformers and capacitors, as adhesives, dedusting agents, pesticide extenders, hydraulic lubricants, flame retardants, cutting oils, heat transfer fluids, paints, sealants, and in carbonless copy paper. Many of these applications led to the direct release of PCBs into the environment. Other applications ended with improper disposal or accidental leakage (Fiedler et al., 1994). Such leaks were extremely common for power pole transformers, which often contained PCBs if they were built before 1977 (Emmett et al., 1988).

PCB contamination is a major health concern to both humans and wildlife for three main reasons: PCBs are widespread due to the variety of applications they were used for, they are difficult to degrade and soluble in fatty tissue so they bioaccumulate, and they are toxic, carcinogenic, and mutagenic (Narquis, 2007). Studies also indicate that some PCBs can bind to receptors intended for estrogen or estradiol, making them potential endocrine disrupters (Wang et al., 2006). Bioaccumulation is a major concern because this process can bring PCBs into higher trophic levels and expose entire food chains (Bernard et al., 2002). The half-lives of PCBs in the human body are 7-10 years (Wang et al., 2006), which allows time for accumulation.

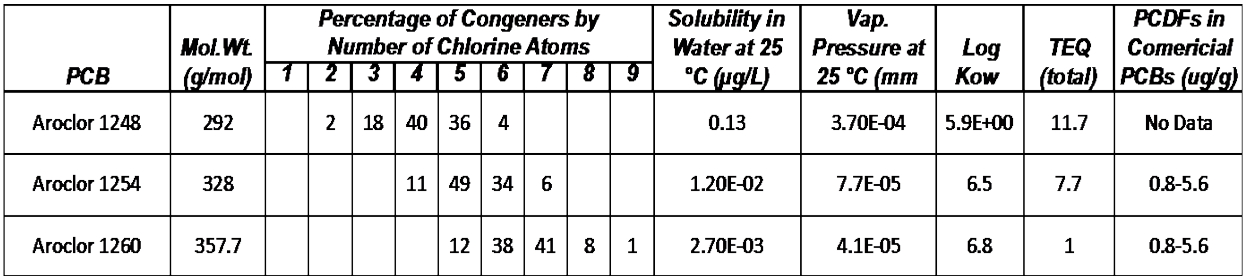
Because each congener has a different toxicity and PCBs are usually in mixtures, the toxicity of PCB congeners is measured as toxic equivalents (TEQ), which are calculated using toxic equivalency factors (TEFs) for each congener (Narquis, 2007). The TEF values of PCB congeners are based on a scale between 0 and 1 (1 being the most toxic) to rate the toxicity of each congener. These values along with the amount of each congener in a sample can be used to calculate the TEQ. These TEQ values are used in risk assessments and regulatory control (Van den Berg et al., 1998).

Another health concern with PCBs is the formation of polychlorinated dibenzofurans (PCDFs), which can occur during the synthesis of PCBs or during pyrolysis or incineration at temperatures below 1200^{o} C (Gibbs, 1997) (a common method of disposing of toxic waste during the time PCBs were used).

The PCBs measured in Area IV were quantitated based on the closest Aroclor product match to the congener concentrations measured. The PCBs analyzed in Area IV most closely match the congener make-up of Aroclors 1248, 1254, and 1260. The composition and physical properties of these PCB mixtures are detailed in **Table 5.1**.

PCDFs may have been formed on the SSFL site as the wildfire burned over soil contaminated with PCBs in 2005. Combustion of PCBs at the top of the soil likely produced some PCDFs. This may have led to higher levels of dioxins in Area IV than before the fire. It may have also caused some dispersal due to volatilization. PCDFs have also been found in low concentrations in commercial PCB mixtures (Table 5.1). PCDFs have significant health concerns because they are known teratogens, mutagens, and suspected human carcinogens (Fiedler, 1997).

Table 5.1. Average molecular weight, congener mixture (Heidelore Fiedler, 1997)**, solubility, vapor pressure, Log Kow** (ASTDR, 2010)**, total TEQ** (Narquis, 2007)**, and PCDFs of each PCB found in the clearly contaminated parts of Area IV at SSFL.**



PCB	Mol.Wt (g/mol)	Percentage of Congeners by Number of Chlorine Atoms									Solubility in Water at 25 °C (µg/L)	Vap. Pressure at 25 °C (mm	Log Kow	TEQ (total)	PCDFs in Comericial PCBs (ug/g)
		1	2	3	4	5	6	7	8	9					
Aroclor 1248	292		2	18	40	36	4				0.13	3.70E-04	5.9E+00	11.7	No Data
Aroclor 1254	328				11	49	34	6			1.20E-02	7.7E-05	6.5	7.7	0.8-5.6
Aroclor 1260	357.7					12	38	41	8	1	2.70E-03	4.1E-05	6.8	1	0.8-5.6

Toxicity equivalency quotients (TEQ) were calculated for each Aroclor mixture as follows:
The TEQ contribution of each congener was calculated by multiplying the individual PCB congener concentration by its toxic equivalency factor, and then these products were summed to obtain the total TEQ of the mixture (Narquis, 2007).

5.2. Abiotic Weathering of PCBs

Due to the extremely low vapor pressure of PCBs, significant volatilization from soils is unlikely. PCB volatilization has been reported in measurable quantities at temperatures of 109^{o}C (Dubey & Dugal, 1977), but soil temperatures at SSFL are unlikely to exceed 30^{o}C. However, some PCB volatilization is likely to have occurred during the 2005 wildfire at the site.

PCBs in contaminated soil are likely to remain stationary in the organic fraction of the soil because of the chemical properties of PCBs, most notably their low solubility in water and high octanol-water partition coefficients (Davis & Wade, 2003). This sequestration in the soil may lead to reduced bioavailability and therefore low biodegradation rates of PCBs in soil (Hyun et al., 2010). Other abiotic degradation processes, including photo-oxidation and chemical degradation, are expected to be negligible for this site based on a study by Sinkkonen & Paasivirta (2000). Photo-oxidation can only affect degradation rates in aquatic environments. Chemical degradation of PCBs is expected to be negligible as well.

5.3. Biodegradation of PCBs

Both bacteria and fungi have been shown to biodegrade complex PCB mixtures. Bacterial degradation of PCBs typically occurs via reductive dechlorination of highly chlorinated PCB congeners under anaerobic conditions (Quensen et al., 1990) followed by aerobic biodegradation of the lightly chlorinated congeners by bacteria using these compounds as a carbon source (Haggblom et al., 2012). These two pathways combined can completely mineralize PCB mixtures. However, this multiple-step process is complex, involving multiple microorganisms, and tends to be slow (Seeger et al., 1997). Additionally, aerobic microbes are fairly selective towards lower chlorinated PCBs, often leaving the higher chlorinated congeners untouched (Pieper, 2004). Fungal biodegradation is typically mediated by species like white-rot fungi which produce lignolytic enzymes which are used to break down the complex organic molecules in lignin (Thomas et al., 1992 and Čvančarová et al., 2012). These enzymes have a broad specificity, and have been reported to biodegrade PCB congeners with 1 to 6 chlorines

(Čvančarová et al., 2012). Bacterial and fungal biodegradation of PCBs are discussed in detail below.

5.3.1. Bacterial Biodegradation of PCBs: Anaerobic Reductive Dechlorination of PCBs

Anaerobic dechlorination displaces a chlorine atom with hydrogen through a biologically-mediated, reductive process. For PCBs, anaerobic dechlorination yields lower chlorinated congeners. This process is typically cometabolic, involving an electron donor (such as organic carbon or hydrogen) which must be available to the bacteria (Quensen et al., 1988). The rate of anaerobic dechlorination decreases as the degree of chlorination increases. The microbial population also has a large impact on which PCBs can be degraded, and how fast (Quensen et al., 1990).

Anaerobic dechlorination does not work equally well for all positions of chlorine atoms on the PCB molecule. Typically, *meta* and *para* chlorines are preferentially dechlorinated, while *ortho* chlorines are more recalcitrant. A study conducted by Quensen et al. (1990), used microbial populations in sediment from the Hudson River, which has been highly contaminated with PCBs for decades, to reductively dechlorinate Aroclor 1242, 1248, 1254, and 1260. After 25 weeks, chlorines in meta and para positions showed significant dechlorination for all of these Aroclors except 1260. Aroclors 1242, 1248, and 1254 showed 85, 75, and 63% removal of chlorines from meta and para positions. Ortho chlorines remained largely unaffected during the 25-week study. The experiment was repeated using sediments from Silver Lake, Massachusetts, and this time showed a 19% decrease in meta and para chlorines (Quensen et al., 1990). However, dechlorination of *ortho* chlorines has been observed for single congeners supplemented with fatty acids (Wiegel & Wu, 2000).

There are currently 8 known microbial PCB dechlorination pathways that attack chlorines at the *meta* and *para* positions. These pathways have been designated M, N, Q, T, H, H', LP, and P (Wiegel & Wu, 2000). **Table 5.2** below shows a list of susceptible chlorines for each of these dechlorination pathways. The pathways of *ortho* dechlorination are not as well understood. **Figure 5.2** shows a generic pathway for both LP and *ortho*-removing processes as observed in microcosms with sediment from Woods Pond, New York. **Figure 5.3** shows Process N with the same sediment *in situ* (Bedard, 2008).

Table 5.2: Susceptible chlorines removed by seven different dechlorination pathways (Wiegel & Wu, 2000)**. The term "flanked" means that there is also a chlorine atom on the carbon adjacent to the chlorine of interest.**

Positions of chlorines removed by each dechlorination process	
Dechlorination Process	**Susceptible chlorines**
M	Flanked and Unflanked *meta*
Q	Flanked and Unflanked *para, meta* of 23-group
H'	Flanked *para, meta* of 23- and 234- group
H	Flanked *para,* doubly flanked *meta*
PN	Flanked *para*
LP	Flanked *meta*
T	Flanked *meta* of 2345- group, in hepta- and octachlorobiphenyls
N	Flanked *meta*

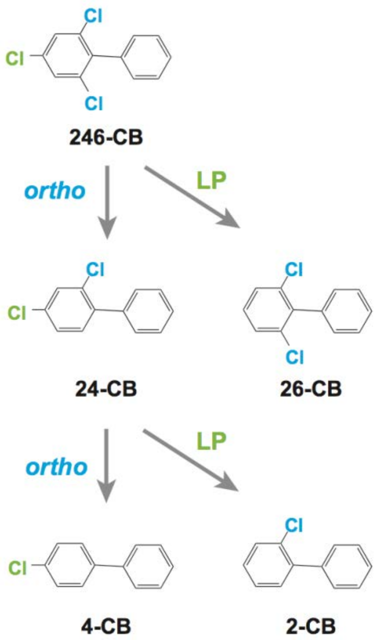


Figure 5.2. Ortho and LP declorination in microcosm incoulated with sediment from Woods Pond (Bedard, 2008)**.**

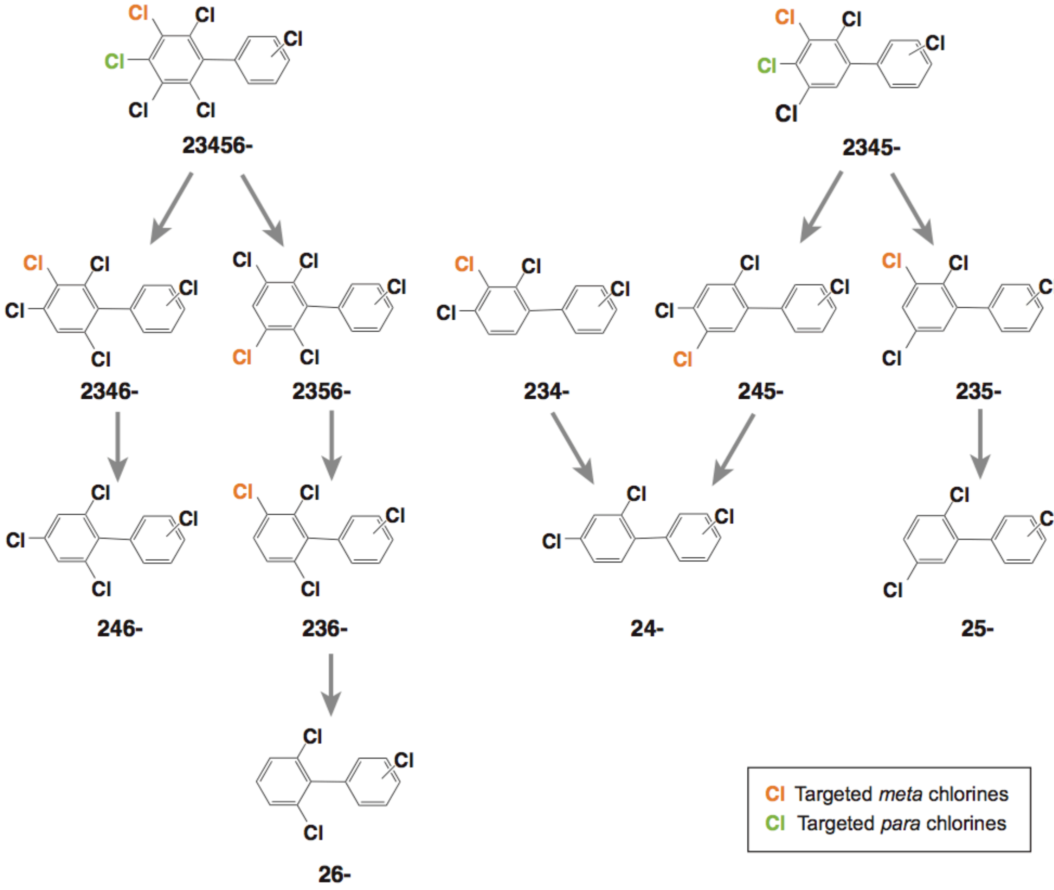


Figure 5.3. PCB biodegradation by Pathway N as observed *in situ* in Woods Pond (Bedard, 2008)**.**

PCB dechlorination rates in the environment are dependent on both biological and physical conditions. **Figure 5.4** shows that the products of dechlorination depend on the starting congener as well as the pathway used by the dechlorinating species of bacteria. The microbial population dictates to some extent if reductive dechlorination is possible and if so by which pathway. In the studies summarized in Figure 5.4, different results were obtained for different sediment sources of bacteria.

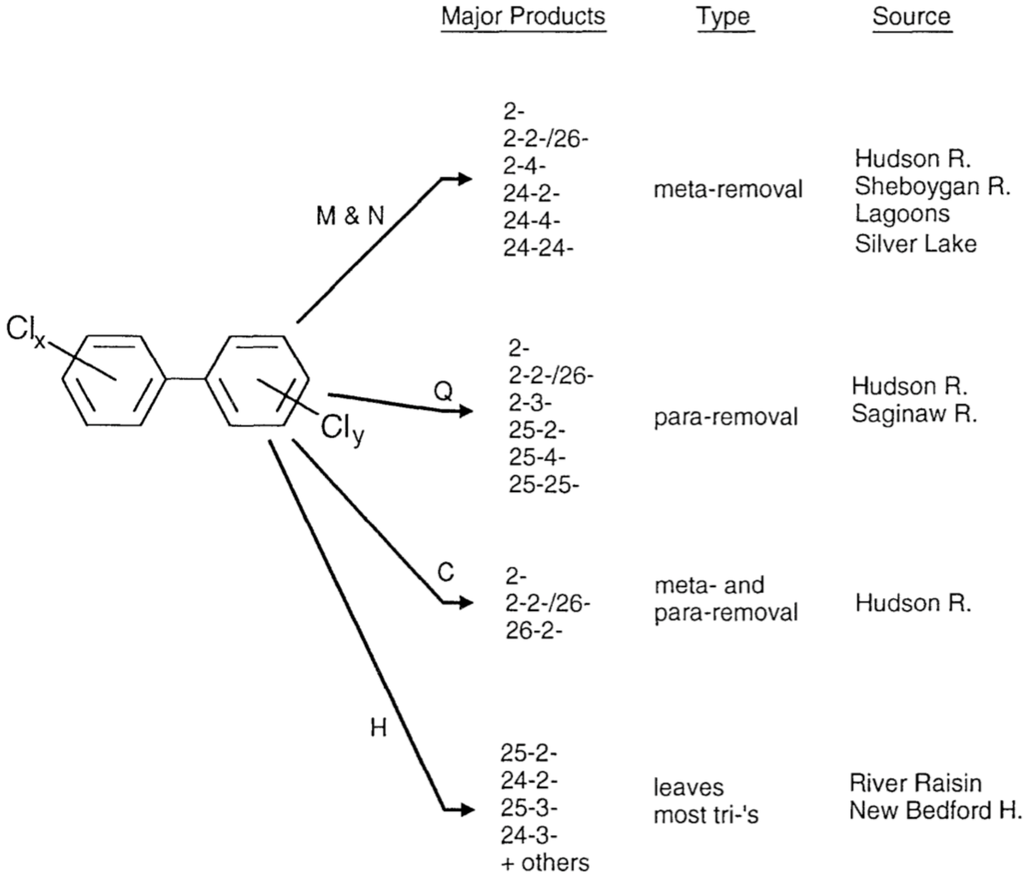


Figure 5.4. The different dechlorination pathways seen in different sediments that have been contaminated with PCBs (Processes M, N, Q, C, and H). The most common products of these pathways are by the remaining chlorine position(s). The source of the sediment is also listed (Tiedje et al., 1993)**. Major products are named in terms of the position of the chlorines remaining on the biphenyl. See Figure 5.1 for location numbers.**

Numerous researchers have isolated bacteria that can reductively dechlorinate PCBs in an anaerobic environment. A summary of the results of these studies is presented in **Table 5.3**. Rates measured in these laboratory experiments varied from 1% in 3 days to 98% in 48 hours. However it should be noted that these are controlled laboratory experiments that usually had high concentrations of PCBs spiked into the soil. Rates measured in the field are not expected to be accurately predicted by these values. It is important to understand that these rates were determined in the conditions stated in these studies, and that varying physical conditions can have a large impact on rates as well (Tiedje et al., 1991). A smaller table with dechlorination rates reported for lab and field studies is presented later.

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
1	*Burkholderia sp*. LB400	100% / 24h	NS	1	Rein et al. 2007
1	*Rhodococcus sp*. SK-1	40% / 4 days	NS	1	Chang et al. 2013
1	*Rhodococcus sp*. SK-3	45% / 4 days	NS	1	Chang et al. 2013
1	*Rhodococcus sp*. SK-4	35% / 4 days	NS	1	Chang et al. 2013
1	*Aquamicrobium defluvii* SK-2	28% / 4 days	NS	1	Chang et al. 2013
2	*Rhodococcus sp*. SK-1	64.4% / 4 days	NS	1	Chang et al. 2013
2	*Rhodococcus sp*. SK-3	64.4% / 4 days	NS	1	Chang et al. 2013
2	*Aquamicrobium defluvii* SK-2	42% / 4 days	NS	1	Chang et al. 2013
3	*Burkholderia sp*. LB400	100% / 22.7h	NS	1	Rein et al. 2007
3	*Rhodococcus sp*. SK-1	66% / 4 days	NS	1	Chang et al. 2013
3	*Rhodococcus sp*. SK-3	60% / 4 days	NS	1	Chang et al. 2013
3	*Rhodococcus sp*. SK-4	50% / 4 days	NS	1	Chang et al. 2013
3	*Aquamicrobium defluvii* SK-2	31.4% / 4 days	NS	1	Chang et al. 2013
4	*Corynebacterium sp*. MB1	87% / 24h at 5uM	NS	2	Bedard et al. 1986
4	*Alcaligenes eutrophus* H850	100% / 24h at 5uM and 25uM	NS	2	Bedard et al. 1986
4	*Rhodococcus sp*. SK-1	14.2% / 4 days	NS	2	Chang et al. 2013
4	*Rhodococcus sp*. SK-3	16% / 4 days	NS	2	Chang et al. 2013
4	*Rhodococcus sp*. SK-4	22% / 4 days	NS	2	Chang et al. 2013
4	*Aquamicrobium defluv*i SK-2	34.7% / 4 days	NS	2	Chang et al. 2013
5	*Corynebacterium sp*. MB1	100% / 24h at 5uM and 25uM	NS	2	Bedard et al. 1986
5	*Alcaligenes eutrophus* H850	100% / 24h at 5uM and 25uM	NS	2	Bedard et al. 1986
5	*Burkholderia sp*. LB400	99% / 20.8h	NS	2	Rein et al. 2007
6	*Rhodococcus sp*. SK-1	33.7% / 4 days	NS	2	Chang et al. 2013
6	*Rhodococcus sp*. SK-3	35% / 4 days	NS	2	Chang et al. 2013
6	*Rhodococcus sp*. SK-4	34.8% / 4 days	NS	2	Chang et al. 2013
6	*Aquamicrobium defluvii* SK-2	34.8% / 4 days	NS	2	Chang et al. 2013
6	*Sphingomonas sp.*	77% / 72h	NS	2	Yong-lei et al. 2011
6	*Pseudomonas sp.*	86% / 72h	NS	2	Yong-lei et al. 2011
8	*Corynebacterium sp*. MB1	100% / 24h at 5uM and 25uM	NS	2	Bedard et al. 1986
8	*Alcaligenes eutrophus* H850	100% / 24h at 5uM and 25uM	NS	2	Bedard et al. 1986
8	*Rhodococcus sp*. SK-1	20% / 4 days	NS	2	Chang et al. 2013
8	*Rhodococcus sp*. SK-3	21% / 4 days	NS	2	Chang et al. 2013
8	*Rhodococcus sp*. SK-4	22% / 4 days	NS	2	Chang et al. 2013
8	*Aquamicrobium defluvii* SK-2	21.3% / 4 days	NS	2	Chang et al. 2013
8	*Sphingomonas sp.*	70% / 72h	NS	2	Yong-lei et al. 2011
8	*Pseudomonas sp.*	87% / 72h	NS	2	Yong-lei et al. 2011

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
9	*Rhodococcus sp.* SK-1	23.6% / 4 days	NS	2	Chang et al. 2013
9	*Rhodococcus sp.* SK-3	26% / 4 days	NS	2	Chang et al. 2013
9	*Rhodococcus sp.* SK-4	21% / 4 days	NS	2	Chang et al. 2013
9	*Aquamicrobium defluvii* SK-2	19.1% / 4 days	NS	2	Chang et al. 2013
9	*Sphingomonas sp.*	51% / 72h	NS	2	Yong-lei et al. 2011
9	*Pseudomonas sp.*	99% / 72h	NS	2	Yong-lei et al. 2011
15	*Corynebacterium sp*. MB1	100% / 24h at 5uM and 25uM	NS	3	Bedard et al. 1986
15	*Alcaligenes eutrophus* H850	61% / 24h at 5uM	NS	3	Bedard et al. 1986
16	*Burkholderia sp*. LB400	91% / 20.2h	NS	3	Rein et al. 2007
16	*Rhodococcus sp*. SK-1	15.9% / 4 days	NS	3	Chang et al. 2013
16	*Rhodococcus sp*. SK-3	17% / 4 days	NS	3	Chang et al. 2013
16	*Rhodococcus sp*. SK-4	17% / 4 days	NS	3	Chang et al. 2013
16	*Aquamicrobium defluvii* SK-2	37% / 4 days	NS	3	Chang et al. 2013
17	*Burkholderia sp*. LB400	97% / 22.2h	NS	3	Rein et al. 2007
17	*Ralstonia sp*. SA-4	70.5% / 261h	CBA, chloride	3	Adebusoye et al. 2008
17	*Pseudomonas sp*. SA-6	86.2% / 261h	CBA, chloride	3	Adebusoye et al. 2008
17	*Sphingomonas sp.*	42% / 72h	NS	3	Yong-lei et al. 2011
17	*Pseudomonas sp.*	74% / 72h	NS	3	Yong-lei et al. 2011
18	*Corynebacterium sp*. MB1	61% / 24h at 5uM	NS	3	Bedard et al. 1986
18	*Alcaligenes eutrophus* H850	100% / 24h at 5uM and 98% / 24h at 25uM	NS	3	Bedard et al. 1986
18	*Burkholderia sp*. LB400	92% / 19.8h	NS	3	Rein et al. 2007
18	*Paenibacillus sp*. KBC101	100% / 3 days	NS	3	Sakai et al. 2004
18	*Rhodococcus sp*. RHA1	98% / 3 days	NS	3	Sakai et al. 2004
18	*Burkholderia xenovorans* LB400T	100% / 3 days	NS	3	Sakai et al. 2004
18	*Pseudomonas pseudoalcaligenes* KF707	10% / 3 days	NS	3	Sakai et al. 2004
18	*Rhodococcus sp*. SK-1	40% / 4 days	NS	3	Chang et al. 2013
18	*Rhodococcus sp*. SK-3	42% / 4 days	NS	3	Chang et al. 2013
18	*Aquamicrobium defluvii* SK-2	32% / 4 days	NS	3	Chang et al. 2013
18	*Ralstonia sp.* SA-4	78.5% / 255h	CBA, chloride	3	Adebusoye et al. 2008
18	*Pseudomonas sp*. SA-6	92.5% / 255h	CBA, chloride	3	Adebusoye et al., 2008
19	*Rhodococcus sp*. SK-1	24.5% / 4 days	NS	3	Chang et al. 2013
19	*Rhodococcus sp*. SK-3	25% / 4 days	NS	3	Chang et al. 2013
19	*Rhodococcus sp*. SK-4	25% / 4 days	NS	3	Chang et al., 2013

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
19	*Aquamicrobium defluvii* SK-2	18.5% / 4 days	NS	3	Chang et al. 2013
19	*Sphingomonas sp.*	49% / 72h	NS	3	Yong-lei et al. 2011
19	*Pseudomonas sp.*	82% / 72h	NS	3	Yong-lei et al. 2011
20	*Burkholderia sp.* LB400	89% / 21.6h	NS	3	Rein et al. 2007
22	*Rhodococcus sp.* SK-1	23% / 4 days	NS	3	Chang et al. 2013
22	*Rhodococcus sp.* SK-3	24% / 4 days	NS	3	Chang et al. 2013
22	*Rhodococcus sp.* SK-4	23% / 4 days	NS	3	Chang et al. 2013
22	*Aquamicrobium defluvii* SK-2	19.2% / 4 days	NS	3	Chang et al. 2013
25	*Burkholderia sp.* LB400	42% / 23.4h	NS	3	Rein et al. 2007
25	*Rhodococcus sp.* SK-1	57% / 4 days	NS	3	Chang et al. 2013
25	*Rhodococcus sp.* SK-3	50% / 4 days	NS	3	Chang et al. 2013
25	*Rhodococcus sp.* SK-4	50% / 4 days	NS	3	Chang et al. 2013
25	*Aquamicrobium defluvii* SK-2	37.8% / 4 days	NS	3	Chang et al. 2013
27	*Sphingomonas sp.*	43% / 72h	NS	3	Yong-lei et al. 2011
27	*Pseudomonas sp.*	85% / 72h	NS	3	Yong-lei et al. 2011
28	*Stenotrophomonas sp.* JSG1	50.3% / 48h	NS	3	Ganesh-Kumar et al. 2013
28	*Corynebacterium sp.* MB1	100% / 24h at 5uM and 98% / 24h at 25uM	NS	3	(Bedard et al., 1986
28	*Alcaligenes eutrophus* H850	76% / 24h at 5uM	NS	3	Bedard et al. 1986
28	*Rhodococcus sp.* SK-1	22% / 4 days	NS	3	Chang et al. 2013
28	*Rhodococcus sp.* SK-3	23% / 4 days	NS	3	Chang et al. 2013
28	*Rhodococcus sp.* SK-4	24% / 4 days	NS	3	Chang et al. 2013
28	*Aquamicrobium defluvii* SK-2	16.8% / 4 days	NS	3	Chang et al. 2013
31	*Stenotrophomonas sp.* JSG1	60.4% / 48h	NS	3	Ganesh-Kumar et al. 2012
31	*Corynebacterium sp.* MB1	86% / 24h at 5uM and 22% / 24h at 25uM	NS	3	Bedard et al. 1986
31	*Alcaligenes eutrophus* H850	99% / 24h at 5uM and 86% / 24h at 25uM	NS	3	Bedard et al. 1986
31	*Burkholderia sp.* LB400	92% / 20.8h	NS	3	Rein et al. 2007
34	*Burkholderia sp.* LB400	80% / 22.2h	NS	3	Rein et al. 2007
40	*Corynebacterium sp.* MB1	100% / 24h at 5uM and 96% / 24h at 25uM	NS	3	Bedard et al. 1986
40	*Alcaligenes eutrophus* H850	99% / 24h at 5uM and 53% / 24h at 25uM	NS	4	Bedard et al. 1986
40	*Pseudomonas sp.*	24% / 72h	NS	4	Yong-lei et al. 2011
41	*Burkholderia sp.* LB400	39% / 22.5h	NS	4	Rein et al. 2007

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
43	*Stenotrophomonas sp.* JSG1	100% / 48h	NS	4	Ganesh-Kumar et al. 2012
44	*Corynebacterium sp*. MB1	100% / 24h at 5uM and 32% / 24h at 25uM	NS	4	Bedard et al. 1986
44	*Alcaligenes eutrophus* H850	98% / 24h at 5uM and 53% / 24h at 25uM	NS	4	Bedard et al. 1986
44	*Rhodococcus sp.* SK-1	31% / 4 days	NS	4	Chang et al. 2013
44	*Rhodococcus sp.* SK-3	30% / 4 days	NS	4	Chang et al. 2013
44	*Aquamicrobium defluvii* SK-2	27% / 4 days	NS	4	Chang et al. 2013
44	*Sphingomonas sp.*	17% / 72h	NS	4	Yong-lei et al. 2011
44	*Pseudomonas sp.*	29% / 72h	NS	4	Yong-lei et al. 2011
45	*Pseudomonas sp.*	44% / 72h	NS	4	Yong-lei et al. 2011
47	*Alcaligenes eutrophus* H850	54% / 24h at 5uM and 5% / 24h at 25uM	NS	4	Bedard et al. 1986
47	*Paenibacillus sp*. KBC101	33% / 3 days	NS	4	Sakai et al. 2004
47	*Rhodococcus sp*. RHA1	83% / 3 days	NS	4	Sakai et al. 2004
47	*Burkholderia xenovorans* LB400T	81% / 3 days	NS	4	Sakai et al. 2004
48	*Stenotrophomonas sp.* JSG1	100% / 48h	NS	4	Ganesh-Kumar et al. 2012
49	*Sphingomonas sp.*	18% / 72h	NS	4	Yong-lei et al. 2011
49	*Pseudomonas sp.*	40% / 72h	NS	4	Yong-lei et al. 2011
52	*Stenotrophomonas sp.* JSG1	100% / 48h	NS	4	Ganesh-Kumar et al. 2012
52	*Corynebacterium sp*. MB1	17% / 24h at 5uM	NS	4	Bedard et al. 1986
52	*Alcaligenes eutrophus* H850	100% / 24h at 5uM and 71% / 24h at 25uM	NS	4	Bedard et al. 1986
52	*Burkholderia sp.* LB400	87% / 22.5h	NS	4	Rein et al. 2007
52	*Paenibacillus sp*. KBC101	72% / 3 days	NS	4	Sakai et al. 2004
52	*Rhodococcus sp.* RHA1	76% / 3 days	NS	4	Sakai et al. 2004
52	*Burkholderia xenovorans* LB400T	100% / 3 days	NS	4	Sakai et al. 2004
52	*Pseudomonas pseudoalcaligenes* KF707	9% / 3 days	NS	4	Sakai et al. 2004
52	*Rhodococcus* sp. SK-1	29% / 4 days	NS	4	Chang et al. 2013
52	*Rhodococcus* sp. SK-3	27% / 4 days	NS	4	Chang et al. 2013
52	*Rhodococcus* sp. SK-4	28% / 4 days	NS	4	Chang et al. 2013
52	*Aquamicrobium defluvii* SK-2	37% / 4 days	NS	4	Chang et al. 2013
52	*Sphingomonas sp.*	17% / 72h	NS	4	Yong-lei et al. 2011
52	*Pseudomonas sp.*	41% / 72h	NS	4	Yong-lei et al. 2011

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
53	*Stenotrophomonas sp.* JSG1	100% / 48h	NS	4	Ganesh-Kumar et al. 2012
56	*Rhodococcus sp*. SK-1	48% / 4 days	NS	4	Chang et al. 2013
56	*Rhodococcus sp.* SK-3	45% / 4 days	NS	4	Chang et al. 2013
56	*Rhodococcus sp.* SK-4	41% / 4 days	NS	4	Chang et al. 2013
56	*Aquamicrobium defluvii* SK-2	25% / 4 days	NS	4	Chang et al. 2013
66	*Corynebacterium sp*. MB1	93% / 24h at 5uM and 64% / 24h at 25uM	NS	4	Bedard et al. 1986
66	*Alcaligenes eutrophus* H850	22% / 24h at 5uM	NS	4	Bedard et al. 1986
66	*Paenibacillus sp*. KBC101	58% / 3 days	NS	4	Sakai et al. 2004
66	*Rhodococcus sp*. RHA1	99% / 3 days	NS	4	Sakai et al. 2004
66	*Burkholderia xenovorans* LB400T	43% / 3 days	NS	4	Sakai et al. 2004
66	*Pseudomonas pseudoalcaligenes* KF707	31% / 3 days	NS	4	Sakai et al. 2004
66	*Rhodococcus sp.* SK-1	36% / 4 days	NS	4	Chang et al. 2013
66	*Rhodococcus sp.* SK-3	37% / 4 days	NS	4	Chang et al. 2013
66	*Rhodococcus sp.* SK-4	33% / 4 days	NS	4	Chang et al. 2013
66	*Aquamicrobium defluvii* SK-2	17% / 4 days	NS	4	Chang et al. 2013
67	*Corynebacterium sp*. MB1	56% / 24h at 5uM and 5% / 24h at 25uM	NS	4	Bedard et al. 1986
67	*Alcaligenes eutrophus* H850	90% / 24h at 5uM and 54% / 24h at 25uM	NS	4	Bedard et al. 1986
67	*Rhodococcus sp.* SK-1	34.5% / 4 days	NS	4	Chang et al. 2013
67	*Rhodococcus sp.* SK-3	32% / 4 days	NS	4	Chang et al. 2013
67	*Rhodococcus sp.* SK-4	36% / 4 days	NS	4	Chang et al. 2013
67	*Aquamicrobium defluvii* SK-2	48% / 4 days	NS	4	Chang et al. 2013
69	*Burkholderia sp.* LB400	26% / 23h	NS	4	Rein et al. 2007
69	*Sphingomonas sp.*	16% / 72h	NS	4	Yong-lei et al. 2011
69	*Pseudomonas sp.*	30% / 72h	NS	4	Yong-lei et al. 2011
70	*Stenotrophomonas sp.* JSG1	61.2% / 48h	NS	4	Ganesh-Kumar et al. 42012
70	*Burkholderia sp.* LB400	57% / 22.1h	NS	4	Rein et al. 2007
70	*Sphingomonas sp.*	13% / 72h	NS	4	Yong-lei et al. 2011
70	*Pseudomonas sp.*	20% / 72h	NS	4	Yong-lei et al. 2011
71	*Rhodococcus sp.* SK-1	57% / 4 days	NS	4	Chang et al. 2013
71	*Rhodococcus sp.* SK-3	51% / 4 days	NS	4	Chang et al. 2013
71	*Rhodococcus sp.* SK-4	54% / 4 days	NS	4	Chang et al. 2013
71	*Aquamicrobium defluvii* SK-2	56% / 4 days	NS	4	Chang et al. 2013

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
74	*Stenotrophomonas sp.* JSG1	100% / 48h	NS	4	Ganesh-Kumar et al. 2012
74	*Rhodococcus sp*. SK-1	45.2% / 4 days	NS	4	Chang et al. 2013
74	*Rhodococcus sp*. SK-3	41% / 4 days	NS	4	Chang et al. 2013
74	*Rhodococcus sp*. SK-4	40% / 4 days	NS	4	Chang et al. 2013
74	*Aquamicrobium defluvii* SK-2	20.3% / 4 days	NS	4	Chang et al. 2013
74	*Pseudomonas sp.*	26% / 72h	NS	4	Yong-lei et al. 2011
75	*Sphingomonas sp.*	14% / 72h	NS	4	Yong-lei et al. 2011
75	*Pseudomonas sp.*	38% / 72h	NS	4	Yong-lei et al. 2011
77	*Corynebacterium sp*. MB1	21% / 24h at 5uM	NS	4	Bedard et al. 1986
77	*Paenibacillus sp*. KBC101	56% / 3 days	NS	4	Sakai et al. 2004
77	*Burkholderia xenovorans* LB400T	6% / 3 days	NS	4	Sakai et al. 2004
82	*Stenotrophomonas sp.* JSG1	35.6% / 48h	NS	5	Ganesh-Kumar et al. 2012
82	*Aquamicrobium defluvii* SK-2	17% / 4 days	NS	5	Chang et al. 2013
85	*Stenotrophomonas sp.* JSG1	21.4% / 48h	NS	5	Ganesh-Kumar et al. 2012
86	*Corynebacterium sp.* MB1	98% / 24h at 5uM and 58% / 24h at 25uM	NS	5	Bedard et al. 1986
86	*Alcaligenes eutrophus* H850	46% / 24h at 5uM	NS	5	Bedard et al. 1986
87	*Alcaligenes eutrophus* H850	59% / 24h at 5uM	NS	5	Bedard et al. 1986
87	*Aquamicrobium defluvii* SK-2	15% / 4 days	NS	5	Chang et al. 2013
91	*Stenotrophomonas sp.* JSG1	23.7% / 48h	NS	5	Ganesh-Kumar et al. 2012
92	*Stenotrophomonas sp.* JSG1	2.3% / 48h	NS	5	Ganesh-Kumar et al. 2012
99	*Stenotrophomonas sp.* JSG1	2.5% / 48h	NS	5	Ganesh-Kumar et al. 2012
99	*Rhodococcus sp*. SK-1	22% / 4 days	NS	5	Chang et al. 2013
99	*Rhodococcus sp*. SK-3	20% / 4 days	NS	5	Chang et al. 2013
99	*Rhodococcus sp*. SK-4	23% / 4 days	NS	5	Chang et al. 2013
99	*Aquamicrobium defluvii* SK-2	11.7% / 4 days	NS	5	Chang et al. 2013
101	*Corynebacterium sp*. MB1	9% / 24h at 5uM	NS	5	Bedard et al. 1986
101	*Alcaligenes eutrophus* H850	70% / 24h at 5uM and 30% / 24h at 25uM	NS	5	Bedard et al. 1986
101	*Burkholderia sp.* LB400	47% / 21.9h	NS	5	Rein et al. 2007
101	*Paenibacillus sp*. KBC101	58% / 3 days	NS	5	Sakai et al. 2004

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
101	*Rhodococcus sp.* RHA1	29% / 3 days	NS	5	Sakai et al. 2004
101	*Burkholderia xenovorans* LB400T	100% / 3 days	NS	5	Sakai et al. 2004
110	*Rhodococcus sp.* SK-1	25% / 4 days	NS	5	Chang et al. 2013
110	*Rhodococcus sp.* SK-3	23% / 4 days	NS	5	Chang et al. 2013
110	*Rhodococcus sp.* SK-4	25% / 4 days	NS	5	Chang et al. 2013
110	*Aquamicrobium defluvii* SK-2	12% / 4 days	NS	5	Chang et al. 2013
129	*Stenotrophomonas sp.* JSG1	1.9% / 48h	NS	6	Ganesh-Kumar et al. 2012
138	*Rhodococcus sp.* SK-1	21.2% / 4 days	NS	6	Chang et al. 2013
138	*Rhodococcus sp.* SK-3	22% / 4 days	NS	6	Chang et al. 2013
138	*Rhodococcus sp.* SK-4	21% / 4 days	NS	6	Chang et al. 2013
138	*Aquamicrobium defluvii* SK-2	34% / 4 days	NS	6	Chang et al. 2013
141	*Stenotrophomonas sp.* JSG1	18.4% / 48h	NS	6	Ganesh-Kumar et al. 2012
141	*Stenotrophomonas sp.* JSG1	2.0% / 48h	NS	6	Ganesh-Kumar et al. 2012
146	*Aquamicrobium defluvii* SK-2	21.9% / 4 days	NS	6	Chang et al. 2013
147	*Rhodococcus sp.* SK-1	22% / 4 days	NS	6	Chang et al. 2013
147	*Rhodococcus sp.* SK-3	24% / 4 days	NS	6	Chang et al. 2013
147	*Rhodococcus sp.* SK-4	22% / 4 days	NS	6	Chang et al. 2013
147	*Aquamicrobium defluvii* SK-2	18.7% / 4 days	NS	6	Chang et al. 2013
151	*Stenotrophomonas sp.* JSG1	12.3% / 48h	NS	6	Ganesh-Kumar et al. 2012
153	*Stenotrophomonas sp.* JSG1	3.5% / 48h	NS	6	Ganesh-Kumar et al. 2012
153	*Alcaligenes eutrophus* H850	16% / 24h at 5uM	NS	6	Bedard et al. 1986
153	*Paenibacillus sp.* KBC101	11% / 3 days	NS	6	Sakai et al. 2004
153	*Burkholderia xenovorans* LB400T	41% / 3 days	NS	6	Sakai et al. 2004
153	*Rhodococcus sp.* SK-1	18.5% / 4 days	NS	6	Chang et al. 2013
153	*Rhodococcus sp.* SK-3	19% / 4 days	NS	6	Chang et al. 2013
153	*Rhodococcus sp.* SK-4	19% / 4 days	NS	6	Chang et al. 2013
153	*Aquamicrobium defluvii* SK-2	31.8% / 4 days	NS	6	Chang et al. 2013
173	*Rhodococcus sp.* SK-1	16% / 4 days	NS	7	Chang et al. 2013
173	*Rhodococcus sp.* SK-3	18% / 4 days	NS	7	Chang et al. 2013
179	*Rhodococcus sp.* SK-1	30.5% / 4 days	NS	7	Chang et al. 2013
179	*Rhodococcus sp.* SK-3	32% / 4 days	NS	7	Chang et al. 2013
179	*Rhodococcus sp.* SK-4	29% / 4 days	NS	7	Chang et al. 2013
179	*Aquamicrobium defluvii* SK-2	17% / 4 days	NS	7	Chang et al. 2013
187	*Rhodococcus sp.* SK-1	27% / 4 days	NS	7	Chang et al. 2013

Table 5.3. PCB-degrading bacterial species

PCB congener #	Microorganism Involved in Degradation	Observed Rate of Degradation	Metabolites	Number of Chlorines	Reference
187	*Rhodococcus sp.* SK-3	26% / 4 days	NS	7	Chang et al. 2013
187	*Rhodococcus sp.* SK-4	25% / 4 days	NS	7	Chang et al. 2013
187	*Aquamicrobium defluvii* SK-2	17% / 4 days	NS	7	Chang et al. 2013
108, 142	*Stenotrophomonas sp.* JSG1	10.3% / 48h	NS	5,6	Ganesh-Kumar et al. 2012
118, 134	*Stenotrophomonas sp.* JSG1	27.8% / 48h	NS	5,6	Ganesh-Kumar et al. 2012
120, 148	*Stenotrophomonas sp.* JSG1	5.8% / 48h	NS	5,6	Ganesh-Kumar et al. 2012
123, 142	*Stenotrophomonas sp.* JSG1	1.5% / 48h	NS	5,6	Ganesh-Kumar et al. 2012
138, 160	*Stenotrophomonas sp.* JSG1	2.8% / 48h	NS	6,6	Ganesh-Kumar et al. 2012
15, 18	*Sphingomonas sp.*	44% / 72h	NS	2,3	Yong-lei et al. 2011
15, 18	*Pseudomonas sp.*	74% / 72h	NS	2,3	Yong-lei et al. 2011
16, 32	*Sphingomonas sp.*	32% / 72h	NS	2,3	Yong-lei et al. 2011
16, 32	*Pseudomonas sp.*	64% / 72h	NS	3,4	Yong-lei et al. 2011
22, 51	*Sphingomonas sp.*	28% / 72h	NS	3,4	Yong-lei et al. 2011
22, 51	*Pseudomonas sp.*	51% / 72h	NS	3,4	Yong-lei et al. 2011
25, 26	*Sphingomonas sp.*	29% / 72h	NS	3,3	Yong-lei et al. 2011
25, 26	*Pseudomonas sp.*	63% / 72h	NS	3,3	Yong-lei et al. 2011
28, 31	*Sphingomonas sp.*	59% / 72h	NS	3,3	Yong-lei et al. 2011
28, 31	*Pseudomonas sp.*	64% / 72h	NS	3,3	Yong-lei et al. 2011
33, 53	*Sphingomonas sp.*	47% / 72h	NS	3,3	Yong-lei et al. 2011
33, 53	*Pseudomonas sp.*	43% / 72h	NS	3,4	Yong-lei et al. 2011
37, 42	*Sphingomonas sp.*	19% / 72h	NS	3,4	Yong-lei et al. 2011
37, 42	*Pseudomonas sp.*	28% / 72h	NS	3,4	Yong-lei et al. 2011
4 and 10	*Sphingomonas sp.*	64% / 72h	NS	2,2	Yong-lei et al. 2011
4 and 10	*Pseudomonas sp.*	90% / 72h	NS	2,2	Yong-lei et al. 2011
42, 64	*Stenotrophomonas sp.* JSG1	50.2% / 48h	NS	3,4	Ganesh-Kumar et al. 2012
56, 60	*Stenotrophomonas sp.* JSG1	40.1% / 48h	NS	4,4	Ganesh-Kumar et al. 2012
60, 93	*Stenotrophomonas sp.* JSG1	1.0% / 48h	NS	4,5	Ganesh-Kumar et al. 2012
77, 109	*Stenotrophomonas sp.* JSG1	1.1% / 48h	NS	4,5	Ganesh-Kumar et al. 2012
87, 101	*Stenotrophomonas sp.* JSG1	55.9% / 48h	NS	4,5	Ganesh-Kumar et al. 2012

Notes: NS: Not stated

Physical and chemical factors can dictate the rate of dechlorination by limiting the microbial growth or the ability of the microbes to uptake and react with the PCBs. PCB concentration is one such factor. The optimum PCB concentration range for dechlorination is between 200 to 1000 ppm (w/w) in sediment (Quensen et al., 1988). Below 50 ppm dechlorination is reported to be halted (Tiedje et al., 1993). Bioavailability of PCBs in the environmental matrix is also a key factor. If PCBs are dissolved in organic phases within the soil or covered by a layer of organic matter the microbes or extracellular enzymes cannot easily access them. Temperature and climate can also affect PCB biodegradation rates. For example, dechlorination of a lower chlorinated PCB mixture (Aroclor 1242) was shown to occur at 25° C, but not at 37° C (Bedard & Wiegel, 1996). Effects of temperature are also apparent in the studies on Woods Pond sediment (Wu et al., 1996, 1997a, 1997b). The temperature that the microcosm was held at (between 4° and 66°C) dictated which pathway the microbes used to dechlorinate the PCB mixture.

Nutrients are also necessary for microbes to thrive. If a population is starved of electron acceptors or carbon sources it will slow the rate of biodegradation. It has been shown that adding carbon sources to anaerobic soil can increase the rate of dechlorination (Nies & Vogel, 1990). Nitrogen and phosphorous are also essential nutrients for bacteria, and are used for both biomass production and metabolism (Thirukkumaran & Parkinson, 2000).

Finally, inhibitors and other contaminants should be considered as potential rate limiting agents. Metals, oils, grease, and solvents are sometimes toxic to microbial populations and may have an effect on their ability to dechlorinate (Tiedje et al., 1993). It has been found that high concentrations of oil and grease are associated with lower dechlorination rates (Tiedje et al., 1993).

5.3.2. Aerobic Pathways of Bacterial PCB Biodegradation

Following anaerobic dechlorination, aerobic bacteria can break down some of the remaining dechlorinated or lightly chlorinated biphenyls. Reported aerobic pathways all start with biphenyl 2,3-dioxygenases (Pieper, 2004). This is known as the upper, or bph pathway. This pathway may be initiated on PCBs that contain low amounts of chlorine, but this depends on the degree and position of chlorination as well as the specificity of these 2,3-dioxygenases (Pieper, 2004). See **Figure 5.5** for the general pathway of unchlorinated biphenyl biodegradation. This pathway can also be used to degrade lightly chlorinated (1-2 chlorines) PCBs (Pieper, 2004). Although the bacteria possess and use this family of enzymes, PCBs are rarely used as carbon or energy sources themselves. Their degradation is usually part of some cometabolism process, and therefore the upper pathway often produces metabolites that are dead-end products (Bedard & Haberl, 1990; Furukawa et al., 1979; and Seeger et al., 1997). The upper pathway remains largely the same for the catabolism of biphenyl and some lower chlorinated biphenyls. For a visual representation of the general pathway for the degradation of biphenyl see **Figure 5.6** below. Many of the bacteria that express these enzymes have beer sequenced, so bacteria that possess the genes for these enzymes can be identified via genetic analysis (Pieper, 2004). The enzyme BphB, which belongs to a family of broad substrate enzymes that are common in degradation of aromatic compounds, is essential to this pathway (Pieper, 2004).

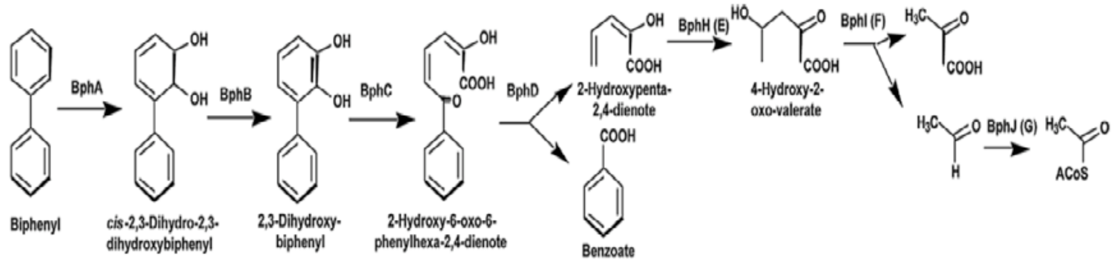


Figure 5.6. General pathway for aerobic biodegradation of non-chlorinated biphenyls (Pieper, 2004)**.**

The lower pathways include the degradation of 2-hydroxypenta-2,4-dienoates and benzoates. The pathway produces acetaldehyde and pyruvic acid (Pieper & Seeger, 2008). Both of these products are commonly used as carbon and energy sources by aerobic bacteria. Benzoate readily biodegrades under most aerobic conditions (Wibbertmann, Kielhorn, Koennecker, Mangelsdorf, & Melber, 2005). However, if it is chlorinated at this time other pathways may be used to degrade it further.

Although some bacterial species have been observed using these enzymes to degrade lower chlorinated biphenyls, it is not always possible. Some of the enzymes in the pathway may not be able to use the chlorinated product of the previous reaction, creating "dead-end" products including dihydrodiols (Brühlmann & Chen, 1999), dihydroxybiphenyls (including 3,4-dihydroxylated derivatives) (Tříska et al., 2004), or chlorinated HOPDAs (Furukawa et al., 1979; Seeger et al., 1997). Although it is difficult, it is not impossible for these to be degraded. It is rare for bactera to express all the correct variations of these enzymes to be able to degrade a lower chlorinated PCB all the way via this pathway (Pieper, 2004 and Seeger et al., 1997).

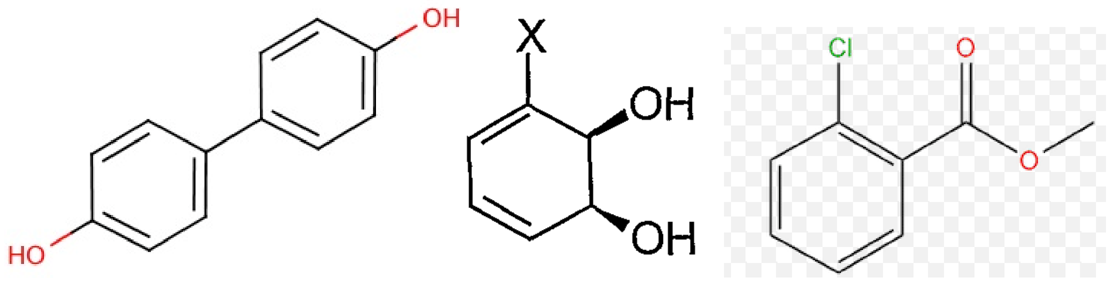


Figure 5.7. Common dead-end product structures. From left to right, the general structures of dihydroxybiphenyl, dihydrodiol, and 2-chlorobenzoate (note that the methyl group on one of the oxygens could be any R group).

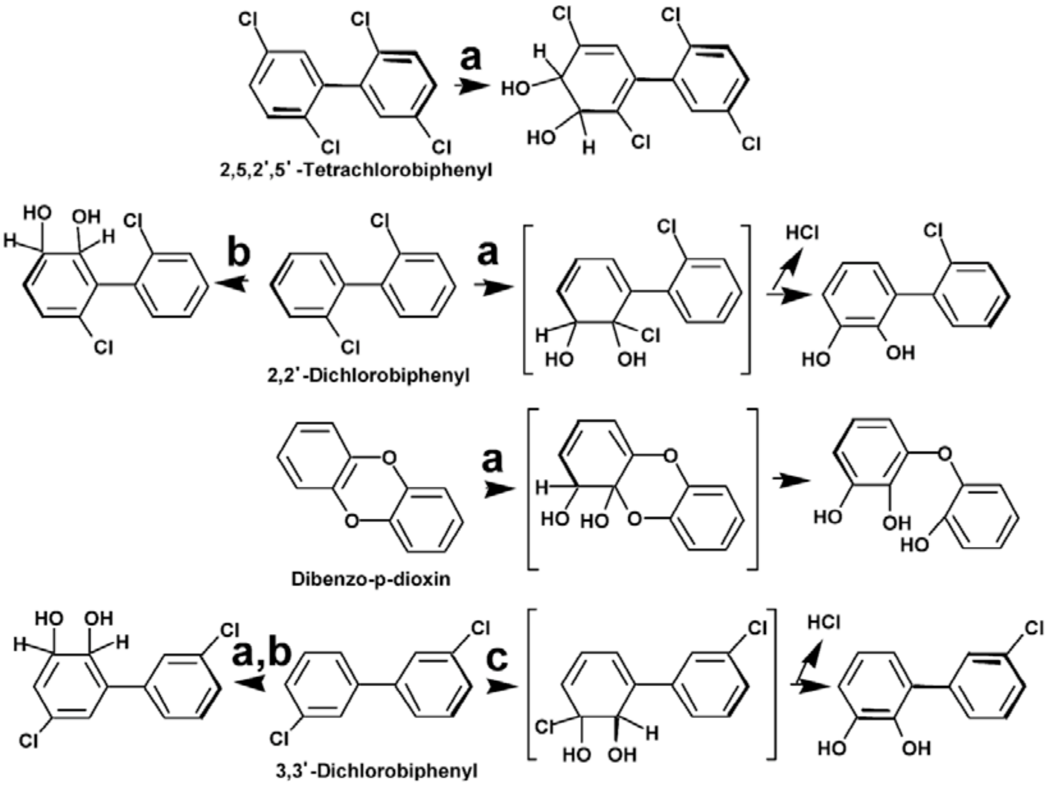


Figure 5.8. Aerobic degradation pathways of selected lower chlorine PCB congeners and dibenzo 2,3-p-dioxin by biphenyl 2,3-dioxygenases found in (a) Burkholderia sp. LB400 (Haddock & Gibson, 1995)**, (b) Pseudomonas pseudoalcaligenes KF707** (Suenaga et al., 2002) **and (c) Phe227Val and Phe377Ala mutants of KF707 dioxygenase** (Suenaga et al., 2002)**. Unstable intermediates are in brackets. (Figure from** (Pieper, 2004)**.**

The "dead-end" products that often result from these reactions can sometimes be degraded via other pathways. One of the most common products of the lower pathways, chlorobenzoate, has been shown to be degradable by multiple species of bacteria through several pathways. These bacteria can be classified into 2 groups: those capable of biodegrading 2-chlorobenzoate (Engesser & Schulte, 1989; Fetzner et al., 1989; Higson & Focht, 1990; Suzuki et al., 2001) and those also capable of degrading either 2,4-dichloro- or 2,5-dichlorobenzoate (Hernandez et al., 1991; Hickey & Focht, 1990; Kozlovsky et al., 1993; Pavlů et al., 1999; Romanov et al., 1993). All these pathways include the catalysis of 1,2-dioxygenation of 2-chlorobenzoate resulting in one of the vic-hydroxyl groups in the cis-dihydrodiol being bound to the same carbon as the chlorine substituent. Note that not all the final products may be readily biodegradable.

5.4. Fungal Biodegradation of PCBs

Several studies have shown that lignolytic fungi can be efficient degraders of a wide range of PCB congeners (Čvančarová et al., 2012; Eaton, 1985; Novotný et al., 2004; Yin et al., 2011). One study showed that these fungi can effectively degrade PCB congeners that have between 1 and 6 chlorines (Čvančarová et al., 2012). By far the most commonly studied lignolytic fungi for biodegradation of PCBs (as well as many other contaminants) is white-rot fungi (*Phanerochaete* sp.). Some pathways for PCB biodegradation by this fungi have been proposed by Čvančarová et al., 2012 (**Figure 5.9**). The degradation products (determined by GC-MS) revealed that the various PCB congeners were most likely being transformed through a reductive pathway, producing chlorobenzoates (Čvančarová et al., 2012). As stated above, these chlorobenzoates are difficult for bacteria to breakdown, and thus are often "dead-end" products of bacterial biodegradation of PCBs (Pieper, 2004). However, lignolytic fungi can degrade these further to produce even less toxic products (Čvančarová et al., 2012). The key to the fungi's success is low substrate specificity extracellular enzymes that they secrete.

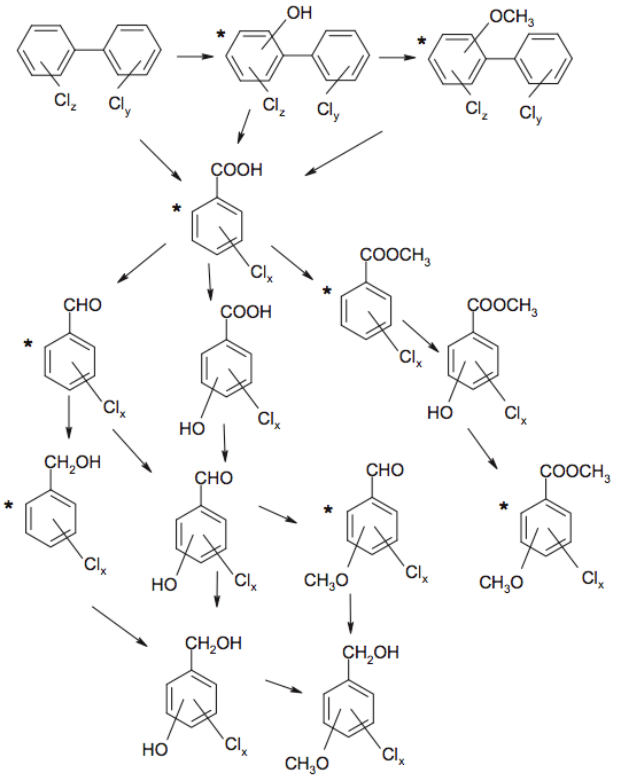


Figure 5.9. Proposed metabolic pathway of PCB degradation by ligninolytic fungi. Structures marked with an asterisk were detected with GC-MS after metabolism by fungi. Hydroxylated chlorobenzaldehyde was not detected and is only hypothesized (Čvančarová et al., 2012).

There are 4 key groups of enzymes used to mediate PCB biodegradation by fungi: lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidases (VP) and laccases, which belong to the phenol oxidase family (Čvančarová et al., 2012). These enzymes are non-specific enough that they will react with a wide range of congeners, in great contrast to bacterial PCB degradation. Several studies suggest reducing PCB concentrations can improve the rate of degradation (about 200 ppm for dichloro congeners, and 1 ppm for hexachloro congeners) (Yin et al., 2011). *Phanerochaete chrysosporium*, *Trametes versicolor* and *Pleurotus ostreatus* are some of the most common lignolytic fungal degraders reported for PCBs (Novotný et al., 2004).

5.5. Reported Natural Attenuation Rates of PCBs

The complex biodegradation pathways of PCBs make it difficult to model their biodegradation rate. The rate is very dependent on the microbial population dynamics and soil conditions, both of which could change over time. Because of this, most PCB biodegradation rates have been determined in laboratory experiments, and expected field rates are extrapolated from there. However, laboratory experiments often overestimate biodegradation rates in the field because laboratory studies often use freshly spiked contaminants which are more bioavailable than weathered contaminants which are sequestered in the soil matrix.

Some published PCB natural attenuation rates are listed in **Table 5.4**. PCB contamination of river sediments has been the focus of most bioremediation studies because of large historical spills in aquatic environments, most notably the Hudson River. While rates of PCB natural attenuation for river sediments are shown in Table 5.4, it should be noted that microbial populations and metabolism in an aquatic environment are expected to differ greatly from those in soil. To provide an analysis of published data most pertinent to SSFL, we focus here on studies done with soils rather than aquatic sediments.

A microcosm study done by Viisimaa et al. (2013) is the most recent PCB study conducted with soil rather than river sediments. They used natural topsoil in microcosms spiked with a mixture of PCBs (commercially known as Clophen A30, which is 19.73% of dichloro-, 48.33% trichloro-, 24.55% tetrachloro-, 6.21% pentachloro- and 1.18% of hexachloro- biphenyls). A 27% reduction in PCB concentrations was observed in just 42 days via natural attenuation (Table 5.4). Larger reductions were observed with biosurfactant and hydrogen peroxide additions (see biostimulation section below). These additional factors combined with the natural microflora resulted in 47-50% removal in 42 days (Viisimaa et al., 2013). However, the PCB mixture used in this experiment was far less chlorinated than the PCB mixtures in the soil at SSFL, and therefore lower biodegradation rates would be expected at SSFL.

In another soil microcosm study, Krumins et al. (2009) observed biodegradation of a pure PCB congener (PCB 116: pentachlorobiphenyl, with all chlorines on one phenyl group) over a much longer duration of 415 days. About 10% of this highly-chlorinated congener was biodegraded after 415 days. Krumins et al. also noted that bioaugmentation or biostimulation with halogenated electron acceptors (haloprimers) increased biodegradation rates significantly. Halopriming alone, with tetrachlorobenzene (TeCB, 25 µM), pentachloronitrobenzene (PCNB, 25 µM), or 2,3,4,5,6-PCB (PCB116, 2.0 µM) resulted in first-order biodegradation rate constants of .007 yr^{-1}, .077 yr^{-1} , and .004 yr^{-1} respectively. PCNB microcosms bioaugmented with *Dehalococcoides ethenogenes* strain 195 at 3×10^6 cells/mL resulted in a rate constant of .069 yr^{-1}. Based on this study, natural attenuation conditions without halopriming or bioaugmentation would be expected to result in very slow biodegradation.

Table 5.4. Biodegradation Rates of PCBs under Natural Attenuation Conditions.

Compound	Matrix	Lab/Field (L/F)	Sediment PCB Concentration (nM)		Length of Study (days)	Biodegradation Kinetics		Reference
			Initial	Final		Percent Degraded	Degradation Rate (mg/kg/day)	
2,3,4,2',4',5'-hexaCBP	river sediment	L	2250	608	450	73	4	Rhee et al., 1993
2,3,4,5,6-penta CBP	river sediment	L	1850	925	450	50	2	Rhee et al., 1993
3,4,3'4' CBP	river sediment	L	--	--	450	0	--	Rhee et al., 1993
2,4,5,2',4',5'-CBP	river sediment	L	525	525	600	0	0	Rhee et al., 1993
2,4,2',4', CBPs	river sediment	L	--	--	450	0	--	Rhee et al., 1993
--			**Soil PCB Concentration (mg/kg)**		--			
PCB mixture	sediment	F	39.4	22.9	73	42	0.23	Harkness et al., 1993
Aroclor 1260	soil	L	264.6	--	200	--	0.008 mol%/day	Kjellerup et al., 2012
PCB mixture	soil	L	2.1	1.9	415	*10*	--	Krumins et al., 2009
Clophen A30	soil	L	52000	37960	42	*27*	*334*	Viisimaa et al., 2013

Notes

[1] Initial and final concentrations estimated from graphs and calculated based on percent degradation, respectively, provided in article.

[2] Initial and final concentrations not provided in article.

[3] Each collected soil sample contained a varying amount of PCBs; the most representative sample was cited here.

[4] Actual percentage removal of PCBs for unamended soils was not provided in article

A third soil microcosm study with potential relevance to SSFL was performed by Kjellerup et al. (2012) to measure the natural attenuation rates of Aroclor 1260 in soil. In this study anerobic dechlorinators were isolated from soil collected from contaminated sites (all prior studies isolated bacteria from anaerobic aquatic sediments rather than soil). The populations of dechlorinating microorganisms found in the soil samples were 2-5 orders of magnitude lower than the number reported for various sediment samples. Dechlorination rates were also lower in soil versus aquatic sediment, even after correcting for microbial population. This is likely due to more favorable localized environmental conditions for bacterial dechlorination in aquatic environments compared to soil. In the Kjellerup et al. soil study, 13 out of 19 samples showed no dechlorination after 200 days, and dechlorinating microbes were detected in these samples using 16S rRNA sequencing. The highest measured rate was .00693 mol%/day and the lowest measurable rate was .00093 mol%/day.

5.6. Biostimulation of PCB Biodegradation

Biological and chemical surfactants are promising remedial amendments for PCB-contaminated soils because they can increase PCB bioavailability. In Viisimaa et al's 2013 study, biosurfactant amendment reduced concentrations of PCB congeners with 6 to 9 chlorines by 10-20%. Federici (2012) observed no significant change in overall PCB concentrations with soybean oil. However, the biosurfactant soya lecithin, resulted in 40% degradation of PCBs in one year in Federici's 2012 study.

Halopriming, a method by which halogenated compounds are added to already contaminated soils, has been shown to improve bioremediation of PCBs (Krumins et al., 2009). For example, the addition of penta-chloro-nitro-benzene to PCB-contaminated soils facilitated a 20% increase in the concentrations of lesser-chlorinated congeners after 415 days of incubation, indicating dechlorination of more chlorinated PCBs.

A review of the literature indicates mixed results regarding the success of nutrient supplementation on PCB degradation. One study showed that nutrient addition effectively enhances biodegradation of lightly chlorinated PCBs (Harkness et al., 1993), while another stated that adequate nutrients were available at the site and added nutrients produced no significant elevated PCB degradation rates (Sudjarid et al., 2012).

The literature review suggests that bulking agents have not been adequately studied as a PCB-contaminated soil remediation technique.

5.7. Bioaugmentation of PCB Degradation

In-situ natural attenuation of PCB-contaminated soils has had unreliable success in many studies (Bedard & May, 1996; Bedard et al., 2005; and Bedard et al., 1998), but a few promising studies indicate that it may be possible through bioaugmentation with dehalogenating organisms. Bedard et al. (2006) indicated that a critical mass of cells was required to initiate reductive dechlorination of Aroclor 1260 and proposed that low populations of dehalorespiring bacteria might be the root cause of insubstantial PCB degradation in the environment. Another study conducted by Krumins et al. (2009) indicated that bioaugmentation with *Delalococcoides ethenogenes* and stimulation with haloprimers penta-chloronitrobenzene and tetrachlorobenzene effectively accelerated PCB degradation, while stimulation with electron donors did not. Augmentation with *D. chlorocoercia* DF1 and *D. chlorocoercia* resulted in 56% by mass reduction of Aroclor 1260 initially at 1.3 ppm (Payne et al., 2011). This study was promising in that both organisms were sustained within the local microflora population for 120 days after initial inoculation, meaning that initial bioaugmentation may lead to self-sustaining populations of dechlorinating bacteria. Another study indicated that the collaborated use of the bacteria *Sphingomonas sp.* and *Pseudomonas sp.* increased degradation rates of PCBs to exceed those of the organisms individually, and degradation of more highly chlorinated PCBs was enhanced (Yong-lei et al. 2011).

Bioaugmentation with fungi is promising because of the wide range of chlorinated substrates metabolized by the lignolytic enzymes they secrete. *Pleurotus ostreatus*, a species of white-rot fungus, was found to facilitate PCB biodegradation PCBs in a study conducted by Zeddel *et al.* (1993). In this study, a PCB-congener mixture of primarily tri- and tetra-chlorinated biphenyls at 2500 ppm was degraded more than 95% after five weeks. In this same study, penta- and hexa-chlorobiphenyls were found to be degradable by 50%. Only 2,2',4,2',5,5'-hexachlorobiphenyl was resistant to biodegradation. This study indicated that *Phanerochaete chrysosporium*, another species of white-rot fungi, was unable to degrade any PCBs except mono- and di-chlorobiphenyl at atmospheric oxygen levels.

5.8. Phytoremediation of PCBs

Both phytoextraction and rhizoremediation have been shown to be operative mechanisms of remediation of PCBs in soil (Aken et al., 2010). Alfalfa and other plant species have been shown by several studies to extract PCBs from soil (Ficko et al., 2010; Greenwood et al., 2011; Liu & Schnoor, 2008; Xu et al., 2010; Teng et al., 2010; Zeeb et al., 2006). However, PCB degradation within the plant does not appear to be the primary mechanism of phytoremediation. Several studies have suggested that rhizoremediation was the primary mechanism of PCB degradation at specific field sites, and in these studies stimulated enzyme activity and increased microbial populations in the root zone supported this hypothesis (Chekol et al., 2004; Y. Li et al., 2013; Xu et al., 2010; and Teng et al., 2010). Rhizodegradation of PCBs may be stimulated by plants in four ways: (1) Root exudates contain compounds readily available for bacterial metabolism, (2) Plants increase the rhizosphere oxygen content, (3) Plants produce pollutant analogs, and (4) Root exudates contain general growth-promoting factors (Campanella et al., 2002). When plants stimulate the rhizosphere in this way the removal of PCBs from the soil can be enhanced.

Field and lab studies reported varying phytoremediation rates for PCBs, with rates ranging from 8.1% removal in 180 days to 77% removal in 122 days. The type of PCB congener, the plant species, and the PCB concentration impact rates of removal. Given the observed rates of removal cited in the literature, phytoremediation for PCBs at a soil concentration of 10 ppm could be effective on a time scale of 2 to 50 years assuming first order kinetics and a final concentration of 0.017ppm (background). This is a very wide range of estimates, so it would be helpful to conduct greenhouse experiments with SSFL soils and plants from the site to narrow down this estimate.

5.9. Other PCB Degradation Pathways

Some aerobic bacteria, as well as birds and mammals, can transform PCBs to various hydroxylated PCBs (Abramowicz, 1995; Connor et al., 1997). The main transformation that occurs with these hydroxylated PCBs is monohydroxylation by non-specific oxidases (Sinjari et al., 1998). These hydroxylated PCBs have estrogenic or anti-estrogenic activities in humans, similar to that of some PCBs (Abramowicz, 1995). These compounds are fairly stable and are therefore excreted by the organism (Kim & Rhee, 1997), and they have been shown to be biodegradable by some organisms (Utkin et al., 1994). It is thought the mechanism used to dechlorinate these compounds in these higher animals is not the same as the bacterial mechanisms of PCB dechlorination (Wiegel & Wu, 2000).

5.10. Potential for Natural Attenuation of PCBs at SSFL

Based on published studies, it is unlikely that abiotic weathering processes such as volatilization or photooxidation will have any significant effect on PCB concentrations in the soils at SSFL. Contaminants at SSFL are already highly weathered, so it is likely that any volatile fractions or surface concentrations have already been subjected to these processes.

Biological PCB degradation under unamended natural conditions at the SSFL site is also unlikely to meet clean up goals, because several factors are likely to severely limit biodegradation of PCBs under natural attenuation conditions. The inherent recalcitrance of the higher chlorinated PCBs identified at SSFL would suggest very low biodegradation rates, even under ideal conditions. The most common congener mixture reported at SSFL is Aroclor 1260, which is highly chlorinated. The two published soil microcosm studies with highly chlorinated PCBs described above indicated very slow biodegradation rates without biostimulation or bioaugmentation. Additionally, soil conditions at SSFL are far from ideal, as the highly weathered contaminants are likely to be sequestered in the soil matrix with low bioavailability. This sequestration problem could potentially be diminished through the use of surfactants (see below).

Another important limitation of bacterial biodegradation of PCBs at SSFL may be the lack of anaerobic soil conditions at the site. Unless anaerobic conditions are present in soil microenvironments, bacterial dechlorination would not be expected. Anaerobic conditions are very unlikely in the surface of the unsaturated soil that is exposed to oxygen, and this region is likely where most of the PCB contamination is located. It is therefore important to survey the soils at SSFL to determine if anaerobic conditions exist. Field testing is planned using soil vapor analyses to provide an estimate of the redox conditions in soils at SSFL. If anaerobic conditions are absent in Area IV soils at SSFL, then fungal biodegradation would be more likely than anaerobic dechlorination.

Lignolytic fungi are typically not present in large enough populations to affect degradation rates. These fungi gain an advantage over other microbes in high lignin environments because most bacteria and fungi cannot use lignin as a carbon source. They tend to grow on dead trees and rotting wood (Tortella et al., 2005). They are likely to be present at SSFL, but not in a large population throughout the soil. Therefore they are not likely to significantly impact natural attenuation of PCBs at SSFL without biostimulation and/or bioaugmentation.

Another problem with bioremediation of PCBs is that the biodegradation process is often incomplete, leading to the production of residual compounds. For example, 2,3,4,6-biphenyl and 2,3,5,6- biphenyl are common residuals from the biodegradation of Aroclor 1260 (Kjellerup et al., 2012), which is similar to the PCB congener mixture at SSFL.

The three microcosm studies with soils described above all reported extremely low biodegradation rates for unamended soil, which would represent natural attenuation conditions. These studies did show some promise for active bioremediation using biostimulation and/or bioaugmentation, which could be applied at SSFL. The most effective biostimulation method for SSFL would likely be addition of surfactants as this could help overcome the problem of contaminant sequestration. Nutrient addition could improve biodegradation rates, but only if the soils at SSFL are nutrient limited. For this reason, assessment of nutrient availability at SSFL is needed to determine whether or not this type of biostimulation would enhance remediation of PCBs. Use of hydrogen peroxide may be useful for pretreatment of PCBs, and biostimulation with haloprimers such as TeCB or PCNB are likely to increase dechlorination rates, but addition of additional contaminants to the site might be perceived negatively. The most promising bioaugmentation method would be to introduce fungi, such as white-rot fungi, because these species do not require anaerobic soil conditions like the dechlorinating bacteria. Fungi are likely to be more suited to soils than to the aquatic sediments most researchers have studied because the lower water tension in soil is more favorable for fungal growth than that of aquatic environments. A companion microcosm study is planned to determine the potential efficacy of these biostimulation and bioaugmentation strategies for the SSFL soils in Area IV.

The potential for natural attenuation of PCBs at the site could be better predicted if the PCB congeners in the soil contamination are determined. By examining the degree of chlorination, some estimation of historical dechlorination could be made. These analyses are planned for early 2014.

While bioaugmentation and biostimulation show some promise for improving PCB biodegradation rates, it is unlikely that even active bioremediation will provide enough reduction in contaminant concentrations to meet the strict clean-up goals for this site. The clean-up goals at SSFL call for reducing PCB concentrations to background levels ranging from 17 to 50 µg/kg (ppb) (**Table 5.5**). These concentrations are orders of magnitude lower than those used in the microcosm studies described above in Table 5.9 and used as a basis for determining the feasibility of PCB biodegradation at SSFL. In fact one study suggested that a minimum PCB concentration of 50 ppm is required to initiate PCB biodegradation, and that below this threshold PCB dechlorination is not likely (J. M. Tiedje et al., 1993).

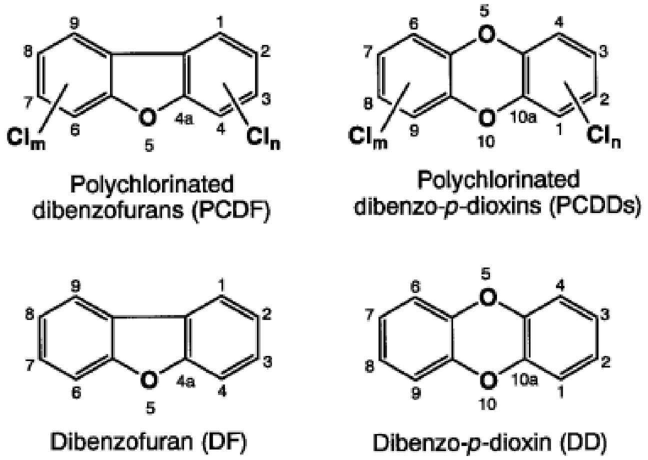
Table 5.5. Background PCB concentrations promulgated as clean-up goals for SSFL Area IV (DTSC).

PCB Mixture	Target Background Concentration µg/kg (ppb)
Aroclor 1016	17
Aroclor 1221	33
Aroclor 1232	17
Aroclor 1262	33
Aroclor 1254	17
Aroclor 1260	17
Aroclor 1268	33
Aroclor 1242	17
Aroclor 1248	17
Aroclor 5432	50
Aroclor 5442	50
Aroclor 5460	50

6.0 Dioxins (polychlorinated dibenzo-*p*-dioxins and dibenzofurans)

6.1. Physical Properties and Toxicity of Dioxins:

Chlorinated dioxins are tricyclic compounds comprised of two families: dibenzo-p-dioxins and dibenzofurans (**Figure 6.1**). Polychlorinated dibenzo-*p*-dioxins (PCDD) have a backbone of two benzene rings connected with two ether linkages and have chlorination possible at 10 different sites, resulting in 75 possible congeners. Polychlorinated dibenzofurans (PCDF) are connected with only one ether linkage and have 9 possible chlorination sites, resulting in 135 possible congeners (because of the reduced symmetry of the PCDF backbone, there are more unique congeners than for PCDD). There are 17 PCDDs having a 2,3,7,8 substitution of chlorination, which results in the most toxicity to higher organisms (Van Den Berg et al., 2013 and Boening, 1998). These are the compounds of greatest concern in the environment.



Polychlorinated dibenzofurans (PCDF)

Polychlorinated dibenzo-*p*-dioxins (PCDDs)

Dibenzofuran (DF)

Dibenzo-*p*-dioxin (DD)

Figure 6.1. Chemical structures of Dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF), showing analogues and numbering conventions (Nojiri et al., 2001)**.**

PCDDs and PCDFs are unwanted byproducts of industrial paper production, herbicide and pesticide synthesis, metal smelting, and waste incineration, all of which are major sources of chlorinated dioxin production (Tuppurainen et al., 2003). In particular, incineration of chlorinated waste has been shown to yield dioxins in quantities of concern for public health (Brzuzy & Hites, 1996). Natural burning processes such as forest fires and volcanic activity can also produce these compounds.

Dioxins are highly hydrophobic. For example, the aqueous solubility of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is 0.019 ppb, and the logarithm of the octanol–water coefficient ($\log K_{ow}$) is 6.5 (Yang et al., 2006). Physical properties of important PCDDs and PCDFs are shown in **Table 6.1**.

Table 6.1. Physical properties of PCDDs and PCDFs (vapor pressure of subcooled liquid [P_L], water solubility [S], octanol-water partitioning coefficient [LogK_{ow}] (adapted from (Haglund, 2007a)**). Reported half-lives ($t_{1/2}$) for biodegradation in soil are also shown.**

Dioxin Congener	$T_{1/2,}$ soil (years)	P_L (Pa)	S (mg/m^3)	Log K_{ow}	Dibenzofuran (DF) congener	$T_{1/2,}$ soil (years)	P_L (Pa)	S (mg/m^3)	Log K_{0w}
DD		2	5000	4.3	DF		0.5	1000	4.3
MCDD		0.2	10	4.6	MCDF		0.07	300	5
DCDD		0.01	10	5.4	DCDF		0.008	4	6.4
TCDD		0.001	0.3	5.8	TCDF		0.0002	0.06	6.5
TeDD	60	0.0002	0.4	6.5	TeDF	100	0.00009	0.02	6.8
PeDD	60	0.00002	0.2	6.5	PeDF	110	0.00001	0.003	7.1
HexDD	70	0.000003	0.01	7.0	HexDF	280	0.000001	0.006	7.8
HepDD	40	0.0000006	0.001	7.4	HepDF	100	0.0000002	0.002	8.0
OCDD	30	0.0000001	0.001	8.0	OCDF	150	0.0000001	0.00007	8.2

PCDDs and PCDFs are of environmental concern due to their well-documented effects on the human endocrine, immune, and reproductive systems (Consonni et al., 2008; Panteleyev & Bickers, 2006; Turyk et al., 2007) as well as their dermal toxicity and carcinogenicity (Van den Berg et al., 1998). Of the 75 congeners of PCDDs and 135 congeners of PCDFs, 30 are considered significant toxins (Peng et al., 2013). The most toxic dioxin is thought to be 2,3,7,8- tetrachlorodibenzo-*p*-dioxin (TCDD), which was found in the adipose tissue of soldiers exposed to Agent Orange herbicide during the Vietnam War (Kang et al., 1991). The 12 congeners of DDs and DFs that have the 2,3,7,8 chlorine substitution pattern are known as the "dirty dozen" because of their extreme toxicity to mammals. Since PCDDs and PCDFs are relatively insoluble in water, they may not be a serious threat to ground water resources, but they can bioaccumulate in the fatty tissue of animals (Kang et al., 1991; O'Kane et al., 2013). As a result, more than 95% of human exposure to DDs, DFs, and dioxin like compounds occurs via consumption of food (Weber et al., 2008).

When dioxins are formed in the environment, a number of different congeners are formed, all with different toxicities (Van den Berg et al., 1998). To collectively quantify the toxicity of dioxin mixtures, the toxic equivalency (TEQ) was developed which provides an estimate of the overall toxicity of such mixtures based on the concentration of each congener in the environmental medium (e.g. soil, air or water). To calculate the TEQ, toxic equivalency factors (TEFs) are used for each congener which is a measure of that congener's toxicity relative to the most toxic congeners. In this system, the most toxic congeners (2,3,7,8 TCDD) are assigned a TEF of one, and all other congeners have been assigned a number proportional to their toxicity (**Table 6.2**). For mixtures of congeners, the TEQ is calculated by multiplying each individual concentration by the TEF for that congener and then summing these products for all congeners.

Table 6.2. Dioxin toxic equivalency factors (TEFs) reported by the World Health Organization (WHO).

		WHO TEF
Dioxins	2,3,7,8-Tetra-CDD	1
	1,2,3,7,8-Penta-CDD	1
	1,2,3,4,7,8-Hexa-CDD	0.1
	1,2,3,6,7,8-Hexa-CDD	0.1
	1,2,3,7,8,9-Hexa-CDD	0.1
	1,2,3,4,6,7,8-Hepta-CDD	0.01
	OCDD	0.0001
Dibenzofurans	2,3,7,8-Tetra-CDF	0.1
	1,2,3,7,8-Penta-CDF	0.05
	2,3,4,7,8-Penta-CDF	0.5
	1,2,3,4,7,8-Hexa-CDF	0.1
	1,2,3,6,7,8-Hexa-CDF	0.1
	1,2,3,7,8,9-Hexa-CDF	0.1
	2,3,4,6,7,8-Hexa-CDF	0.1
	1,2,3,4,6,7,8-Hepta-CDF	0.01
	1,2,3,4,7,8,9-Hepta-CDF	0.01
	OCDF	0.0001
Coplanar PCBs	3,3′,4,4′-TCB (77)	0.0001
	3,4,4′,5-TCB (81)	0.0001
	3,3′,4,4′,5-PeCB (126)	0.1
	3,3′,4,4′,5,5′-HxCB (169)	0.01
Mono-*ortho*-PCBs	2,3,3′,4,4′-PeCB (105)	0.0001
	2,3,4,4′,5-PeCB (114)	0.0005
	2,3′,4,4′,5-PeCB (118)	0.0001
	2′,3,4,4′,5-PeCB (123)	0.0001
	2,3,3′,4,4′,5-HxCB (156)	0.0005
	2,3,3′,4,4′,5′-HxCB (157)	0.0005
	2,3′,4,4′,5,5′-HxCB (167)	0.00001
	2,3,3′,4,4′,5,5′-HpCB (189)	0.0001

6.2. Abiotic Weathering Effects on Dioxins

Abiotic processes which could potentially contribute to weathering and natural attenuation of dioxins include volatilization, photo-oxidation and sequestration in soil (Vasquez et al., 2004), and these processes are considered in this section. Biological weathering processes such as biodegradation are described below in Section 6.3.

Volatilization of dioxins from soil is a potential pathway for natural attenuation, but volatilization rates are reported to be very slow (Trapp & Matthies, 1997), as expected from the low vapor pressure of dioxins. For highly contaminated soils, dioxins may be volatilized from soils and deposited onto plant tissue (Trapp & Matthies, 1997), but this is not likely to be significant at SSFL.

Early studies suggested that chlorinated dioxins are not photodegraded in soils (Isensee & Jones, 1975), but later studies implicated ultraviolet radiation in the photodegredation of dioxins, accounting for up to 10% of degradation rates observed in the field. However, this UV effect was only observed in the top 5-6 mm of the soil as UV light penetration is blocked in the deeper soils (Kieatiwong et al., 1990). Another study estimated that most of the photolytic activity occurs at soil depths of only 0.06-0.13 mm (Miller et al., 1989).

Dioxins have long been considered to be immobile in soils (Isensee & Jones, 1975) due to their adsorption to organic material in soils. This adsorption leads to sequestration in the soil matrix which greatly reduces bioavailability of these compounds to biodegradation (Cornelissen et al., 2005). After deposition on soil, the pollutants will be redistributed from weak adsorption sites to stronger, from the surface to the interior of the soil, and even further into the finer pores, a process generally referred to as aging. The contaminants become very recalcitrant to biodegradation in soils with finer soil particles such as clay and silt. On the positive side, this reduction in bioavailability could also lead to decreased toxicity (Alexander, 1995). Also, the hydrophobic nature of dioxins limits their solubility in water and thus contamination of groundwater and rainwater runoff do not pose serious threats.

Biodegradation of dioxins, as described below, typically leads to a reduction of concentrations of easily degraded components of dioxin mixtures, leaving the more recalcitrant fractions and more sequestered components to persist in the soil. This weathering process leads to a decrease in dioxin concentrations and toxicity over time, which eventually reaches a lower limit (Hatzinger & Alexander, 1995).

6.3. Biodegradation of Dioxins

PCDDs, PCDFs, and other dioxin-like chemicals are part of the natural chlorine cycle, which leads to their eventual biodegradation in the environment by either bacteria or fungi. Similar to PCBs, dioxins with fewer chlorine substitutions are typically biodegraded aerobically by bacteria, while chlorinated dioxins with more than four chlorines are not known to be biodegraded by aerobic bacteria (Sakaki & Munetsuna, 2010). However, these higher chlorinated dioxins can be reductively dechlorinated by anaerobic bacteria, resulting in their transformation to lower chlorinated dioxins which are then amenable to aerobic biodegradation (Sakaki et al. 2010). Thus, bacterial biodegradation of dioxins may be mediated by a complex consortium of bacteria in anaerobic and aerobic zones of sediments or soils. The higher chlorinated compounds tend to persist longer in the environment due to decreased rates of degradation, with half-lives reported from 12 to 170 years (Kjeller & Rappe, 1995). Bacterially-mediated anaerobic dechlorination and aerobic biodegradation are described separately below in Sections 6.3.1 and 6.3.2, respectively.

Lignolytic fungi have also been shown to biodegrade dioxins. Specifically, the lignin-degrading peroxidases secreted by fungi such as white-rot fungi have been shown to cometabolically biodegrade dioxins (Field & Sierra-Alvarez, 2008a). Fungal biodegradation of dioxins is described in Section 6.3.3 below.

Bacteria and fungal species reported to biodegrade dioxins are listed in **Table 6.3**.

Table 6.3. Dioxin-degrading microorganisms cited in literature. Initial and final concentrations were used to determine degradation rates.

Compound	Microorganism Involved in Degradation	Dioxin Concentration (ppm)		Length of Study (days)	Metabolites	Biodegradation Kinetics		Reference
		Initial	Final			Percent Degraded	Rate Constant (mg/kg/day)	
Dibenzo-p-dioxin (DD)	*Rhodococcus sp. strain p52*	250	12.5	2.5	Ethers, Hydrocarbons	*95*	*9.50E+01*	Peng et al., 2013
DD	*Sphingomonas wittichii RWI*	55	2.75	0.67	NS	*95*	*7.84E+01*	Wilkes et al., 1996
DD	*Beijerinckia sp. B8/36*	500	29.4	1	1,2-dihydroxydibenzo-p-dioxin	*94*	*4.71E+02*	Klečka & Gibson, 1980
DD	*Pseudomonas veroniiPH-03*	219	20.4	2.5	Catechol	*91*	*7.94E+01*	Hong et al., 2004
DD	*Rhodococcus opacus SAO101*	1	0.02	7	monohydroxy dibenzo- p-dioxin	*98*	*1.40E-01*	Kimura & Urushigawa, 2001
DD	*Klebsiella sp. strain HL1*	8.7	2.6	0.33	NS	*70*	*1.83E+01*	Fukuda et al., 2002
DD	*Sphingomonas sp. HL7*	8.7	0	0.17	NS	*100*	*5.22E+01*	Fukuda et al., 2002
DD	*Coprinellus disseminatus TUFC11148*	NS	NS	14	NS	99	--	Suhara et al., 2003
DD	*Coprinellus disseminatus TUFC34534*	NS	NS	14	NS	78	--	Suhara et al., 2003
DD	*Coprinellus disseminatus TUFC30081*	NS	NS	14	NS	62	--	Suhara et al., 2003
DD	*Pseudomonas sp. Strain HH69*	10	NS	NS	1-hydroxy dibenzo-p-dioxin	--	--	Harms et al., 1991
DD	*Phanerochaete chrysosporium*	833	416	1	2,3-dihydroxydibenzo-p-dioxin	*50*	*4.17E+02*	Joshi & Gold, 1994
DD	*Cordyceps sinensis strain A*	30	15	4	2,2',4,5-tetrahy-droxydiphenyl ether,Catechol	*50*	*3.75E+00*	Nakamiya et al., 2005

Table 6.3. Dioxin-degrading microorganisms cited in literature. Initial and final concentrations were used to determine degradation rates.

Compound	Microorganism Involved in Degradation	Dioxin Concentration (ppm)		Length of Study (days)	Metabolites	Biodegradation Kinetics		Reference
		Initial	Final			Percent	Rate	
DD	*Staphylococcus auriculans DBF63*^{c}	500	NS	NS	1-hydro-1,la-dihydroxy-D	--	--	Monna et al., 1993
1-CDD	*Sphingomonas wittichii RW1*	55	22	0.67	3-Chlorocatechol	*60*	*4.95E+01*	Wilkes et al., 1996
1-CDD	*Beijerinckia sp. B8/36*	500	137	1	None	*73*	*3.63E+02*	Klečka & Gibson, 1980
1-CDD	*Pseudomonas veronii PH-03*	219	25.6	2.5	3-Chlorocatechol	*88*	*7.74E+01*	Hong et al., 2004
1-CDD	*Rhodococcus opacus SAO101*	1	0.08	7		*92*	*1.31E-01*	Kimura & Urushigawa, 2001
2-CDD	*Sphingomonas wittichii RW1*	55	33	0.67	4-Chlorocatechol	*40*	*3.30E+01*	Wilkes et al., 1996
2-CDD	*Beijerinckia sp. B8/36*	500	136.5	1	None identified	*73*	*3.64E+02*	Klečka & Gibson, 1980
2-CDD	*Sphingomonas sp. strain KA1b*	NS	NS	7	NS	96	--	Habe et al., 2002
2-CDD	*Pseudomonas veronii PH-03*	219	46.9	2.5	4-Chlorocatechol	*79*	*6.88E+01*	Hong et al., 2004
2-CDD	*Burkholderia sp. JB1*	0.1	0.005	1	4-Chlorocatechol	*95*	*9.50E-02*	Parsons et al., 1998
2-CDD	*Klebsiella sp. strain HL1*	8.7	4.35	0.33	NS	*50*	*1.31E+01*	Fukuda et al., 2002
2-CDD	*Sphingomonas sp. HL7*	8.7	0	0.17	NS	*100*	*5.22E+01*	Fukuda et al., 2002
2-CDD	*Pseudomonas resinovorans CA10a*	10	0.3	5	4-Chlorocatechol	*97*	*1.94E+00*	Habe et al., 2001
2-CDD	*Terrabacter sp. DBF63*	10	2.5	0.83	4-Chlorocatechol	*75*	*9.00E+00*	Habe et al., 2002
23-CDD	*Dehalococcoides sp. CBDB1*	6.32	2.97	28	2-CDD	*53*	*1.20E-01*	Bunge et al., 2003

Table 6.3. Dioxin-degrading microorganisms cited in literature. Initial and final concentrations were used to determine degradation rates.

Compound	Microorganism Involved in Degradation	Dioxin Concentration (ppm)		Length of Study (days)	Metabolites	Biodegradation Kinetics		Reference
		Initial	Final			Percent	Rate	
23-CDD	*Pseudomonas sp. strain CA10a*	1	0.11	5	4,5-Dichlorocatechol	*89*	*1.78E-01*	Habe et al., 2001
23-CDD	*Sphingomonas sp. strain KA1b*	NS	NS	7	NS	70	--	Habe et al., 2002
23-CDD	*Beijerinckia sp. B8/36*	500	422.5	1	None	*16*	*7.75E+01*	Klečka & Gibson, 1980
23-CDD	*Rhodococcus opacus SAO101*	18.4	14.2	7	NS	*23*	*6.00E-01*	Kimura & Urushigawa, 2001
23-CDD	*Sphingomonas sp. HL7*	10	0	0.33	2-methyl-4H-chroman-4-one	100	*3.00E+01*	Fukuda et al., 2002
23-CDD	*Terrabacter sp. DBF63*	10	2	0.83	4,5-Dichlorocatechol	*80*	*9.60E+00*	Habe et al., 2002
23-CDD	*Pseudomonas sp. EE41*	0.3	0.09	63	NS	*70*	*3.33E-03*	Du et al., 2001
2,7-DCDD	*Sphingomonas wittichii RW1*	50	23.5	4	4-Chlorocatechol	*53*	*6.63E+00*	Hong et al. 2002
2,7-DCDD	*Coprinellus disseminatus TUFC34534*	0.3	0.25	14	monomethoxy-DCDD	*17*	*3.57E-03*	Suhara et al., 2011
2,7-DCDD	*Coprinellus disseminatus TUFC11148*	0.3	0.26	14	monomethoxy-DCDD	*13*	*2.86E-03*	Suhara et al., 2011
2,7-DCDD	*Coprinellus micaceus TUFC30081*	0.3	0.15	14	monomethoxy-DCDD	*50*	*1.07E-02*	Suhara et al., 2011
2,7-DCDD	*Phlebia lindtneri*	12.6	8.8	14	monomethody-diCDD	*30*	*2.71E-01*	Kamei & Kondo, 2005
2,7-DCDD	*Erwinia sp.*	5	3.6	1	NS	*28*	*1.40E+00*	Liaw & Srinivasan, 1990
2,7-DCDD	*Phanerochaete chrysosporium*	2	1	27	4-chloro-1,2-benzoquinone 2-hydroxy-1,4-benzoquinone	*50*	*3.70E-02*	Valli, Wariishi, & Gold, 1992
2,7-DCDD	*Pseudomonas sp. CA 10*	10	7.5	5		*25*	*5.00E-01*	Habe et al., 2001

Table 6.3. Dioxin-degrading microorganisms cited in literature. Initial and final concentrations were used to determine degradation rates.

Compound	Microorganism Involved in Degradation	Dioxin Concentration (ppm)		Length of Study (days)	Metabolites	Biodegradation Kinetics		Reference
		Initial	Final			Percent	Rate	
2,8-DCDD	*Rhodococcus opacus SAO101*	25.3	21.3	7	NS	*16*	*5.71E-01*	Kimura & Urushigawa, 2001
123-TrCDD	*Dehalococcoides sp. CBDB1*	28.9	11.6	57	23-/13-DCDD, 2-MCDD	*60*	*3.04E-01*	Ballerstedt et al., 2004
123-TrCDD	*Pseudomonas resinovorans CA10a*	1	0.61	5	NS	*39*	*7.80E-02*	Habe et al., 2001
123-TrCDD	*Sphingomonas wittichii RW1*	10	8	5	trichlorotrihydroxydiphenyl ether	*20*	*4.00E-01*	Hong et al., 2002
123-TrCDD	*Pseudomonas sp. EE41*	1.2	0.83	21	NS	*31*	*1.76E-02*	Du et al., 2001
124-TrCDD	*Dehalococcoides sp. CBDB1*	17.2	7.7	57	13-DCDD, 2-MCDD	*55*	*1.67E-01*	Bunge et al., 2003
237-TCDD	*Pseudomonas sp. EE41*	0.3	0.18	63	NS	*40*	*1.90E-03*	Du et al., 2001
237-TCDD	*Cordyceps sinensis strain A*	300	150	4	MCC & DCC	*50*	*3.75E+01*	Nakamiya et al., 2005
237-TCDD	*Sphingomonas wittichii RW1*	59	19.4	5	345-TCC	*67*	*7.92E+00*	Nam et al., 2006
1234-TeCDD	*Dehalococcoides sp. CBDB1*	14.9	11.3	28	124-TrCDD, 13-/23-DCDD, 2-MCDD	*24*	*1.29E-01*	Bunge et al., 2003
1234-TeCDD	*D. ethanogenes strain 195*	9.9	0.99	40	124-TrCDD, 13-DCDD	*90*	*2.23E-01*	Fennell et al., 2004
1234-TeCDD	*Sphingomonas wittichii RW1*	50	31.5	4	3456-TCC, 2-methoxy-3456-tetrachlorophenol, 45-dichlorocatechol	*37*	*4.63E+00*	Hong et al. 2002
1234-TeCDD	*Phanerochaete sordida YK-624*	0.05	0.03	14	NS	*40*	*1.43E-03*	Takada et al., 1996
1234-TeCDD	*Pseudomonas sp. EE41*	1	0.62	21	3456-tetrachlorocatechol	*38*	*1.81E-02*	Du et al., 2001
1234-TeCDD	*Pseudomonas veronii PH-03*	322	264	5	NS	*18*	*1.16E+01*	Du et al., 2001

Table 6.3. Dioxin-degrading microorganisms cited in literature. Initial and final concentrations were used to determine degradation rates.

Compound	**Microorganism Involved in Degradation**	**Dioxin Concentration (ppm)**		**Length of Study (days)**	**Metabolites**	**Biodegradation Kinetics**		**Reference**
		Initial	**Final**			**Percent**	**Rate**	
1368-TeCDD	*Phlebia brevispora*	16.1	8.1	90	NS	*50*	*8.89E-02*	Kamei et al., 2009
2378-TeCDD	*Bacillus megaterium*	0.005	0.002	244	None identified	*60*	*1.23E-05*	Iii & Matsumura, 1983
2378-TeCDD	*Phanerochaete chrysosporium*	NS	NS	NS	NS	--	--	Bumpus et al. 1985
2378-TeCDD	*Pseudomonas sp. EE41*	0.3	0.06	21	NS	*80*	*1.14E-02*	Du et al., 2001
2378-TeCDD	*Pseudallescheria boydii*	3.2	0.66	0.83		*79*	*3.05E+00*	Ishii et al. 2009
2378-TeCDD	*Pseudomonas testosteroni G1036*	0.3	NS	NS	HTeCDD	--	--	Philippi et al., 1982
12378-PeCDD	*Dehalococcoides sp CBDB1*	NS	NS	84	2378-TCDD, DCDD, 237-TrCDD	75	--	Bunge et al., 2003
123478-HCDD	*Mixed culture containing D. ethanogenes 195*	186	167.4	200	1378-/1248-TCDD	*10*	*9.30E-02*	Liu & Fennell, 2008
123478-HCDD	*Sphingomonas RW1*	50	36	5	TeCC and 2-methoxy-3456-tetrachlorophenol	*28*	*2.80E+00*	Nam et al., 2006
123478-HCDD	*Phanerochaete sordida YK-624*	0.05	0.012	14	NS	*76*	*2.71E-03*	Takada et al., 1996
OCDD	*Cordyceps sinesis strain A*	300	150	4	MCC, DiCC, TrCC	*50*	*3.75E+01*	Nakamiya et al., 2005

6.3.1. Bacterial Anaerobic Reductive Dechlorination

Many researchers have demonstrated the ability of microorganisms to dechlorinate dioxins through the process of reductive dehalogenation (Ballerstedt et al., 2004; Bunge et al., 2003). *Dehalococcoides* has been the most extensively studied genus of bacteria with the ability to reductively dechlorinate dioxins. These bacteria grow under anaerobic conditions, using multiple dehalogenase enzymes to remove chlorine from dioxin congeners through a cometabolic process which requires an external electron donor such as sugars, hydrogen or lactate. Successful dechlorination of higher chlorinated compounds may depend on consortia of different microorganisms rather than a single species (Beurskens et al., 1995; Bunge et al., 2008; Pelz et al., 1999; Wittich et al., 1999).

Anaerobic dechlorination of 1234-tetrachloro dibenzo-p-dioxin (TCDD) has been demonstrated by *Dehalococcoides sp.* CBDB1 (Bunge et al., 2003) and *Dehalococcoides ethenogenesstrain* 195 (Fennell et al., 2004). Both bacterial species produce the same end product, 2-chloro dibenzo-p-dioxin, which can then be further metabolized aerobically by different microorganisms.

Studies suggest that the enzymes involved in dehalogenation are located on the inner membrane of the bacterial cells. The enzymes involved are thought to be PCE reductive dehalogenase (Neumann, Wohlfarth, & Diekert, 1996) and ortho-chlorophenol dehalogenase CprA (Pas et al., 1999), both of which have been purified from cell membranes. They form clusters on the membrane involved in electron transfer to the active site of cobalamin (Vitamin B structure) (Sakaki & Munetsuna, 2010).

Several researchers have studied the mechanisms of reductive dehalogenation (Ahn et al., 2005; Ballerstedt et al., 2004; Beurskens et al., 1995; Bunge et al., 2003; Bunge et al., 2001; Fennell et al., 2004; Vargas et al., 2001). An example pathway for dechlorination of 1234-TCDD is shown in **Figure 6.2**.

Anaerobic dechlorination has been augmented at some sites by introducing dehalogenating bacteria collected from other sites such as contaminated anaerobic sediments. Using enrichment cultures, studies showed that spore-forming and non-spore-forming bacteria produced different intermediates using different mechanisms. This suggests that a larger group of bacteria may participate in the dehalogenation of higher chlorinated dioxins (Field & Sierra-Alvarez, 2008a).

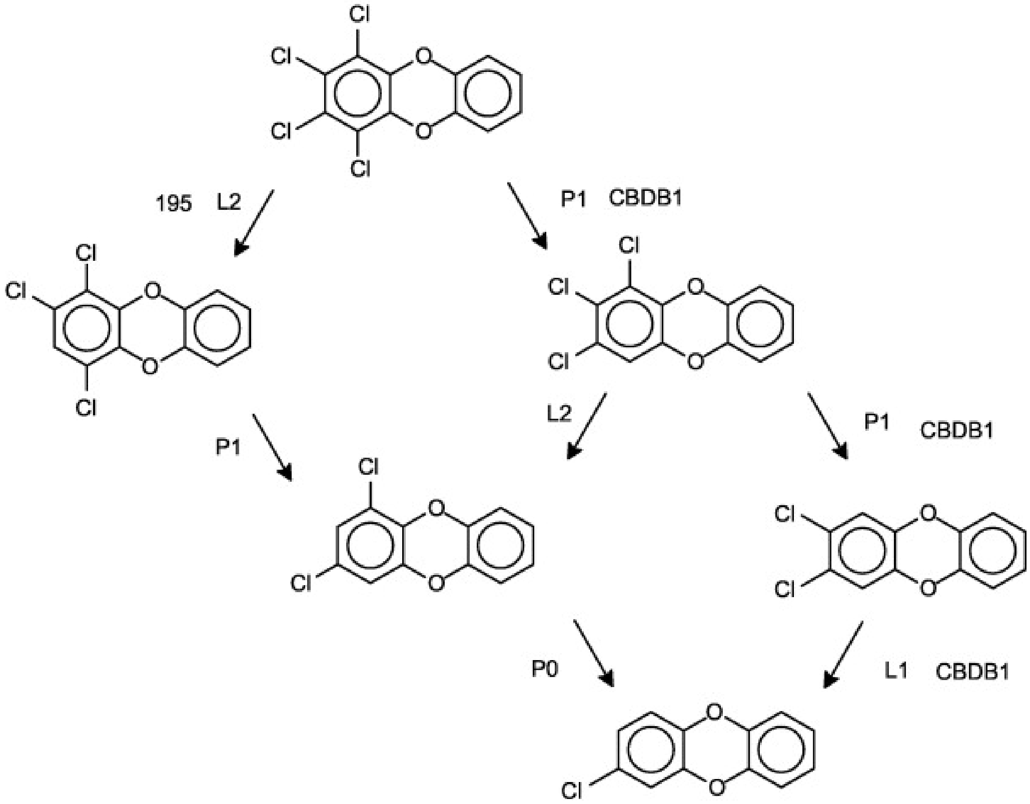


Figure 6.2. Anaerobic reductive dechlorination pathway of 1234-TeCDD by pure cultures and enrichment cultures of *Dehalococcoides* spp. (Field & Sierra-Alvarez, 2008a)**.**

6.3.2. Bacterial Aerobic Mechanisms for Degradation of Lower Chlorinated Dioxins

Aerobic biodegradation of lower chlorinated dioxins, dibenzo-furans, and dibenzo-dioxins occurs via many pathways, which can lead to a wide range of intermediates and products. Biodegradation of these lightly chlorinated dioxins has been documented for bacteria in the genera of *Sphingomonas, Pseudomonas and Burkholderia* (Nam et al., 2006). The two major enzyme systems used by microorganisms to degrade lower chlorinated contaminants are dioxygenase and cytochrome P450 (Sakaki & Munetsuna, 2010).

Dioxygenase enzyme pathways for aerobic dioxin biodegradation are shown in **Figure 6.3**, as reported by (Field & Sierra-Alvarez, 2008a). Biodegradation yields different products depending on if the reactant is a monochlorinated dibenzofuran (DF) or a dibenzodioxin (DD).

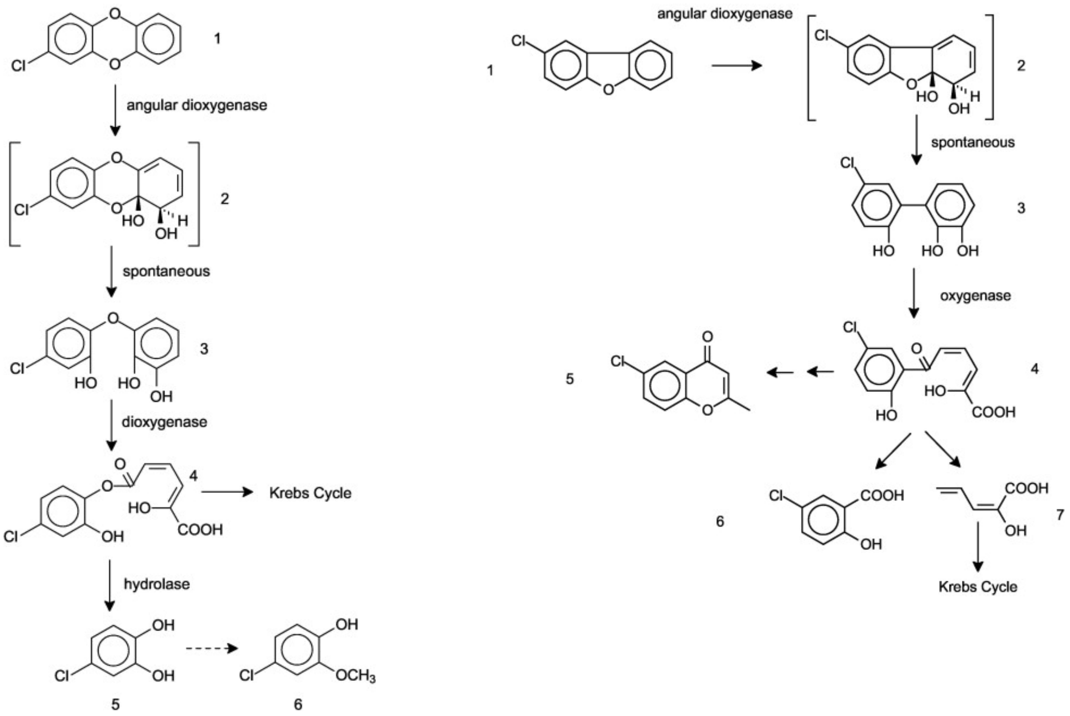


Figure 6.3. The aerobic biodegradation pathways of monochlorinated PCDD (A) and PCDF (B) by bacteria (J. A. Field & Sierra-Alvarez, 2008b)**.**

PCDF and monochlorinated dibenzofurans (MCDF) and PCDDs can serve as the sole carbon and energy sources for some aerobic bacteria which can completely mineralize the non-chlorinated aryl ring (Hong et al., 2004; Wittich, 1998). In contrast, di- to tetra-chlorinated congeners are attacked co-metabolically during growth of the respective bacteria on another utilizable substrate such as PCDF or PCDD. Often this scenario leads to accumulation of salicylic acid or chlorocatechols as intermediates (Habe et al. 2001 and Hong et al. 2002). This accumulation can be avoided if the compounds are converted through co-cultivation with bacteria such as *Burkholderia* that can degrade the chlorosalicylate excreted by known PCDD/F degraders (Arfmann et al., 1997). Thus, the complete metabolism of dioxin-like compounds may be dependent on several different microbial populations to degrade the metabolites produced by biodegradation (Arfmann et al., 1997; Bunge et al., 2008; Field & Sierra-Alvarez, 2008a; Holliger et al., 1992).

6.3.3. Fungal Biodegradation of Dioxins: White-rot fungi

White rot fungi have exhibited promising rates of biodegradation of PCDDs in numerous studies (Takada et al., 1996). White-rot fungi have developed complex enzyme systems to degrade lignins, and these same enzyme systems can biodegrade many ordinarily recalcitrant compounds. *Phanerochaete chrysosporium,* the most studied of the white rot fungi, has been shown to biodegrade several dioxins (Bumpus et al., 1985 and Valli et al., 1992) including highly chlorinated dioxins (Takada et al., 1996) as well as chlorinated DD (Joshi & Gold, 1994). In addition to *P. chrysosporium,* several other fungal species have been shown to biodegrade dioxins. *Coprinellus spp., Phlebia lindtneri, Pseudallescheria boydii*, and *Cordyceps sinensis*, along with other fungi have been associated with effective biodegradation of PCDDs and dioxin metabolites (Ishii et al. 2009; Kamei et al. 2005; Nakamiya et al. 2005; and Suhara et al. 2011).

Dioxin biodegradation is mediated by a number of complex enzyme-mediated mechanisms, which are described only briefly here. More detailed descriptions of fungal biodegradation mechanisms are available in the literature (Bumpus et al., 1985; Sakaki & Munetsuna, 2010; Valli et al., 1992). *P. chrysosporium* has been shown to secrete two heme peroxidases, lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP). The LiP can also degrade various environmental pollutants including PCDDs (Sakaki & Munetsuna, 2010). MnP can only react with these molecules after the cleavage of the dioxin ring (Valli et al., 1992). Therefore, the first step for degradation is the cleavage of the ring by LiP, forming chlorinated benzoquinone derivatives (**Figure 6.4**). These intermediates are further degraded by LiP and MnP through various steps and eventually produce β-ketoadipic acid, which is easily degraded to CO_2 (Valli et al., 1992). The free-radical mechanism that degrades these compounds is highly nonspecific and non-stereoselective, making white-rot fungi great candidates for sites contaminated with many compounds.

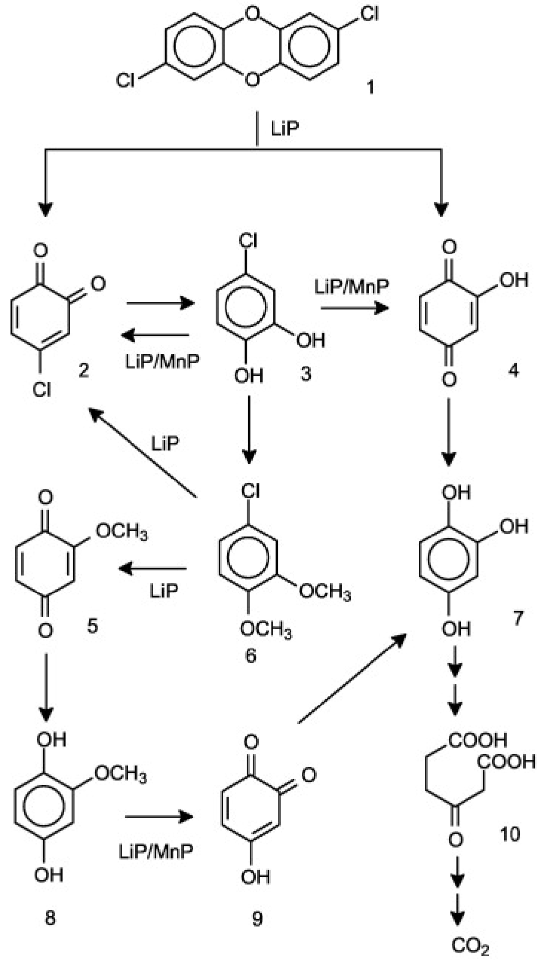


Figure 6.4. Proposed pathway of degradation of 2,7-dichlorodibenzo-*p*-dioxin (2,7 DCDD) by the white-rot fungi *Phanerochaete chrysosporium* (Valli et al., 1992)**.**

White-rot fungi may also mediate dioxin biodegradation using cytochrome P450 enzymes. It has been shown that *P. chrysosporium* possesses 148 CYP genes, which suggests that cytochrome P450 enzymes may be involved in these processes (Kasai et al., 2010).

6.4. Methods of Active Bioremediation of Dioxins

A variety of methods for facilitating biodegradation of dioxins are available, such as biostimulation through the addition of nutrients, cometabolites or surfactants and bioagmentation with known dioxin-degrading microorganisms. Another method of biostimulation is the addition of other easily-degraded chlorinated compounds to stimulate biodegradation in a strategy referred to as "halopriming," as described above for PCBs Phytoremediation of dioxins is likely to be limited, but potential phytoremediation strategies are also described here. Additionally, there are physical-chemical means of enhancing the removal of dioxins from contaminated soil.

6.4.1. Biostimulation of Dioxin Biodegradation

Biostimulation of existing microbial communities that are actively degrading dioxin contaminants can be achieved by adding surfactants, nutrients, electron acceptors and/or donors, and co-substrates. Due to the propensity for dioxins to become sequestered in soils, it is likely that surfactant addition would increase dioxin bioavailability and accelerate its biodegradation, although little research has been done in this area (Nam et al., 2006).

Takada et al. (1996) investigated glucose addition as a co-substrate for dioxin biodegradation. These researchers compared the biodegradation of tetra- to octa-chlorinated dibenzo-*p*-dioxins and dibenzofurans by glucose-supplemented *P. chrysosporium* and *P. sordida*. Glucose-supplemented *P. chrysosporium* consistently degraded all congeners but 2, 3, 7, 8, Tetrachlorodibenzofuran (TCDF) more efficiently than *P. sordida* at rates ranging from 27.3 to 64.9% over 7 days. *P. sordida*'s degradation rates of the same contaminants ranged from 14.2 to 50.4% in the same time period. *P. sordida*'s ability to degrade tetra- and octa-chlorinated dibenzo-p-dioxins is significant for bioremediation because the more dioxin congeners an organism can degrade, the higher its likelihood of aiding cleanup of soil contamination.

Another example of biostimulation with a co-substrate is the addition of more easily biodegraded chlorinated compounds to stimulate growth and enzyme expression of potential dioxin-degrading microorganisms. This approach is called "halopriming," because of the addition of halogenated (chlorinated) compounds. The addition of smaller chlorinated compounds (such as chlorobenzene) can increase the rate of dechlorination of dioxins (Bunge et al., 2003; Fennell et al., 2004).For example, addition of 1,2,3,4-tetrachlorobenzene as a haloprimer increased the dechlorination rate of 1,2,3,4-TCDD by native microbial communities compared to samples with only a growth substrate (Ahn et al., 2008).

Yet another approach to improving remediation of dioxin-contaminated soils is the production of enzymes to mediate dehalogenation. For example, researchers have created designer enzymes based on cytochrome P450 for the bioremediation of PCDDs in contaminated soil (Sakaki & Munetsuna, 2010).

6.4.2. Bioaugmentation

Bioaugmentation has been used to increase biodegradation rates of dioxins by adding either bacteria or fungi. Bioaugmentation of dioxin-contaminated soils has been researched for several aerobic bacteria: *Sphingomonas wittichii* RW1, *Sphingomonas* sp. strain KA1, *Pseudomonas resinovorans* CA10*,* and *Terrabacter* sp. strain DBF63. All strains degraded DD, DF, and their low-chlorinated congeners in soil slurry systems (Habe et al. 2001 and 2002; Halden et al. 1999; and Megharaj et al. 1997). The success of these lab studies does not guarantee success in the field because field conditions were not mimicked in the lab: soils were artificially contaminated with dioxins and augmented with pure cultures. It is likely that "fresh" dioxin introduced to microcosms is much more bioavailable than dioxin contamination that remains in soil after long periods of weathering such as those at SSFL. Repeated inoculation of soils has also been shown to enhance dioxin degradation; the use of *S. wittichii* RW1 enhanced toxic PCDD removal by up to 10.3% (Nam et al. 2005). The bioaugmentation of soil microcosms with certain bacteria has yielded promising results. In many cases, published bioaugmentation degradation rates have greatly exceeded those of control microcosms or soils (Habe et al., 2002).

Amendment of dioxin-contaminated soils with wood chips seeded with white rot fungi may be a promising treatment option. The wood chips could serve as a bulking agent to increase aeration (Rastegarzadeh et al., 2006), and the fungi could aid in biodegradation because white-rot fungi have been shown to degrade dibenzo-p-dioxins ranging from low to high degrees of chlorination (Takada et al., 1996).

6.4.3. Phytoremediation of Dioxins

Dioxins are similar to PCBs in their chemical structure and properties but limited research has been done on dioxin phytoremediation. Studies done with zucchini (*Cucurbita pepo*) have shown that this species can efficiently phytoextract dioxins from soil (Campanella & Paul, 2000; Huelster et al., 1994). Plants can stimulate rhizosphere dioxin degradation in the same way they stimulate PCB rhizosphere degradation (Campanella et al., 2002). The degradation of dioxins through phytoremediation has not been adequately studied to assign a remediation time scale. However, if dioxins are degraded in a similar fashion as PCBs, their remediation time scale could be similar.

6.4.4. Photodegredation, Irradiation and Soil Washing

Application of UV radiation may be effective at increasing dioxin remediation through enhanced dechlorination reactions. Isosaari, et al. (2005) observed increase dioxin degradation by applying UV light in combination with vegetable oils to dioxin-contaminated soils. It was speculated that the oil acted as a surfactant to bring the dioxin out of the soil pores, so it could then be exposed to the UV light. Further, researchers have concluded that UV radiation may only penetrate the top 5-6 mm of soil (Kieatiwong et al., 1990 and Zhong et al., 1994) or only 0.06-0.13 mm as described above (Miller et al., 1989). Thus, the soil would need to be tilled occasionally to expose it to sunlight, making it a very labor intensive and disruptive process.

Photocatalytic degradation of polychlorinated dibenzo-p-dioxins has also been studied with the use of TiO_2 films under UV light ($\lambda > 300$ nm).The photocatalytic degradation rates of PCDDs decreased with the number of chlorines and were described by first-order reaction kinetics with half-lives of 5.8, 3.9, 0.71, and 0.38 hours for OCDD, HpCDD, TCDD, and MCDD, respectively (Choi, Hong, Chang, & Cho, 2000). These types of hybrid catalytic systems in combination with other remediation efforts may prove effective, but application to the entire contaminated soil column is problematic.

Gamma irradiation was also considered for dioxin soil treatment. With a Cobalt-60 source and addition of 2% surfactant, Hilarides et al. (1994) demonstrated up to 99% removal with artificially contaminated soil and 75% with weathered soil.

Rinsing soil with compounds that can solvate dioxins has been seriously considered by other researchers. (Lee et al., 2005) used 99.5% ethanol to wash a reference soil and removed over 95% of removal of dioxin content after using 7 extraction cycles. On a large scale, it would be extremely impractical to treat soils with this pure ethanol, and treatment would require special collection of the surfactant to avoid it mixing with ground water. The process may be extremely labor intensive and costly.

6.4.5. Field Studies and Natural Attenuation Rates of Dioxins in Soil

Natural attenuation and remediation rates for dioxins in soils are tabulated in **Table 6.4**. Some studies indicate that chlorinated dioxins do not biodegrade in soil, while others suggest biodegradation may be a viable means of natural attenuation. For example, Wilson et al. (1997) reported no decrease in soil dioxin concentrations after 260 days of monitoring. Conversely, a microcosm study indicated 37-44% removal of 2378-TeCDD at concentrations ranging from 1-100 ppm (Kearney et al., 1972). Other studies reported dioxin reductions of 2 to 86%. Clearly, the rates of biodegradation will depend on contaminants' chemical composition and environmental conditions.

Generally, dehalogenation of higher chlorinated dioxins is very slow, and is usually insufficient to rely on for bioremediation. Estimations for half-lives of dioxins in the soil range from 1-120 years depending on the type of compound (Haglund, 2007b; Isosaan et al., 2004; and Sinkkonen & Paasivirta, 2000). Rates obtained in laboratory studies are thought to be an overestimate of actual degradation rates that may be occurring at a site (Sinkkonen & Paasivirta, 2000) because fresh contaminants are less sequestered in the soil. Unfortunately, there are few long-term studies reported for natural attenuation of dioxins in the field, because of the length of time needed to observe such changes.

Table 6.4. Summary of reported rates of dioxin biodegradation under natural attenuation conditions. Initial and final concentrations are listed in in mg/kg or ppm, and kinetics have been calculated.

Compound	Matrix	Lab/Field (L/F)	Soil Dioxin Concentration (mg/kg)		Length of Study (days)	Biodegradation Kinetics		Reference
			Initial	Final		Percent Degraded	Rate of Degradation (mg/kg/day)	
PeCDF	anaerobic river sediment	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
PeCDF	anaerobic aquifer sediments	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
PCDF	soil	F	180	170	260	*6*	*3.85E-02*	Wilson et al., 1997
PCDD	soil	F	140	68	260	*51*	*2.77E-01*	Wilson et al., 1997
HeCDF	anaerobic river sediment	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
HeCDF	anaerobic aquifer sediments	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
HeCDD	anaerobic river sediment	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
HeCDD	anaerobic aquifer sediments	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
HCDD	anaerobic river sediment	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
HCDD	anaerobic aquifer sediments	F	100	--	--	--	--	Adriaens & Grbicgalic, 1994
TCDD	soil	L	1	0.46	350	*54*	*1.54E-03*	Kearney, et al., 1972
TCDD	soil	L	1	0.46	350	*54*	*1.54E-03*	Kearney, et al.,1972
TCDD	soil	L	10	4.3	350	*57*	*1.63E-02*	Kearney et al., 1972
TCDD	soil	L	10	3.7	350	*63*	*1.80E-02*	Kearney, et al.,1972
TCDD	soil	L	100	44	350	*56*	*1.60E-01*	Kearney, et al.,1972
TCDD	soil	L	100	29	350	*71*	*2.03E-01*	Kearney, et al.,1972
TCDD	soil	L	91	81	90	*11*	*1.11E-01*	Kearney, et al.,1972
TCDD	soil	L	88	96	90	--	--	Kearney, et al.,1972
TCDD	soil	L	92	84	90	*9*	*8.89E-02*	Kearney, et al.,1972
TCDD	soil	L	98	90	90	*8*	*8.89E-02*	Kearney, et al.,1972
TCDD	soil	L	93	94	90	--	--	Kao et al., 2001
TCDD	soil	L	90	94	90	--	--	Kao et al., 2001

Table 6.4. Summary of reported rates of dioxin biodegradation under natural attenuation conditions. Initial and final concentrations are listed in in mg/kg or ppm, and kinetics have been calculated.

Compound	Matrix	Lab/Field (L/F)	Soil Dioxin Concentration (mg/kg)		Length of Study (days)	Biodegradation Kinetics		Reference
			Initial	Final		Percent Degraded	Rate of Degradation (mg/kg/day)	
TCDD	soil	L	94	85	90	*10*	*1.00E-01*	Kao et al., 2001
TCDD	soil	L	90	88	90	*2*	*2.22E-02*	Kao et al., 2001
TCDD	soil	L	91	89	90	*2*	*2.22E-02*	Kao et al., 2001
TCDD	soil	L	95	90	90	*5*	*5.56E-02*	Kao et al., 2001
TCDD	soil	L	97	84	90	*13*	*1.44E-01*	Kao et al., 2001
TCDD	soil	L	94	89	90	*5*	*5.56E-02*	Kao et al., 2001
DF	soil	L	1000	790	84	*21*	*2.50E+00*	Wang & Oyaizu, 2011
DD	soil	L	20	17.6	84	*12*	*2.86E-02*	Wang & Oyaizu, 2011
2,8-DCDF	soil	L	20	17.6	84	*12*	*2.86E-02*	Wang & Oyaizu, 2011
2,7-DCDD	soil	L	20	17.6	84	*12*	*2.86E-02*	Wang & Oyaizu, 2011
2,4,8-TCDF	soil	L	20	17.6	84	*12*	*2.86E-02*	Wang & Oyaizu, 2011
2,3,7,8-TeCDD	soil mixed with anaerobic sludge	L	0.096	0.013	90	*86*	*9.22E-04*	Kao et al., 2001
1,2,4-TCDD	soil	L	20	17.4	84	*13*	*3.10E-02*	Wang & Oyaizu, 2011
1,2,3,4-TCDD	anaerobic reservoir sediment slurries	L	15.5	7.9	120	*49*	*6.33E-02*	Ahn et al., 2008
1,2,3,4-TCDD	soil	L	20	16.2	84	*19*	*4.52E-02*	Wang & Oyaizu, 2011
1-CDD	soil	L	20	18	84	*10*	*2.38E-02*	Wang & Oyaizu, 2011

Notes

[1] Initial measured concentration exceeds final measured concentration. No degradation rate could be extrapolated.

[2] No final concentration given in paper; contaminant half-lives provided. No calculations performed.

NS = Not Specified

6.5. Potential for Dioxin Natural Attenuation at SSFL Site

Dioxins in the soils at SSFL are thought to have originated from burning of chlorinated wastes on site as well as the Topanga Wildfire in 2005, as both of these processes are known to produce dioxins (Tuppurainen et al., 2003) Dioxin concentrations in Area IV soils of the SSFL range from 2.68 to 650 parts per trillion (ppt). The current clean-up goal for the site is to reach background levels of contaminants as defined by the DTSC. For dioxins the DTSC applied the World Health Organization's 2,3,7,8-TCDD toxicity equivalence approach, and set a goal of a 2,3,7,8-TCDD TEQ of 0.912 pg/g (= 0.912 ppt). Thus, for some locations only a three-fold reduction in dioxin concentration would be required, while for other locations a 700-fold reduction would be required.

Soils with higher dioxin concentration are not likely to be sufficiently remediated by natural attenuation alone because of the long half-lives expected for biodegradation rates as described above. The soils with lower concentrations would be more amenable to biodegradation, particularly if methods of accelerating dioxin degradation are employed (Field & Sierra-Alvarez, 2004).

It is difficult to accurately predict chlorinated dioxin remediation times required for soils at SSFL via natural attenuation because most of the small numbers of degradation rates reported in literature were observed under anaerobic conditions. As described above (Table 6.4), reported first-order rate constants range from approximately 2.2×10^{-4} to 6.9×10^{-1} day^{-1} in field studies. The higher degradation rates were observed under anaerobic conditions, while lower ones were observed under aerobic conditions. To make a conservative calculation, only rates observed in field studies were used, not the higher rates reported for lab studies. Using the field-derived rate constants and the range of TCDD TEQ concentrations measured in SSFL soils, we calculated the time required to reach a background level of 9.12 pg/g as the specified goal in the DTSC "look-up tables." This calculation was performed for a range of dioxin concentrations, from 10 to 650 pg/g for illustration purposes. A sample calculation is shown in **Table 6.5**.

Clearly, for dioxins anaerobic degradation occurs at a much higher rate than aerobic degradation (half lives measured in days instead of years in **Table 6.6**). Because site conditions are yet to be confirmed, it will be important to determine the redox conditions in the soil column at the SSFL site to help estimate the potential for anaerobic and aerobic natural attenuation processes at the site, as redox conditions have been shown to change degredation rates drastically (Kao et al., 2001). Based on conversations with CDM Smith, it is likely that the soils at the site are mostly aerobic. These aerobic conditions may preclude anaerobic dechlorination processes, which could severely limit reductive dechlorination of the more highly chlorinated dioxins. In this case, fungi may play a more significant role than bacteria for natural biodegradation of dioxins at the site. Field tests are scheduled to measure soil vapor concentrations of oxygen and methane so that the redox conditions in the vadose zone of the soil can be estimated.

Table 6.5. Sample calculation of time required to reach background TCDD TEQ concentration.

Assumptions:

1. First-order rate of decay is assumed for all cited studies. Based on initial and final concentrations, a first-order rate constant was derived in order to calculate a time to reach background TCDD TEQ concentration of 9.12 pg/g.
2. No rate-limiting factors inhibit dioxion degradation (e.g. sufficient nutrients are available, including oxygen, and weathering does not impede contaminant degradation).

Calculations:

For first-order biodegradation, concentration (C) decreases exponentially:

$$C = C_0 e^{-kt}$$

where:

C_0 = initial concentration at t = 0 (sample: 650 pg/g)
k = first-order decay rate constant (sample: k = $2.2\text{x}10^{-4}$ day^{-1})
C_f = background TCDD TEQ level as outlined by DTSC = 9.12 pg/g
t = time required to reach C_f, days

Taking the natural logarithm and rearranging, solve for t:

$$t = \frac{ln\left(\frac{C}{C_0}\right)}{-k} = \frac{\ln\left(\frac{9.12}{650}\right)}{-2.2\ x\ 10^{-4}\ day^{-1}} * \frac{year}{365\ days} = 53.1\ years$$

Table 6.6. Estimated time required to reach TCDD TEQ background level (9.12 pg/g) assuming first-order kinetics using minimum and maximum reported rate constants.

Dioxin Concentration (TEQ ppt)	Time Required to Reach Background Level			
	Anaerobic (days)		Aerobic (years)	
Initial	Minimum[1]	Maximum[2]	Minimum[1]	Maximum[2]
10	0.1	0.5	0.1	1.1
50	2.5	10.0	1.7	21.2
100	3.5	14.1	2.4	29.8
200	4.5	18.2	3.0	38.5
300	5.0	20.5	3.4	43.5
400	5.5	22.2	3.7	47.1
500	5.8	23.6	3.9	49.9
600	6.0	24.6	4.1	52.1
650	6.2	25.1	4.2	53.1

[1] based on highest reported rate constant
[2] based on lowest reported rate constant

The soil moisture content of the soils at SSFL should also be measured to determine if the moisture levels are suitable for biodegradation. Soil moisture of about 15% is ideal for the biodegradation of some contaminants (Chokshi & Nelson, 2003). There appears to be healthy vegetation at the site, which could be beneficial in providing conditions for a healthy rhizosphere environment. Several studies have demonstrated the increased capacity for removal of contamination due to interactions between plants and certain microbial communities (Wang & Oyaizu, 2011).

The topsoil at SSFL Area IV appears to be a sandy loam with the size distribution shown in **Table 6.7**. Since the soil particle sizes are relatively large, less sequestration of dioxins would be expected than for clay soils. This improves the chances of active dioxin biodegradation at the site.

Table 6.7. Sieve analysis of Area IV soil at SSFL
(tested November, 2013).

Size fraction	Weight %
>2mm	~25%
2mm - 0.425mm	~33%
0.425mm - 0.075 mm	~34%
<0.075 mm	~6.6%

The Santa Susana site averages 273 sunny days per year ("Climate in Santa Susana, California," 2012) making the site ideal for natural photodegredation. However, much of the dioxin is located under the surface soil at depths which UV light cannot penetrate. Thus, photodegradation alone is not a viable mechanism for natural attenuation of dioxins.

The outlook for natural attenuation processes to reduce dioxin concentrations to background levels at SSFL is not promising, particularly with the low target concentrations currently set. Dioxins are very recalcitrant contaminants, and may require more active remediation approaches to reach background levels. Areas with lower levels of dioxin contamination may be amenable to biostimulation and/or bioaugmentation, while areas with higher concentrations may need to be remediated through excavation.

A companion study is underway to determine if fungi growing in the soil at the site are capable of biodegrading dioxins, and to use DNA analyses to determine if known dioxin-degrading bacteria (e.g. *Dehalococcoides*) are present in the SSFL soils.

Laboratory experiments are also planned to measure biodegradation rates under natural conditions at the site. These experiments are essential for providing an accurate estimate of the time that would be required to reach background levels of dioxins under natural site conditions. The microcosm experiments will also be used to test the efficacy of biostimulation (surfactants and nutrients) and bioaugmentation with white-rot fungi (*Phanerochaete*), which is known to biodegrade dioxins under a variety of conditions. These later tests will provide valuable information on the feasibility of using these approaches to increase natural biodegradation rates to provide for adequate bioremediation.

7.0. Perchlorate

7.1. Physical Properties and Toxicity of Perchlorate

Perchlorate (ClO_4^-) is a soluble anion which has contaminated groundwater and soil in some locations, primarily as a result of manufacturing of rocket fuel and explosives. Perchlorate was used as the exothermic oxidant for rockets because it formed products such as water and gaseous N_2 and O_2 that left no significant residue (Brown & Gu, 2006). However, improper disposal and management of perchlorate has led to environmental contamination. Because perchlorate does not degrade easily, it can accumulate in aquifers. Its high mobility and low rate of adsorption cause it to follow the flow of water, leading to its detection in some drinking water supplies (Brown & Gu, 2006). Perchlorate also accumulates by natural processes in nitrate-rich fertilizers, as observed in California, Nevada, New Mexico, and some southern states (Logan et al., 2001). In California, perchlorate was first detected in drinking water in Sacramento in 1997 near an Aerojet facility that used ammonium perchlorate salts as a solid rocket propellant (US EPA, 2007).

Research has shown that perchlorate can interfere with iodine uptake, which in turn affects thyroid regulation. Specifically, perchlorate is a competitive inhibitor with iodide due its comparable ionic size, and this impedes function of the Na^+/I^- symporter needed to pump iodide into the thyroid (Nagle & Duman, 2005). If the thyroid is unable to signal the release of hormones, many organ systems will be affected. Perchlorate consumption is of particular concern for infants, who rely on a healthy thyroid to secrete hormones for bone development and metabolic activity (Nagle & Duman, 2005). Perchlorate levels are now regulated in U.S. drinking water, with a concentration of 6 µg/L set as the safe drinking standard (US EPA, 2007).

Due to its high solubility in water, perchlorate leaches easily through soils. Because perchlorate does not adsorb to soil particles, it is often displaced by hydroxide and phosphate ions (Urbansky & Brown, 2003). As water travels through soil, perchlorate salts are dissolved in the surface soil layer and travel into deeper layers depending on the flow and volume of ground water. Because of this process, perchlorate becomes more of a groundwater concern rather than a soil issue. However, if evaporation rates equal or exceed the rate of migration then perchlorate precipitates and becomes trapped in soil (Urbansky & Brown, 2003). Studies on perchlorate biodegradation in the vadose zone have indicated that perchlorate becomes an environmental concern when low soil moisture keeps the anion from migrating into groundwater (Gal et al., 2008).

7.2. Abiotic Processes Affecting Perchlorate

As a highly stable, strong oxidant, perchlorate is resistant to most reactions when other charged ions are present. Due to its high solubility in water, perchlorate can move quickly through soil in the aqueous phase. As a polar molecule with a low charge density, perchlorate tends to form ionic bonds with cations equal in charge density (Urbansky & Brown, 2003). With a standard potential of 1.389 volts (**Eq 7.1**), perchlorate is a stronger oxidant than oxygen or chlorine. The oxidation state of chlorine atoms in perchlorate is +7. Thermodynamically, perchlorate thus should be easily reduced, but its reduction reaction is restricted by slow kinetics because of the high activation energy for the abiotic reaction. Therefore, perchlorate is considered chemically nonreactive (Cao et al., 2005). However, biological processes can utilize enzymes as catalysts to overcome the high activation energy and allow the reduction reaction to occur more rapidly (see below).

$$ClO_4^- + 8H^+ + 8e^- = Cl^- + 4H_2O \quad E^0{}_H = 1.389\ V$$

Equation 7.1. Standard potential of perchlorate (from Cao et. al., 2005).

Perchlorate is considered a non-complexing anion and a poor nucleophile. Certain metals (i.e. Vanadium II and III, Titanium III) have been identified as being able to reduce perchlorate when using an acid catalyst. Vanadium II can reduce perchlorate with a half-life of 11.3 years and Titanium III with 0.83 years, however these metals are typically present in aqueous environments in such minuscule concentrations that they do not affect perchlorate in the environment (Cao et al., 2005).

Nanoscale iron was reported as being an electron donor for perchlorate reduction, with the reaction shown below occurring at temperatures between 25-75°C (**Eq. 7.2**). The rate of reduction was also influenced by temperature, as the perchlorate-reduction rate increased as with incremental temperature increases.

$$ClO_4^- + 4Fe^0 + 8H^+ \rightarrow Cl^- + 4Fe^{2+} + 4H_2O \quad \Delta G^o = -1,387.49\ kJ/mol$$

Equation 7.2. Free energy of perchlorate reduction with iron (from Cao et. al, 2005)

Abiotic removal of perchlorate from soil has been investigated by the process of electrokinetic injection. Although usage has been primarily developed for removing perchlorate in groundwater, electrokinetic studies have also been done in soil media. In one study (Jackson et al., 2004), organic substrates such as glycine and lactate were injected into laboratory soil columns and led into electrode reservoirs to maintain neutral pH. Electrodes were placed at the cathode and anode with a voltage between 60-90 V. A net flux of cations flowed towards the positive cathode and a net flux of anions to the negative anode provide an electrical gradient for ionic species to migrate through the matrix. Pore fluid in the matrix moved by electroosmosis towards the cathode. Through electrokinetic remediation technology, perchlorate can be removed by precipitation and ion exchange at the electrodes. The combination of electrokinetic extraction with electrokinetic injection of organic compounds has resulted in feasible transport rates despite low permeability in soils. The efficacy of perchlorate transport depends on the buffering capacity of the soil. Migration of ions will be dominated by acidic H^+ ions unless other properties of the soil delay this process (Acar & Alshawabkeh, 1993).

Experiments by Jackson et al. (2004) were also conducted for removal of perchlorate with electrokinetic injection to increase the rate of biodegradation. In supplying an electric gradient within the soil, perchlorate migrated towards the anode and was able to be physically removed, as shown in **Figure 7.1** (Jackson et al., 2004). Natural soil was analyzed solely with electrokinetic extraction to establish initial rates of degradation and then with electrokinetic injection of organic compounds to test for an increase in rate of removal. In injecting soil with lactate and glycine, perchlorate was reduced with a jump from 44% to 94.2% in biodegradation rate for a Texan soil plot and from 0.3% to 36.6% for another Texan soil sample (Jackson et al., 2004). Saturated soil was also more amenable to the removal of perchlorate compared to dry, oxidized soil (Jackson et al., 2004).

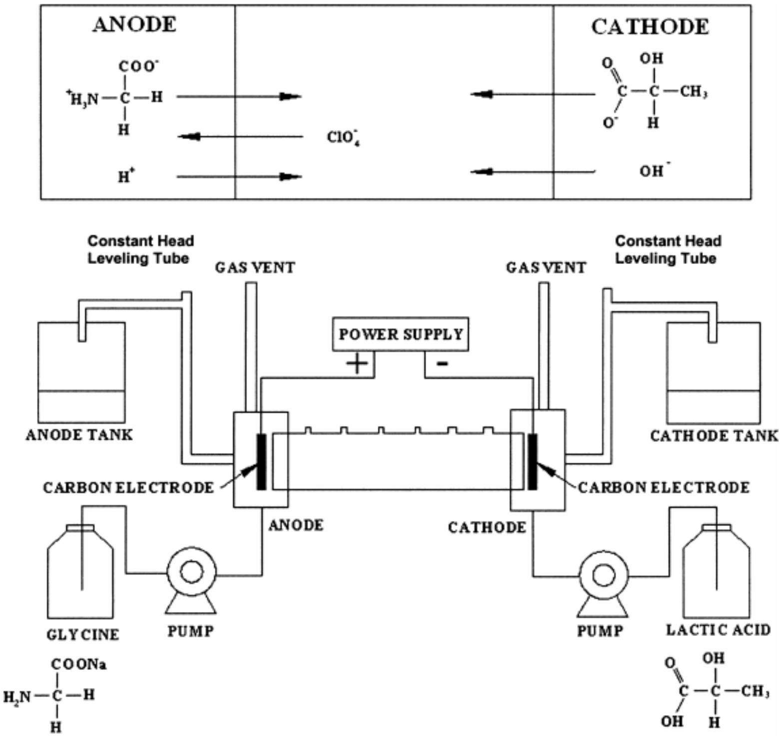


Figure 7.1. Schematic of electromigration of perchlorate from cathode to anode as represented in an electrokinetic experiment by Jackson et. al., 2004. Glycine was pumped through the anode and lactic acid was pumped through the cathode to establish the electric gradient.

7.3. Microbial Reduction of Perchlorate

In biological reactions perchlorate acts as an electron acceptor (oxidizing agent) to transfer electrons from another compound (an electron donor), while the perchlorate becomes reduced. Electron donors are consumed by bacteria as a food source, and the electrons are transferred to perchlorate in the process. In this way perchlorate is reduced to form harmless end products such as chloride, water, and carbon dioxide (Sellers et al., 2006). For microbes, electron donors are typically carbon-based compounds, although hydrogen has also been used as an electron donor for perchlorate reduction.

For bacteria, oxygen and nitrate are preferable electron acceptors for metabolism. Therefore, perchlorate is typically only used as an electron acceptor after oxygen is depleted from the environment. Although bacteria possess the enzymes necessary to reduce perchlorate, the bacteria will preferentially use O_2 and NO_3^- as electron acceptors before reducing ClO_4^-.

Biological perchlorate reduction thus requires anaerobic conditions. Perchlorate is, however, preferred by bacteria as an electron acceptor over sulfate.

In a laboratory-controlled experiment with acetate as the electron donor, *Proteobacteria* and *Dechlorosoma* were shown to significantly reduce perchlorate to chloride due to the presence of chlorite dismutase in their membrane to mediate the reduction (Chaudhuri et al, 2002). In another study biologically-mediated redox reactions coupled the oxidation of acetate to the reduction of perchlorate with chloride as the main product in soil samples (Coates et al., 1999). When oxygen was introduced to the environment, rates of reduction significantly decreased, indicating that microbes switched to aerobic mechanisms to obtain energy. Other studies have indicated that the reduction to chlorate is inhibited when oxygen is present (Chaudhuri et al., 2002). In fact, when cultures were grown in aerobic environments, the reducing enzyme was not active in bacterial membranes, suggesting that perchlorate reduction would not occur in the surface layer of soil due to high oxygen levels. This means that contaminated soil would need to be anoxic by means of flooding or another method for biological perchlorate reduction to be possible. It has also been reported that the bacteria should be in log phase of growth to show enough enzyme activity to accelerate biodegradation (Chaudhuri et. al., 2002). A somewhat diverse microbial population is also needed in the soil to facilitate perchlorate reduction, as some species have the chlorite dismutase enzyme but cannot reduce perchlorate (Gal et al., 2008).

The presence of the metal molybdenum can enhance the growth of perchlorate-reducing bacteria along with the transformation of perchlorate (Chaudhuri et al., 2002). For the species *Dechloromonas aromatica*, molybdenum was a nutritional requirement to encode a chaperone gene to form the enzymes chloride dismutase and perchlorate reductase (Chaudhuri et al., 2002). Molybdenum would most likely be present in more basic soils as low pH limits availability of molybdenum salts.

Nitrate has been reported to compete with perchlorate as an electron acceptor, and can inhibit perchlorate reduction (Coates et al., 1999). As described above, under anoxic conditions reducing bacteria prefer to use nitrate, followed by perchlorate, and then sulfate as electron acceptors. When perchlorate-grown bacteria were plated with perchlorate and nitrate substrates, microbes reduced nitrate to obtain energy (Chaudhuri et al., 2002). Although bacteria possessed enzymes specifically for perchlorate reduction, nitrate was the preferred energy choice. Thus, complete nitrate reduction (denitrification) improves perchlorate reduction as this process removes the competing nitrate ions (Chaudhuri et al., 2002). Research by Chaudhuri et al. (2002) suggests that high levels of nitrate in soil could dramatically inhibit natural attenuation of perchlorate.

Rates of perchlorate reduction in soils were assessed in a microcosm study using perchlorate-contaminated soils in Israel (Gal et al., 2008). This study investigated perchlorate in unsaturated soil (vadose zone) 40 m deep, and determined that perchlorate was utilized by microbes when no external carbon source was added to soil samples, reducing perchlorate at 0.45 mg/day per 4.5 mL slurry (Gal et al., 2008). When acetate was added to the soil as a carbon source, the reduction rate increased to 7.2 mg/day per 4.5 mL slurry (Gal et al., 2008). Microbial populations were suggested to have adapted to high perchlorate conditions after 30 years of exposure, even though concentrations of 300 mg/L were considered toxic (Gal et al., 2008). Bacterial strains were therefore able to adapt to the oxidative damage caused by perchlorate with maximum concentrations of 200 mg/L.

Various bioremediation methods have been explored for actively increasing perchlorate reduction rates at contaminated sites, typically involving the injection of electron donors. One promising approach is the injection of gaseous electron donors as an *in situ* technique for perchlorate bioremediation. Hydrogen can be added as an electron donor to the soil at variable depths using injection wells paired with vapor extraction wells (Cai et al., 2010). Past experiments showed that hydrogen injection resulted in the complete reduction of nitrate and perchlorate when adequate soil moisture was available (Logan et al., 2001). Hydrogen gas is effective because it is easily diffused and therefore has high mobility in the gas state. It can also be readily taken up by bacteria due to its small size. In a microcosm experiment, hydrogen gas was found to be preferable to other electron donors as it resulted in the shortest lag phase of 7 days, meaning bacteria did not need as much time to adapt to perchlorate reduction (Cai et al., 2010). In this microcosm experiment, the first-order rate constant for perchlorate reduction was about equal with low and high hydrogen concentrations, indicating that abundance of the electron donor does not significantly affect the microbes' ability to reduce perchlorate (Cai et al., 2010). This is an added benefit for bacteria needing to reduce perchlorate in deep soil layers where conditions for growth are less favorable.

Soil moisture content is an important condition for perchlorate reduction. The microcosm study by Cai et al. (2010) found that perchlorate was reduced most efficiently at 16% soil moisture, and no perchlorate reduction was observed at 7% moisture. Complete nitrate reduction was observed at all tested soil moisture contents, reinforcing that nitrate is the more preferred electron donor.

7.4. Phytoremediation of Perchlorate

Phytoremediation is an attractive option for perchlorate remediation due to its comparatively low cost, landscape aesthetics, and easy maintenance. Phytoremediation of perchlorate was investigated in a soil microcosm study using perchlorate-contaminated soils from the Longhorn Army Ammunition Plant (LAAP) in Texas. This study showed that root products of poplar trees enhanced perchlorate reduction by microbial strains (Shrout et al., 2006). This site in Texas was chosen for its previous activity with missile construction. Soil samples were treated with root homogenate (suspended root fragments in a uniform mixture) from hybrid poplar tree cuttings and inoculated with bacterial strains known to reduce perchlorate (Shrout et al., 2006). Results showed that bacteria were able to use root products as a food source to reduce perchlorate. Additional tests showed that roots introduced to soil with no previous perchlorate exposure were still able to act as a carbon source for perchlorate-reducing bacteria, indicating that trees do not need to have been grown in a perchlorate-rich environment to be conducive to this process (Shrout et al., 2006). Plants can therefore be used to create an environment suitable for bacterial growth and consequent perchlorate reduction. Application of phytoremediation to the LAAP site showed 45% reduction in perchlorate within a 2-year period by planting 425 poplar trees (Shrout et al., 2006).

Perchlorate salts can also be recycled through soil by plants extending into the groundwater region, such as willow and cottonwood (Urbansky & Brown, 2003). In Las Vegas, the salt-cedar tree was able to dispel perchlorate salts from its leaves through transpiration, which changed the chemistry of the organic soil layer (US EPA, 2003). Phytoremediation may not be as feasible for perchlorate in deeper zones of soil where roots cannot penetrate.

7.5. Published Biodegradation (Reduction) Rates of Perchlorate

Table 7.1 summarizes the results of published studies of perchlorate degradation in soils and soil slurries. These results have been classified by matrix investigated (soil vs. slurry), removal process, initial and final perchlorate concentrations, and reported rates of removal. Experimental conditions are also specified. Significant reductions of perchlorate concentrations were observed, with some studies reporting 100% removal in as little as 30 days (Table 7.1). A wide range of rates were observed, depending on experimental conditions.

7.6. Potential for Natural Attenuation of Perchlorate at SSFL

Perchlorate concentrations in soils in Area IV of SSFL have been reported over a wide range, from 99.6 ppb to 21.3 ppm (**Table 7.2**). Due to the pattern of transport of perchlorate in soil, this anion is likely to percolate down to the groundwater, and therefore will become less of a soil problem.

It is difficult to estimate the potential natural attenuation rate of perchlorate in Area IV via biodegradation because of a lack of information about the conditions in the soil. Most of the reported data for biodegradation of perchlorate comes from studies on wet soils or slurries under anaerobic conditions (Table 7.1). If the soils in Area IV are aerobic, then perchlorate biodegradation under natural conditions is unlikely. Therefore it is recommended that soil vapor analyses be conducted to survey the redox conditions in the soils to determine if anaerobic conditions exist that would facilitate perchlorate reduction.

If favorable conditions are found in Area IV, it may be feasible to enhance perchlorate degradation through biostimulation methods such as amending the soil with electron donors. Additionally, soil should be tested for molybdenum, which aids in the formation of enzymes for perchlorate-reducing bacteria.

Phytoremediation may be a plausible approach of remediating perchlorate in SSFL soils based on the success of phytoremediation of perchlorate-contaminated soils in Texas (Shrout et al., 2006). It has been reported that root exudates may serve as an electron donor for the reduction of perchlorate. Because root products can easily be regenerated in a short time period, carbon stores can be replenished for perchlorate-reducing microbes. However, phytoremediation is not expected to reduce perchlorate in a short time due to the competitive inhibition of nitrate as an electron acceptor. Most of the studies indicate that perchlorate can only be reduced once nitrate has been eliminated from the area, or simultaneously with electron transfer to perchlorate. Because nitrate is generally preferred as an electron acceptor, this will significantly add to the length of time needed for perchlorate remediation. Therefore, it would be important to determine nitrate levels in the soils at SSFL to further assess the feasibility of microbially-mediated perchlorate reduction at the site.

Table 7.1. Reported rates of perchlorate degradation through natural attenuation processes and stimulated conditions in various experimental conditions.

Matrix	Process	Perchlorate Concentration		Rate	Conditions	Reference
		Initial	Final			
Soil slurry	Microbial metabolism	70 mg/L	-	0.45 mg/day per 4.5 mL slurry	Natural organic matter as carbon source	(Gal et al., 2008)
Soil Slurry	Microbial metabolism	70 mg/L	-	5 – 7.2 mg/day per 4.5 mL slurry	Acetate as carbon source and electron donor	(Gal et al., 2008)
Soil	Bio-remediation	5.7 mg/kg	0.04 mg/kg	0.189 mg/kg/day	Homogenized soil, neutral pH, 16% soil moisture, H_2	(Cai et al., 2010)
Soil	Phyto-remediation	100 mg/L	5 mg/L	95 mg/L per 60 days	Anaerobic, JDS4 bacteria, root homogenate	(Shrout et al., 2006)
Soil slurry		5 ppm	-	0.03 – 1.42ppm/ day	Prior ClO_4^- exposure, anaerobic	(Tan et al., 2004)
Water	nanoparticles	200 mg/L	81.86 mg/L	59.07% reduction, 0.013 mg/g/hr	mechanical agitation, 28 day observation, 20g/L iron	(Cao et al., 2005)
Water	nanoparticles	200 mg/L	21 mg/L	90% reduction, 1.52 mg/g/hr	75 ^{o}C, mechanical agitation, 1 day observation, 10 g/L iron	(Cao et al., 2005)
Saturated soil	Nonreactive tracer	100 mg/L	0 mg/L	100 mg/L per 64 days	Columbia loam, air-dried, crushed, 25°C	(Tipton et al., 2003)
Saturated soil	Bio-degradation	180 mg/L	0 mg/L	180 mg/L per 30 days	Yolo loam air-dried, crushed, 25°C	(Tipton et al., 2003)

Table 7.2. Reported concentrations of perchlorate at Santa Susana Field Laboratory for various locations in Area IV.

Location	Perchlorate Concentration	Area (ft^2)	Depth (ft)	Volume (yd^3)
Building 4015 Field Fill	45.4 ppm	52, 357	4	7,760
Building 4010	21.3 ppm	4,441	8	1,320
FSDF Southeast	3.6 ppm	5,972	5	1,870

Electrokinetic processes could also be considered for perchlorate remediation in some of the highly contaminated soils of Area IV. This would be particularly useful for deeper soils which are not reached by plant roots and which might have lower microbial populations. However, this method works better on saturated soils (Jackson et al., 2004), which may limit its use at SSFL. Also, electrokinetic methods are expensive because of the cost of equipment and maintenance.

8.0. Mercury

Mercury is a contaminant of interest in Area IV soils at the SSFL site due to its frequency of detection. It is unique among the contaminants addressed in this study because it is a metal which does not biodegrade. Nonetheless, there are processes affecting mercury in soils which could contribute to natural attenuation of the mercury contamination over time. Such processes include volatilization, methylation, uptake by plants, and leaching. These processes could be modified to provide active remediation along with more intensive methods such as thermal desorption.

8.1. Physical Properties and Toxicity of Mercury

Mercury exists in the environment as elemental mercury (Hg^0), ionic mercury (Hg^{2+} or Hg+), and methylmercury (MeHg). The ionic forms of mercury are found in precipitates such as mercuric hydroxide ($Hg(OH)_2$), and mercuric sulfide (HgS) (Yao et al., 2011).

Elemental mercury is a liquid at room temperature, with a melting point of -38.8°C and a specific gravity of 13.5. The vapor pressure of elemental mercury at various temperatures is shown in **Table 8.1**. At 25°C, the vapor pressure of mercury is about 0.0017 mm Hg (Huber et al., 2006).

Table 8.1. Vapor Pressure of Mercury (CRC, 2003)**.**

Vapor Pressure	1 Pa	10 Pa	100 Pa	1 kPax	10 kPa	100 kPa
Temperature (°C)	42	77	120	176	250	356

Mercury is one of the most toxic metals found in the environment and has a variety of health implications for humans. Mercury is a powerful neurotoxin and can bioaccumulate in the food chain because of its solubility in fatty tissue, and it is recognized as mutagenic and teratogenic (Tchounwou et al., 2003). It has also been implicated in cancer of the kidneys (Tchounwou et al., 2003). Mercury also is cause for ecological concern because of its adverse effects on aquatic plants, invertebrates, fish and birds (Boening, 2000; Wolfe et al., 1998).

Methylmercury is the most toxic form of mercury and of the greatest concern to human health (Tchounwou et al., 2003). It is formed biologically under anaerobic conditions with a low pH, so it forms predominantly in anaerobic aquatic environments. When it forms in these aquatic environments, it can be absorbed into the muscle tissue of fish and results in bioaccumulation up the food chain (Monteiro & Costa, 1996). For these reasons, mercury is on the US EPA list of priority pollutants (Cameron, 1992).

Several natural processes can reduce mercury concentrations in the environment:
1) volatilization of elemental mercury, 2) methylation and subsequent volatilization, and
3) phytoextraction (hyperaccumulation in plants). These processes can also be enhanced to form the basis of active remediation through methods such as genetic modification of plants to enhance phytoremediation, soil washing to remove mercury from the soil, electrokinetic treatment, and thermal desorption (Mulligan et al., 2001). These processes are reviewed below

and an assessment is made of the potential for these processes to contribute to the natural attenuation of mercury contamination in the soils at SSFL.

8.2. Volatilization and Methylation of Mercury

Mercury in its elemental form (Hg^0) is volatile and thus volatilization can remove mercury from soils into the air, although the rate is slow. Schluter (2000) reported mercury evaporation rates from background soils of less than 0.2 μg/m^2/hr, although significantly higher rates could be expected for "mercury-enriched mineralized areas" (Schluter, 2000). Unit conversions of this reported rate suggests an evaporation rate of 1.8 mg/m^2/year, which could result in significant removal of mercury over the long term. However, mercury volatilization as a method of remediation may be problematic because of the toxicity of mercury vapors (Essa et al., 2002). Some researchers have observed bacterially-mediated reduction of mercury salts to elemental mercury (von Canstein et al., 1999) and subsequent volatilization, and this has been explored as a bioremediation strategy (Essa et al., 2002). However, toxicity of mercury vapors is still an issue with this approach.

Methyl mercury can be formed by the transformation of Hg^{2+} through natural biological processes, and due to its volatile nature, lead to the removal of mercury from soils. Sulfate-reducing bacteria and iron reducing bacteria have been shown to methylate mercury under anaerobic conditions, particularly in aquatic environments (Yu et al., 2012). This biomethylation process increases volatility and provides for greater mobility which allows the mercury to evaporate and also enter into groundwater (Mulligan et al., 2001). However, since it is even more toxic than elemental mercury, air and groundwater pollution with methyl mercury could be seriously problematic. Methylation of mercury can also thwart phytoremediation attempts as it is toxic to plants (Meagher & Heaton, 2005).

8.3. Phytoremediation of Mercury

Since metals cannot be biodegraded, phytoextraction is the primary mechanism of metal phytoremediation (Raskin et al., 1997). Certain plants are well known to take up metals into their tissue and store them (Baker & Brooks, 1989). One plant uptake mechanism is chelation. Plants produce binding proteins such as metallothioneins or phytochelatins and these proteins can bind to metals and form a complex thereby increasing the bioavailability of the contaminant which can then be more readily taken up into the plant (Mejáre & Bülow, 2001).

If a plant accumulates metals in the foliage or stems, those sections can be harvested and the metals disposed of safely. The plant can then grow back, accumulate more metals, and be harvested until the surroundings have been remediated (McGrath & Zhao, 2003). However, such a removal mechanism is not natural attenuation because active harvesting and disposal of plant material is required. In addition to phytoextraction, phytovolatilization has been shown to occur for mercury, however, the natural volatilization rate is extremely low (Heaton et al., 1998; Lewis et al., 1966).

Certain plants termed "hyperaccumulators" are of particular interest for use in phytoremediation. Although the exact criteria used to define metal hyperaccumulators is under discussion (Ent et al., 2013), metal hyperaccumulators are plants capable of storing metals in their tissue at concentrations that are much higher than the surrounding environment (Baker & Brooks, 1989 and Memon & Schröder, 2009).

Many studies have looked at which plant species are most effective at removing mercury (Meagher, 2000; Pilon-Smits, 2005; and Rugh et al., 1998). In China in 2009, researchers investigated absorption and accumulation characteristics of plants in soil contaminated with mercury near Huainan mining areas. The researchers tested eleven different plant species at three major coal-mining areas to test for how well the plants accumulated mercury (Yao et al., 2011). Carrots, beans, garlic sprouts, Jerusalem artichoke, wheat, fleabane, daylily, geranium, wild artemesia, liriope, and clover were all tested for effectiveness at removing mercury. The mercury accumulation of the plants was divided into two categories – stem/leaves and roots (Yao et al., 2011). The mercury in the soil at the mining sites tested ranged from 0.089 to 0.184 mg/kg. Of the eleven crops tested, the fleabane was by far the most effective at accumulating mercury and can be considered a hyper-accumulator of mercury (**Table 8.2**). For most of the mining sites tested, the mercury concentration in hyper-accumulators was higher in the roots than in the stems/leaves, suggesting that the mercury was being absorbed from the soil and not the atmosphere (Yao et al., 2011).

Table 8.2. Mercury concentrations in plants grown in mercury-contaminated soil at a coal mine in China (Yao, 2011).

Mining Site Tested By Yao	Plant	Stem-Leaf Mercury Concentration (mg/kg)	Root Mercury Concentration (mg/kg)
1	Fleabane	0.088	0.077
	Wild Artemisia	0.059	0.087
2	Geranium	0.096 ± 0.055	0.146 ± 0.048
	Wild Artemesia	0.090 ± 0.034	0.134 ± 0.051
	Fleabane	0.095 ± 0.021	0.083 ± 0.019
4	Geranium	0.097 ± 0.043	0.118 ± 0.042
	Wild Artemesia	0.072 ± 0.028	0.104 ± 0.032
5	Fleabane	0.271	0.191

The main barriers to Hg uptake are lack of Hg bioavailability and poor translocation of Hg from the roots to the aerial portion of the plant (Heaton et al., 1998). Mercury in most soils is often bound to organics or in sulfide minerals, which limits its uptake by plants (Schuster, 1991). One way to increase bioavailability of mercury is to add chelating agents to the soil (Ashraf-Khorassani & Taylor, 1999; Wang et al., 2012), and this is described below in Section 8.4 on active remediation methods for mercury.

8.4. Active Remediation of Soils Contaminated with Mercury

Many active methods of remediating metal-contaminated soils have been proposed, including metal chelation, soil washing, thermal desorption and electrokinetic processes (Mulligan et al., 2001).

Chelation to Enhance Mercury Uptake by Plants: Chelation of mercury by chemicals such as iodide, EDTA and ammonium thiosulfate has been successfully used to increase the bioavailability of mercury and increase plant uptake (Subirés-Muñoz et al., 2011). In 2012, a group of Italian researchers from the National Research Council researched the "modified bioavailable contaminant stripping" method using common European plants (*Brassica juncea, Poa annua, Helianthus annus*) as a means of remediating contaminated industrial soil containing 15.1 ppm mercury (Pedron et al., 2013). *Brassica juncea* and *Poa annua* were selected because of historic success in mercury accumulation, and *Helianthus annus* was selected because of high biomass production with reported metal tolerance (Pedron et al., 2013). These researchers added ammonium thiosulfate, $(NH_4)_2S_2O_3$, to soil to increase the bioavailability of mercury. The thiosulfate serves as a ligand that chelates the mercury and enhances the capacity of roots to absorb mercury as well as transport it to the above-ground tissues of the plant, thus removing it from the soil (Subirés-Muñoz et al. 2011; Evangelou et al. 2007; Wang et al. 2012). Plants were grown in a greenhouse for 60 days prior to conducting testing. Mercury removal from the soil was indicated by the available mercury fraction. When no available mercury fraction could be further removed from the soil, the residual mercury in the soil was considered safe (Pedron et al., 2013). The ammonium thiosulfate addition proved to be effective: plants treated with the ammonium thiosulfate had a 14 times higher available mercury fraction than that in untreated soil. As a result, all the plants accumulated more mercury. After one growth cycle, the plants successfully removed 95.7% of the bioavailable mercury from the soil. The remaining mercury in the soil was considered inert by the researchers, as there was no significant uptake by the ammonium thiosulfate nor the plants when researchers conducted a second growth cycle in the soil (Pedron et al., 2013). This study indicates that chelation by ammonium thiosulfate is a promising method of facilitating mercury remediation.

Genetic modification to enhance uptake by Plants: Researchers have genetically modified plants to make them more adept at mercury phytoremediation (Meagher, 2000; Meagher & Heaton, 2005; and Mulligan et al., 2001). Certain plants, such as willows or tobacco, can be genetically altered to express the *mer*A gene which enables a greater resistance to mercury contamination and a greater rate of Hg^0 volatilization (Rugh et al., 1998; C. L. Rugh et al., 1996). *Mer*B is another gene that encodes a mercury-processing enzyme called organomercurial lyase. This enzyme catalyzes the breaking of carbon-mercury bonds in methyl-mercury (MeHg) and produces Hg^{2+} which is then used by the mercuric reductase enzyme and volatilized (Heaton et al., 1998). Both *mer*A and *mer*B genes also help protect the plant cells from organic mercury (Ruiz & Daniell, 2009). While these experiments are promising, genetic modification will not likely play a role in the remediation of SSFL because introducing genetically modified plants would not be acceptable.

***Soil Washing of Mercury*:** A technique with past success in removing mercury is soil washing, a method that uses chemical leaching, physical separation, or physiochemical procedures to remove mercury from soil. Potential mercury soil washing agents include iodide, thiosulfate, and EDTA. These agents act as chelating agents and can remove about 30% of the mercury in soil (Wang et al., 2012). However, when these agents are used, the soil's weak acid soluble fraction may be increased, creating a greater environmental risk because of increased mercury mobility (Subirés-Muñoz et al., 2011). Advantages of using soil washing include possible permanent removal of mercury from the soil, being able to return the processed soil to the site, and a short time frame. However, this method uses large quantities of water to make the washing solutions and metal-chelant complexes form in these solutions so solutions must be treated before they can be safely discharged (Wang et al., 2012).

Thermal Desorption of Mercury: Thermal desorption of mercury is a process that uses heat to increase the volatility of the mercury to remove it from the soil without actually combusting the soil. Mercury in the form of mercury(II) compounds, like HgS, HgO, and $HgCO_3$, can be converted to elemental mercury when the soil temperature is between 600 and 800°C (Wu et al., 2012). Since elemental mercury is extremely toxic, some sort of collection mechanism is necessary to prevent the mercury from entering the atmosphere. In an experiment by Kucharski et al. (2005), a scrubber was used to collect these vapors. These researchers heated contaminated soil for ten days at 167^{o}C and reduced water-soluble, exchangeable, and elemental mercury to undetectable levels (less than 0.05 mg/kg) and reduced total mercury levels by about 550 mg/kg (Kucharski et al., 2005). **Table 8.3** shows the results of this experiment. In a different study by Huang et al. (2011), thermal desorption was effectively used to reduce the mercury concentration from 1320 mg/kg to 6 mg/kg by heating the soil with a fixed bed reactor at 550°C for an hour. Through various rounds of testing, this study concluded that heating soil above 400°C can lower the mercury content of the soil below 20 mg/kg (Huang et al., 2011). Thermal desorption effectively removes mercury from soil but requires large amounts of energy to do so. It has also only been proven effective for high soil concentrations of mercury (Wang et al., 2012).

Table 8.3. Mercury Concentrations before and after thermal desorption
(Kucharski et al., 2005).

Mercury Fraction	**Soil Mercury Concentration (mg/kg)**	
	Before Heating	**After Heating**
Water-Soluble	18.62 ± 363	Not Detected
Exchangeable	222.5 ± 65.5	Not Detected
Elemental	308.6 ± 34.8	Not Detected
Fulvic/Humic Acids	170.5 ± 26.1	165.9 ± 26.6
Organic and Sulfide	617.4 ± 64.3	621.4 ± 64.8
Residual	386.9 ± 42.8	391.5 ± 42.3
Total Mercury	1738 ± 132.4	1187 ± 89.1

8.5. Potential for Natural Attenuation of Mercury at the SSFL Site

The volatilization of elemental mercury from soils in Area IV is a possible mechanism of natural attenuation at this site, but this process is typically slow and it is difficult to predict volatilization rates without knowing the speciation of mercury in the soil. Also, since mercury vapors are toxic, volatilization could cause air quality problems. Mercury methylation could also lead to removal of mercury from soils, but this biological process requires anaerobic conditions. At this time, the soils in Area IV are thought to be aerobic, in which case methylation would not be likely. Soil vapor analyses are planned to determine the degree of aeration of the soils at SSFL. If methylation did occur at the site, it could potentially lead to leaching of methylmercury into the groundwater and/or production of methylmercury air pollution. Since methylmercury is the most toxic form of mercury, this would be a serious problem. Tests are currently underway at the University of California Riverside to determine the speciation of mercury in soils at the SSFL site, and results of these tests could improve our understanding of the natural attenuation processes which might reduce mercury concentrations in the soil.

Phytoremediation is an unlikely mode of natural attenuation of mercury contamination at SSFL, although active processes could possibly be used to make phytoremediation possible. Removal of mercury by phytoextraction would require harvesting and disposal of plants, and thus is considered an active process (not natural attenuation). Phytovolatilization is a possible mode of natural attenuation, but this process is expected to be slow, and it would require active uptake through plant roots. Such uptake is expected to be slow without the use of chelating agents to facilitate uptake through the roots (Heaton et al., 1998). Without active management, phytoremediation of mercury could also be limited because of by sporadic plant growth and possibly reduced biomass production caused by toxic metals in the soil (Mendez & Maier, 2008).

Mercury phytoremediation could still be pursued as an active bioremediation strategy. There is an abundance of studies that show uptake of heavy metals by plants, and it is estimated that over 500 plant taxa are hyperaccumulators of metals (Ent et al., 2013). Phytoremediation can be effective given enough time, proper harvesting, and adequate planting density/speciation, and any necessary amendments such as chelating agents. However, the specific time for complete remediation can be highly variable depending on these factors. A companion study is currently underway at Cal Poly to investigate possible mercury uptake by selected native plants growing at the SSFL site. Greenhouse experiments with these plants will help ascertain the time required for this method of remediation and also evaluate the potential for enhancing phytoremediation with chelating agents.

Other active methods of remediation may provide opportunities for more rapid mercury removal at SSFL, but they are expensive and disruptive. Thermal deposition has been effective for mercury remediation, but it requires heating the soil to high temperatures, which could potentially damage the soil. Mercury vapors would need to be collected during this process to avoid mercury air emissions. Soil washing is another option that has been reported with positive results, but it requires large quantities of water. Both thermal deposition and soil washing would involve extensive disturbance of soils at the site and would require removal of vegetation. Phytoremediation is the most promising mercury remediation process investigated because it can remove mercury from soil through plant harvesting with less disturbance.

The feasibility of natural attenuation processes and active remediation strategies will depend on the speciation of mercury in the soil. A companion study is currently being conducted by U.C. Riverside to determine the speciation of mercury in Area IV soils at SSFL. After results of that study are available, a more detailed assessment of potential natural attenuation of mercury can be made.

9.0 Conclusions

9.1. Potential for Natural Attenuation of COIs at SSFL

Logical reasoning suggests that natural attenuation processes are highly unlikely to reduce soil contaminant concentrations in Area IV soils to background levels specified by the DTSC in the near future. Most of the contaminants were deposited in the soil several decades ago, and therefore natural attenuation processes have already been acting on the soils for decades. Based on the mechanisms of natural attenuation identified in this literature review, there is no reason to expect a sudden increase in natural attenuation to result in precipitous decreases in contaminant concentrations over the next few years. To the contrary, most natural attenuation processes theoretically follow first-order kinetics, which means that the rates of natural attenuation would decrease over time as the contaminant concentrations decrease. In fact, biodegradation typically slows down even more than expected from first-order kinetics over time as contaminants become sequestered in the soil and the most easily biodegraded components of the contaminants have biodegraded early in the weathering process.

Natural attenuation processes have been identified which are expected to reduce the concentrations of the contaminants in Area IV soils at SSFL to some degree. These processes include abiotic weathering by volatilization, leaching, and photooxidation, as well as biodegradation by bacteria and fungi, and phytoremediation by plants. The important questions are: 1) To what extent can COI soil concentrations be reduced, and 2) How long will this take? Since each of the COIs have very different chemical properties, they are affected in different ways by each of these processes, and thus the potential for natural attenuation of each COI is described individually below.

Abiotic natural attenuation processes are expected to act on each of the COIs, but the rates of these processes are limited. Also, the COIs in SSFL soils are highly weathered because of the length of time they have been in the soil. This weathering has most likely already led to volatilization of the lighter components, leaving the less volatile components in the soil at the present time. Similarly, photoxidation of contaminants near the soil surface has most likely already oxidized the contaminants amenable to this weathering process. This means that the contaminants remaining in the soil at SSFL are not likely to be significantly further reduced in concentration by abiotic weathering.

Of the natural attenuation processes investigated, bacterial and fungal biodegradation appear to be the most likely to contribute significantly to reductions in concentrations of the COIs. One exception to this is mercury and other metals, which do not biodegrade, and in this case phytoremediation may be possible. Biodegradation processes have been researched for each of the COIs, and lists of microorganisms capable of mediating biodegradation have been provided for each COI. In addition, tables of published biodegradation rates under natural attenuation conditions have also been provided in this report for each COI.

As described above, natural attenuation processes are expected to be very slow at SSFL. In addition, there are some important limitations to biodegradation of the contaminants under natural, unamended conditions. First of all, the appropriate microorganisms must be present in the soils. Genetic testing is planned in a companion study to determine if such microorganisms are present in Area IV soils. Also, each contaminant has some propensity to become sequestered in the soil matrix, reducing its bioavailability for biodegradation over time. Another important consideration is the "low-hanging fruit" theory - that easily biodegraded fractions biodegrade first, leaving the more recalcitrant fractions in weathered soils. For example, lighter PAHs with fewer aromatic rings are biodegraded first, leaving the larger PAHs like benzo-a-pyrene. Similar scenarios are described for the other contaminants below. Nutrients must also be available in the soil to support growth of microbes able to degrade COIs. Another limitation could be the toxicity of metal contaminants in the soil, which could inhibit microbial growth at some locations.

Anaerobic soil conditions are required for microbially-mediated reductive dechlorination of the highly chlorinated dioxins and PCBs. Similarly, biological reduction of perchlorate is only possible under anaerobic conditions. However, redox conditions in the soils at SSFL have not yet been determined, so it is not known if such conditions exist. Tests are currently planned to analyze soil vapors to determine the concentrations of oxygen in the soils at Area IV.

Rates of each natural attenuation process are highly dependent on site conditions, such as soil moisture, aeration, and temperature, as well as the disposition of the contaminants. Effort was made to include studies that matched the SSFL climate and soil type, but site-specific conditions should be investigated in more detail. As described above, an important consideration is if anaerobic conditions exist anywhere in the SSFL soils. Therefore, recommendations have been made for site characterization as well as specific laboratory studies to determine the feasibility of bioremediation and phytoremediation strategies. These are summarized in more detail below in Section 9.2. Once the results of these studies are complete, a better assessment of the potential for natural attenuation at SSFL can be made.

The focus of this report is on natural attenuation without altering the environment, but recommendations are also made for promising active bioremediation methods such as biostimulation of microbial activity with surfactants and/or nutrients, bioaugmentation by adding known degraders of the COIs, and phytoremediation with native plants. Given the long time frame for natural attenuation, more active remediation approaches are recommended. Laboratory microcosm experiments are planned in a companion study at Cal Poly to assess the feasibility of these remediation strategies for soils at SSFL, and these studies will help guide future decisions on remediation strategies.

9.1.1. Natural Attenuation of Petroleum Hydrocarbons

Most petroleum hydrocarbons are amenable to biodegradation under natural attenuation conditions like those at SSFL. Numerous common soil bacteria and fungi are known to biodegrade petroleum hydrocarbons – in fact too many to list in the tables in this report. However, some hydrocarbon compounds are more difficult to biodegrade than others. For example, longer-chain hydrocarbons biodegrade more slowly than short-chain hydrocarbons, and there are some constituents of petroleum hydrocarbons which are particularly recalcitrant. Reported half-lives for biodegradation of petroleum hydrocarbons in soil range from days to several years, and reported first-order rate constants in field studies range from 3.8×10^{-4} to 3.3×10^{-2} day^{-1}. This wide range of rates is likely due to the varied chemical compositions of the petroleum-hydrocarbon contamination studied and soil conditions in the various studies.

The published first-order rate constants were used to estimate the time required for biodegradation to reduce the TPH concentration of the soils at SSFL to the specified background concentration of 5.7 ppm. Depending on the initial TPH concentration and the first-order rate constant used in the calculation, estimates range from 0.42 to 69 years. Since hydrocarbon contaminants at SSFL are highly weathered, the most rapid rates would not be expected for natural attenuation at SSFL. Microcosm experiments with SSFL soil would provide data for much more accurate prediction of site specific rates. In addition, soil analyses as described below should be done at SSFL to better ascertain the rates of biodegradation of petroleum hydrocarbons at SSFL.

The time for biodegradation of petroleum hydrocarbons could be reduced via active bioremediation strategies such as biostimulation or bioaugmentation. A few treatments that have shown promise for increasing biodegradation rates are additions of fertilizer, bulking agents, surfactants, and microbes.

9.1.2. Natural Attenuation of PAHs

A large number of aerobic PAH-degrading bacteria and fungi have been reported, but their ability to biodegrade PAHs is highly dependent on the chemical nature of the PAH, in particular the number of aromatic rings. In general, biodegradation rates are slowest for PAHs with the greatest number of aromatic rings, such as benzo-a-pyrene, although published biodegradation rates vary widely for all PAHs. Half-lives of 60 days to 3 years have been reported for PAH mixtures in soil. These published rates are very encouraging, especially because the rapid rates were reported for a site in Spain with a similar Mediterranean climate to southern California. However, weathering of soil contaminants at SSFL may have greatly reduced their bioavailability, and this could reduce biodegradation rates, particularly at lower concentrations.

The time estimated to reach the background levels specified for PAHs at the site (2.5 - 5.6 ppb) range from 5 to 15 years based on comparison to relevant published field studies. But again, it may take much longer if the PAHs are strongly sequestered in the soil.

Natural attenuation biodegradation rates could likely be accelerated by amending soils with surfactants to increase the bioavailability of the sequestered PAHs, by adding nutrients to accelerate biodegradation, or adding specific species of bacteria and/or fungi which are known to biodegrade PAHs. Phytoremediation has also been successful for PAHs, and data from one study suggests that the PAHs in Area IV soils could be remediated in 1.5 to 2.7 years. PAH sequestration could limit phytoremediation, as it might for microbial biodegradation but it may be possible to combine phytoremediation with surfactant amendments. There are biological surfactants available which might be better accepted by the public than commercial chemical surfactants.

9.1.3. Natural Attenuation of PCBs

Due to the low volatility of PCBs, it is unlikely that volatilization would contribute significantly to their natural attenuation. Further, PCBs at SSFL are already highly weathered, so it is likely that any volatile fractions have already evaporated.

PCB biodegradation is more complex than hydrocarbon biodegradation, often requiring a complex consortium of bacteria operating under a combination of anaerobic and aerobic conditions. Complete bacterial PCB degradation requires two steps: anaerobic dechlorination followed by aerobic biodegradation, and therefore requires two separate environments and different species of bacteria. Only a few species of bacteria have been identified with the ability to reductively dechlorinate PCBs, and these were found mostly in aquatic sediments. Reported rates of PCB biodegradation are extremely low, even under ideal conditions. In fact, a half-life of 40 years was reported for Aroclor 1260, which is the predominant PCB contaminant found in Area IV.

Fungal biodegradation of PCBs may be more promising at SSFL than bacterial biodegradation, because fungi do not require anaerobic conditions. Although the fungal populations at SSFL are likely to be low, these populations could be augmented with additional fungi. Microcosm experiments are underway in a companion study to test the efficacy of this approach.

Other methods which could be used to accelerate PCB biodegradation include surfactant addition, and the use of oxidizing agents like hydrogen peroxide. Halopriming with more biodegradable chlorinated compounds could also improve PCB biodegradation rates.

Phytoremediation of PCBs is a possibility for soils at SSFL. Field and lab studies reported PCB phytoremediation rates ranging from 8.1% removal in 180 days to 77% removal in 122 days. These removal rates could be sufficient for some of the soils with lower PCB concentrations. However, the phytoremediation ability of the plants on site has not been determined, so experiments would be necessary to assess the feasibility of phytoremediation of PCBs at SSFL. Such experiments are currently underway at Cal Poly.

9.1.4. Natural Attenuation of Dioxins

Like PCBs, chlorinated dioxins are biodegraded through a complex combination of anaerobic and aerobic processes, often involving co-metabolism. If anaerobic conditions are not found in the SSFL soils, then significant dioxin biodegradation by bacteria would not be expected. Many of the dioxins found on the site are highly chlorinated and found in relatively high concentrations, and these factors further decrease the likelihood of biodegradation under natural attenuation conditions.

TEQs for dioxin in Area IV range from 2.68 to 650 ppt, while the target background level is 0.912 ppt TEQ for 2,3,7,8-TCDD. Biodegradation of these dioxin concentrations would take 1 to 50 years under natural attenuation conditions based on published studies. Soils with lower concentrations could be amenable to biodegradation, particularly if methods of accelerating dioxin degradation are employed. As described for the other contaminants, methods of biostimulation and/or bioaugmentation could improve biodegradation rates.

Bioaugmentation with fungi could improve biodegradation rates, but laboratory experiments would be needed to make this assessment (as described below). Although the population of ligninolytic fungi on the site may not be large enough to support significant dioxin biodegradation rates, adding additional fungi could accelerate the rate. A companion study is underway to use DNA analyses to assess if fungi growing in the soil at the site are known to be capable of biodegrading dioxins. Laboratory experiments are also underway to measure biodegradation rates of dioxin under natural conditions at the site, as well as test various active bioremediation strategies.

Limited research has been done on phytoremediation of dioxins, but some researchers suggest that its effectiveness for dioxins might be similar to that for PCBs. Laboratory testing using SSFL soils and SSFL plants is currently underway to assess the feasibility of using phytoremediation to accelerate natural attenuation of dioxins.

9.1.5. Natural Attenuation of Perchlorate

Leaching into the underlying groundwater is likely to be an important mechanism of soil perchlorate natural attenuation because of its high solubility in water. This would be expected to reduce soil concentrations of perchlorate while possibly increasing groundwater concentrations of perchlorate.

Biodegradation of perchlorate requires anaerobic conditions. As is the case for PCBs and dioxins, if anaerobic conditions are not present in SSFL soils, then no biodegradation of perchlorate would be expected. Fungal biodegradation of perchlorate has not been reported.

Phytoremediation may enhance perchlorate remediation in SSFL soils based on the success of a phytoremediation study in Texas. However, the Texas study most likely involved saturated soil, and thus may not be applicable to SSFL.

Another active method of perchlorate remediation is electrokinetic removal using electrodes in the soil to migrate perchlorate to one electrode for removal. However, this method also works better on saturated soils, and thus may not be applicable to SSFL soils.

9.1.6. Natural Attenuation of Mercury

Volatilization of elemental mercury and/or methyl mercury is a possible natural attenuation mechanism for mercury removal from SSFL soils, but this process is likely to be very slow, and it could create air pollution issues.

Phytoremediation of mercury is a potential method of removing mercury from the soil, although this would not be a natural attenuation method since it would involve active removal of plants from the site. It is unlikely that plants at SSFL will take up mercury into their roots unless the mercury is first chelated. Greenhouse experiments are underway to test the use of a chelating agent to facilitate mercury uptake by plants from SSFL in a companion study.

Other more invasive methods of mercury remediation, such as thermal desorption, could be feasible at SSFL. Soil washing is another option, but it requires large quantities of water. Both of these methods would be very disruptive to the ecosystem.

9.2. Recommendations

The times estimated in this report for natural attenuation of the various contaminants vary tremendously, from less than a year to many decades. This uncertainty is a result of the wide range of natural attenuation rates reported in the literature, and this variability is inherent because of the variety of conditions employed in the published field and laboratory studies. Laboratory microcosm experiments using SSFL soil and incubated under SSFL site conditions would greatly narrow the range of predicted times required for natural attenuation. A companion study is currently being initiated to conduct m microcosm experiments with three different soil samples collected from Area IV at SSFL. The control groups of these microcosms will be incubated under conditions mimicking conditions at SSFL, such as soil temperature, moisture content and oxygen availability. Data from these microcosm tests will then be used to make more precise estimations of natural attenuation rates expected at SSFL.

A number of field tests should be conducted to better understand the conditions for natural attenuation at the site. This will help to provide better comparisons to published studies and also to guide experimental conditions for the proposed microcosm studies. Most important is the testing for anaerobic conditions in the soils since such conditions are required for bacterial dechlorination of PCBs and dioxins, and microbial reduction of perchlorate. Redox conditions can also affect the methylation of mercury. Soil concentrations of nitrate, phosphate and potassium should be determined because these nutrients are essential for bacterial and fungal growth. Concentrations of the trace metal molybdenum should also be determined because this micronutrient can affect phytoremediation. Temperature and moisture content also affect biodegradation rates, so these parameters should be measured at the site. The native microbial populations should be characterized using analysis of DNA extracted from the soil and also using traditional culturing techniques. The species identified can then be compared to known degraders of the COIs.

Detailed chemical analyses of the contaminants at the site should be done to provide information about the nature and extent of the weathering of the contaminants, and this could provide valuable insights for the potential of natural attenuation of each contaminant. It would be particularly useful to examine historical trends in the chemical composition of the contaminants, but even a present-day snapshot would be useful. In the case of petroleum hydrocarbons, chromatographic information could be used to determine the extent of alkane biodegradation which has already occurred as well as the nature of the remaining hydrocarbons. For hydrocarbons, the ratio of concentrations of specific biodegradable compounds to more recalcitrant compounds can provide an indication of the degree of biodegradation which has occurred. For PCBs and chlorinated dioxins, the degree of chlorination is an indication of the degree of anaerobic dechlorination. So for example if there is a high concentration of highly chlorinated PCB congeners at the site it would indicate that not much anaerobic dechlorination has occurred and this process would not likely contribute much to natural attenuation. A similar approach applies to chlorinated dioxins. Determination of mercury speciation could provide guidance for predicting mercury natural attenuation and this will be helpful for assessing potential phytoremediation processes (a companion study is currently being conducted by U.C. Riverside to determine the speciation of mercury in Area IV soils at SSFL). Tests such as selective extractions are also available to ascertain the extent of sequestration and bioavailability of contaminants in the soils.

It is unlikely that natural attenuation alone will be a viable option to remediate the SSFL soils to background contaminant levels. Therefore, active bioremediation methods should be considered. Several methods of biostimulation and bioagmentation were identified in this report for each of the COIs. The most promising biostimulation strategies identified were addition of surfactants, chelating agents and nutrients. These amendments should be tested in controlled laboratory microcosm experiments. Addition of lignolytic fungi may be the best bioaugmentation option for the more recalcitrant PAHs, PCBs and dioxins, especially if anaerobic conditions are not found at the site. Bacterial dechlorinators of these compounds require anaerobic conditions, but fungi do not. Fungal bioaugmentation may be best using fungi isolated from the site, since these fungi are best adapted to site conditions and would have a better survival rate compared to alien microbes (Yateem et al., 1998). Microcosm experiments in a companion study will test the effect of bioagmentation of SSFL soils with white-rot fungi.

10.0. References

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Abbreviations

AI	Atomic International
AOC	Administrative Order of Consent
ASTDR	Agency for Toxic Substances and Disease Registry
BTEX	Benzene, toluene, ethylbenzene, xylenes
CBP	Chlorinated Biphenyl
COI	Contaminant of Interest
DD	Dibenzodioxin
DF	Dibenzofuran
DOD	Department of Defense
DOE	Department of Energy
DTSC	Department of Toxic Substances
EDTA	Ethylenediaminetetraacetic acid
EPA	Enviromental Protection Agency
ETEC	Energy Technology Engineering Center
F	Field
kg	kilogram
K_{ow}	Octanol Water Partition Coefficient
L	Lab
LiP	lignin peroxidase
MCDF	Monochlorinated Dibenzofurans
mg	milligram
mm	millimeters
MnP	Manganese-dependent Peroxidase
NAA	North American Aviation
NASA	National Aeronautics and Space Admisistration
NS	Not Stated
NSD	Not Significantly Different
PAH P	olyaromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Diobenzodioxin

PCDFs	Polychlorinated Dibenzofurans
pg	picograms
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RMHF	Radioactive Materials Handling Facility
SSFL	Santa Susana Field Laboratory
STIG	Soil Treatability Investigation Group
TCDD	Tetrachloro Dibenzodioxin
TeCDD	Tetrachloro Dibenzodioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TPH	Total Petroleum Hydrocarbon
ug	micrograms
USAF	United States Air Force
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
V	Volts