IMPACT OF COMPOSTING STRATEGIES ON THE TREATMENT OF SOIL CONTAMINATED WITH DIESEL FUEL

BY

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A Thesis

Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
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DOCTOR OF PHILOSOPHY

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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ABSTRACT

Soil contamination from leaking underground storage tanks and accidental surface spills are major problems faced by environmental practitioners today. Of the several forms of treatment for hydrocarbon-contaminated soils, composting bioremediation has advantages over other technologies. Advantages of composting include relative simplicity of operation and design, and relatively high treatment efficiency. The major objective of this study was to evaluate the potential of utilizing composting, a substrate dense, self-heating waste treatment method, as a technique for the bioremediation of diesel fuel contaminated soil in a bench-scale reactor. The study was divided into two major phases.

The first phase was initiated to determine the effect of soil loading and texture on microbial activity during the active (thermophilic) phase of composting of diesel fuel contaminated soil. The thermophilic composting phase was simulated using bench scale (28L) reactors under controlled temperature and aeration conditions. Soil loads (% of total wet weight) investigated ranged from 0% to 64%. As a measure of composting microbial activity, the relative heat generation and volatile solids removal were measured. The results found that a change in thermophilic microbial activity had occurred at sand and silt loads above 40% and 20%, respectively. The volatile solids reduction was poorly correlated to sand and silt loads. The lack of observed trend may have resulted from sample variability. While the thermophilic microbial activity observed was not affected by sand or silt load, the thermophilic microbial activity was affected at the higher clay load (40%). This study concluded that soil load and texture

must be considered in any composting study in order to maintain microbial activity during the active phase of composting.

The second phase was to investigate the effect of co-substrates and operating temperatures on composting of diesel fuel contaminated soil. A sandy soil contaminated with diesel fuel (20,000 mg kg^{-1} dry soil) and spiked with labeled phenanthrene 9-^{14}C was used in this study. The contaminated soil was mixed with either fresh feedstock material or with finished compost and incubated using either thermophilic or mesophilic temperature patterns. After 18 weeks of composting, while no mineralization of ^{14}C phenanthrene was detected in the soil without co-substrate addition, soils receiving co-substrate had significant mineralization. Finished compost at mesophilic temperatures resulted in the highest mineralization rate of phenanthrene (42%). Extractable ^{14}C-phenanthrene was less than 11 % for all treatments, however, the soil without co-substrate addition still had more than 65% extractable ^{14}C-phenanthrene by the end of the experimental period. Extractable diesel range organics (EDRO) were reduced below the acceptable levels of 2000 mg kg^{-1} mandated by local authorities, in the soil mixed with fresh feedstock at the thermophilic or finished compost at the mesophilic. However, the acceptable level was achieved more rapidly from soil mixed with fresh feedstock. Although the composting at either fresh feedstock or finished compost was highly effective in eliminating ^{14}Cphenanthrene and extractable diesel range organics, as compared to soil without co-substrates, earthworm and seed germination assays indicated there was still significant toxicity associated with the soil. Other trace contaminants, besides the diesel fuel compounds, have caused the toxicity.

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Definition of Terms

Biodegradation: is the breakdown of organic contaminants that occurs due to microbial activity.

Bioremediation: refers to the application of biodegradation reactions to the practical clean up of a compound or compounds.

Bound residues: are those chemical species that are unextracted by a defined method, whereby the method used does not significantly change chemical nature of the residues.

Covalent Bonding: the means by which the atoms in organic molecules customarily complete their outer-shell or valence-shell octet is by sharing electrons with other atoms. It is a high-energy bond compared to hydrogen bonds or Van der Waals forces.

Extracellular Enzymes: are enzymes produced by some organisms, and instead of being held within the cell, they are excreted into the medium. It capable of break down large molecules into smaller ones that can pass through the cells wall and used as nutrients for growth.

Humic acids: the fraction of humic substances that is not soluble in water under acidic conditions (pH < 2) but is soluble at higher pH values. They can be extracted from soil by various reagents and which is insoluble in dilute acid. Humic acids are the major extractable component of soil humic substances. They are dark brown to black in color.

Fulvic acids: the fraction of humic substances that is soluble in water under all pH conditions. They remain in solution after removal of humic acid by acidification. Fulvic acids are light yellow to yellow-brown in color

Humin: the fraction of humic substances that is not soluble in water at any pH value and in alkali. Humins are black in color.

Humification: incorporation of contaminants into the soil organic matter, or humus, through binding (polymerization) reactions of plant and microbial enzymes.

Humus: organic material that has undergone enough degradation and transformation to make the parent material unrecognizable. It is largely composed of polymerized substances: aromatics, polysaccharides, amino acids, acid polymers, and phosphorus containing compounds.

Mineralization: is complete biodegradation of the organic contaminants, such as phenanthrene, to carbon dioxide and water.

Mesophilic Temperature: temperature range of 25 to 45°C.

Thermophilic Temperature: temperature range of 45 to 65°C.

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DEDICATION

This thesis is dedicated to:

My parents, Khadija and Mabrouk, and my aunts, Najia and Aisha, for their wisdom and support

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My wife, Entisar, and my children, Amel, Aya, and Mohammed who supported and accompanied me in this journey

1. Introduction

1.1 Statement of Problem

Contamination of the soil environment by petroleum hydrocarbons is a widespread problem facing environmental practitioners today. These contaminants are introduced into soils mainly through improper disposal methods, accidental surface spills, and leaking underground storage tanks. Once released into the soil, there are number of fates for the compounds in the environment. The contaminants may become sorbed to inorganic and organic fraction of soils. Sorption may cause immobilization of the contaminants within the soil and subsequently reduce exposure potential to humans. However, there is still a potential for the contaminant to migrate to the groundwater or surface water and pose a risk to humans and animals. For example, Manitoba Hydro has identified many sites where diesel fuel and other petroleum hydrocarbons have been leaked or spilled resulting in contamination of the subsurface soil and groundwater (Man, 1998).

There are a variety of remedial options available for cleaning up a contaminated site including incineration, solidification/stabilization, soil vapor extraction, low temperature thermal stripping, soil washing, and soil flushing. These methods can be relatively expensive because the extracted contaminants or incinerated soil must often be further treated or disposed of, adding to the overall treatment costs. This study focuses on biological remediation.

Bioremediation employs microorganisms to biologically degrade organic compounds into ultimately harmless byproducts of microbial metabolism such as carbon dioxide, water, and other inorganic substances. These biological reactions can be carried out in undisturbed soil in the ground (in-situ), or above ground reactors with excavated soil (ex-situ). The in-situ bioremediation of soil involves the injection of air and nutrient into the contaminated zone. Ex-situ bioremediation (including landfarming, composting, and bioslurry) use tilling, turning or continuously mixed slurries to apply oxygen and nutrients, and is performed in a prepared bed or reactor. This study focuses on the composting bioremediation system.

Composting is the aerobic biological decomposition and stabilization of organic substrates under conditions that allow development of thermophilic temperature (45 to 65 °C) as a result of biologically generated heat (Haug, 1993). Composting involves the addition of bulking agents and organic amendments, such as woodchips, animal, and vegetative waste, to enhance the porosity of the mixture to be decomposed. There are three basic process designs used in composting: (1) windrow system, (2) static pile system and (3) in-vessel system. In the windrow composting system, the contaminated soil is usually mixed with a bulking agent to facilitate air permeation through the soil. The static pile composting system uses forced aeration to maintain aerobic decomposition in a much large pile mass than with the windrow system (Cookson, 1995). Aeration is typically provided by a system of perforated pipes installed under the static pile(s). In the in-vessel composting system, the soil compost mixture placed inside enclosed reactors (plug flow or agitated-bed reactors) where the actual composting takes place.

All composting systems share similar unique characteristics that make them well suited to bioremediation. First, composting systems have high microbial diversity, with microbial populations much larger than both fertile and highly contaminated soils (US EPA, 1998). Secondly, heat of microbial metabolic activity causes the composting matrix to increase in temperature. High temperatures achieved during the composting process may increase the rate of release of contaminants into the aqueous phase (Williams and Keehan, 1992). Release of contaminants into the aqueous phase should increase hydrocarbon biodegradation rates and shorten the treatment time.

Composting has been shown to be effective for the degradation of a wide range of common environmental contaminants such as soil contaminated with explosives (Williams and Keehan, 1992), pesticides (Liu and Cole, 1995), and chlorophenol (Laine and Jorgensen, 1997; Benoit and Barriuso, 1995). Soil composting of hydrocarbons has been studied in experiments ranging from bench scale to large pilot studies (Al-Daher et al. 1998; Joyce et al. 1998; Wischmann and Steinhart, 1997; Beaudin et al. 1996; Civilini et al. 1996; Mahro and Kästner, 1996; Mahro and Kästner, 1993; Qiu and McFarland, 1991). Many bench-scale studies have been carried out to (1) elucidate the fate of hydrocarbons pollutants on a single contaminant and complex mixture of hydrocarbons, and (2) to investigate the fate of hydrocarbons with the addition of either fresh feedstock or mature compost. No study compared the benefits of amending diesel fuel contaminated soil with finished compost to that of fresh feedstock.

Many factors can have an impact on the performance of composting systems (pH, pore space oxygen, carbon to nitrogen ratio, moisture content, and free air space, and

operating temperatures). Another factor that must be considered when soil is added to the feedstock is the soil to compost ratio. Soil loading that is too high may cause microbial inhibition (depending on the toxicity of contaminated soil) and/or the biodegradable carbon source may be diluted to the point where self-heating is reduced or eliminated (Ziegenfuss and William, 1991). The influences of different soil to compost ratios on the thermophilic microbial activity and removal of petroleum hydrocarbon have been investigated (Wytrykush et al. 2002; William et al. 1997; Hupe et al; 1996; Stegmann et al. 1991). Various mix ratios for optimum composting performance have been suggested in these studies. Discrepancies could be related to soil particle size. As such, a study is needed to quantify the effect of various soil particles and loads on microbial performance during composting.

Operating temperature is another factor to be considered. Composting can be operated within either mesophilic (25 to 45 °C) or thermophilic temperature range depending on substrate and type of bacteria that are present to degrade the contaminants. The benefit of the thermophilic temperature phase on the composting bioremediation of petroleum hydrocarbons has been considered in some studies (Haderlein et al.1999; Semple et al. 1998; Beaudin et al. 1996; Hogan et al. 1989). These studies were different in terms of the benefit of thermophilic temperatures. For example, Hogan et al. (1989) found that both thermophilic and mesophilic temperature provided similar removal of aliphatic hydrocarbons (e.g. octadecane) and polyaromatics hydrocarbon (e.g. phenanthrene). Haderline et al. (1999) found that mesophilic temperature resulted in more removal of aliphatic (e.g. hexadecane) and polyaromatic hydrocarbons (e.g. pyrene). Another study by Semple et al. (1998) found thermophilic temperatures resulted in a greater removal of

benzene, toluene, ethylbenzene and xylenes (BTEX) compounds as compared to mesophilic temperatures. Here too, a study is needed to verify the effect of temperature.

In short, there are several issues need to be addressed in soil composting. First, the effect of soil load and particle size on the thermophilic stage of composting has not yet been documented. Second, there is lack of information on the benefit of amending diesel fuel contaminated soil with finished compost compared to that of fresh feedstock. Third, it is not clear whether thermophilic temperatures are beneficial to diesel fuel treatment.

1.2 Research Hypotheses

The experimental hypotheses for this study were: (1) thermophilic microbial activity within compost system will cease at high soil loading rates; (2) thermophilic microbial activity within soil compost system will be impacted differently by different soil textures; (3) thermophilic operating period should increase the biodegradation of reference diesel fuel components (extractable diesel range organics and phenanthrene); (4) addition of organic substrate to soil during composting should increase the size of the non-extractable fraction (bound residue) of the diesel fuel as compared to soil alone, and (5) composted contaminated soil will not be toxic to earthworms and plants.

1.3 Research Objectives

The main objective of the study was to evaluate the potential of utilizing composting, a substrate dense, self-heating waste treatment method, as a technique for the bioremediation of diesel fuel contaminated soil. The study was divided into two major phases.

The specific objective of the first phase was to investigate the effect of soil loading and texture on the microbial activity during the active (thermophilic) phase of composting diesel fuel contaminated soil. To achieve this objective, an experimental program was divided into two sub-phases: Phase 1A & Phase 1B. Phase 1A determined the effect of sand or silt soil on the thermophilic phase of composting. Phase 1B confirmed the effect of soil texture on the thermophilic phase of composting. The microbial activity was monitored through volatile solids removal and relative heat generation.

The specific objective of second phase was to investigate the effects of co-substrates and operating temperatures on the performance of composting treatment of diesel fuel contaminated soil. In order to achieve this objective, an experimental program was divided into two sub-phases: Phase 2A & Phase 2B. Phase 2A observed the performance of soil composting process by measuring the reduction in target diesel fuel concentrations (phenanthrene and extractable diesel range organics). Phase 2B observed the performance by assessing the treatment's ability to lower soil toxicity. Plant and earthworm bioassays were used to evaluate the efficacy of compost treatment to reduce toxicity associated with contaminated diesel soil.

1.4 Anticipated Significance of Research

It is expected that this study will provide information (1) to identify the maximum amount of contaminated soil that should be added in composting mix for optimum composting performance; (2) to better understand the fate of diesel fuel hydrocarbon components in fresh feedstock and mature compost materials; and (3) to assess the potential of application of mature compost for bioremediation of diesel fuel contaminated

soil. Generally, it is expected that it will be beneficial to establish an effective treatment design in terms of treatment efficiency and time scale of this bioremediation strategy for diesel-contaminated soil.

2. Diesel Fuel Degradation

2.1 Diesel Fuel Composition

Diesel fuel No.2 is a complex mixture of over two hundreds petroleum hydrocarbon compounds each with its own physical and chemical properties (Millner et al. 1992; Block et al. 1993). Despite this complexity in diesel fuel composition, diesel fuel may be separated into two broad categories of compounds including aliphatic hydrocarbons and aromatic hydrocarbons (Block et al. 1991). The relative compositions of the diesel fuel components are shown in Table 2-1.

Table 2-1. Composition of diesel fuel no. 2 (After Riser-Robert, 1992).

Carbon Number	Concentration (% Volume)		
	Aliphatics	Cycloaliphatics	Aromatics
C_{10}	0.9	0.6	0.4
C_{11}	2.3	1.7	1.0
C_{12}	3.8	2.8	1.6
C_{13}	6.4	4.8	2.8
C_{14}	8.8	6.6	3.8
C_{15}	7.4	5.5	3.2
C_{16}	5.8	4.4	2.5
C_{17}	5.5	4.1	2.4
C_{18}	4.3	3.2	1.8
C_{19}	0.7	0.6	0.3
Totals	45.9	34.3	19.8

Aliphatic hydrocarbons constitute the bulk of diesel (65 –85%) and may further subdivided into normal, branched and cyclic alkanes (cycloaliphatics). The more

predominant chromatograph peaks are normal and branched alkanes. The branched alkanes are predominantly monomethyl, dimethyl, and trimethyl substitute alkanes. Ratios of compounds in this category are used to identify the source and extent of weathering of fuel spill at site. Cycloalkanes are present in low concentrations in diesel fuel, and as they tend to exhibit poor gas chromatograph analytical behavior, are difficult to quantify (Block et al. 1991). Toxicity of cycloalkanes is not high, however, normal alkanes have been identified as a potential carcinogens (Millner et al. 1992). Alkenes tend not to be present in diesel fuel, however, they may be incorporated into diesel fuel during catalytic cracking processes (Block et al. 1991).

The aromatic hydrocarbons make up approximately 10 to 30% of the compounds present in diesel fuel. They include simple monoaromatic and polyaromatics. Monoaromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene, due to their toxicity, are frequently included as target analytes when investigating petroleum hydrocarbon impacted sites (Block et al. 1991). Polyaromatic hydrocarbons (PAHs) which consist of 2 or more benzene rings such as phenanthrene, fluoranthrene, pyrene, benz(a)anthracene, and chrysene are particularly of concern because some of which are potentially carcinogenic (Millner et al. 1992; Eweis et al. 1998).

2.2 Petroleum Degrading Microbial Population

Biodegradation of an organic compound in soil is dependent on both microbial population density and the activity of microbial population. Most natural environments have a high diversity of microorganisms that have the ability to degrade petroleum products (Englert et al. 1993). Individual organisms in pure culture may be found

incapable of mineralizing the target compound as a sole source of carbon. Assemblages of mixed populations with overall broad enzymatic capacities are required to degrade complex mixtures of hydrocarbons in the soil matrix (Bossert and Bartha, 1984). The genera of hydrocarbon-degrading bacteria and fungi isolated from soil are presented in Table 2-2. The most prevalent bacterial hydrocarbon degraders belong to *Pseudomonas*, *Achromobacter, Flavobacterium, Nocardia, and Arthrob*acter (Atlas and Cerniglia, 1995; Englert et al. 1993). The group of bacteria found with the highest frequency consists of those belonging to the genus *Pseudomonas (*Englert et al. 1993; Cookson, 1995). *Pseudomonas* consists of gram-negative, aerobic chemoheterotrophic organisms (Cookson, 1995). Many species of pseudomonas such as *Pseudomonas stutzeri, Pseudomonas aeruginosa, and Pseudomonas chrysosporium* have been identified as capable of degrading diesel hydrocarbons, including phenanthrene, as their sole carbon and energy source (Balashova et al. 1999; Aitken et al. 1998; Tongpim and Pickard, 1999).

Fungi tend to have more significant roles than do bacteria in the biodegradation of hydrocarbons, especially, highly condensed polycyclic aromatic hydrocarbon (Wolter et al. 1997). Fungal species consistently isolated from hydrocarbon-contaminated soil include, in decreasing order, *Trichoderma, Penicillium, Aspergillus, and Mortierella* (Englert et al. 1993).

Thermophilic hydrocarbon degrader organisms are uncommon in polluted soil environments. These organisms, with a growth temperature optimum between 45 and 80°C, are extremely diverse group including bacteria, fungi, protozoa, and algae.

Examples of thermophilic degradation of petroleum compounds are listed in Table 2-3. Thermophiles are found in nature in certain restricted areas. For example, soils subjected to full sunlight are often heated to temperature above 50°C at midday, although a few centimeters under the surface the temperature is much lower (Madigan et al. 2000). Other environments in which temperatures are hospitable to thermophiles are compost piles, which usually reach temperatures of 60 to 65°C.

Thermophilic organisms thrive at high temperature because their enzymes and other proteins are much more stable to heat than those of mesophiles. Possible explanation for thermostability in these enzymes include enhanced hydrophobic interactions, increased hydrogen bonding, internal salt bridges (ionic bonds between the positive and negative charges of various amino acids), and associations with cellular structures such as the cell wall (Madigan et al. 2000). In addition to enzymes and other protein in the cell, thermophiles have membrane lipids rich in saturated fatty acids. Saturated fatty acids form a much stronger hydrophobic environment than do unsaturated fatty acids, which helps accounts for the membrane stability (Madigan et al. 2000).

A variety of hydrocarbon degraders have been identified to occur in soil environment. Besides the variety of bacterial and fungal species that degrade hydrocarbons, there are significant differences in mechanism of hydrocarbon metabolism used by fungi and bacteria (Atlas and Cerniglia, 1995).

2.3 Metabolism of Diesel Fuel Components

2.3.1 Bacterial Metabolism

Different hydrocarbon compounds have different biodegradation pathways. Alkanes are

Table 2-2. Genera of hydrocarbon-degrading bacteria and fungi isolated from soil (after Englert et al. 1993)

Bacteria	Fungi
Achromobacter	*Acremonium*
Acinetobacter	*Aspergillus*
Alcaligenes	*Aureobasidium*
Arthrobacter	*Beauveria*
Bacillus	*Botrytis*
Brevibacterium	*Candida*
Chromobacterium	*Chrysosporium*
Corynebacterium	*Cladosporium*
Cytophaga	*Cochliobolus*
Erwinia	*Cylindrocarpon*
Flavobaeterium	*Debaryomyces*
Micrococcus	*Fusarium*
Mycobacterium	*Geotrichum*
Nocardia	*Gliocladium*
Proteus	*Graphium*
Pseudomonas	*Humicola*
Sarcina	*Monilia*
Serratia	*Mortierella*
Spirillum	*Paecilomyces*
Streptomyces	*Penicillium*
Vibrio	*Phoma*
Xanthomonas	*Rhodotorula*
	Saccharomyces
	Scolecobasidium
	Sporobolomyces
	Sprotrichum
	Spicaria
	Tolypocladium
	Torulopsis
	Trichoderma
	Verticillium

Table 2-3. Example of thermophilic degradation of aromatic compounds

Organisms	**Compound Degraded**	**Optimal Temperature**	**Reference**
Thermus Sp.	BTEX	60 °C	Chen & Taylor (1997a)
Thermus aquaticus	BTEX	70 °C	Chen & Taylor (1995)
Bacillus thermoleovorans	Naphthalene	60 °C	Annweiler et al. (2000)
Bacillus stearothermophilus	n-alkanes	60 °C	Margesin & Schinner (2001)

degraded primarily through the oxidation of the terminal methyl group, followed by the cleavage of the molecule between the second and the third carbon in the chain. Alkane microbial degradation uses a monooxygenase enzyme; hence there is a requirement for the presence of molecular oxygen in order for the reaction to occur. The addition of oxygen to the terminal carbon yields an alcohol. The resulting alcohol then can be oxidized to an aldehyde and fatty acid (Figure 2-1a). An alternate pathway is sub-terminal oxidation where the process occurs on a mid-chain carbon and proceeds as for β-oxidation. Either way, the further degradation of fatty acid can be processed by β-oxidation (successive removal of two carbon units) which ultimately results in complete oxidation of hydrocarbon (Figure 2-1b) (Baker and Herson, 1994; Eweis et al. 1998). Cycloalkanes are usually degraded by oxidase attack to produce a cyclic alcohol which is dehydrogenated to a ketone (Eweis et al. 1998).

Aromatic compounds require a more complex transformation process due to their fused ring structure. Bacteria initiate the oxidation of monoaromatic hydrocarbons by incorporating the atoms from molecular oxygen and two hydrogen atoms into an aromatic ring to produce cis-dihydrodiol. This reaction is catalyzed by an aromatic ring dioxygenase. These dioxygenase reactions have been shown to occur for benzene and toluene, for example (Gibson, 1988). Dihydrodiols are further oxidized by bacteria to dihydroxylated derivatives such as catechol (Figure 2-2). Once catechol is formed, the aromatic ring of catechol is converted to aliphatic products (Eweis et al. 1998; Atlas and Bartha, 1993; Cerniglia, 1984).

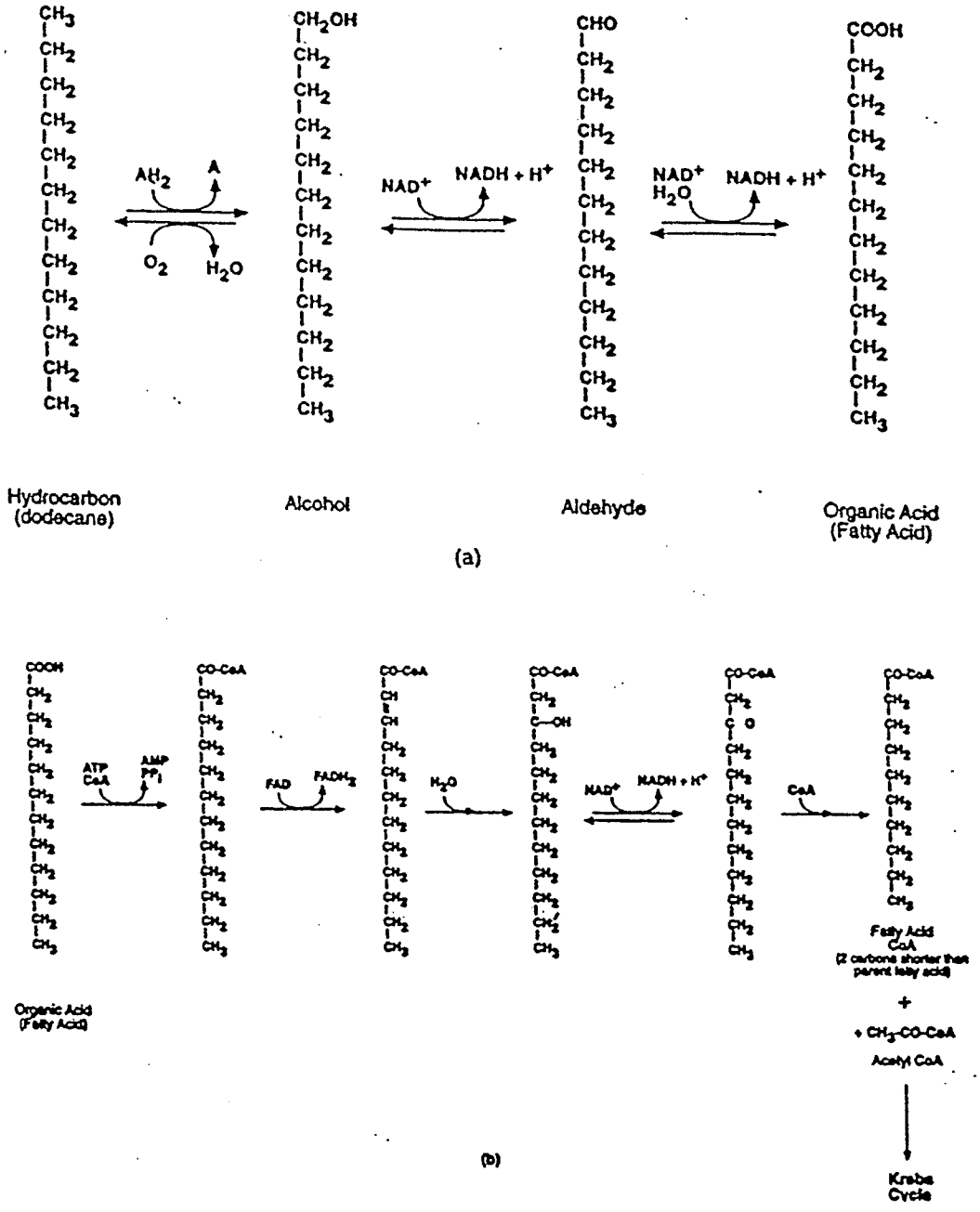


Figure 2-1. Metabolism of normal alkanes (After Baker and Hesron, 1994)

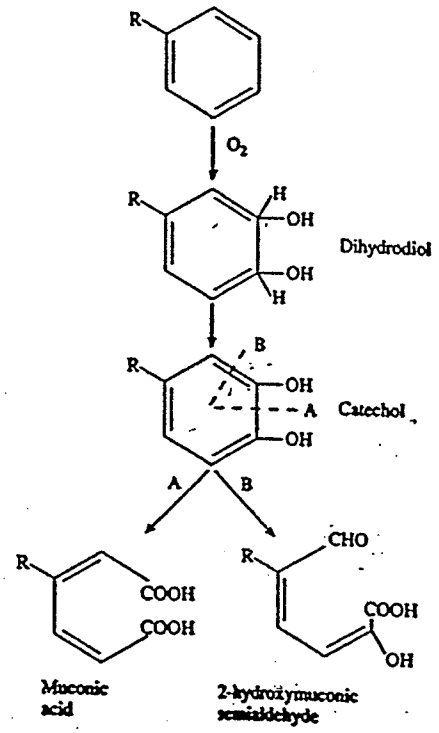


Figure 2-2. Metabolism pathways of monoaromatic compounds (after Eweis et al. 1998)

The polycyclic aromatic hydrocarbons (PAHs) are degraded by similar mechanisms as the ones used for monoaromatic compounds. In the case of phenanthrene, the degradation pathways involve conversion to a dihydrodiol using monoxygenase enzyme. The dihydrodiol produced by bacteria may vary depend on the initial site of bacterial enzymatic attack on phenanthrene. Pseudomonas oxidize phenanthrene at carbons 1,2- and 3,4-position to form phenanthrene cis-1,2- and 3, 4 -dihydrodiol (Cerniglia et al. 1989). Attack at the 9,10 position to form phenanthrene trans-9,10-dihydrodiol has been reported in *Streptomyces flavovirens* (Sutherland et al. 1995). These three isomers of dihydroxy dihydrophenanthrene would be followed by further metabolized through naphthalene and from here, through salicyclic acid to catechol (Sutherland et al. 1995) where ring cleavage follow the same degradation in monoaromatics. A broad range of bacteria are able to metabolize phenanthrene, although the exact range and extent of metabolism for phenanthrene seems to be unique to the particular bacterial isolates (Aitken et al. 1998). Figure 2-3a & 2-3b shows the metabolic pathways for the degradation of phenanthrene as proposed by Sutherland et al. (1995). Phenanthrene and other PAHs that have a high molecular weight they tend to be more stable and their degradation are thought to occur through the process of co-metabolism by most bacteria (Atlas and Bartha, 1993).

2.3.2 Fungal Metabolism

In addition to bacterial degradation of PAHs, a group of fungi, commonly called white-rot fungi such as *Phanerochaete chrysosporium,* are known to have extensive biodegradative capabilities associated with the production of extracellular enzymes, involved in lignin biodegradation. The production of lignin degrading enzymes, known

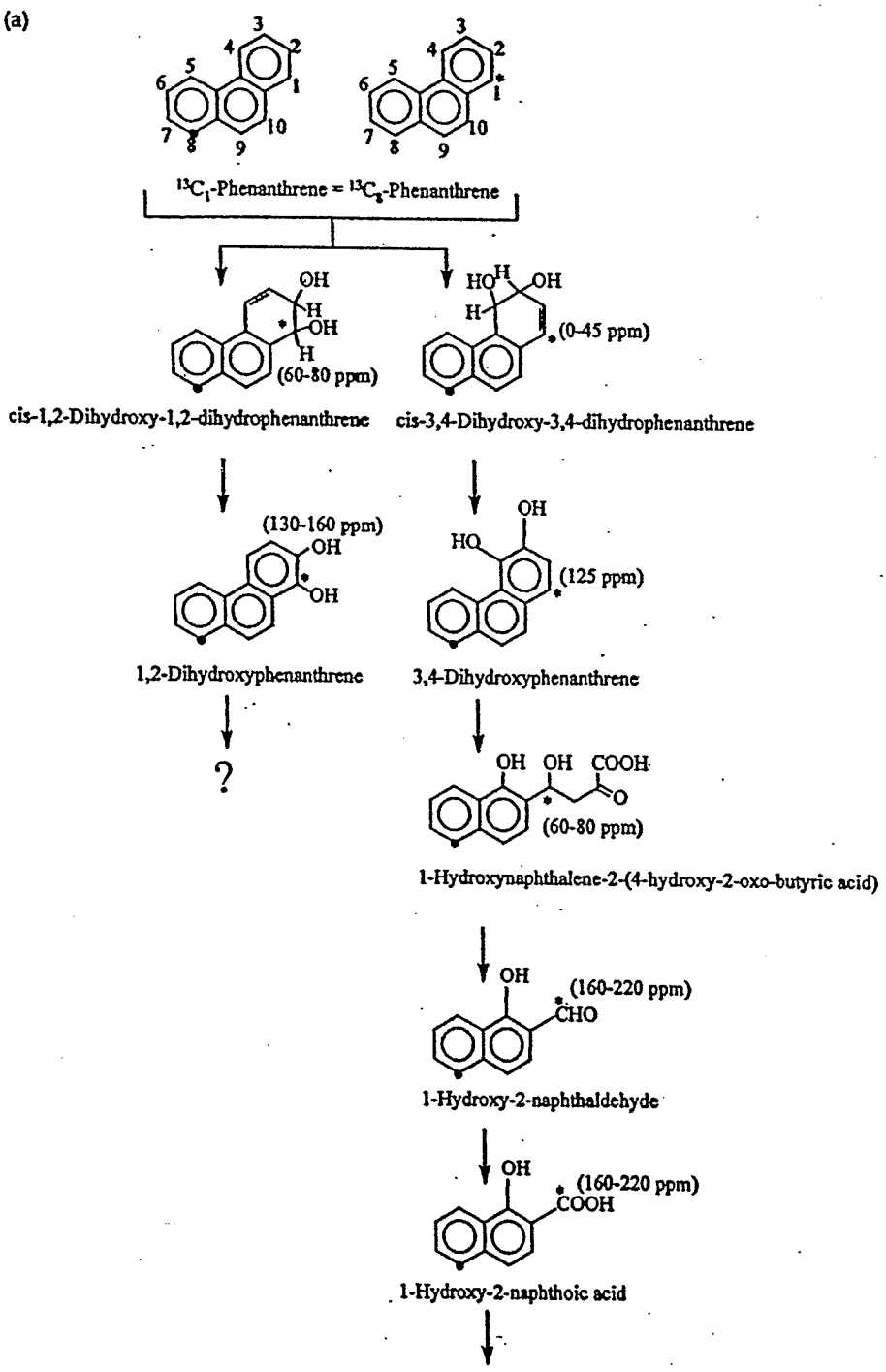


Figure 2-3 (a,b). Metabolic pathway for the degradation of phenanthrene in soil (after Sutherland et al. 1995).

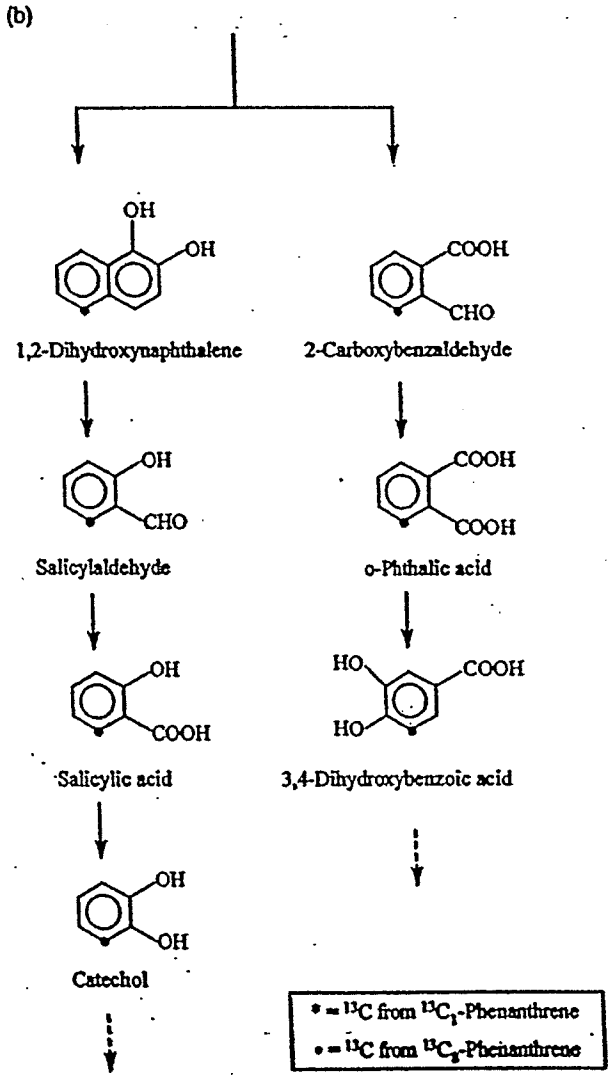


Figure 2-3. (*continued*).

as lignin peroxidases, is dependent on the presence of a primary growth substrate such as glucose or cellulose (Baker and Herson, 1994).

One way to understand the nonspecific ability of white rot fungi to degrade pollutants is to consider the complexity of lignin. Lignin is a three-dimensional polymer consisting non-repeating phenyl propanoid units linked by various carbon-carbon and ether bonds. The heterogeneity and potential for repeating structures in the molecule, render lignin very resistant to biodegradation because most of microorganism do no possess enzyme capable of degrading molecules which lack structural and stereoregularity (Bumpus and Aust, 1986). The mechanism that give fungi the ability to degrade lignin also allow them to degrade the non-water soluble compounds such as PAHs even if adsorbed to solids (Kästner and Mahro, 1996). Some organic compounds which have been degraded by white rot fungi, *Phanerochaete chrysosporium*, are benzo(a)pyrene, phenanthrene, anthracene, and fluorene (Baud-Grasset et al. 1993; Atlas and Cerniglia, 1995). Figure 2-4 shows the pathway for phenanthrene degradation by *Ligninolytic P. chrysosporium* grown in malt extract-glucose broth as demonstrated by Sutherland et al. (1991).

In addition to ligninolytic fungi, other species of nonligninolytic fungi (fungi that do not attack lignin) can initially metabolize PAHs using a membrane bound enzyme, cytochrome P-450 containing monooxygenase, to form an unstable arene oxide, which is immediately either hydrated by epoxide hydrolase to trans-dihydrodiol or rearranged non enzymatically to a phenol (Cerniglia et al 1992). The trans-dihydrodiol may be dehydrogenated to form a catechol (Atlas and Cerniglia, 1995). Secondary metabolism may lead to the detoxification of phenol and trans-dihydrodiol by conjugation with other

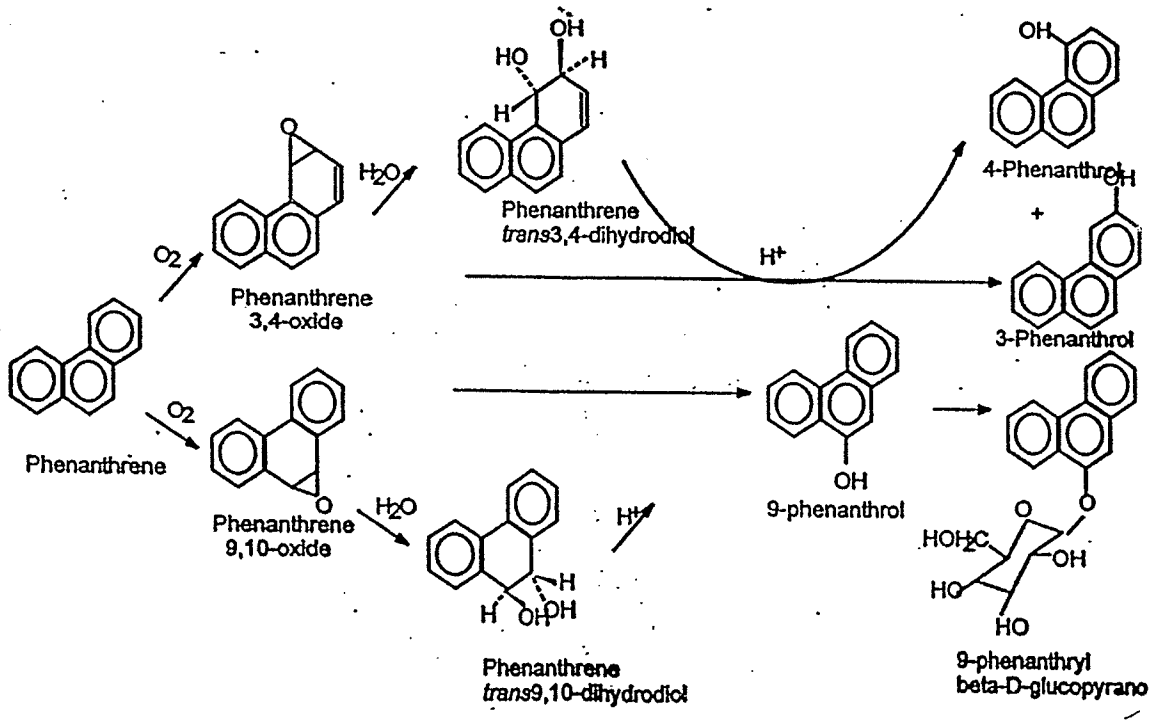


Figure 2-4. Metabolic pathway of phenanthrene in cultures of P. chrysosporium (after Sutherland et al. 1991)

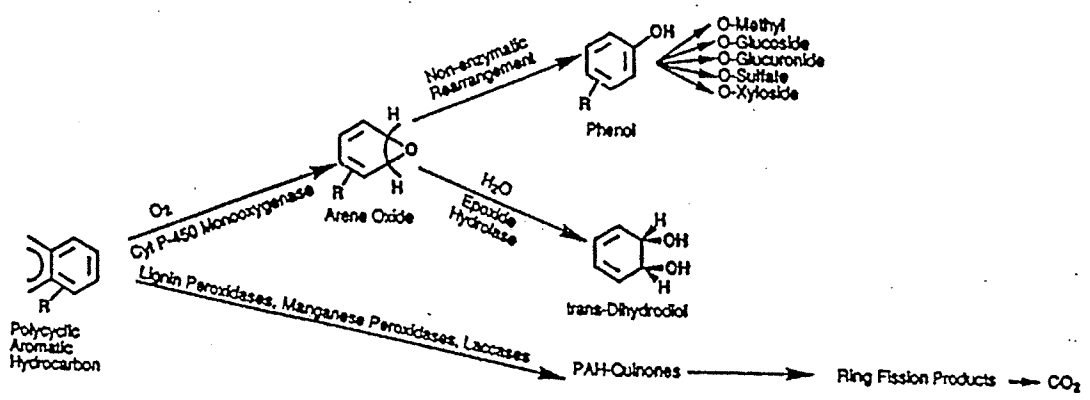


Figure 2-5. Pathways for the fungal metabolism of polycyclic aromatic hydrocarbon (after Cerniglia, 1997)

molecule such as sulfates, glucoronides, and xylosides.

Generally, several species of fungi oxidize PAHs to phenol, trans-dihydrodiols, and quinines metabolites. Trans 9, 10-dihydrodiol has been reported as the metabolites produced from phenanthrene by white rot fungi (Sutherland et al. 1991; Bezalel et al. 1996b). Various mechanisms of aromatic hydrocarbon by the action of different fungi are shown in Figure 2-5. Only a few fungi appear to have the ability to metabolize them completely to carbon dioxide. *Phanerochaete chrysosporium*, for example, produced carbon dioxide from benzo(a)pyrene, phenanthrene, and fluorene in pure culture (Gibson, 1977; Atlas and Cerniglia, 1995). Generally, fungi produce phenanthrene metabolites with high water solubility, which could enhance the mineralization of these compounds by indigenous soil bacteria (Cerniglia, 1997). Except for the white-rot basidiomycetes, all fungi transform PAHs into trans-dihydrodiol intermediates under co-metabolic conditions (Kästner and Mahro, 1996; Cerniglia et al. 1989).

2.3.3 Co-metabolism

Co-metabolism is another factor to be considered in diesel fuel degradation. Co-metabolism is the transformation of the organic compound by a microorganism that does not utilize the substrate as a source of energy for growth (Alexander, 1999). In co-metabolism, an enzyme produced for degradation of a growth supporting compound also degrades another compound that is not used, nor is essential for growth. Co-metabolism has been identified as a major mechanism in the transformations of petroleum compounds such as toluene, benzene, phenanthrene, o-xylene and cyclohexane (Alexander, 1999). Co-metabolism of phenanthrene by Rhodococcus strain during growth on anthracene as

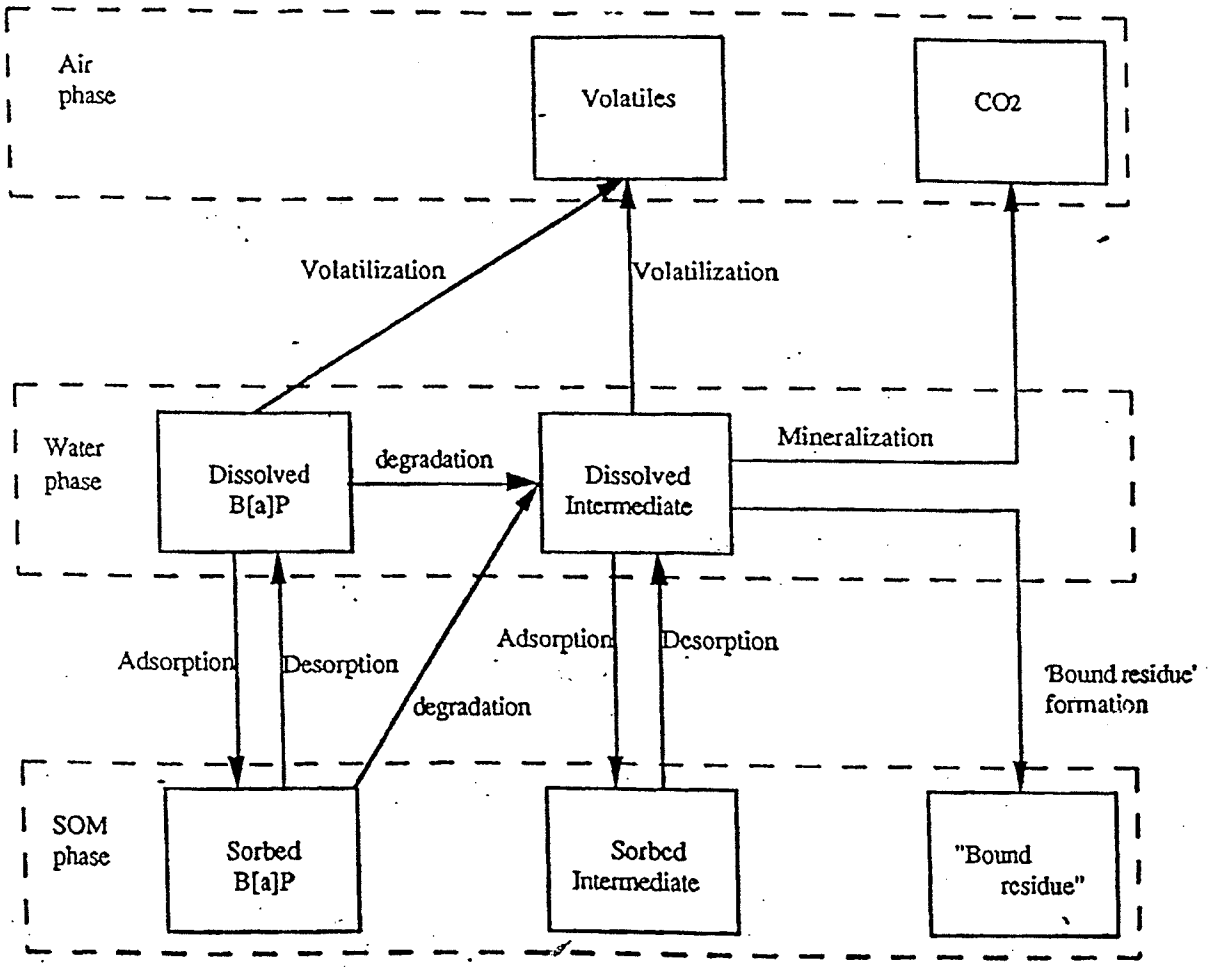
sole source of carbon has resulted in the accumulation of trans-9,10-dihydrodiol in growth medium. This microorganism uses cytochrome p-450 monooxygenase to co-metabolize phenanthrene at the carbon 9, 10 position to trans-9,10-dihydrodiol. Phenanthrene trans-9,10-dihydrodiol has also been reported as metabolite produced from phenanthrene by white rot fungus *Pleurotus ostreatus* (Bezalel et al. 1996b; Cerniglia et al. 1989). In general, the organisms shown to carry out co-metabolism reactions for PAHs in the laboratory media include species of *Pseudomonas, Acinetobacter, Nocardia, Bacillus, Mycococus, Achromobacter, and Arthrobacter among the bacteria and Penicillium and Rhizoctonia* among the fungi (Alexander, 1999).

2.4 Fate of Diesel Fuel Components

Possible fate of diesel fuel and their metabolites entering soil environment include biodegradation, sorption to soil and volatilization to air. The fate and behavior of organic compounds within the soil environment are dependent on a complex array of processes include biodegradation, sorption, humification and volatilization (Figure 2-6).

2.4.1 Biodegradation

The susceptibility of hydrocarbons to biodegradation varies with types of the compounds. Alkanes, major constituents of diesel fuel, are the most readily degraded in a petroleum mixture (Baker and Herson 1994; Atlas and Cerniglia, 1995). All n-alkanes in diesel oil mixture between C_{10} and C_{20} were observed to degrade at the same rate, regardless of the chain length of the alkanes (Jonge et al. 1997). The presence of branching within the alkanes may render the hydrocarbons refractory to microbial degradation. However, it



2-6. Fate of organic compounds in various soil compartments (after Qiu and Mcfarland, 1991)

has been reported that biodegradation can occur in branched alkanes, but n-alkanes disappear faster than the branched alkanes (Britton, 1984; Baker and Herson, 1994; Jonge et al. 1997).

The biodegradation of aromatic hydrocarbons has been shown to be widespread among naturally aerobic microorganism (Baker and Herson, 1994). Many species of bacteria inoculated into mineral salt media were capable of degrading mono and polycyclic aromatics hydrocarbons (Ellis, 1994; and Boldrin et al. 1993; Weissenfels et al. 1990). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene, pyrene and fluoranthene, however, tends to decrease with increased numbers of rings and with increasing numbers of alkyl substitutes. Studies performed by Weissenfels et al. (1992) on PAHs degradation indicated that the highest degradation rates were found for phenanthrene, fluorene, fluoranthene, and pyrene, whereas the lowest degradation rates were for benz(a) anthracene and benzo(a)pyrene. In part, the decreased degradation with the number of rings is a result of the lower solubility and hence the lower bioavialability. Normally, PAHs do not serve as amenable substrates for microorganisms (Eweis et al. 1998; Baker and Herson, 1994). The enzymes required for the degradation of high molecular PAHs can be induced by the presence of lower molecular weight PAHs. Lower molecular weight PAH such as anthracene has been found to serve as co-metabolic substrate for phenanthrene degraders (Tongpim and Pickard, 1999).

Microorganisms are capable of degrading the petroleum hydrocarbons found in diesel fuel. However, successful biodegradation of hydrocarbons can only occur if proper environmental conditions, including moisture content, temperature, and nutrient

concentration, has been met. The significance of soil water content for microbial activity has been recognized for years. Liu (1998) found that 60% to 80% moisture content was the optimum for biodegradation of phenanthrene. Soil water content affects microbial activity by influencing aeration, substrate diffusion and mobility of microorganisms. With decreasing water content, the activity of bacteria appears to be limited mainly because of the decreasing proportion of water filled pores resulting in reduced mobility of bacterial cells and limited availability of substrate. As the water content of soils increases, the percentage of air filled pores decreases resulting in a decreased O_2 content in the soil and the development of anaerobic or facultative anaerobic activity (Cookson, 1995). However, some of aromatic hydrocarbons are not appreciably altered under anaerobic conditions because their degradation involves direct incorporation of O_2 into the aromatic rings to achieve structural cleavage (Cookson, 1995). Therefore, degradation by native organisms occurs when oxygen is available.

Soil temperature also influences petroleum biodegradation in both direct and indirect ways. Directly, microbial activity can be related to temperature, with rates of metabolic reaction increasing with increasing temperature. Indirectly, soil temperature can have profound effects on physicochemical states of the contaminants, such as solubility, and composition of the microbial community (Margesin and Schinner, 2001). Haines et al. (1993) reported increased No. 2 fuel oil and crude oil biodegradation rates as a result of increasing temperature from 12 to 20°C. Margesin and Schinner (2001) found BTEX depletion increased with increasing temperature. For example, the 25% loss of BTEX measured in soil at 10°C was significantly lower than the 76% BTEX loss measured at 20°C.

In sub-soil system, the extent of degradation of hydrocarbon compounds is dependent on one or more nutrients needed for microbial growth. Most sub-soil systems are nutrient poor, therefore may limit the capacity of microorganisms to degrade contaminants in soils. The addition of nutrients such as nitrogen and phosphour can play a large role in the degradation of hydrocarbons. The addition of nitrogen and phosphorus resulted in a doubling of biodegradation rates of diesel oil in soils (Atlas, 1991). Manilal and Alexander (1991) found that the addition of phosphate increased the rate of phenanthrene mineralization. Another study by Carmichael and Pfaender (1997) conducted to assess the impact of inorganic and organic nutrients on the mineralization of ^{14}C phenanthrene showed nutrient addition decreased or had little effect on mineralization although the addition of nutrient increased microbial populations. The authors postulated that might have resulted from preferential use of nutrients as a carbon source over ^{14}C phenanthrene. Another study by Johnson and Scow (1999) also found that phenanthrene mineralization rates in soil either unaffected or slightly depressed by added nutrients. The authors postulated that the bioavialability of added nutrients and carbon substrate might have affected microbial metabolism of phenanthrene in soil. Table 2-4 present a number of reported studies on mineralization of phenanthrene under controlled soil environmental conditions.

Given that 1) an effective microbial consortium, which can degrade a contaminant, exists, 2) environmental conditions favorable to biodegradation are within acceptable levels, and 3) bioavialability of added and nutrients is not limited, many compounds that would normally be quickly degraded by microorganisms may not easily be degraded by microorganisms in soil environment. This is because organic compounds may become

Table 2.4. Aerobic bioremediation rates of phenanthrene in soil

% ^{14}C Phenanthrene Mineralized	Soil Type	Incubation Temperature (°C)	Inoculant added	References
1.3	NA[1]	30	Yes	Morgan et al. '91
7.2	Clay loam	20	No	Berry, '95
19	Sandy loam (0.8% OM)	20	Yes	Ortega et al.'97
5.1	Sandy loam (37% OM)	20	Yes	Ortega et al.' 97
0.23	Silty loam	20	No	Liu '98
2.3	Clay loam	20	No	Maurice '98
4.5	Sand	20	No	Maurice '98
4 -5	Sandy Loam	25	No	Johnson &Scow,'99
35	Clay Suspension	22	No	Laor et al. '99

Notes: [1] not available

sorbed to soil matrix and/or entrapped within the physical matrix of the soil. In these zones, where contaminants reside, removal of the contaminant becomes impossible because they may not be in the form that is readily available for the microorganisms. The following section elucidates the effect of sorption process on fate of hydrocarbons in soil environments.

2.4.2. Sorption

Sorption is another important factor for evaluating the fate of organic compound in the environment. The term sorption relates to molecules that are adsorbed by the surface of soil or absorbed within the mass of the soil matrix without the formation of covalent bonds. The term sorption is used to include both adsorption and absorption. Surface

adsorption is considered quite rapid and reversible (Scow, 1993; Verstraete and Devliegher, 1996). Different types of sorption processes include van der Waals attractive forces, hydrogen bonding, hydrophobic sorption (or partitioning), ligand exchange and ion excahnge reactions. These sorption processes are strongly dependent upon the properties of the organic chemicals and the properties of the soils (Alexander, 1999; Scow, 1993; Lyman et al. 1992;). The properties of some of petroleum hydrocarbons are shown in Table2-5.

Table 2-5. Physiochemical properties of selected petroleum hydrocarbons (After Liu, 1998).

Compound	Water solubility (mg/l) at 25°C	Vapor pressure (atm) at 25°C	Soil adsorption coeffiecient Log Koc	Octanol-water Partition coeffecient Log Kow
Decane	$3.8x\ 10^{-2}$	1.75×10^{-3}	NA[1]	NA
p-Xylene	$1.79\ X\ 10^{+2}$	$0.177x\ 10^{-2}$	NA	NA
Naphthalene	$3.0 \times 10^{+1}$	1.09×10^{-4}	3.36	3.11
Phenanthrene	$0.1^{2} \times 10^{+1}$	2.67×10^{-7}	4.52	4.36
Anthracene	4.5×10^{-2}	2.6×10^{-7}	4.45	4.27
Pyrene	1.35×10^{-1}	3.33×10^{-9}	5.09	4.81

Notes: [1] not available ; [2] phenanthrene solubility in water has been reported by another sources as 0.06 mg/l

The inorganic and organic fraction of soils, such as clay minerals, organic matter, and metal oxides, are responsible for the sorption of many compounds. The extent of the sorption is directly correlated with octanol-water partition coefficient, which is expressed as the K_{ow} value (a measure of hydrophobicity of chemicals), and the percentage of organic carbon in the soils; the more organic matter present in the soil phase, the more

the hydrophobic molecule is sorbed to solid surface. Sorption of nonpolar hydrocarbons to clay minerals and metal oxides and oxyhydroxides are maintained by physical forces or van der Waals forces (Dragun, 1988; Choudhry, 1983). The larger the size of the organic molecule, the greater the van der Waals forces of attraction, and the greater the extent of sorption (Gevao et al. 2001). When there is organic matter in the soil (more than 1%), the organic fraction would provide a hydrophobic environment for the accumulation of nonpolar hydrocarbons, such as phenanthrene, and sorption due to minerals surface area will play a minor role in bioavailability (Scow, 1993). The bond formed between the organic contaminants and the organic matter on which the contaminants accumulated or partitioned is called a hydrophobic bond (Scow, 1993). In some instances in which organic compounds interact with organic matter, or humic material of soil, the interaction is not really sorption. Instead, the change involves the formation of stable linkages. These linkages may sometimes be covalent linkages between organic molecules and the humic substances. These types of interactions that lead to bound residue formation will be discussed in section 2.4.3.

Sorption onto clay minerals has been shown to control the rates of biodegradation of petroleum component. The capacity of clay to affect biodegradation differs according to clay type. The extensive sorption of petroleum hydrocarbons to clay minerals, especially montmorillonite has been reported in the literature (Berry and Burton, 1997; Dragun, 1988; Brown and Thomas, 1984). Clay minerals such as montmorillonite, in which the lattice structure can expand, may sorb petroleum hydrocarbon on both external and internal surfaces. Cassidy (1995) found the extent of diesel fuel biodegradation was obstructed in the presence of 2:1 clay minerals and humic substances as compared to

quartz sand and kaolinite (nonexpanding clay). He also observed that relative amount of straight alkanes biodegraded was less than branched alkanes in the presence montmorillonite than in presence of sand or kaolinite clay. This study postulated that straight alkanes were strongly sorbed onto the internal surface of montmorillonite. The availability of organic compounds sorbed between expanding layers of montmorillonite would be far different from those compounds sorbed on the surface of clay (Alexander, 1999). Apitz et al. (1996) found the microbial biodegradation of n-alkanes of diesel fuel was insignificant on the illite clay, while n-alkanes in the sand was significantly degraded by microorganism. On both sand and illite, however, total PAHs degraded to 80 % and 90 %, respectively. This study implied that PAH components of petroleum products may be weakly sorbed (hydrogen bonds or van der Waals forces) to soil minerals, thereby, they could be easily desorbed and become available. Generally, the bioavialability of sorbed organic compounds will depend on the sorption mechanisms. Figure 2-7 illustrates the different forms the organic compounds can have in soil.

Organic matter associated with the soil plays a large role in the fate of many organic compounds in the environment. PAHs and other nonpolar compounds are sorbed mainly by the organic matter rather than by the clay constituents of soil. The extent of this retention is directly correlated with the K_{ow} value and the percentage of organic carbon in the soil; the more organic matter in the soil, the more the hydrophobic molecule is sorbed. Manilal and Alexander (1991) compared the mineralization rates of phenanthrene in four soils, and found a clear reduction in mineralization rates in a soil with high organic matter content (37%). Similar results by Ortega-Calvo et al. (1997)

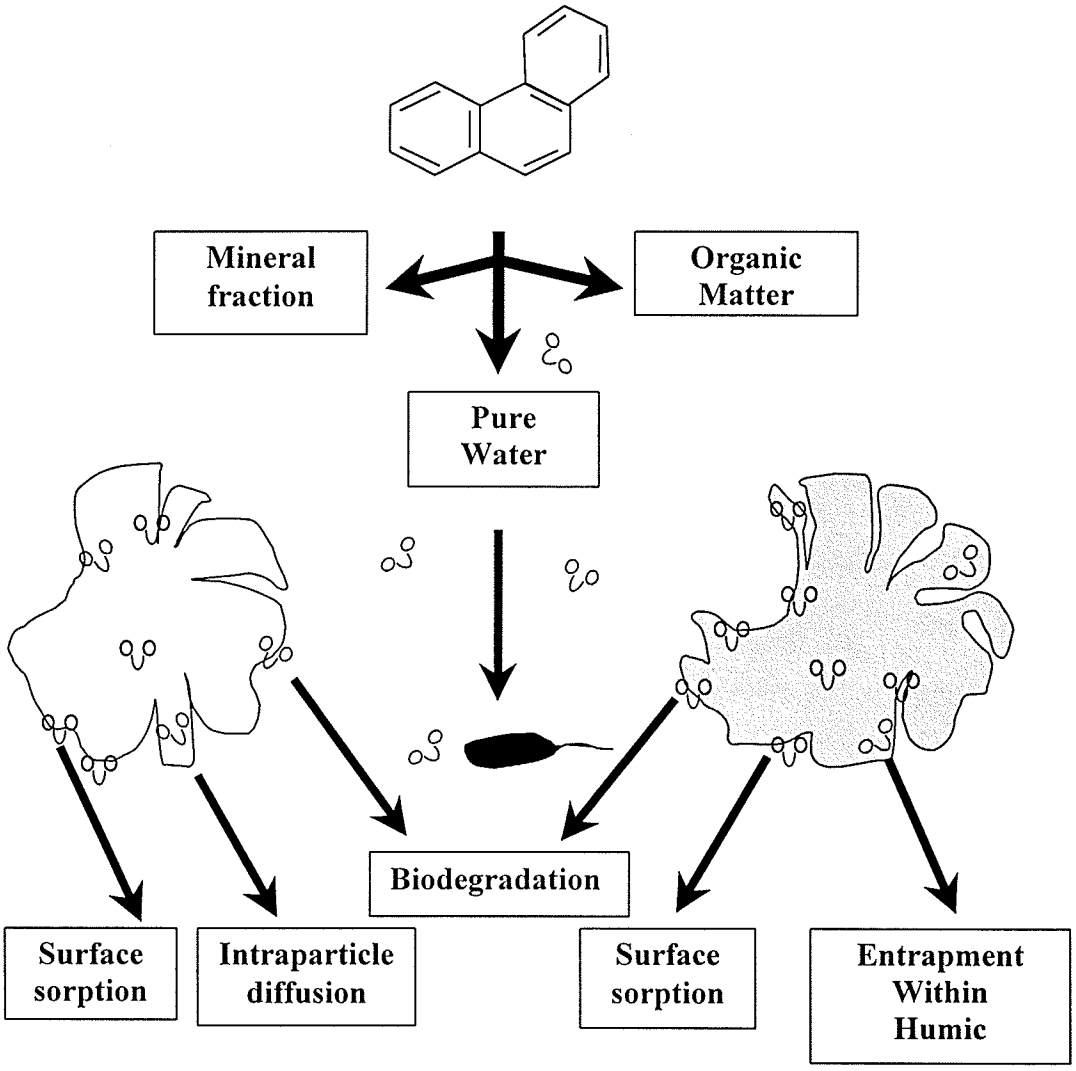


Figure 2-7. Different form of organic compounds in soil (After Reid et al. 2000)

found that an increase in organic matter content in soils reduced the phenanthrene mineralization. Further, these authors demonstrated that phenanthrene sorbed to the fulvic-clay complex was more easily degraded than that sorbed to humic-clay complex. The author related that due to the fact that fulvic acid is less hydrophobic than humic acid and therefore its interaction with phenanthrene would have been weaker. Generally, these studies concluded that sorption of the phenanthrene to the soil organic matter was the cause for the reduction in mineralization.

Organic compounds may diffuse into spatially remote areas, such as soil macro and micro pores. Physical entrapment within soil organic matter may also occur with the aging of chemicals in soil (Semple et al. 2001; Alexander, 1999). Pignatello and Xing (1996) in their review of sorption mechanisms referred to these phenomena as slow sorption or sequestration. Although sequestrated chemicals are inaccessible due to their location in microsites in the soil matrix, they still can be extracted from soil with organic solvents (Gevao et al. 2001). Generally, the sequestration or slow sorption reduces chemicals bioavailability and promote the formation of non-bioavailable residues. It has been observed that as the length of time that an organic chemical, such as phenanthrene and pyrene, remains in contact with the soil increases, the ability of that chemical to be degraded by microorganisms decreases (Carmichael et al. 1997; Parker and Burgos, 1999).

The ultimate result of sorption process is the movement of compounds from accessible soil compartments into less or inaccessible compartments, the result of which is the reduction in bioavailability. It is generally recognized that three soil-associated chemical

pools exist after sorption: (1) a fraction which can be rapidly desorbed; (2) a fraction which is more slowly desorbed, and (3) a fraction which has been termed ' bound residue' or 'non-extractable' (Reid et al. 2000; Semple et al. 2001). Bound residues represent the compounds in soils, plants or animals which persist in the matrix in the form of the parent compound or its metabolites (Semple et al. 2001; Alexnder, 1999). The following section discusses the process involved in the persistent of hydrophobic contaminants such as phenanthrene in soil.

2.4.3 Humification

Soil organic matter consists of a mixture of plant and animals products in various stages of decomposition together with substances synthesized biologically and/or chemically from breakdown products as well as microorganisms and small animals and their decomposing remains (Choudhry, 1984). Organic matter is usually divided into two chemical groups: non humic and humic substances.

Non humic substance include a large number of relatively simple, discrete compounds belonging to groups of polysaccharides and sugars, proteins, amino acids, fats simple organic acids, and other low molecular weight organic substances. In general these substances are relatively easily metabolized by microorganisms in soil and have a relatively short turnover time (Choudhry, 1984). The bulk of the organic matter in most soils consists of humic substances. Humic substances are defined by MacCarthy et al. (1990) as being " a naturally occurring biogenic, heterogeneous organic substances that generally can be characterized as being yellow or black, hydrophilic, acidic, polydisperse substances of high molecular weight ranging from several hundreds to tens of thousands,

and refractory which formed through the microbial degradation of plant and animal remains in soil".

Humic substances can be divided into three fraction, based on their solubility in water as a function of pH: 1) humic acid, which is not soluble in water under acidic conditions but is soluble at higher pH values; 2) fulvic acid, which is soluble in water under both acidic and basic conditions; and 3) humin, which is not soluble in water at any pH value (Choudhry, 1984), and therefore not extractable from soils in aqueous solutions.

Humic substances are composed primarily of carbon, oxygen, and hydrogen, with smaller amounts of nitrogen and phosphorus (MacCarthy et al. 1990). Figure 2-8 shows a hypothetical structure for a fraction of a humic molecule. The hydrophobic nature of humic substances provides a hydrophobic environment for the partitioning of organic contaminants in soil by the hydrophobic force. Although it is considered reversible, partition into humic substances has been shown to control the rates of biodegradation (See section 2.4.2).

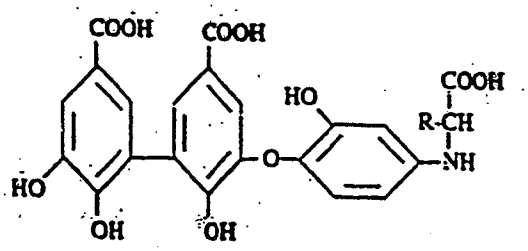


Figure 2-8. Hypothetical structure for a fraction of a humic molecule (after Stevenson, 1985).

The presence of a great variety of chemical reactive groups, such as carboxyl, hydroxyl, carbonyls and phenolic, in humic substances renders them able to form irreversible covalent bonds (ester, ether, carbon-carbon bonds) with aromatic intermediates.

Structure of covalent humic acid bound phenanthrene metabolites as postulated by Käcker et al. (2002) is outlined in Figure 2-9. Chemical or enzymatic catalysts, leading to stable mostly irreversible incorporation into the soil, often mediate the formation of covalent bonds between organic chemicals and/or their metabolites and soil humic substances. The incorporation of organic chemicals into the soil organic matter, or humus, through binding (polymerization) reactions of microbial enzymes is called humification (Rittmann and McCarty, 2001).

Humification results in the formation of complex compounds that cannot be removed by a standard method, such as soxhlet solvent extraction, because it does not significantly change the chemical nature of the residues. These unextractable residues are usually referred as " bound residues" (Kohl and Rice, 1998). The formation of soil-bound residue has been reported for many PAHs. Kohl and Rice (1998) found a significant portion of ^{14}C naphthalene, phenanthrene and benzo[a]pyrene applied to a soil is irreversibly bound to soil humic substance and the majority (> 70%) of bound residue was located with the humin fraction. Richnow et al. (1999) investigated the transformation of the ^{13}C anthracene in close soil bioreactor system and found that 50% of the anthracene formed bound residue with humic substances in the soil and transformation of anthacene into non extractable residues was predominantly influenced by biotic rather than abiotic processes. Miller and Clark (1998) reported that humic acids were more effective in binding PAHs than fulvic acids because of their higher aromatic and lower content of functional groups.

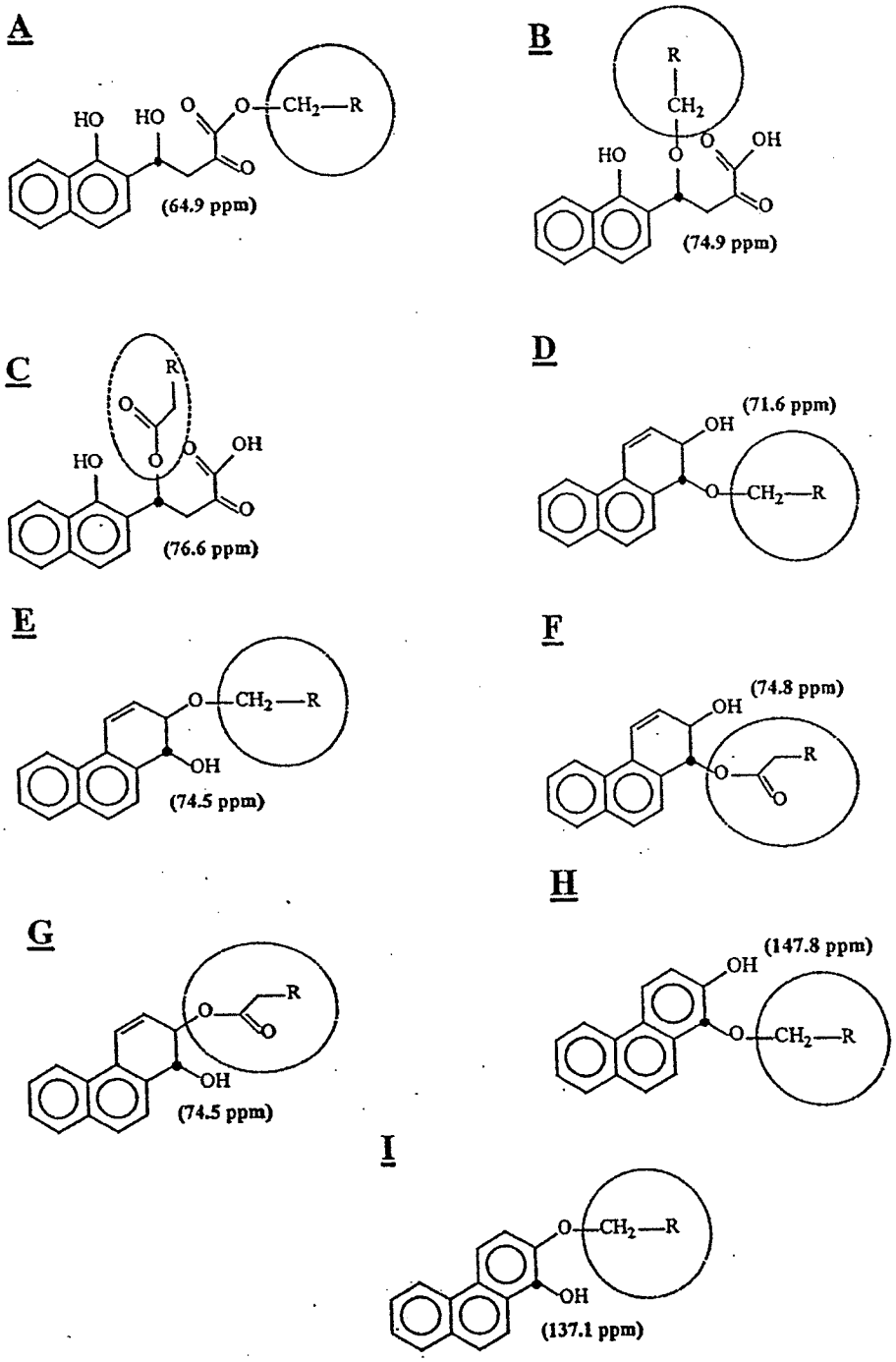


Figure 2-9. Postulated structures of covalent humic acid bound phenanthrene metabolites (A-C: 1-Hydroxynaphthalene 2-(4-hydroxy-2oxo-butyric acid); D-G: 1,2-dihydroxy-1,2-dihydrophenanthrene; H and I: 1,2-dihydroxyphenanthrene), with consideration of 13C label (•) and simplified structure of humic material is encircled (After Käcker et al. 2002)

Bound residue formation results from the synthesis of a relatively labile bond, such as an ester, which is usually readily hydrolyzed microbiologically or chemically, creating a low, long-term stabilization of soil bound residue in bioremediated soil (U.S. EPA, 1998). On the other hand, formation of ether linkages between humic materials and metabolites results in relatively long-term stabilization of the metabolite in a form of low bioavialability (U.S. EPA, 1998). Käcker et al. (2002) found ester- and ether bound phenanthrene metabolites such as 1-hydroxy-2-naphthoic acid residues in soil. However, 50 to 70% of phenanthrene metabolites were ester bound via their carboxyl groups. Richnow et al. (1998) also found a large part of the labeled anthracene and its metabolites were irreversibly immobilized in the non extractable bound residue fraction via ester bonds. Richnow et al. (2000) investigated the transformation of labeled phenanthrene in soil bioreactor and found that parent phenanthrene and metabolites products, such as acidic, hydroxylated, and aromatic alcohols are incorporated into the soil humic matrix via ester and ether bonds.

In summary, humification has been shown to occur with PAHs. In instances where considerable incorporation of contaminants into the soil organic matter takes place it is difficult to distinguish between reductions in extractable contaminants due to biodegradation and from incorporation into humic substances. Humification is thought to be an irreversible process, however, the fate of humified PAHs has not been sufficiently investigated.

2.4.4 Volatilization

Volatilization is the process whereby chemicals move from either a liquid or a solid phase to a gas phase. The petroleum volatilization rate depends on the vapor pressure of the volatile constituents. Low vapor pressure that is less than 10^{-10} atm indicates that there are high intermolecular attraction and negligible amounts of the constituents of interest in the atmosphere or soil air. Alternatively, if the vapor pressure exceeds the 10^{-5} atm most of the constituents will show high tendency to be present in the atmosphere or soil air. Those chemicals having vapor pressures between these two values may have a tendency exist in both the atmosphere or in soil air as well as in liquid or solid form (Dragun, 1988). The vapor pressure of the majority of diesel fuel hydrocarbons is between 10^{-5} and 10^{-10} (Table 2-5). Therefore, during an initial spill there may be significant loss of contaminant as the diesel pools on top of the soil. Over time, the contaminant usually enters the soil system where the volatilization potential decreases.

Soil type and soil textures also affect the volatile losses of petroleum hydrocarbons to the atmosphere. Sandy soils have a low specific area for molecular retention, and the pore size tends to be large on average. Organic matter and clay content tends to cause the most adsorption of petroleum hydrocarbons that results in significant reduction in volatilization rate (Bossart and Bartha, 1984; Park et al. 1990).

2.5 Toxicity of Hydrocarbons

Hydrocarbon partitioning to soil organic carbon and within soil pore space reduces bioavailability of hydrocarbon contamination. The extent of partitioning would increase with the increasing the soil organic carbon content, the organic carbon partitioning

coefficient, and the time that has elapsed since contamination. All of these soil-contaminated interactions make it very difficult to predict the toxicity of soil contaminants or to determine possible environmental effects based on the chemical analysis (Chang et al. 1997; Juvonen et al. 2000; Cook et al. 2002). For proper assessment of efficacy of composting process, chemical analysis and toxicity data should be combined.

Toxicity tests can integrate the effect of all toxicants in the soil into a single toxicological response. Several different soil toxicity bioassays include bacterial, animals, and plant tests have been recommended when evaluating the ability of bioremediation to reduce the adverse effects of contaminants in soil (Marwood et al. 1998). In general, at least two major types of toxicity bioassay should be conducted (Duncan, 1993). Bacterial toxicity tests, such as bioluminescence (measure the change in the light emission by *Vibrio fischeri*) and SOS Chromotest (measure the intensity of colour reaction with the amount of DNA damage to *E. coli*) are fast, simple, and inexpensive, and only a small amount of sample is needed to perform the test.

Soil animals and plants toxicity tests are the most reliable and relevant tests to assess the possible harmful effect of toxic substances on soil fauna and flora (Chang et al. 1997; Juvonen et al. 2000; Phillips et al. 2000; Kapanen and Itävaara, 2001). Earthworms, however, are more sensitive than plants to assess soil toxicity (Bierkens et al. 1998; Dorn et al. 1998).

2.5.1 Earthworms Tests

Earthworms are attractive as an experimental animal model when evaluating the possible harmful effects of toxic substances on soil fauna, because they are common in many soils and easy to handle because of their relatively large size. Earthworms have a particularly intimate contact with the soil, consuming large quantities and having little external barrier to the soil solution. Thus, earthworms come in immediate contact with chemical sorbed to soil particles and dissolved in soil water (Reid et al. 2000; Kapanen and Itävaara, 2001). Earthworms respond to many chemicals in several ways, e.g., increase in body weight, decrease in mortality, and overall decrease in activities normally associated with viable earthworm populations (Charrois et al. 2001). Thus, earthworm posses key characteristics necessary for environmental monitoring.

Several species of earthworms have been used in toxicity experiments: *Eisenia fetida, Eisenia andrei, Allolobophora caliginosa, Lumbricus terrestris, Aporrectodea tuberculat*a. *Eisenia fetida,* recommended by U.S. Environmnetal Protection Agency (USEPA) and the Organization for Economic and Cooperative Development (OECD), has been used as a typical earthworm in many studies (Kapanen and Itävaara, 2001). This is because *E. fetida* have a wide temperature tolerance and can live in organic wastes with a range of moisture content (Edwards, 1998). Unlike the more commonly used red worm, *E. fetida, L. terrestris*, a soil inhabiting worm to Canada, is as applicable and reliable as *E. fetida* for toxicity assays (Cook et al. 2002). Comparative tests of the two earthworm species have indicated that the sensitivity of *E. fetida* to hydrocarbons is sufficiently comparable to that of *L. terrestris* in spite of the ecological differences

between these species (Potter et al. 1999; Sayles et al. 1999). Other researchers, however, reported that *L. terrestris* were more sensitive to other chemical toxicant, such as the heavy metal lead and insecticides chlorpyriphos, than *E. fetida (*Chang et al. 1997; Eijsackers, 1998).

The toxicity of aromatic hydrocarbons to the earthworm species has been reported to increase with the increase of proportion of contaminated soil in tested sample. For example, Charrois et al. (2001), using 14-day earthworm survival bioassay on three weathered creosote contaminated soils, found that exposure of *E. fetida* to sample soil containing 100% contaminated soil (a final total PAH 1320 mg/kg) resulted in 100% mortality in 2 days compared to 3% in control. Potter et al. (1999) observed that exposure of *L. terrestris* and *E. fetida* to 100% composted creosote-contaminated soil (final total PAH ranged from 888 to 1556 mg/kg) resulted in 50% mortality to both species of earthworms. Another study by Phillips et al. (2000), however, found no mortality when *E. fetida* was exposed to 100% treated creosote PAH and petroleum hydrocarbon with a final total PAHs and TPH of about 600 and 1000 mg/kg, respectively. A similar study by Sayles et al. (1999) also showed that land treated PAH contaminated soil (a final TPH of 1160 mg/kg) was not toxic (i.e. 100% of worm survived in 100% test soil) to both the *L. terrestris* and *E. fetida species.*

2.5.2 Plant Tests

The sensitivity of plant to chemicals in the environment varies considerably. Plants sensitive to harmful substances can be used as bioindicators and more resistant plant are useful in bioremediation. According to Kapanen and Itavaara (2001), the plant tests used

in environmental analysis can be classified into several groups: 1) biotransformation (change in the concentration of chemicals caused by plant), 2) food chain uptake (amounts and concentrations of toxic chemical enter food chain via plant uptake), 3) phytotoxicity (toxicity and hazard posed by pollutants to the growth and survival of plants), and 4) sentinel (monitoring the pollutant by observing toxicity symptoms displayed by plants).

The Phytotoxicity test has received the most attention. The inhibition of seed germination and effects on root elongation or plant growth are the main areas of interest in studies on phytotoxicity (Kapanen and Itavaara, 2001). Some of the plant species recommended for growth by U.S. Environmnetal Protection Agency (USEPA) and the Organization for Economic and Cooperative Development (OECD) are Cress (*Lepidium sativum*), lettuce (*Lactuca sativa*), Oat (*Avena sativa*), Radish (*Raphanus sativus*), Red clover (*Trifolium pratense*), and Ryegrass (*Lolium perenne*).

Plant growth and germination are one of the commonly used tests when studying the composting and detoxification of contaminated soils. A large number of studies have been carried out with different plant species to assess compost detoxification to soil contaminated with hydrocarbons. Baud-Grasset et al. (1993) treated a soil contaminated with a mixture of PAHs and the decrease in toxicity determined with lettuce, oat, and millet seed was closely correlated with the decrease in parent PAH compounds. This study concluded that different plant species would differ in their sensitivity to parent compounds and their metabolites after treatment. Phillips et al. (2000) and Sayles et al. (1999) also found that the land treatment of PAHs contaminated soil showed no residual

toxicity to lettuce and oat seeds despite a soil remaining concentration of 600 to 1200 mg/kg total PAH. However, Marwood et al. (1998) found an increase in toxicity of diesel fuel (final TPH of 5000 mg/kg) to lettuce seed following the land treatment of four soils of different physical compositions. In contrast, the authors found the toxicity in two of the four soils decreased when the solid phase Microtox test was used to conduct direct toxicity testing of soil samples using *Photobacterium phosphoreum*. Wang and Bartha (1990) found that bioremediation of soil that had been contaminated by fuel spill reduced the toxicity to soybean and ryegrass seeds. However, toxicity of the fuel increased in the order of jet fuel < heating oil < diesel oil. This study indicated that toxicity of fuel spills would be correlated to hydrocarbon composition of fuels.

Results from these studies suggest that seed germination can be a potential toxicity test for PAHs contaminated soils. However, the variety of results generated from the plant and earthworm toxicity assays emphasizes the need for conducting several different soil toxicity bioassays.

2.6 Summary

The literature indicated microorganisms are capable of degrading the petroleum hydrocarbons found in diesel fuel, including PAHs, leading to formation of dehydroxyl metabolites which is either further metabolized or accumulate in culture media. However, many compounds that would normally be quickly degraded by microorganisms in culture liquids may not be easily degraded by microorganisms in soil if the environmental and nutritional conditions favorable to biodegradation doe not exist within acceptable levels. Given that all favorable conditions exist, sorption and humification of

parent hydrocarbon compounds or their metabolites in soil matrix has been reported to control the rate of biodegradation. Thus, one might expect that diesel fuel hydrocarbons may retain on the soil surface or in interparticle space if the soil has very high clay or organic contents. On the other hand, a sandy soil may allow rapid infiltration.

A review of the literature indicates a variety of bioremediation technologies exist for hydrocarbon contaminated soils. The following chapter will provide a critical review of the existing literature on hydrocarbon bioremediation using composting technology.

3. Composting Literature Review

3.1 General Description of Composting

Composting is the aerobic biological decomposition and stabilization of organic substrates under conditions that allow development of thermophilic temperatures as a result of biologically produced heat (Haug, 1993). Traditionally, composting has been used primarily to decompose agriculture waste, yard and kitchen waste, municipal solid waste, and sewage sludge. The primary reasons for composting these materials are to reduce moisture content and volume, to destroy pathogens and odour producing nitrogen and sulfur containing compounds for ultimate disposal or use as a soil organic amendment. Recently, attempts have been made to use composting process as an ex-situ biological technology for many hazardous wastes (William and Keehan, 1992).

In contaminated soil composting, soil is mixed with suitable organic materials and placed in a pile or enclosed vessel. The organic material serves to improve the soil texture by reducing bulk density and increasing porosity and to provide sufficient nutrition and energy source to rapidly establish a large microbial population (Epstein, 1997). The large microbial population results in the production of a large amount of heat upon consuming the supplied co-substrates.

The heat produced from organic decomposition during the composting process causes an increase in the temperature within the composting mass into the thermophilic range (Figure 3-1). At this temperature range, growth is restricted to a few species of thermophilic microorganisms. After the rapidly degradable components are consumed,

microbial activity and temperatures gradually decline. At the end of this stage, the material is no longer self-heating and the piles cool, resulting in an increase in mesophilic microorganisms (William and Keehan, 1992; Semple et al. 2001). Substantial changes occur in microbial populations and species abundance during the various temperature stages. Mesophilic bacteria and fungi are dominant in the initial warming period, thermophilic bacteria, especially *Actinomycetes*, during the thermophilic phase, and mesophilic and fungi during curing phase (US EPA, 1998).

The use of composting process for bioremediation, where temperature can exceed 60°C, should prove advantageous by directly increasing rates of compound mass transfer to aqueous phase (Figure 3-2). In cases in which the limiting factor to biodegradation is the bioavialability, the increase in aqueous solubility could lead to an appreciable increase in metabolism. Another favorable factor inherent to compost-based remediation is that large amounts of organic matter are added to the system. Organic matter is considered a major pool of organic compounds (See section 2.4.2). Thus, while composting makes a fraction of pollutants transfer to the aqueous phase (by thermal desorption) and thus more readily biodegradable, an additional fraction is made recalcitrant by strongly associating with residual organic matter (Semple et al. 2001). The rich microorganisms present in the composting material can support both of these processes. Microorganisms, on the one hand promote biodegradation, while on the other favor pollutants association with organic matter through humification (See section 2.4.3). The end result is a soil-compost mixture that contains reduced pollutant concentration and /or a matrix that has less pollutant in a bioavailable form (Semple et al. 2001).

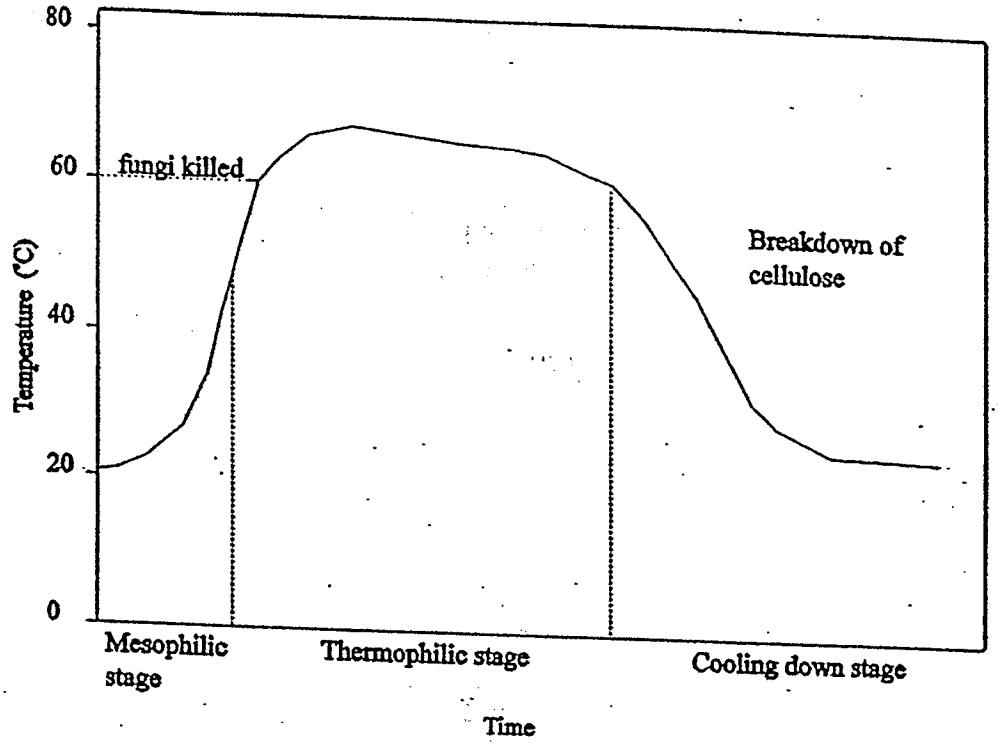


Figure 3-1. Typical temperature profile in a compost heap (after Beaudin, 1995)

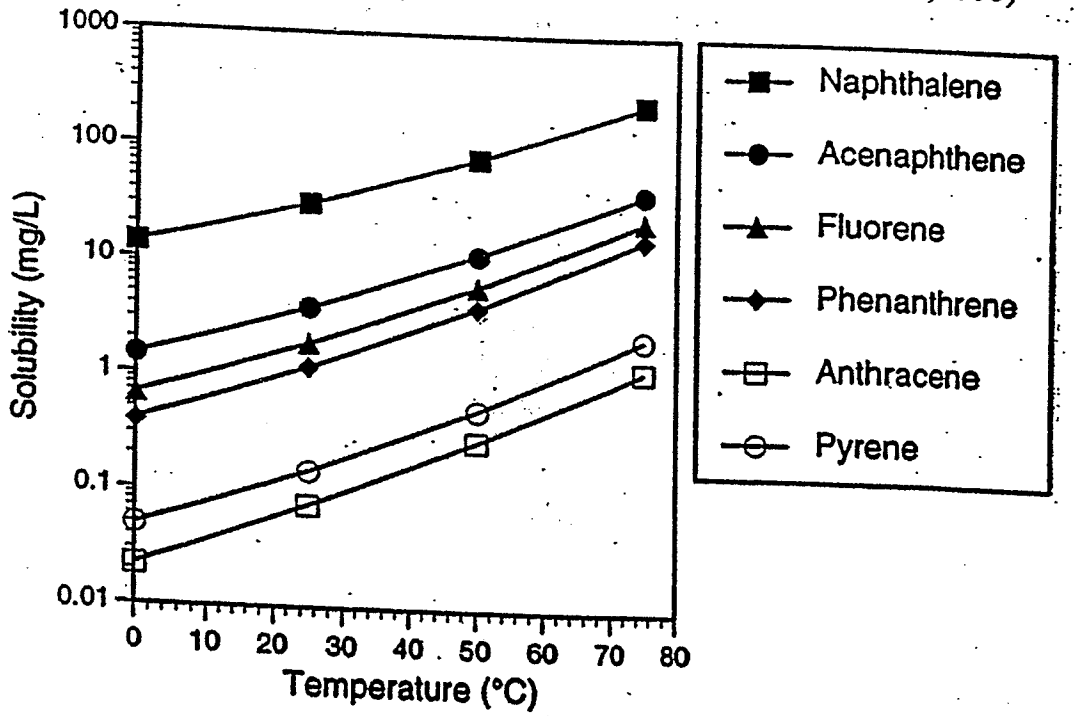


Figure 3-2. Observed solubilities in water of various PAHs over a range of temperatures (after Landau, 2000)

3.2 Composting of Hydrocarbons Contaminated Soil

A wide range of common environmental contaminants degraded rapidly during composting such as explosives (William and Keehan,.1992), chlorophenols (Laine and Jorgnsen, 1997; Valo and Salkinoja-Salonen, 1986), pesticides (Michel et al. 1995), aromatic hydrocarbons (Joyce et al. 1998; Semple et al. 1998; Wischmann and Steinhart, 1997; Mahro and Kästner, 1993; McFarland and Qiu, 1995) and petroleum hydrocarbons (Al-Daher et al. 1998). Soil composting of hydrocarbons has been studied in experiments ranging from bench scale to large pilot studies. In bench-scale studies, many studies have been carried out to: (1) elucidate the fate of hydrocarbons pollutants on a single contaminant and complex mixture of hydrocarbons, and (2) to investigate the fate of hydrocarbons with the addition of mature compost

To allow for elucidation of the fate processes for specific single pollutant, Qiu and McFarland (1991) investigated the degradation rate of labeled ^{14}C benzo(a) pyrene in both sandy loam and silt loam soils. Radiolabeled ^{14}C benzo(a) pyrene spikes were added at 40.32-$\mu Ci\ kg^{-1}$ dry soil. Both soils received corncob as a carbon and energy source and fungal inoculum. The amendment soil reactors were placed in constant room temperature (20°C) in the dark to minimize photodegradation. The removal of the labeled ^{14}C was monitored for 120 days period. After 120 days of incubation, they found that 37 % and 15% of ^{14}C were bound as non-extractable residues in silt loam and sandy loam soils, respectively. The high bound residue formation in silt loam soil may be due to the higher clay content of soil.

McFarland and Qui (1995) investigated the fate of benzo[a]pyrene in soil under composting system in the presence and absence of *Phanerochaete chrysporium.* This study showed that although the benzo[a]pyrene appeared to be removed, there was no appreciable difference (after 95 days) between the uninoculated and inoculated systems with 65.6 and 62.8% removal, respectively, although initial rates of removal were faster in inoculated incubations. Interestingly, analysis of gaseous traps indicated that there was no loss through volatilization or mineralization and that nearly 100% of the bezo[a]pyrene removed was attributed to bound residues as the parent compound (~60%) or as chemical intermediates. Further, this study highlighted that the presence of the fungus increased the rate of bound residue formation in the first 30 days of the composting study, where the rate went from 0.73 mg kg^{-1} day^{-1} in the absence of the fungus to 1.58 mg kg^{-1} day^{-1} in the presence of the fungus.

There are few studies that consider the implication of pollutant mixtures. Civilini et al. (1996) considered a composting process using municipal solid wastes and fertilizer, to clean up PAHs present in creosote-contaminated soil (soil type was not discussed). The compost was organic matter from municipal solid waste, which were mixed with the PAH contaminated soil (soil to compost ratio was not discussed). At 45°C, it was found more than 97% removal was achieved for naphthalene, acenaphthene, fluorene and phenanthrene, as well as more than 80% removal for slowly degradable PAHs such as benzo(a)anthracene and chrysene, after 15 days. However, although the authors account for volatilization, which was found to be less than 10% for all the PAHs with the exception of acenaphthene (~54%), this study based the removal of the PAHs on total extractability of the PAHs and did not consider any fraction which is non-extractable.

From the data described, it can be seen that as the molecular size and weight increased, there was a decrease in recovery, suggesting that a fraction of the PAHs may have become sorbed, sequestered within the compost matrix.

Similarly, Beaudin et al. (1996) investigated the feasibility of composting to cleanup a soil contaminated with a complex mixture of a weathered crude oil. The soil was a sandy soil (83.5% sand) contained 17,00 mg kg^{-1} mineral oil and grease (MOG) on average. The compost consisted of leaves (mostly maple), alfalfa, and mature compost (90 day old). The soil/compost mixtures were filled into 8-L aerated reactors. The compost temperature was limited to about 50°C by increasing aeration rate once that temperature had been achieved. They found that after 105 days the extractable mineral oil and grease (MOG) concentration decreased from 17,800 mg kg^{-1} to 7500-mg kg^{-1} dry compost (i.e., 50% of the MOG originally in the soil had been degraded). Overall MOG fractionation demonstrated that 83 % of polar compounds, 60% of aliphatics, and 54% of aromatics were degraded.

Joyce et al. (1998) investigated the fate of a mixture of three and four ring PAHs (fluorene, anthracene, phenanthrene, pyrene, benz[a]anthracene) under composting conditions with municipal solid waste monitored over a 60-day period (30 days of active composting followed by 30 days of compost curing). The fate of the PAHs was also monitored in mercury (II) chloride ($HgCl_2$)-treated systems to compare the impact of biotic and abiotic processes. The results of this study showed that the loss processes occurred during the active phase of composting. Anthracene, phenanthrene, and pyrene were removed effectively during composting process by a combination of biotic and

abiotic processes, principally dominated by the biotic processes. Fluorene proved to be too volatile and so most of compound (75%) was removed abiotically in the gas phase. Additionally, benz[a]anthracene proved to be resistant to biodegradation throughout the composting incubation with between approximately 40 to 45% being lost abiotically.

The potential of using mature compost (the resultant product of composting) for bioremediation purposes have been investigated. Mahro and Kästner et al. (1993) investigated the fate of pyrene in soil and soil-compost mixtures over a period of 100 days. A starting soil to compost ratio of 1:5 (dry weight) was used. The soil/compost mixture was filled into 3-L aerated reactors. The reactors were incubated at room temperature (21 ± 2°C) for 100 days. They found that the degradation of pyrene was enhanced significantly with the addition of mature compost with >80% removed after 20 days in the presence and <5% removed in the absence of the compost. Further Kästner et al. (1995) investigated the impact of mature compost addition on the fate of ^{14}C-labeled anthracene in diesel fuel mixed into sandy loam soil. In soil compost mixtures, 23% of the labeled anthracene transformed into $^{14}CO_2$ after 103 days. After the same time, 50% of the added radioactivity was recovered in the soil-compost mixture, of which only 5 % could be recovered as liquid extract using either organic or humic acid extraction. The remaining 45% of the activity were considered irreversibly sequestered/bound to the soil compost matrix as non-extractable bound residues. In soil without compost, however, more than 90% of ^{14}C was recovered by either of two applied extraction methods with the formation of bound residues being less significant. This study suggested that the mature compost addition enhanced $^{14}CO_2$ mineralization and stimulates the formation of non-extractable bound residue. Kästner and Mahro (1996) followed this work up by

investigating the degradation of napththalene, anthracene, fluoranthene, pyrene and phenanthrene in soil and soil compost mixtures. The soil was contaminated with 500-mg naphthalene kg^{-1} dry soil and with 100-mg kg^{-1} dry soil each of fluoranthene and pyrene, phenanthrene and anthracene. The soil was mixed with mature compost and sterilized compost in a wet weight ratio of 3:1. The soil and soil/compost mixtures were filled into the 1.5-L reactors. The reactors were then incubated at 25°C for 25 days. In soil/compost mixture, it was found that naphthalene, phenanthrene and anthracene were completely removed after 25 days while fluoranthene and pyrene complete removal was finished after 35 days. In contrast to soil/compost mixture, sterilized soil/compost mixture showed no significant removal of any PAH except naphthalene. The authors found that the presence of the organic matrix of the compost and microorganisms enhanced removal of PAHs as compared to the control soil.

Wischmann and Steinhart (1997) also investigated the removal of 18 PAHs and the formation of PAH metabolites products in creosote-contaminated soil with and without compost amendments. The soil type and mature compost materials used were not discussed in this paper. The soil was placed into 1-L jars and then spiked with 2 mL PAH standard solution, resulting in total PAH concentration of 1,749 mg kg^{-1} dry weight. The jars with soil and soil/compost mixture were kept at ambient temperature in the dark for 25 weeks. PAHs and PAHs metabolites products fate were also monitored in autoclaved and mercury (II) chloride ($HgCl_2$)-treated systems. PAHs were extracted with 50 ml dichloromethane and identified and quantified using gas chromatography equipped with a mass spectrophotometer (GC-MS). In soil with no compost, only PAHs up to three aromatics rings were degraded over 25 weeks; however, there was enhanced

elimination of the parent PAH compounds with the addition of compost with approximately 100% of fluoranthene and pyrene, more than 90% of benz[a]anthracene and chrysene and approximately 70% of benzo[a]pyrene removed from the soil mixed with the compost during 25 weeks. Further, this study found substantial accumulation of PAHs metabolites products such as 9-fluorenone, anthracene-9,10-dione, and benza[a]anthracene-7,12-dione in soil with no compost and poisoned soil treated system, suggesting a substantial contribution of abiotic oxidation processes. However, in soil with compost, such metabolites products were found in the first two weeks in the compost amended soil, followed by rapid decrease in the following weeks. This study indicated the addition of the compost had significantly enhanced the removal of PAHs and their metabolites compared to soil with no compost.

In pilot scale studies of bioremediation of petroleum contaminated soil, Al-Daher et al. (1998) investigated the composting of calcareous sandy soil contaminated during the Gulf War in sixteen composting soil piles. The composting piles were amended with woodchips, and either with dried sewage sludge, mature compost, and petroleum degrader bacteria (only added after 3 months of composting). Samples were collected monthly from each pile to look at the degradation of two components, total extractable matter (TEM) with dichloromethane-soxhlet extraction and total PAHs. Gas chromatography equipped with a flame ionization detector (GC-FID) was used to measure TEM. PAHs were identified and quantified using gas chromatography equipped with a mass spectrophotometer (GC-MS). After 8 months, the results showed that the windrow soil pile systems resulted in 45% to 59 % reduction in TEM and 54% to 60% reduction of PAH content in the composting soil piles. PAHs with five or more rings

were resistant to degradation during the composting process. This study also observed no significant differences in the degradation rates between sewage sludge and mature compost. The addition of petroleum-degrading bacteria had no significant effect on hydrocarbon removal. This study lacked information related to operation conditions within the compost, for example, temperature conditions (i.e. mesophilic or thermophilic), oxygen concentration, and starting soil to compost ratio. This study, however, highlighted the significance of using either fresh feedstock or mature compost in the composting systems. Another study by O'Reilly et al. (1997) investigated the use of composting to bioremediate soil contaminated with petroleum hydrocarbons at an oil refinery site. The composting piles were made from fresh feedstock consisted of woodchips, agricultural fertilizers, and activated sludge from a wastewater treatment plant. The compost piles were operated for 6 months, and found concentrations of alkanes and PAHs were significantly reduced (85% and 60%, respectively). This study lacked information on the type of soil and starting soil to compost ratio used. This study concluded that composting the soil with fresh feedstock could be a competitive technology to remediate this site.

The above studies have successfully applied composting methods to clean up soil that was contaminated with a variety of hydrocarbon compounds. Chemical data, however, are usually not sufficient to evaluate the toxic effect, because it is impossible to analysis all the chemical compounds contributing to toxicity (Chang et al. 1997; Cook et al. 2002). For proper assessment of efficacy of composting process, a few studies considered the use of toxicity data to complement chemical data. Potter et al. (1999) evaluated the efficacy of composting to reduce toxicity of creosote PAH contaminated

soil in bench scale reactors using chemical analysis and toxicity tests. Five compost amendment conditions were formulated from different nutrient or amendments to the reactor mixtures. Each of the test conditions utilized a 70% soil and 30% corn cob mixture on a dry weight basis. Other conditions included the addition of standard nutrients (N & P) and up to 5% (w/w) activated sludge. After 12 weeks, composting resulted in a final concentration of PAH ranged from 888 to 1556 mg kg^{-1} in the reactors. Several toxicity bioassays in earthworms and plants were used. Earthworms (*L. terrestris and E. fetida*) were exposed to samples of composted soil mixed with artificial soil (10% peat, 20% kaolinite clay, and 70% fine sand by weight) in 0% to 100%dilutions and survival was assessed after 14 days. Seed germination and root elongation tests were evaluated in lettuce and oats, and genotoxicity testing was performed in onion. This study found composting decreased, but not eliminate, toxicity to both earthworms and Lettuce and oats elongation. The authors, however, found that compost treatment eliminated toxicity for the anion. This study concluded that composting PAH contaminated soil could reduce PAH concentration but the toxicity of the soil varied between bioassays.

Juvonen et al. (2000) composted a heterogeneous oily waste from an old landfill of oil refinery in windrows for 4 months. Ten to twenty percent of the total amount of the waste was heavy oil fractions. The rest of the waste consisted of fly ash, metals, and sulfur catalysts used in the oil refinery. Three compost windrows ($50m^3$) with different proportions of oily waste were constructed from the sewage sludge and coniferous bark. Samples from windrows for chemical analysis and toxicity tests were taken at interval time of 0, 32, 60, 74, and 123 days. Toxicity tests conducted were the bioluminescent

test, Met plate, Toxi-Chromotests, Bio Tox (bacterial toxicity tests), earthworm survival test, and seed germination test. The toxicity tests were carried out on 100% of the contaminated sample. During the 4 months of composting both toxicity and oil concentration were reduced during composting. The total oil hydrocarbon concentration decreased from 86,000 to 1400 mg kg^{-1} (98% removal) and PAHs concentration decreased from 135 to 23.5 mg kg^{-1} (83% removal). The total concentrations of heavy metals (675 mg kg^{-1}) did not decrease during composting. Toxicity of the compost sample varied between bioassays; however, most of the tests showed significant decrease in toxicity during the composting process. Seed germination tests were more sensitive than earthworm and bacterial tests. According to seed germination test, the sample collected at day 0 inhibited red clover germination almost completely (98.7%). The inhibition decreased gradually during the composting, but after 123 days it was still greater than 40%. The earthworm species (*Enchytraeus albidus*, *Enchytraeus Sp.*) demonstrated increasing survival and reproduction in the soil samples taken on day 60 and afterward (>90% survival). The study concluded that composting microorganisms reduced the concentration of different oil fractions, including PAHs.

Ahtiainen et al. (2002) composted creosote-contaminated soil in 5 and 100 m^3 windrows for 4 months. Degradation of PAHs was followed by chemical analysis and toxicity tests. The potential toxicity of the compost sample was determined using the bioluminescent bacteria test (*Vibrio fischeri*) and enzyme synthesis inhibition (Toxi-Chromopad test, *E. coli*). After 4 months of composting, the amount of PAHs was reduced significantly (90%) and toxicity decreased as well. Cajthaml et al. (2002) demonstrated composting decreased toxicity of PAH to bioluminescent bacteria *Vibrio fischeri*. However, a

toxicity test on mustard-seed germination did no reveal any significant changes during composting.

The above studies indicated the combination of both chemical analysis and toxicity bioassay data will be best to assess toxic potential of composted soil. Although, there are many potential toxicity tests for testing the toxicity of composted materials, the above studies demonstrated that both seed germination and earthworm survival tests are sensitive to change in PAH and TPH concentrations in soil compost samples.

3.3 Factors Affecting Composting of Hydrocarbons

The objective in composting contaminated soil is to create a favorable environment in which the indigenous microorganisms will biodegrade the petroleum contaminants in soil to innocuous end products. Before the composting operation can begin, many factors must be considered to ensure biodegradation of hazardous waste. These factors include temperature, oxygen level, pH, carbon/nitrogen ratio, moisture content, bulking agent, and soil to compost ratio.

3.3.1 Temperature

The temperature attained during the composting process is critical in soil composting due to the fact that there are different optimum temperatures for different microorganisms above which major changes will take place within the microbial population (Peramaki and Blomker, 1997). Normally, composting is operated within either the mesophilic range or the thermophilic range depending upon substrate. Mesophilic and thermophilic

temperatures as a factor in the biodegradation of petroleum fractions was considered in many investigations.

Hogan et al. (1989) composted representative aliphatic and polyaromatic hydrocarbons at both 35 and 50°C, and found that after 35 days both temperatures provided similar removal of aliphatic hydrocarbons (e.g. octadecane) and polyaromatics hydrocarbon (e.g. phenanthrene). Beaudin et al. (1999) investigated composting of mineral oil and grease (MOG) at temperatures ranging from 23°C to 50°C. They found that after 30 days of composting MOG degradation decreased with increasing temperature from 23°C (56%) to 40°C (33%) and began to increase to attain 47% at 50°C. They also found that maintaining co-compost temperature at 50°C for 30 days resulted in more MOG degradation (70%) than that obtained at 23°C. This study indicated thermophilic temperature would be preferable to mesophilic temperatures if the length of thermophilic phase extended to the point where microorganisms had sufficient time to adapt to temperature shift. Semple et al. (1998) studied the extent of [U-^{14}C] benzene mineralization in spent mushroom compost after a 3-months enrichment period in the presence of a variety of BTEX compounds. It was found that as the incubation temperature was raised from 18°C to 37°C to 50°C, there was an increase in the mineralization of [U-^{14}C] benzene over 14 days. Haderline et al. (1999) found that mesophilic temperatures resulted in more removal of aliphatic (e.g. hexadecane) and polyaromatic hydrocarbons (e.g. pyrene) relative to thermophilic temperatures.

Generally, the results of the above studies were inconclusive regarding the benefit of thermophilic temperature on hydrocarbon degradation. The difference may be related to substrate type, hydrocarbon type, composting temperature, and incubation time.

3.3.2 Oxygen Concentration

Composting is the aerobic biological decomposition of organic substrate. As such, sufficient oxygen content in the interstitial spaces (free air space, FAS) of compost pile is required to enhance the biological treatment of soils contaminated with hydrocarbons. Miller and Clark (1998) and Rhykerd et al. (1999) reviewed a number of studies on oil bioremediation in soil, and found oxygen concentrations of more than 10% in FAS were required to effectively degrade petroleum hydrocarbon compounds. However, Peramaki and Blomker (1997) in their review of applicability of composting as bioremediation technique, reported that some herbicides are degraded at oxygen concentrations of approximately 6% in interstitial space and oxygen concentrations of less than 6% were also effective. The same researchers (Peramaki and Blomker, 1997) also reported that PAH mineralization occurred at a faster rate with interstitial oxygen concentration of 2, 5, and 10 % when compared to an oxygen concentration of 21 %. Hupe et al. (1998) has shown that the maximum mineralization of diesel fuel in soil/compost mixture was achieved at oxygen concentration of 5% in the inlet air. Haug (1993) indicates that oxygen level in the windrow composting pile should be maintained between 5 and 15% for rapid decomposition of municipal waste and extended thermophilic activity. Excess amounts of aeration can cause a loss of heat from the compost matrix and that will affect the temperature regime, but too little can result in anaerobic conditions (Epstein and Alpert, 1980).

Generally, the above studies are conflicting on optimum oxygen level for achieving maximum composting bioremediation effectiveness. The optimum oxygen level seems to be contaminant specific. However, they are almost in general agreement regard to the minimum level that should be maintained inside the composting pile. As low as 5 percent in FAS of composting pile would be enough to effectively degrade petroleum hydrocarbons.

3.3.3 pH

The pH during the composting process changes with time. This is because during the first stages of the biodegradation process organo-nitrogen compounds are broken down which release NH_3 and causes the pH to rise. This is followed by the gradual increase in microbial activity producing CO_2 which cause the pH to decrease. The optimal pH of a composting system was found to lie somewhere between 5.5 and 8 (Day et al. 1998). One study found that most hydrocarbon degradation occurred when the soil pH was adjusted between 5 and 7.8 (Dibble and Bartha, 1979).

3.3.4 Carbon to Nitrogen Ratio (C:N)

The most important nutrient balance in the composting process is the carbon to nitrogen ratio (C:N). Carbon is necessary for microbial growth and energy, while nitrogen is essential for microbial protein synthesis and reproduction. During the microbial growth 25 to 30 parts of carbon is needed for every part of nitrogen (Epstein, 1997). The excess nitrogen present will be converted to ammonia that can volatilize in the high temperature of the compost pile, causing odour problems. If the C:N is greater than optimum levels the growth of the microorganisms will be slowed down due to low level of nitrogen.

This, in turn, will result in a loss in heat and a subsequent lower temperature. Overall, the composting process slows (NRAES, 1992; Epstein, 1997).

In municipal waste composting a C:N ratio of 20 to 40 has been recommended. Larsen and McCartney (2000) found that C:N between 18 and 29 gave the highest rates of composting of pulp and paper biosolids. In the case of petroleum bioremediation, there is no consensus on the optimum C:N ratios and a wide range of values are found in the literature. Dibble and Bartha (1979) found that the optimum C:N ratio was 60. Baker (1994) reported an optimum ratio of 25. Beaudin et al. (1999) reported that an optimum C:N ratio of 15, and increasing of C:N ratio to more than 40 resulted in no hydrocarbon degradation. Miller and Clark (1998) reported that that McMillen and Gray (1994) found C:N ratio of 20:1 worked well for degrading petroleum waste.

The optimum C:N ratio is likely to be materials specific, and related to carbon availability. For example, fresher feedstock will have a high concentration of readily degradable organics than the finished compost. Generally, C:N ratio in the range of 15:1 to 40:1 is recommended at the start of composting.

3.3.5 Moisture Content

During composting, moisture content must be high enough to assure adequate rates of biological stabilization. However, excessive moisture content can result in reduced free air space (FAS). This will reduce the rate of oxygen transfer throughout the composting materials and in turn the rate of microbial activity. Moisture contents of 40 to 60% (wet basis) have been shown to be optimal in many applications (Ziegenfuss and Williams, 1991). At moisture contents below 40% the microbial activity is restricted, whereas

above 60% aerobic microbial metabolism process is slowed down because the soil free air space is reduced and transfer of oxygen is inhibited (Epstein and Alpert, 1980; Haug, 1993).

3.3.6 Free Air Space (FAS)

Oxygen is essential for microbial activity in composting since it is an aerobic process. Composting systems need to keep enough porosity and FAS in the compost materials for aeration. Municipal refuse, agricultural residue, and animal manure are examples of substrates commonly used as composting materials (Haug, 1993). Because of their high moisture content (greater than 60%) and very limited FAS, the use of these as substrate in composting becomes impractical since sufficient air can not penetrate the compost matrix, either by diffusion or forced aeration, and that may impact microbial activity. To provide aerobic conditions, bulking agents are required for these materials (Epstein and Alpert, 1980; Rhykerd et al. 1999).

There are different types of materials that can be used as bulking agents. The ideal bulking agent is one that provides ample porosity, has absorbent property for moisture, resists compaction, degrades slowly, and can be easily recovered from the composted matrix and subsequently recycled (Rhykerd et al. 1999).

Bulking agents such as wood chips, straw and rice hulls may be added to the compost to provide structural support for wet substances, create free air space within the voids between the particles, and increases the size of the pore spaces (Haug, 1993). Although straw and rice hulls provide porosity initially, they may decompose quickly in the compost environment, loosing their structural support before contaminant levels have

been reduced to acceptable levels (Matthew et al. 1997). Woodchips are one of the most commonly used bulking agents, along with straw and rice hulls (Haug, 1993). Woodchips have been proven to maintain a higher FAS within a municipal biosolids compost matrix as compared to straw and leaves (McCartney and Chen, 2001).

Bulking of contaminated soils with chopped hay, sawdust, and vermiculite has been reported to enhance the biodegradation of crude oil contaminated sandy soil (Rhykerd et al. 1999). They found that all of the bulking agents significantly enhanced the reduction of total petroleum hydrocarbons (TPH). The reduction of TPH was higher in the bulked soil (82%) compared to the control soil (33%). However, the type of bulking agents affected the reduction of total petroleum hydrocarbon. Soil bulked with hay resulted in higher reduction of TPH as compared to the soil bulked with vermiculite or sawdust. The addition of hay to contaminated soil resulted in a greater consumption of O_2 compared to other bulking agent. The difference among the bulking agents were attributed to the fact that hay was more readily degraded and the increased microbial population likely increased the degradation of TPH. This study, however, did not report the difference in the FAS of soil bulked with different bulking agents.

3.3.7 Soil to Compost Ratio

The influence of different soil to compost ratios on removal of petroleum has been investigated. Stegmann et al. (1991) evaluated degradation of diesel fuel at soil to compost ratios of 2:1, 4:1, 8:1, and 16:1 on the basis of dry weight. The soil had a high proportion of sand and humic substances. The compost used in this study was a mature biowaste that had been composted at a small windrow plant. They found the highest

biodegradation occurred at a 2:1 ratio. This study indicates that increasing of soil loading level in compost system would effect biodegradation. Too high a soil loading rate may cause microbial inhibition (depending on the toxicity of contaminated soil) and/or the biodegradable carbon source may be diluted to the point where self-heating is reduced or eliminated (Ziegenfuss and William, 1991).

Hupe et al. (1996) investigated the degradation of diesel fuel at soil to compost ratios of 2:1, 4:1, and 8:1 (on the basis of dry weight) for 13 days at 22°C. The soil used was loamy sand soil consisting of 78.1% sand, 15.4% silt and 6.4% clay. The soil was contaminated artificially with 1% (percent by dry weight of soil) diesel fuel before the compost was added. The compost added was kitchen and garden waste composted at a small windrow plant for 13 months. This study found that at a soil to compost ratio of 2:1 the cumulative oxygen caused by the diesel degradation increased significantly as compared to 4:1 and 8:1 soil to compost ratios.

William et al. (1997) investigated the effect of organic compost on the degradation of the most recalcitrant PAHs, benzo(a)pyrene. The soil used was loamy sand soil consists of 66.6% sand, 17.8% silt and 15.6% clay. The soil was spiked with 100 mg kg^{-1} benzo(a)pyrene and inoculated with bacterial consortia (10^{10} per ml) isolated from PAH contaminated soil. The soil then mixed with organic compost, compro®, to yield soil to compost weight ratios of 3:1, 2:1, 1:1. 1:2, 1:3 (the ratio was not stated if based on dry or wet weight). After an incubation period of 90 days at 37°C, the percentages of benzo(a) pyrene extracted from the soil only and soil to compost ratios of 1:1, and 3:1 were not significantly different. However, as the compost ratio increased, i.e. compost two to

three times the soil, the extractable bezo(a)pyrene in amended soil was much less (39.68%) than that in the umamended soil (was 64.19%). This study indicated that as compost content increased, a disappearance of bezo (a) pyrene increased, and soil bound residue formation might have been the major mechanism of disappearance.

Liu and Cole (1995) investigated the effect of additions of compost on degradation of pesticides contaminated soil. The contaminated soil used consists of 27% sand, 32% silt, 19% clay, and 22% gravel. The compost used was mature yard waste that had undergone thermophilic composting. The compost addition to the soil was ranging from 0 to 40% by weight. They found that pesticides degradation was effective at compost rates of 20% or more. Compost when added at less than 20% by weight, was not effective in stimulating the microbial activity and pesticides degradation.

William and Keehan (1992) composted explosives contaminated soil using a static pile compost system. The compost used was a mixture of sawdust, apple pomace, chicken manure and chopped potato waste, which were mixed with the explosives contaminated soil. The soil type used was not discussed in this paper. The amount of contaminated soil added to the compost ranged from 7 to 40 % by volume. After 90 days of composting, they found that the highest removal of 2, 4, 6 trinitrotoluene (TNT) occurred at 30% soil by volume. However, for Royal Demolition Explosives (RDX), the highest removal occurred at 7% by volume. Generally, this study observed no removal of RDX and TNT occurred at 40% contaminated soil.

Another study by Wytrykush et al (2002) investigated the effect of soil loading rate on the thermophilic microbial activity of the compost system during the active composting

phase. The microbial activity within the compost was measured as the relative heat generation of the compost and the volatile solids removal from the compost mixture. The compost mixture consisted of woodchips and dewatered municipal biosolids, which was mixed with diesel fuel contaminated clay soil (clay content 86%). The amount of clay soil added to the compost mixture ranged from 0 to 30% by wet weight. It was found that at up to 24% contaminated clay soil by wet weight could be mixed with compostable material and still achieve thermophilic conditions. When the amount of contaminated soil exceeded the 24% the thermophilic microbial activity sharply decreased. This study indicated increasing of clay soil above 24% resulted in anaerobic conditions within the compost matrix.

These studies suggested that the addition of organic amendments could increase the degradation rate of target contaminants, but might inhibit the degradation rate when an excessive amount of organic amendment added. These studies have also suggested various different soil loads for optimum composting performance. As such, a study is needed to quantify the effect of various soil texture and loads on microbial performance during composting.

3.4 Summary

Composting using either fresh feedstock or mature compost enhanced the removal of hydrocarbon compounds by either simulating microbial degradation, sorption to compost organic matter or, by incorporation into organic matter. The end result is a soil-compost mixture that contains reduced pollutant concentration and/or a matrix that has less parent contaminants or its metabolites in a bioavailable form. However, the treatment efficiency

of composting can be impacted by many factors such as pH, pore space oxygen, carbon to nitrogen ratio, moisture content, and free air space, and operating temperatures. Another factor that must be considered to enhance treatment efficiency is the soil to compost ratio. The effect of amount of soil incorporated into compost mixture on thermophilic microbial phase of composting and degradation rates has been considered in many studies. These studies suggested that the addition of compost could increase the degradation rate of target contaminants, but might inhibit the degradation rate when an excessive amount of compost materials added. These studies have also suggested various soil loads for optimum composting performance.

Operating temperature is another factor to be considered. Composting can be operated within either mesophilic or thermophilic temperature range depending on substrate and type of bacteria that are present to degrade the contaminants. The benefit of the thermophilic temperature phase on the composting bioremediation of petroleum hydrocarbons has been considered in some studies. These studies were different in terms of the benefit of thermophilic temperature on hydrocarbon degradation.

In short, there are several issues need to be addressed in soil composting. First, the effect of soil load and particle size on the thermophilic stage of composting has not yet been documented. Second, there is lack of information on the benefit of amending diesel fuel contaminated soil with finished compost compared to that of fresh feedstock. Third, it is not clear whether thermophilic temperatures are beneficial to diesel fuel treatment. In order to investigate the above-mentioned issues, an experimental program was divided into two major phases. The first phase investigated the effect of soil load and texture on

microbial activity during the active phase of composting. The second phase investigated the effect of co-substrate type and operating temperature on the performance of composting diesel fuel contaminated soil.

4. Effect of Soil Load & Texture on Thermophilic Phase of Composting

4.1 Summary

This study investigated the effect of soil load and texture on microbial activity during the active phase (thermophilic) of composting diesel fuel contaminated soil. The thermophilic composting phase was simulated using bench scale (28 L) reactors under controlled temperature and aeration conditions. Soil loads (% of total wet weight) investigated ranged from 0% to 64%. As a measure of composting microbial activity, the relative heat generation and volatile solids removal were measured. The results found that a change in thermophilic microbial activity had occurred at sand and silt loads above 40% and 20%, respectively. The volatile solids reduction was poorly correlated to sand and silt loads. The lack of observed trend may have resulted from sample variability. While the thermophilic microbial activity observed was not affected by sand or silt load, the thermophilic microbial activity was affected at the higher clay load (40%). This study concluded that soil load and texture must be considered in any composting study in order to maintain microbial activity during the active phase of composting.

4.2 Introduction

The persistence of organic contaminants in the environment is a matter of significant public, scientific and regulatory concern because of the potential toxicity. These concerns continue to drive the need for the development and application of remediation techniques. Bioremediation, use of biochemical abilities of microorganisms, is the most popular strategy to detoxify and degrade environmental contaminants. Microorganisms

have huge ability to interact with soil organic contaminants leading to a structural change or a complete degradation of the target molecule.

However, a common constraint to efficient bioremediation of petroleum hydrocarbons is the low aqueous solubility of many of hydrocarbon contaminants. Limited contaminant solubility results in reduced degradation rates and lower total amounts of contaminants degraded. The use of composting process for bioremediation, where temperature can exceed 60°C, may increase the rate of release of contaminants into the aqueous phase (William and Keehan 1992). In cases in which the limiting factor to biodegradation is the bioavailability, the increase in aqueous solubility could lead to an appreciable increase in contaminants degradation rates.

The amount of soil incorporated into compost mixture can impact the performance of composting systems. Too high a soil loading rate may cause microbial inhibition (depending on the toxicity of contaminated soil) and/or the biodegradable carbon source may be diluted to the point where self-heating is reduced or eliminated (Ziegenfuss and William, 1991). The influence of different soil to compost ratios on thermophilic phase of composting and degradation rates have been investigated. Williams and Keehan (1992) found that 40% contaminated soil by volume (soil type was not discussed) in the compost mix significantly lowered microbial activity and reduced the degradation of explosives. Liu and Cole (1995) found that more than 80% contaminated soil by weight significantly effected thermophilic microbial activity and the pesticides degradation. The soil used consisted of 27% sand, 32% silt, 19% clay, and 22% gravel. Another study by Wytrykush et al. (2002) found that increasing of clay soil loading above 26% by wet

weight resulted in sharp decrease in thermophilic microbial activity during composting of diesel fuel contaminated soil. A variety of mix ratios for optimum composting performance have been suggested in these studies. As such, a study is needed to quantify the effect of various soil particles and loads on microbial activity during composting.

4.3 Experimental Hypotheses & Objectives

The experimental hypotheses were: (1) thermophilic microbial activity within compost system will cease at high soil loading rates, and 2) thermophilic microbial activity within soil compost system would be impacted differently by different soil textures. The specific objective was to investigate the effect of soil load and texture on the thermophilic microbial activity phase of composting.

In order to achieve the objective, this phase of study was divided into two sub-phases. Phase 1A observed the effect of sand and silt loading on the thermophilic microbial activity during the composting of diesel fuel contaminated soil under simulated windrow composting conditions. Phase 1B confirmed the effect of sand or clay on the thermophilic phase of composting. It is expected that this phase of study would provide information on the maximum amount of diesel fuel contaminated soil that should be added to a compost mix.

4.4 Experimental Methods and Materials for Phase 1A

4.4.1 Reactor Apparatus

Airtight reactors with a working volume of 28-L were used in this study. The schematic

diagram of a reactor apparatus is illustrated in Figure 4-1. Each reactor was insulated with 5cm thick pink fiberglass and aluminum-reflecting blanket to minimize the heat loss (Figure 4-2). The insulating reactors were then placed in a temperature-controlled chamber. The reactors were designed as described in Larsen and McCartney (2000) such that the compressive loading could be applied to the material, oxygen could be supplied to the reactors, and the temperature could be controlled and measured.

4.4.2 Soil Material Preparation and Analysis

Two soils were used in the study. Commercially available white silica sand and silt were chosen to represent coarse-grained soil and fine-grained soil, respectively. The sand was sieved to obtain a particle size range of >0.29 to ≤0.42 mm. The silt was pure washed silt purchased from LSL Contracting and Materials, Winnipeg, Manitoba, Canada. Maximum moisture holding capacity for the silica sand and silt was determined following the method described by Carter (1993). The maximum moisture holding capacity was determined to be about 17 and 25% (wet basis) for the sand and silt, respectively.

To approximate field conditions, the silica sand and silt moisture contents were determined and adjusted with distilled water to 50 % of the maximum moisture holding capacity. The moisture contents were determined using the oven dry method (Carter 1993). Diesel fuel, spiked with labeled octadecane1-^{14}C (supplied by the Sigma Chemical Co., St. Louis, MO) at activity of 8 μci/g of diesel fuel, was added to the sand or silt to yield 5000 mg kg^{-1} dry soil. Before the spiked diesel fuel added to the sand or silt, weighed dry samples were placed into a container and distilled water was added to bring the sand or silt to the desired moisture content. The spiked diesel fuel was then

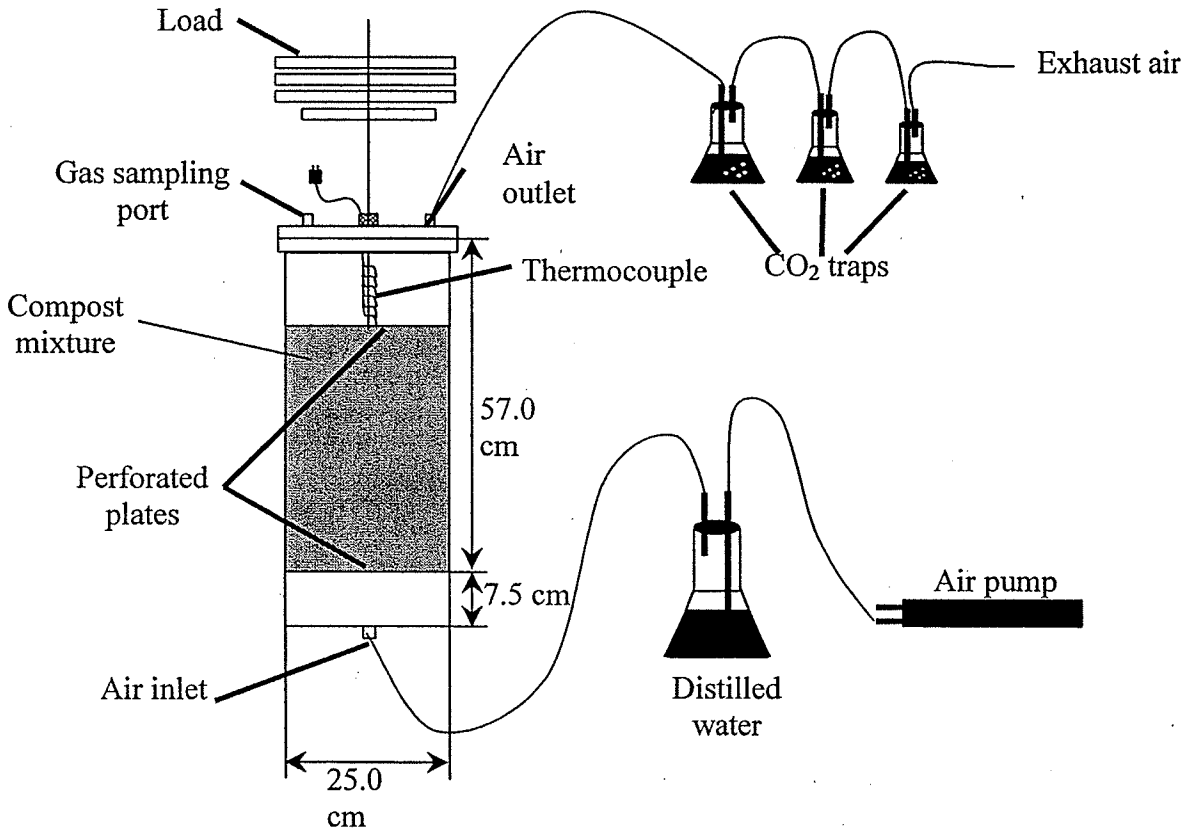


Figure 4-1. Schematic of a reactor used for testing.

Figure 4-2. Photograph of insulating reactors used for testing.

added to the container. While the sand and silt were continuously mixed for 4 minutes, the diesel fuel was added using a plastic dropper. To prevent the moisture loss and volatilization of fuel hydrocarbons after mixing, the contaminated sand and silt were placed either in glass or plastic containers, covered with aluminum wrap, and stored at 4°C for a maximum three hours until ready for use. The addition of water and diesel fuel to sand or silt was carried out in a steel pastry-blending machine, model AS-200-FDT (Hobart MGF CO) at speed of approximately 75rpm.

4.4.3 Feedstock Material Sampling, Preparation, and Analysis

Two materials, biosolids and woodchips were used as the feedstocks. The biosolids were collected from the city of Winnipeg's North End Water Pollution Control Center, and consisted of primary and secondary sludges that had been anaerobically digested at 37°C for 17 days and then centrifugally dewatered. The biosolids were placed in airtight plastic container and stored at 4°C in order to minimize decomposition. The woodchips were collected from Red River Soils, a Division of Consolidated Envirowaste Industries, Inc., Winnipeg, Manitoba. The woodchips were sieved to a particle size range of 4.75mm to 12.5mm. The sieved woodchips were stored in airtight plastic containers at 4°C to maintain their natural moisture content and to minimize decomposition.

Before storing the biosolids and woodchips in airtight containers, samples were taken for analysis. From each container a 1-liter sample was collected and mixed using a steel pastry-blending machine at speed of approximately 75rpm. After mixing, the biosolids or woodchips were spread evenly over a plastic sheet and quartering was carried out until 40 to 50 grams sample of each was obtained. The representative samples were then placed

in sealed bags at 4°C until analysis was performed.

The biosolids and woodchips materials were analyzed for moisture content (MC), total solids (TS) and volatile solids (VS) using the APHA standard method 2540-G. Organic carbon contents (OC) and carbon to nitrogen ratios (C:N) were calculated using the equation presented in NRAES (1992). Wet (D_{wb}) and dry (D_{db}) bulk densities were determined using the Core method (Carter, 1993). The Total Kjeldahl nitrogen (TKN-N) was determined using semi-Micro Kjeldahl method (Carter, 1993). All samples were processed prior to TKN analysis following the procedures provided by Chen (1998) with the exception of using a wet sample to prevent ammonia stripping rather than drying the sample at air temperature. Except where specified otherwise, all measurements were performed in five replicate analyses. A summary of material characteristics is presented in Table 4-1.

Based on the MC values in Table 4-1, the ratio between woodchips and biosolids was calculated using the equation provided in NRAES (1992) to achieve a starting MC of 55% (wet basis). The result was a ratio of woodchips to biosolids of 0.4 gram of woodchips per gram of biosolids (wet basis). The ratio of biosolids to woodchips resulted in C:N ratio of approximately 20. Wet and dry bulk density was measured for the feedstock mixture. The initial FAS of feedstock mixture was calculated using the equation provided by Eftoda and McCartney (2002). The resulting bulk densities and FAS values of the feedstock are summarized in Table 4-2.

Table 4-1 Summary of the individual feedstock characteristics. All values are means of five replicate analyses.

Parameter	Biosolids	Woodchips
MC (% wet basis)	70.20 ± 0.002	17.02 ± 1.20
OC (% dry basis)	24.82 ± 0.002	53.51 ± 0.004
TKN-N (% dry basis)	3.49 ± 1.10	0.80 ± 0.065
C: N	7.1	66.9
D_{wb} (kg m^{-3})	934.8 ± 6.92	197.4 ± 5.81

Table 4-2 Summary of mixed feedstock characteristics (biosolids & woodchips mixture; 0.4 kg woodchips per kg biosolids (wb)). Particle density and bulk densities represent the mean of triplicate analyses.

Parameter	Feedstock Mixture
Dry bulk density (kg m^{-3})	193 ±7.05
Wet bulk density (kg m^{-3})	430. ± 8.03
Particle density (kg m^{-3})	1.83 ±0.135
FAS (cm^3 cm^{-3})	0.55

4.4.4 Microbial Activity Testing

To investigate the effect of sand and silt loading on the thermophilic microbial activity, varying amount of sand and silt was added to the reactors at 2% or 4% increments until a significant impact on thermophilic phase of composting was observed. The amount of sand added ranged from 0% to 64% on a wet weight basis, while the amount of silt ranged from 0% to 48% on a wet weight basis. The amount of feedstock (biosolids and woodchips) was kept constant at 6.5 kg wet weight at the start of each run. Each experimental run included four reactors with each reactor representing one soil load. Each reactor ran for two weeks. The reactors were insulated with 5cm thick pink fiberglass and aluminum reflecting blanket to trap the heat generated and maintain thermophilic temperatures during the experiment. The insulated reactors were placed in a temperature-controlled chamber and a compressive load of 5.6 kPa, which simulated the compressive load at the core of 2 m high compost pile, was applied to each reactor. Preliminary experiment found that under this compressive load, the feedstock mixture FAS value was above the minimum FAS (30%) suggested by Haug (1993) for adequate natural ventilation in a compost pile. The effect of compressive load on FAS can be found in Appendix A.

In order to prevent oxygen from becoming a limiting factor in the composting process, each reactor was connected to an air supply pump. Aeration was conducted from the bottom to the top. The feed air was passed through a 6 L Erlenmeyer flask filled with distilled water to ensure the air was hydrated before oxygen flowing into the reactors. To minimize heat loss from the reactors, the flask was located inside the temperature control chamber to heat the air to the same temperature as the environmental chamber. The

reactors were aerated for 15 minutes each hour at rate of 1100 ml min^{-1} using a Chron Trol XT timer and Masterflex speed controlled 6-600 rpm pump (Cole Parmer Instrument Company). The 15 minutes aeration cycle was found to be enough to maintain oxygen levels above 5% in the headspace at all time. Oxygen concentration measurements were done by injecting a 1 ml headspace sample into a GOW MAC model 550 gas chromatograph equipped with a Poropak Q 80/100 mesh column and a thermal conductivity detector.

Each reactor was equipped with a thermocouple. The temperature was measured using a Fluke 52 K/J a thermometer. At start-up (time zero) the temperatures of control chamber and reactors were measured. The chamber temperature was then set at 45°C. During the experimental run, the reactors and chamber temperatures were measured daily for a period of 14 days testing. The experimental run was considered complete when reactor temperatures dropped below the chamber temperature for a 24 hour span or after 14 days had elapsed.

The exhaust gas of each reactor was collected in a series of three CO_2 traps. The series of CO_2 traps consisted of sorption vessels filled with 4 N NaOH solution. The CO_2 traps were removed and replaced once during the 14-day experimental run.

4.4.5 Microbial Activity Analysis

Microbial activity was measured using the relative heat generation and volatile solid reduction. In addition, to trace the mineralization of diesel hydrocarbons, $^{14}CO_2$ release from reactors was monitored in some of the reactors.

4.4.5.1 Relative Heat Generation

The temperature monitoring results for each reactor were used to construct relative heat generation values (RHG). To obtain these values, each reactor temperature was subtracted from the chamber temperature on that day. RHG was summed over the experimental run and expressed as the degree-days of the reactor above the chamber temperature. Cumulative RHG for each reactor was plotted against the sand and silt loading.

4.4.5.2 Volatile Solids Reduction

For each reactor, the mass of VS was determined at the start and end of each run. This allowed VS mass balance calculation to be completed. To determine the initial VS at start of each experimental run, a sample of about 30 grams was collected as a grab sample from each reactor. The four samples obtained were mixed together to form a composite sample. Then the quartering method was used to obtain five representative samples for determining the initial VS. Individual reactor was not quartered due to the difficulty of re-mixing the reactor content by hand, which is required for quartering method. At the end of composting process, the quartering method was carried out on individual reactor contents until five representative samples of about 30 to 40 grams each were obtained. The representative samples were placed in sealed bags at 4°C for further analysis. Once the samples were ready for analysis, they were removed from the bag and measured for VS.

4.4.5.3 $^{14}CO2$ Production

The total $^{14}CO_2$ captured in NaOH traps were also monitored. The total $^{14}CO_2$ concentration resulting from the mineralization of ^{14}C-labeled octadecane was measured by collecting from each CO_2 trap a 0.1-ml sample of the NaOH. The sample was pipetted into a scintillation bottle containing 7 ml of liquid scintillation cocktail (Ultima Gold XR, LSC Cocktail) and analyzed with a Beckman LS 7500 liquid scintillation counter. Measurement of $^{14}CO_2$ was conducted on a daily basis throughout the test period. Samples from each trap were collected in duplicate, capped, labeled and mixed with 7 ml liquid scintillation cocktail and stored for 48 hours before counting. The results were corrected for the dilution and the background radioactivity.

4.5 Experimental Methods & Materials for Phase-1B

4.5.1 Reactor Apparatus

Reactors with a capacity of 28-L were used. The reactors used in this experiment were described in details in Phase 1A.

4.5.2 Soil Material Preparation and Analysis

The soils used in this study were silica sand and clay. The sand used was commercially available white silica sand passed through a 0.42mm sieve. Additional sand characteristics can be found in the previous phase of the study. Clay was collected from a site in Winnipeg previously excavated to a depth approximately 2 m below original grade. Representative soil samples were analyzed for extractable diesel range organics (C_{10} to C_{19}), grain size distribution, nitrogen content, organic matter and moisture content. The results of the extractable diesel range organics showed that the clay had not

been previously impacted by hydrocarbon contamination. Grain size analysis indicated that the clay soil has a high clay size fraction (75% clay, 24% silt, and 1% sand). The organic carbon content and nitrogen content of the clay soil was negligible (< 0.1% dry weight basis). The hydrometer method (ASTM D-422-63) was used to obtain the approximate particle size distribution of clay soil. Ignition at low temperature (375°C) and oxidizable methods (Hesse, 1971) were used to estimate organic matter content. Semi-micro Kjeldahl method was used to determine total nitrogen content (Carter, 1993).

The soil was prepared as described in Phase 1A except that instead of drying the clay soil and adjusting its moisture content to 50% of its maximum moisture capacity, wet clay soil (moisture content of 37.7% wet weight basis) as collected was used. Diesel fuel was added to the soil to yield 5000 mg kg^{-1} dry soil.

4.5.3 Feedstock Material Preparation and Analysis

Dewatered municipal biosolids and woodshavings were used as the feedstocks. The biosolids were collected from the city of Winnipeg's North End Water Pollution Control Center. The woodshavings (Ponderosa Pine Bedding) were purchased from a pet store. The biosolids and woodshavings materials were analyzed for moisture content (MC), total solids (TS) and volatile solids (VS) using the APHA standard method 2540-G. Organic carbon contents and C:N ratios were calculated using methods presented in NRAES. (1992). Wet (D_{wb}) and dry (D_{db}) bulk densities were determined using the Core method (Carter, 1993). The Total Kjeldahl nitrogen (TKN-N) was determined using semi-micro Kjeldahl method (Carter, 1993). Except where specified otherwise, all

measurements were performed in five replicate analyses. A summary of material characteristics is presented in Table 4-3.

To achieve starting moisture content of 55 % (wet weight basis) the woodshavings were mixed with biosolids at ratio of 0.27:1 (wet weight basis). The ratio of biosolids to woodshavings resulted in C:N ratio of 23 and free air space (FAS) of 0.50 cm^3 cm^{-3}. The FAS of feedstock mixture was tested using the method described in Phase 1A. A summary of feedstock characteristics is presented in Table 4-4.

4.5.4 Microbial Activity Testing

To investigate the effect of sand and clay texture on microbial activity, two experimental runs were performed. Each experimental run included four reactors with each reactor representing one sand or clay load. A total of two replicates of each sand or clay load were run. The amount of sand or clay load added to each reactor was 20% and 40% (wet weight). The amount of feedstock was kept constant at 6.5 kg (wet weight) at the start of each experimental run. Four batches of 6.5 kg feedstock were mixed in a steel pastry-blending machine (Hobart MGF CO) at speed of about 75rmp. These batches were combined in a large vessel and mixed manually using a shovel. The large batch was then separated into four batches of 6.5 kg and mixed with the appropriate amount of sand or clay soil using the steel pastry-blending machine. The feedstock mixtures were then filled into the insulted reactors and the reactors were placed in a temperature-controlled chamber (45°C) and a compressive load of about 4.38 kPa was applied to each reactor. Each reactor ran for two weeks.

Table 4-3 Summary of the individual feedstock characteristics. All values are means of five replicate analyses.

Parameters	Biosolids	Woodshavings
% MC (wb)	67.18 ± 0.23	9.66 ± 0.13
% TKN (db)	2.75 ± 0.82	0.054 ± 0.01
% OC (db)	22.92 ± 0.1	55.41 ± 0.05
C:N	8.33	1026
% VS	41.25 ± 0.13	99.74 ± 0.01

Table 4-4 Summary of mixed feedstock characteristics. (biosolids & woodshaving mixture; 0.27 kg woodshaving per kg biosolids (wb)). Particle density and bulk densities represent the mean of triplicate analyses.

Parameter	Feedstock Mixture
Dry bulk density (kg m^{-3})	191.30 ± 2.93
Wet bulk density (kg m^{-3})	425.10 ± 6.51
Particle Desity (kg m^{-3})	1697 ± 18.91
FAS (cm^3 cm^{-3})	0.50
C:N	23

Air was supplied to each reactor to ensure oxygen was not limiting the reactions. The reactors were maintained under aerobic conditions as described in Phase 1A.

During the experimental run, the temperature and headspace gases were measured daily for a period of 14 days. The temperature was measured using a Fluke 52 K/J a thermometer. Headspace gas samples of each reactor were analyzed daily for oxygen and methane concentrations using Gowmac model 550 gas chromatograph equipped with a thermal conductivity detector, Porapak Q and 80/100 mesh column. Standard percentages of oxygen and methane were run before running samples. Samples (1 ml) of the headspace gas in each reactor were withdrawn from the reactors five minutes before the aeration time started.

4.5.5 Microbial Activity Analysis

The microbial activity within the reactors was measured using the relative heat generation, and volatile solid removal.

4.5.5.1 Relative Heat Generation

The temperature monitoring results for each reactor were used to construct relative heat generation (RHG) using the method described in Phase 1A. The RHG values for each reactor were plotted against soil load.

4.5.5.2 Volatile Solids Reduction

For each reactor, the mass of VS was determined at the start and end of each run. This allowed VS mass balance calculation to be completed. To eliminate the spatial variability in VS determination observed during Phase 1A, a random sampling method

with discrete samples was employed for initial and final volatile solids determination. The number of samples collected from the each feedstock mixture was 20. The method used to determine the minimum number of samples required achieving a certain level of precision at a confidence level of 95% and variability of 5% from the mean is described in Appendix B. Once the number of the sample required was estimated, the feedstock mixture was thoroughly mixed. The completely mixed feedstock (28 L reactor volume) was spread onto clean plastic sheet, approximately square, to a depth of approximately 2 cm. A plastic frame, divided into a grid with each grid size equal to size 7.5cm x 7.5cm, was placed on top of the entire compost. The grid sections were assigned numbers from 1 to 100. The sampling points (20 samples) were randomly selected using the random number generator in Corel Quattro Pro. The representative samples collected in the grid chosen were placed in sealed bag and stored at 4°C for further analysis. Once the samples were ready for analysis, they were removed from the bag and measured for volatile solids.

4.6 Experimental Results & Discussion for Phase 1A

The experimental objective of this phase was to observe the effect of sand and silt loading on the thermophilic microbial activity during the composting of diesel fuel contaminated under simulated windrow composting conditions. Microbial activity was measured through relative heat generation and volatile solids removal. The data collected during the experiment is presented in complete form in Appendix C. The results are summarized in the following sections.

4.6.1 Relative Heat Generation

The reactor and chamber temperatures raw data are included in Appendix C-1. Figure 4-3 and 4-4 show the composting temperature profiles at various sand and silt loads.

In the first two days, the temperatures in all of the reactors rose rapidly. The maximum temperature observed was about 60°C. The maximum temperature observed during the composting process remained only for one day. Starting on day 4, all reactor temperatures observed was about 60°C. The maximum temperature observed during the composting process remained only for one day. Starting on day 4, all reactor temperatures dropped slightly and then begin to level out at around 50°C between days 11 and 14 which is about 3.5 degrees above the chamber temperature. Generally, no clear difference in the reactor temperatures was observed following an initial period of rapidly increasing temperatures.

To provide valuable information on the effect of soil loading on degree of microbial activity, the reactor temperatures were used to construct relative heat generation (RHG) values. The RHG values for sand and silt soils are presented in Figure 4-5 and Figure 4-6, respectively.

For feedstock mixed with sand, the results showed that no significant change in RGH in the data points between sand loading rate of 0 to 40%, indicating that the composting process generates significant amounts of heat similar to heat generated at 0% sand loading. However, a declining trend in RHG was observed in data points between sand loading of 40 to 64%. This suggests that a change in microbial activity had occurred when contaminated sand loading exceeded 40% by wet weight.

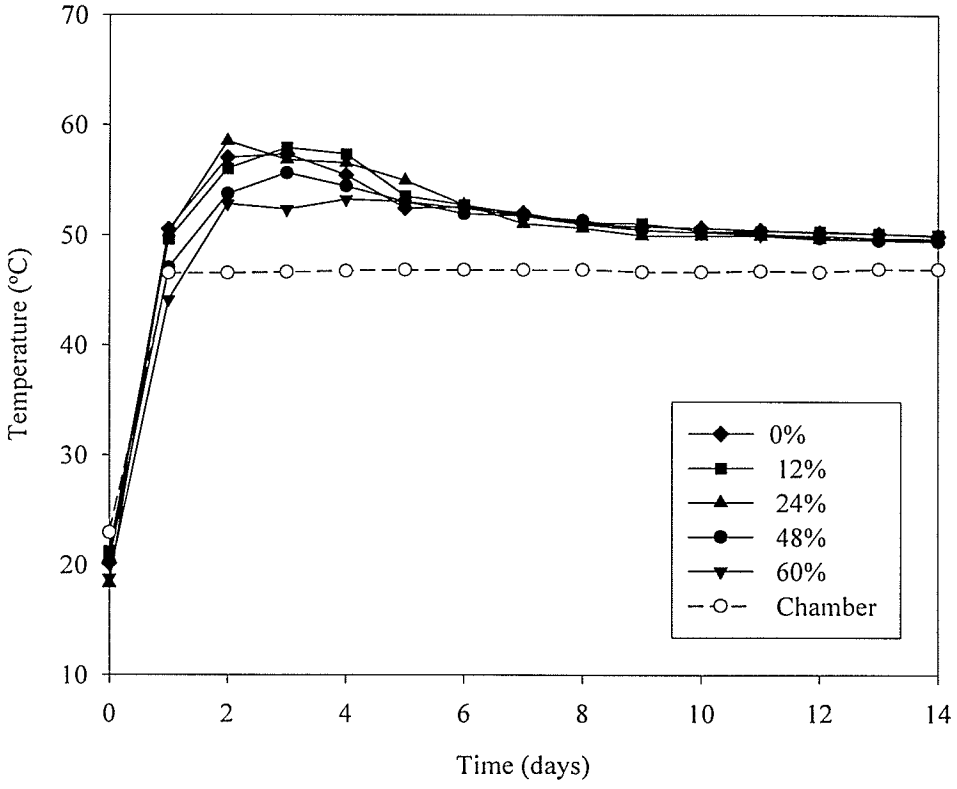


Figure 4-3. Representative temperature profiles for sand loading reactors with environmental chamber temperature profile shown.

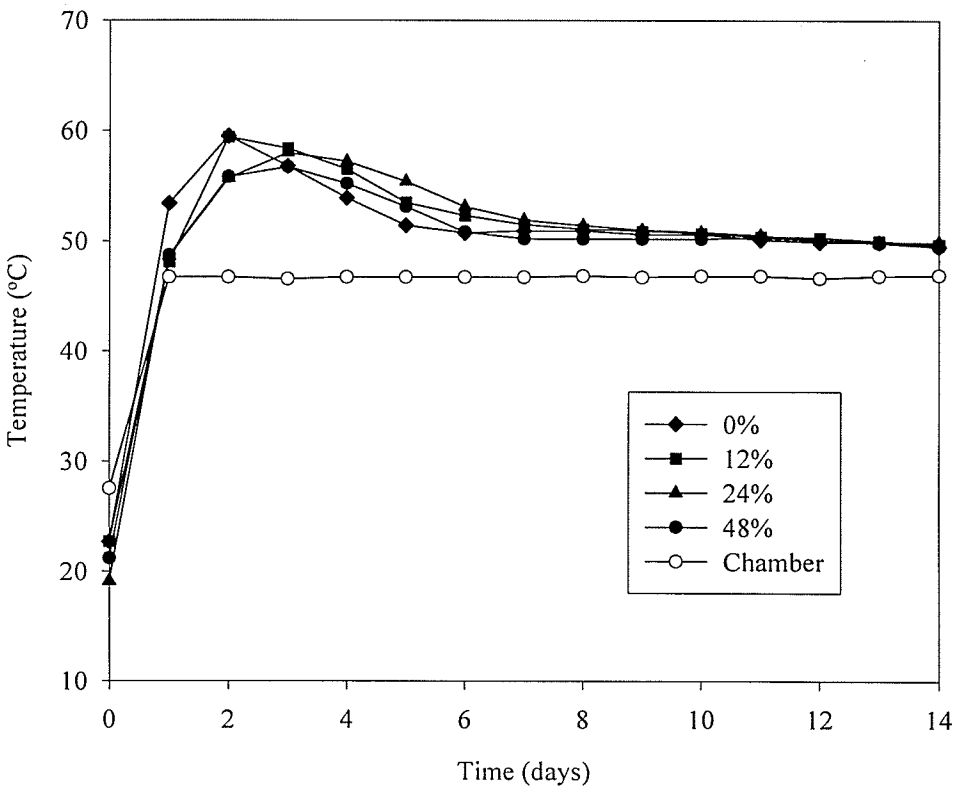


Figure 4-4. Representative temperature profiles for silt loading reactors with environmental temperature profile shown.

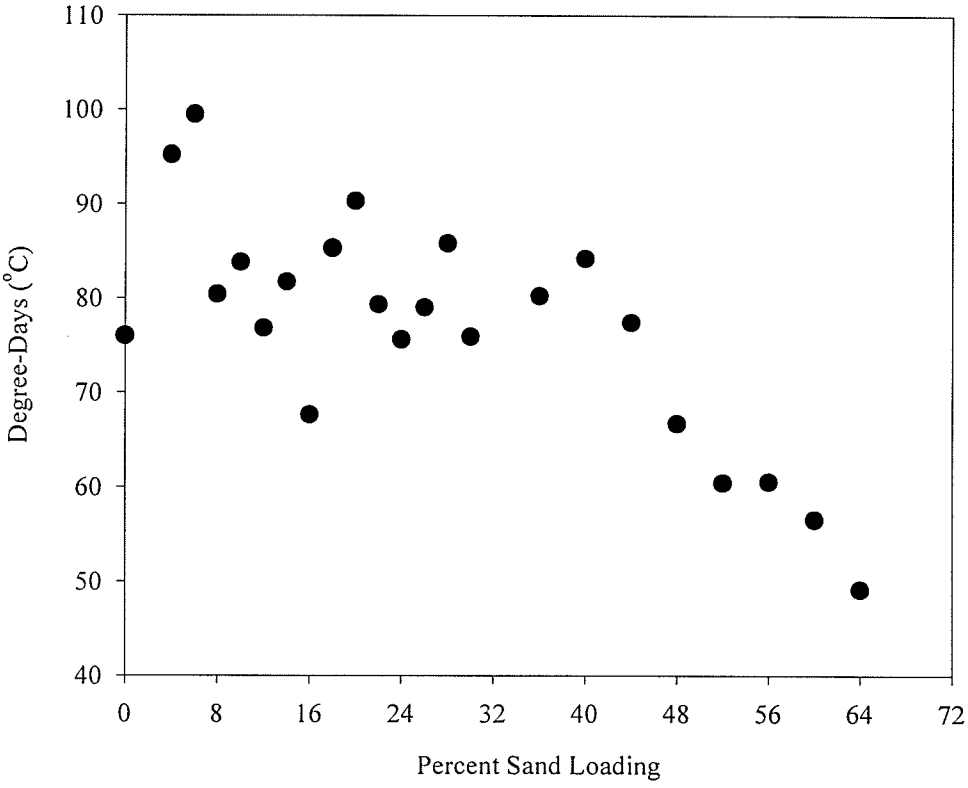


Figure 4-5. Effect of sand loading on the relative heat gerneration.

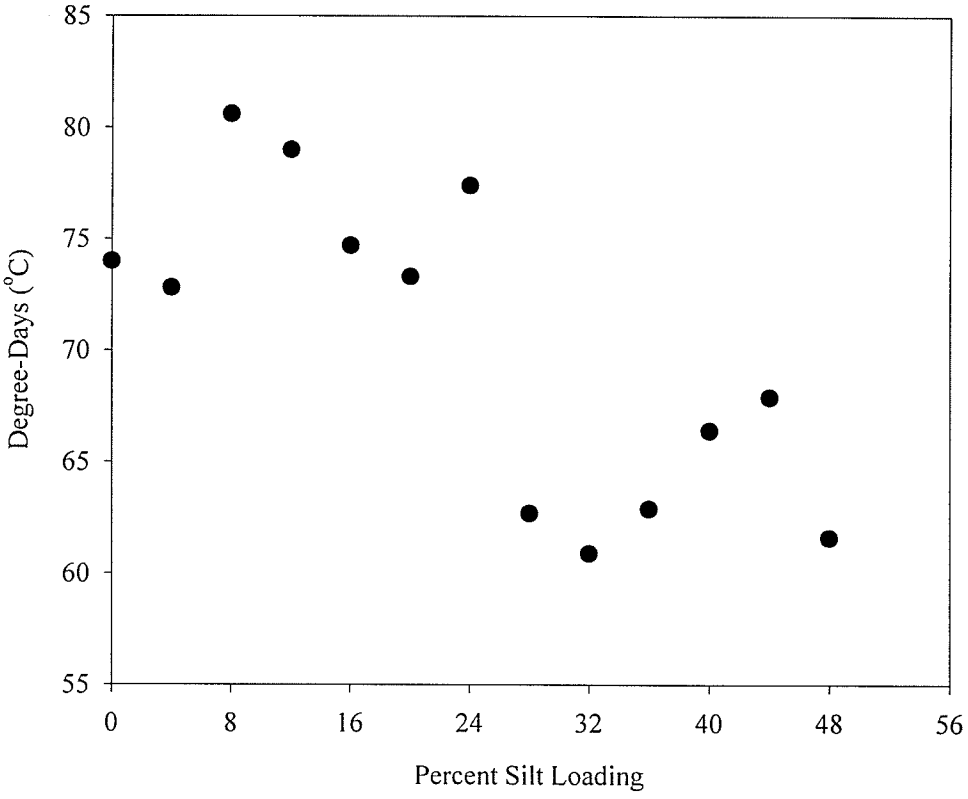


Figure 4-6. Effect of silt loading on the relative heat generation.

For Feedstock mixed with silt, the results showed no significant change in RHG in the data points between silt loading rate of 0 to 20%, indicating that the composting process generates significant amounts of heat similar to heat generated at 0% silt loading. However, a declining trend in RHG was observed in data points between silt loading of 20 to 48%. This suggests that the change in microbial activity had occurred when contaminated silt loading exceeded 20% by wet weight.

Although the composting process started to generate less heat at sand and silt loads above the 40% and 20%, respectively, this study found that 64% sand and 48% silt loads still achieve thermophilic conditions when mixed with feedstock. This is significantly greater than the 24% clay loading observed to be the limit for thermophilic condition to occur by a previous study (Wytrykush et al. 2002).

4.6.2 Volatile Solids Removal

The results of volatile solid and the percentage removal of volatile solids for each reactor are presented in Appendix C-2. The variability in VS data was determined using equations presented in Appendix C-3. The percentage of VS removal as a function of sand and silt loads in the feedstock mixture is presented in Figure 4-7 and Figure 4-8, respectively. The maximum VS removal observed for feedstock mixed with sand and silt was 18% and 16%, respectively. The VS removal, however, was poorly correlated to soil load. Also, the VS removal observed did not correspond with the relative heat generation values observed. The variability in volatile solids removal values probably resulted from errors in sampling the heterogeneous material.

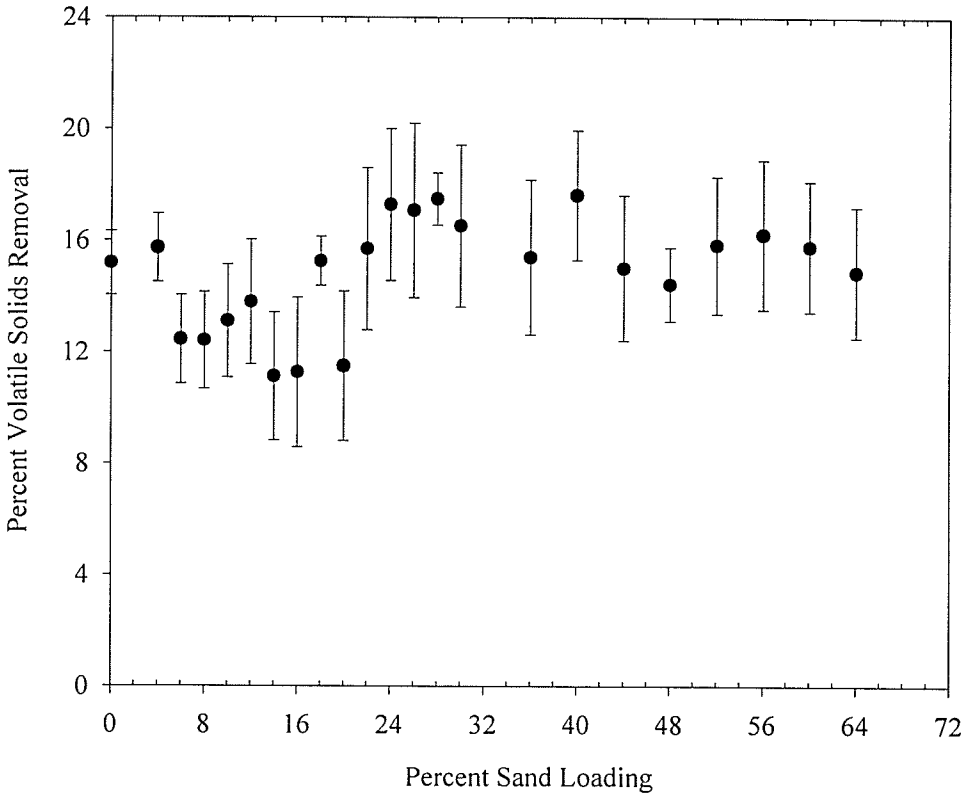


Figure 4-7. Effect of sand loading on volatile solid removal. Each data point is the mean of five replicate samples. The error bar represent the standard error.

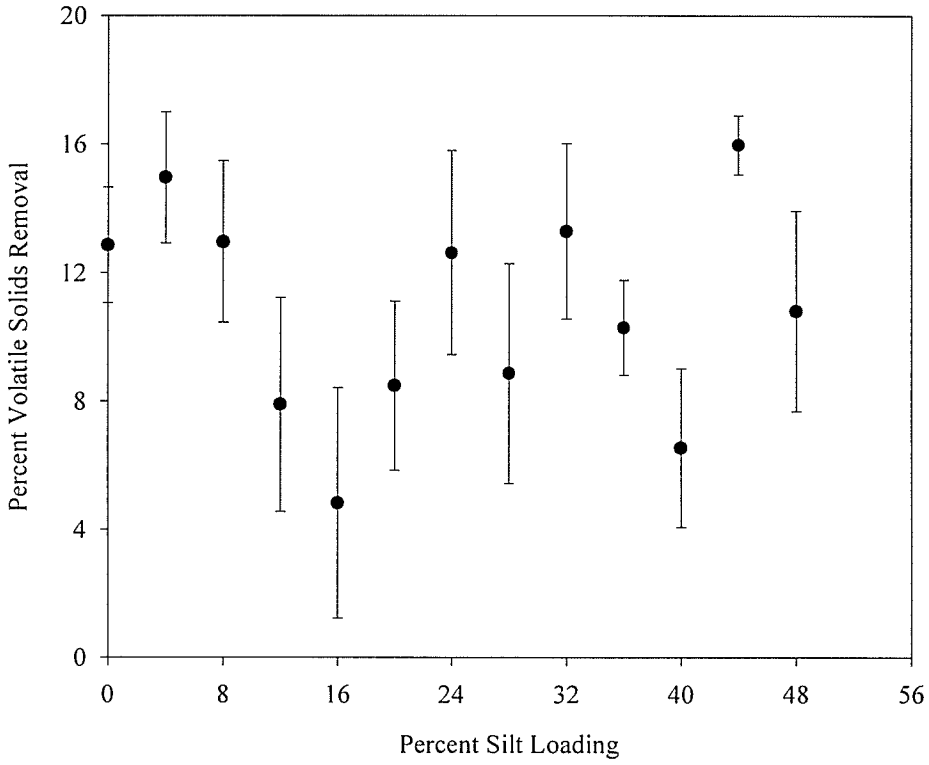


Figure 4-8. Effect of silt loading on volatile solids removal. Each data points is the mean of five replicate samples. The error bars represent standard error.

4.6.3 Mineralization of Labeled Octadecane

The production of $^{14}CO_2$ from feedstock mixtures was minimal. The total $^{14}CO_2$ released from feedstock mixtures was 0.1% and 0.03% of that initially in the feedstock mixture for both sand and silt, respectively. The findings of this study were supported by the findings obtained in the literature. Wytrykush et al. (2002) in lab scale composting of diesel fuel contaminated clay soil found that no release of ^{14}C octadecane as $^{14}CO_2$ after 14 days of thermophilic activity. Another study by Kästner et al. (1995) observed a lack of $^{14}CO_2$ production from 9-^{14}C-anthracene during the first 12 days of composting of diesel fuel contaminated sandy soil. However, after 103 days of composting, 23% of the ^{14}C anthracene transformed into $^{14}CO_2$. Since little $^{14}CO_2$ released during the two weeks of composting, no further investigation of ^{14}C degradation was considered at this stage of investigation.

4.7 Experimental Results & Discussion for Phase 1B

The results of Phase 1A indicated that microbial activity during the active phase of composting was not well correlated to sand or silt load tested. These results were significantly different from that found in the previous work carried out in our laboratory for clay soil. Therefore, this phase of study was sought to confirm the effect of sand or clay on the thermophilic phase of composting. Microbial activity was measured through relative heat generation and volatile solids removal. The results are summarized in the following sections.

4.7.1 Relative Heat Generation

The reactor and chamber temperature raw data are included in Appendix C-4. The temperature profiles over time for sand and clay soil are plotted in Figure 4-9. The reactors loaded with 40% clay soil had the lowest operating temperature; however, none of the reactors achieved a temperature of greater than 55°C.

To provide valuable information on the effect of soil load on degree of microbial activity, the reactor temperatures were used to construct relative heat generation (RHG) values. The RHG values for reactor mixed with sand or clay is shown in Figure 4-10. For feedstock mixed with sand, there was no significant difference ($p \geq 0.05$) in RHG values between the feedstock mixed with 20% and 40% sand. This suggests that no significant change occurred as sand load increased. For feedstock mixed with clay, the composting process generated less heat as clay soil load increased from 20% to 40%. There was a statistically significant difference ($p \leq 0.05$) between the clay loads investigated. The RHG values of the reactor mixed with 20% clay was not significantly different from the RHG of the reactor mixed with sand.

4.7.2 Volatile Solids Removal

The results of volatile solids and the percentage removal of volatile solids for each reactor are presented in Appendix C-5. The percentage of VS removal as a function of sand and clay loads in the feedstock mixture is presented in Figure 4-11. For feedstock mixed with sand, there was no significant difference ($p \geq 0.05$) in volatile solids reduction between the feedstock mixed with 20% and 40% sand. This suggests that no significant change occurred as sand load increased. However, compared to Phase 1A, the

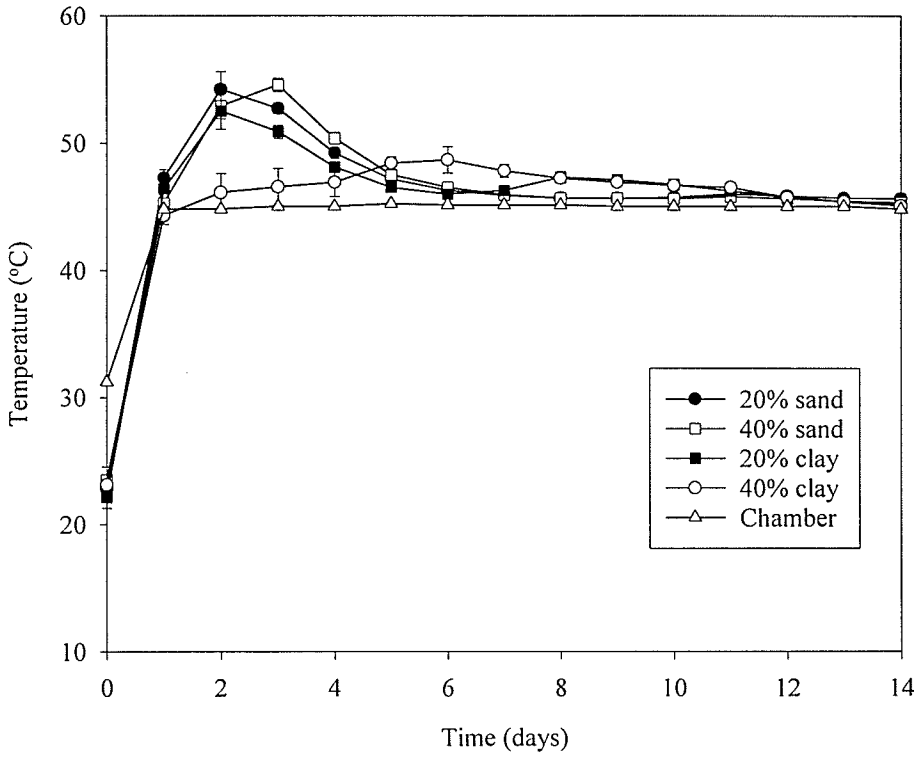


Figure 4-9. Temperature profiles for 20% and 40% sand and clay soil loadings. Each data point is the mean of two replicate reactors. The error bars represent stantard error.

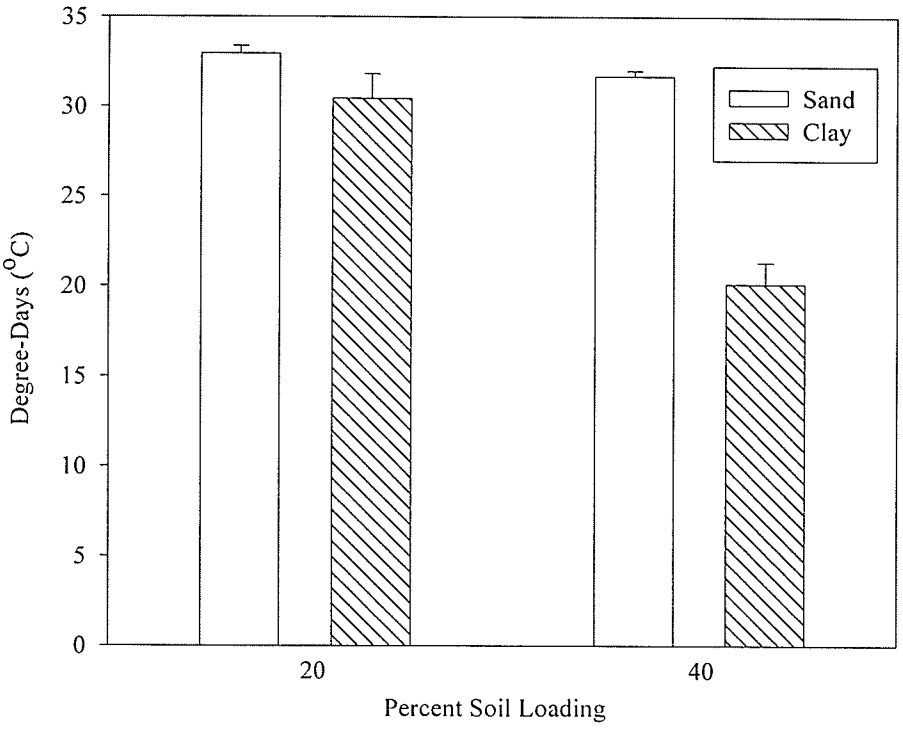


Figure 4-10. Relative heat generation at 20% and 40% sand and clay load. The error bars represent standard error.

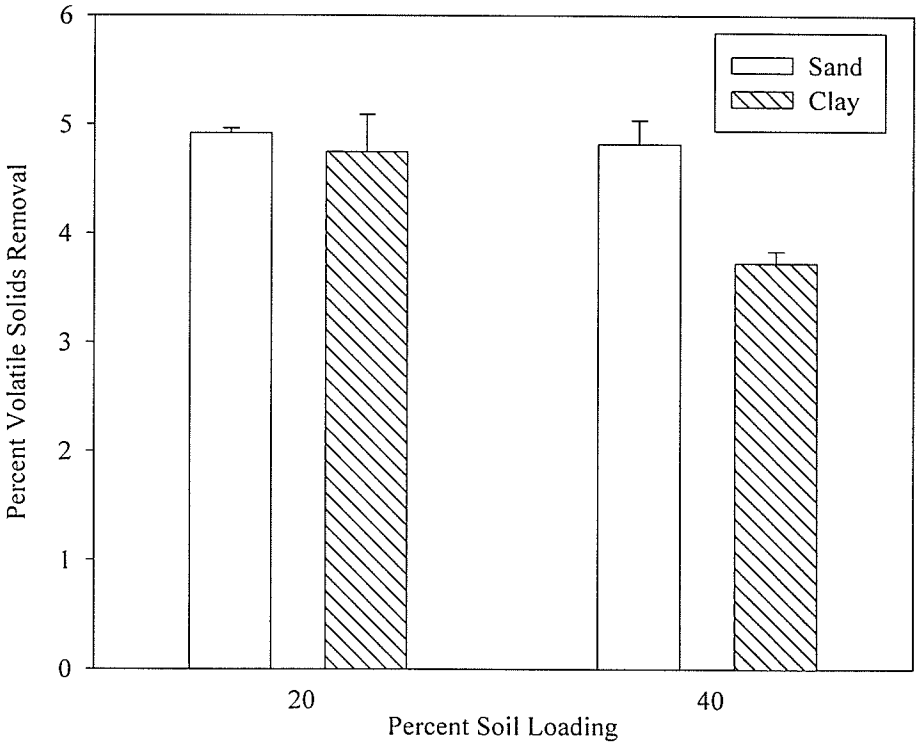


Figure 4-11. Volatile solid removal at 20% and 40% sand and clay load. The error bars represent standard error.

VS values obtained with sand were considerably lower. It was speculated that the amount of available nitrogen and carbon sources in woodshavings and biosolids material may have been lower than in the material used in Phase 1A.

For feedstock mixed with clay, there was a significant difference ($p \leq 0.05$) between the clay loads. The reduction in relative heat generation and volatile solids removal occurred at 40% clay loading possibly due to the presence of clumps of clay-compost and clayey film that coated the biosolids and woodshavings (See Figure B-1 in Appendix B). Although forced aeration was used to maintain aerobic condition, the presence of clay as both clayey film and clumps may have created pockets of anaerobic activity. Generally, compared to the findings observed by Wytrykush et al. (2002), the clay loads used in this study did not impeded the thermophilic microbial activity.

4.8 Summary & Conclusions

The purpose of Phase 1 was to investigate the effect of soil loading and texture on the microbial activity during the active phase of composting of diesel fuel contaminated soil. Phase 1A observed the effect of sand and silt loading on the thermophilic microbial activity during the composting of diesel fuel contaminated under simulated windrow composting conditions. Phase 1B confirmed the effect of sand or clay on the thermophilic phase of composting. Microbial activity was measured through relative heat generation and volatile solids removal. To determine whether the microorganisms were utilizing the diesel fuel within the soils, a radiolabeled compound (octadecane-1-^{14}C) was added to some of the soils and $^{14}CO_2$ production from the reactors was monitored through NaOH traps.

4.8.1 Phase 1A

A change in thermophilic microbial activity, as measured by relative heat generation, occurred at sand and silt loads above 40% and 20%, respectively.

The volatile solids reductions observed for feedstock mixed with sand and silt were 18% and 16%, respectively. However, the reduction in volatile solids was poorly correlated to soil loads. Also, the volatile solids reduction observed did not correspond with the relative heat generation values observed. Sample variability may have played an important role in these observations.

The production of $^{14}CO_2$ from both the sand and silt feedstock mixtures was minimal after 14 days of composting. The total $^{14}CO_2$ released from feedstock mixtures was 0.01% and 0.003% of that initially in the feedstock mixture for both sand and silt, respectively.

4.8.2 Phase 1B

Microbial activity, as measured by the RHG and VS removal, was not affected by sand loads tested and low clay load. However, microbial activity was affected at the high clay soil load (40%). This study confirmed the significant affect of clay soil observed by other researchers.

Based on the results, the following conclusions can be made:

1. Thermophilic microbial activity was not correlated to the sand or silt loading tested.
2. The thermophilic microbial activity was affected at high clay soil load (40%).

3. To maintain maximum level of microbial activity during the active phase of composting, this study recommended maximum soil loading of 20% clay and 40% sand for composting of diesel fuel contaminated soils.

4. Longer co-composting time should be attempted to observe the $^{14}CO_2$ production pattern as the composting process progresses.

5. Effect of Co-Substrate and Operating Temperature on Composting of Diesel Fuel Contaminated Soil

5.1 Summary

A laboratory study was conducted to investigate the effect of co-substrate and operating temperature on composting of diesel fuel contaminated soil. A sandy soil contaminated with diesel fuel (20,000 mg kg^{-1} dry soil) and spiked with labeled phenanthrene 9-^{14}C was used in this study. The contaminated soil was mixed with either fresh feedstock material or with finished compost and incubated using either thermophilic or mesophilic temperature patterns. After 18 weeks of composting, while no mineralization of ^{14}C phenanthrene was detected in the soil without co-substrate addition, soils receiving co-substrate had significant mineralization. Finished compost at mesophilic temperatures resulted in the highest mineralization rate of phenanthrene (42%). Extractable ^{14}C-phenanthrene was less than 11 % for all treatments, however, the soil without co-substrate addition still had more than 65% extractable ^{14}C-phenanthrene by the end of the experimental period. Extractable diesel range organics (EDRO) were reduced below the acceptable levels of 2000 mg kg^{-1} mandated by local authorities, in the soil mixed with fresh feedstock at the thermophilic or finished compost at the mesophilic. However, the acceptable level was achieved more rapidly from soil mixed with fresh feedstock. Although the composting at either fresh feedstock or finished compost was highly effective in eliminating ^{14}Cphenanthrene and extractable diesel range organics, as compared to soil without co-substrates, earthworm and seed germination assays indicated there was still significant toxicity associated with the soil. Other trace contaminants, besides the diesel fuel compounds, have caused the toxicity.

5.2 Introduction

Diesel fuel is a complex mixture of over two hundreds petroleum hydrocarbon compounds each with its own physical and chemical properties (Millner et al. 1992). Of several form of treatments sites with petroleum hydrocarbon impacted soils, this study was initiated to shed some light on the best conditions for compost bioremediation of unweathered diesel fuel contaminated soil. Of particular interest was the potential benefits of the thermophilic operating period associated with using fresh feedstocks. Many remote communities in Canada typically rely on diesel fuel generators as primary or secondary power sources and diesel fuel spills and leaking tanks often result in the need for remediation of these sites. Typically, the contaminated soil is excavated and treated using landfarming methods. Composting bioremediation is an emerging technology offering several technical advantages over landfarming, e.g. higher hydrocarbon mineralization rates, and lower extractable hydrocarbons in the treated soil. Successful composting bioremediation studies have used either fresh compost feedstock material or finished compost material (Kästner et al. 1995; Wischmann and Steinhart, 1997; U.S. EPA, 1998; Potter et al. 1999). Thermophilic temperatures have been cited as being useful in the bioremediation of hydrophobic compounds, such as PAHs, possibly due to increased desorption kinetics at higher temperatures (William and Keehan, 1993). According to Peramaki and Blomker (1997), the temperature attained during the composting process is critical in soil composting due to the fact that there are different optimum temperatures for different microorganisms. Progressing from mesophilic temperatures (25 to 45°C) to thermophilic temperatures (45 to 65°C), and then back to mesophilic temperatures, ensures significant diversity in the microbial population.

A comparison of mesophilic and thermophilic temperatures on the biodegradation of petroleum fractions has been considered by other researchers. Hogan et al. (1989) investigated composting of aliphatic and polyaromatic hydrocarbons using mature compost at both 35 and 50°C, and found that after 35 days both temperatures provided similar removal of aliphatic hydrocarbons (e.g. octadecane) and polyaromatic hydrocarbons (e.g. phenanthrene). Beaudin et al. (1996) investigated composting of mineral oil and grease (MOG) in fresh feedstock at temperatures ranging from 30°C to 50°C. They found that after 30 days of composting hydrocarbon degradation achieved was 56, 33, and 47% at temperatures of 23, 40, and 47°C, respectively. They also found that maintaining a temperature of 50°C for 30 days resulted in more MOG degradation (70%) than was obtained at 23°C (also over 30 days). Their study indicated thermophilic temperatures would be preferable to mesophilic temperatures if the length of thermophilic phase extended to the point where microorganisms had sufficient time to adapt to a temperature shift. Semple et al. (1998) studied the extent of [U-^{14}C] benzene mineralization in spent mushroom compost after a 3-months enrichment period in the presence of alkyl benzenes such as benzene, toluene, ethylbenzene and xylenes (BTEX) compounds. They observed a higher mineralization rate of [U-^{14}C] benzene when the soil was incubated at 50°C as compared to 18°C and 37°C over the 14 days experiment period. Using mature compost, Haderlein et al. (1999) observed lower mineralization rates of hexadecane and pyrene at 55°C as compared to 35°C. These studies were different in terms of the benefits of thermophilic temperatures. The reasons for different results may be related to compost substrate type, hydrocarbon type, composting temperature, and incubation time.

Generally, the results of the above studies suggested that temperature and type of co-substrate may play a role during compost bioremediation of diesel fuel contaminated soil. Further work is needed to clarify some issues. First, which material would be most beneficial: finished compost or fresh feedstock. Second, it is not clear whether thermophilic temperatures are beneficial to diesel fuel treatment.

5.3 Experimental Hypotheses & Objectives

The experimental hypotheses were: (1) a thermophilic operating period should increase the total biodegradation of reference diesel fuel components (extractable diesel range organics and phenanthrene); (2) the addition of organic substrate to soil during composting should increase the size of the non-extractable fraction (bound residue) of the diesel fuel as compared to soil alone; and (3) composted contaminated soil will not be toxic to earthworms and plants.

The specific objective was to investigate the effects of co-substrates and operating temperatures on the composting of diesel fuel contaminated soil. To investigate the objective, this phase of study was divided into two sub-phases. Phase 2A observed the performance of the soil composting process by measuring the reduction in target diesel fuel concentrations (phenanthrene and extractable diesel range organics). Phase 2B observed the performance by assessing the treatment's ability to lower soil toxicity.

5.4 Experimental Materials & Methods for Phase 2A

5.4.1 Preparation of the Soil and Co-Substrate Materials

A sandy soil obtained from the Assiniboine Delta Aquifer, Carberry, Manitoba was used. The soil moisture contents were adjusted with distilled water to 50 % of the maximum moisture holding capacity (17% wet basis). Diesel fuel, spiked with phenanthrene labeled carbon compound in [9-^{14}C] (supplied by the Sigma Chemical Co., St. Louis, MO) at activity of 0.15 μci/g of diesel fuel, was added to the soil to yield 20,000 mg kg^{-1} dry soil.

Two co-substrate materials were used in this study. Both co-substrates consisted of a ratio of 4.59 kg biosolids, 1.14 kg leaves and 0.77 kg woodshavings, yielding feedstock moisture content of 60% (wb) and total free air space of 0.75 cm^3 cm^{-3}. Either fresh feedstock material or finished compost (prepared by thermophilically composting the same fresh feedstock material for thirty days before use) was mixed with the contaminated soil. Each mixture contained 4.33 wet kg of soil and 6.5 wet kg of either fresh feedstock or finished compost.

5.4.2 Set-up of Experiment

A total of ten reactors were used in the study. Four reactors contained finished compost and soil mixture, with two subjected to a mesophilic temperature control pattern and two subjected to a thermophilic temperature control pattern. Two reactors contained fresh feedstock and soil mixture and were subjected to a thermophilic temperature control pattern. Two sets of controls were also used. Two reactors contained contaminated soil, but no co-substrate material and were subjected to thermophilic temperature pattern.

Two reactors contained fresh feedstock mixed with uncontaminated soil and were subjected to a thermophilic temperature pattern. A summary of treatment and control reactors tested are presented in Table 5-1. Each reactor was coded with the designation X-Y-Z, where X designated whether soil was contaminated (C) or uncontaminated (U); Y designated the type of co-substrate (F= fresh feedstock, C= finished compost, and N= no co-substrate); and Z designated the temperature pattern used (M= mesophilic and T= thermophilic).

Table 5-1. Treatments and control reactors tested.

Designation	*Description*	*Replicates*
	Treatments	
CFT	Contaminated soil plus fresh feedstock; thermophilic	2
CCT	Contaminated soil plus finished compost; thermophilic	2
CCM	Contaminated soil plus finished compost; mesophilic	2
	Controls	
CNT	Contaminated soil with no co-substarte; thermophilic	2
UFT	Uncontaminated soil plus fresh feedstock; thermophilic	2

Using a temperature controlled environmental chamber, all the reactors were subjected to either to a thermophilic or to a mesophilic temperature pattern. The thermophilic pattern was designed to simulate a typical thermophilic composting period. The reactor contents were pre-heated to 35°C, then each day, as the reactor temperatures increased; the chamber temperature was set one degree below the lowest temperature observed in the reactors containing fresh feedstock material (CFT and UFT). When reactor temperatures decreased to 50°C, the chamber temperature was maintained at 50°C until a run time of

four weeks was reached. After four weeks, the chamber temperature was again adjusted to one degree below the reactor temperature (CFT and UFT). As the reactor temperature leveled out at 35°C, the chamber temperature was kept at 35°C for the reminder of the run. In the case of mesophilic temperature pattern, the chamber temperature was set at 35°C for the entire experimental run. All reactors were run for a total of 18 weeks.

The reactors used in this study were as described in Phase 1. Briefly, the reactors had a capacity of 28-L and were insulated with 5cm thick pink fiberglass and aluminum-reflecting blanket to minimize the heat loss. A compressive load of about 3.87 kPa was applied to each reactor. This load simulates the load that would be experienced at the core of 2 m high windrow. More details on the needs for compressive load in bench-scale biocell reactors can be found in McCartney and Chen (2001). The reactors were aerated for 15 min hr^{-1} by moistened air with a flow rate of 1100 ml min^{-1}. The exhaust gas of each reactor was passed through in a vessel containing 15g Amersorb 563, which was present to trap any volatile hydrocarbons released from the reactor contents. The exist gas was then passed through three CO_2 traps in series. The CO_2 traps consisted of sorption flasks filled with 300 ml 3N NaOH solution for complete capture of all evolved CO_2. Glass beads were used in the flasks to minimize gas bubbling.

5.4.3 Sampling of Compost

Samples were collected once every two weeks to track extractable ^{14}C-phananthrene, extractable diesel range organics (EDRO), moisture content, and volatile solids. Samples were taken by opening the reactors and spreading the reactor contents on a plastic sheet. Ten samples were collected randomly from each reactor. These samples were mixed

together to form one composite sample, and then the quartering technique was used to obtain one sample (140 g wet weight). A method for compost sampling presented in Phase1B had shown that this sampling procedure would allow representative sampling of feedstock materials with low variability from the mean (<1%). The sampling from the reactors resulted in about a 10% reduction of starting material total soil compost weight over the period of the study, and was accounted for in all calculations.

5.4.4 Moisture Content and Volatile Solids

During each sampling period, reactor moisture contents were measured and adjusted if necessary before being replaced into the reactors. For reactor moisture content maintenance, the moisture content was measured using a rapid method described in McCartney and Tingley (1998). For calculations, moisture and volatile solids contents were determined using three replicate samples of approximately 10 g each dried at 105°C and ashed at 550°C.

5.4.5 Mineralization of [9C-^{14}C] Phenanthrene

NaOH traps were exchanged and sampled weekly in order to determine the total amount of $^{14}CO_2$ concentration resulting from the mineralization of 9C-^{14}C-labeled phenanthrene. Nineteen ml of scintillation cocktail (Ultima Gold XR, LSC Cocktail) was added to 1 ml of the sampled NaOH solution and mixed vigorously. Quenching was minimized by storing vials in the dark 24 hour before liquid scintillation counting. The radioactivity in disintegration per minute was measured using a liquid scintillation counter (Model LSC 7500). Final results were corrected for background radiation, adjusted for the amount of radioactivity that was present in the entire trap, and then related to the initial radioactivity

added to give the percent of mineralization of phenanthrene in diesel. Samples for analyses from each trap were collected in duplicate.

5.4.6 Volatilization of Phenanthrene & Diesel Range Organics

To test for any volatile organic carbon that may have evolved when the experiment was completed, five grams were removed from the ambersorb traps and extracted with 25 ml of hexane/acetone solvent in a 50-ml extraction vial. The extract was subjected to analysis for ^{14}C phenanthrene and diesel range organics using a scintillation counter and gas chromatograph, respectively. Samples for analyses were collected in duplicate.

5.4.7 Sequential Extractable ^{14}C-Labeled Phenanthrene Residues

Residual ^{14}C was extracted to determine the properties of the remaining fraction during 18 weeks of composting. A step-wise extraction using water, methanol, and methylene chloride was used as described in Maurice (1998) with the exception of the sample for methylene chloride extract being placed on a platform shaker for 24 hours instead of using a soxhlet extraction apparatus. A preliminary test found a platform shaker is as efficient as soxhlet extraction method. The water extractable ^{14}C would indicate the possible free phenanthrene and degradation products in soil-compost mixture. These fractions would then be considered as in the mobile phase. The methanol extract would contain the weekly associated phenanthrene and degradation products sorbed to soil-compost mixture. This fraction would be considered no longer bioavailable and have some potential for desorption. The methylene chloride fraction would contain the highly sorbed and stable ^{14}C (Maurice 1998). Briefly, a 10 g wet sample (stored at 4°C for 7 days prior to extraction) was added to a 50-ml vial and extracted with 25 ml water,

methanol, and methyl chloride for 24 hours in step-wise extractions. The sample was then filtered through 0.45um non sterile-nylon filter units to separate the solid and solvent. Extraction recovery efficiency of ^{14}C phenanthrene from freshly prepared samples (1-hour old) yielded 99.86% ±1.94 recovery from the soil alone and 90.15% ±0.88 recovery from the soil with co-substrate. Nineteen ml of scintillation cocktail (Ultima Gold XR, LSC Cocktail) was added to 1 ml of the sampled NaOH solution and mixed vigorously. The radioactivity in disintegrations per minute was measured using a scintillation counter (Model LSC 7500). Samples for analyses from each solvent were collected in duplicate and the final results were summed and presented as total percent extractable of ^{14}C phenanthrene of the total radioactivity initially added. The non-extractable ^{14}C was calculated as the difference between the total radioactivity added and the total recovery (i.e. phenanthrene mineralized and extracted).

5.4.8 Extractable Diesel Range Organics (EDRO)

Extractable diesel range organics (C_{10} to C_{19}) were determined according to US EPA Method 8015B using a hexane/acetone solvent (U.S. EPA, 1993). Briefly, a 10-g wet sample (stored at 4°C for 7 days prior to extraction) was mixed with 25 ml of solvent in a 50-ml extraction vial. The vial was placed on a platform shaker (New Brunswick Scientific, model classic C_2) for 24 hours at 250 rpm then filtered through 0.45um non sterile-nylon filter units to separate the solid and solvent. Extraction recovery efficiency of EDRO from freshly prepared samples (about 1-h old) yielded 102.45% ±5.18 and 90.75% ±0.86 recovery from the soil alone and the soil with co-substrate, respectively. A 1-µl extract was injected into a gas chromatograph (Hewlett Packard Model 5890), a flame ionization detector (FID) and HP-1 column (15 m length x 0.53 mm internal

diameter x 2.65 μm methyl silicone film thickness). Hydrogen was used as the carrier gas and nitrogen as the make-up gas. The initial temperature was set at 100°C for 1.5 minutes. The ramp program then increased the oven temperature at 12°C min^{-1} to 250°C for five minutes. The ramp temperature then increased the oven temperature to a final temperature of 325°C at 12°C min^{-1}. The final oven temperature was then maintained for 3 minutes. Concentrations of remaining EDRO in soil compost mixtures was corrected for moisture content and expressed on a dry weight basis. Due to the high biodegradable content of some of the samples, EDRO concentrations were based on the ash content of the sample.

5.4.9 Carbon Re-supplement for Further Phenanthrene Mineralization

The objective of this treatment was to determine whether the re-supplement of carbon source could trigger release of phenanthrene as $^{14}CO_2$ during the plateau stage of compost treatment. At the completion of the composting treatment (18 weeks of experimental run), a representative sample from composted soil with fresh feedstock (CFT) was collected. After collection, the reactor material was mixed with fresh feedstock material at ratio of 1:0, 1:1 and 1:2 or with glucose at 2000 μg glucose g^{-1} dry composted soil. The samples was then placed in microcosms (500 ml glass jars with air-tight metal lids) and incubated at either 35°C or 50°C for 10 weeks. Within each microcosm jar, two 20 ml borosilicate scintillation vials were placed alongside the 50 ml Pyrex beaker containing the soil-compost to be treated. The first scintillation vial contained 10 ml acidified water (pH 3), which was used to maintain humidity within the jar without sorbing carbon dioxide. The second scintillation vial contained 15 ml sodium hydroxide (3 M NaOH), which was used to trap $^{14}CO_2$ released from the soil.

To maintain aerobic conditions, the microcosms were opened three times a week for 30 seconds to bring the headspace air to ambient air condition. At two weeks intervals, the NaOH trap was changed and the production of $^{14}CO_2$ was measured to determine any further mineralization of ^{14}C phenanthrene induced due to addition of carbon sources. At the time of trap change, the beaker containing the composted soil was also removed from the microcosm, weighed and re-wet if necessary to keep the sample moist at approximately 40% wet weight basis.

5.4.10 Statistical Analysis

The analysis of Variance Test (ANOVA) was used to ascertain whether change in phenanthrene and/or extractable diesel range organics concentrations between treatments were statistically significant ($p \leq 0.05$) over the course of the experiment. All values are expressed as the mean of two replicates followed by their standard error. The letters attached to each value indicate the results of the ANOVA test, and means with a common letter were not significantly different.

5.5 Experimental Material & Methods for Phase 2B

5.5.1 Preparation of Composted Soil Samples

A 4 kg composite sample from each soil-compost mixture was collected at the end of the composting study and was stored frozen until used for toxicity bioassays. Just before beginning the plant and earthworm toxicity bioassays, the composted soil samples (Table 5-2) were stored at 4°C over night. Each of the samples was tested for pH and moisture content. The pH for all composted soil samples were similar and within the optimal range of 5 to 8 for earthworms. The moisture content was 40% (wet basis).

Table 5-2. Treatments and control samples used for toxicity.

Designation	Description
	Treated Samples
CFT	Contaminated soil plus fresh feedstock; thermophilic
CCT	Contaminated soil plus compost; thermophilic
CCM	Contaminated soil plus compost; mesophilic
UFT	Uncontaminated soil plus fresh feedstock; thermophilic
	Untreated Samples (Controls)
CF	Contaminated soil plus fresh feedstock, not treated
UF	Uncontaminated soil plus fresh feedstock, not treated
PS	Potting soil

5.5.2 Seed Toxicity Assay

The seed germination assay was performed according to CCME guidelines (Can/BNQ, 1996), with the exception of lettuce being used as test seed in addition to the cress and radish. Lettuce was selected due to its reported sensitivity to metals and PAHs (Cook et al. 2002). Composted soil samples that were treated either at thermophilic or mesophilic temperature pattern were used in this study. To provide a comparable reference material for the effects testing, three sets of controls were also prepared. The test was conducted by placing 150 g of moist control or test soil in disposable petri dishes. Ten seeds of radish, cress or lettuce were pressed into the composted soil samples in each dish. Test samples were hydrated to 75% of water holding capacity with de-ionized water. Petri dishes were then covered to prevent drying. Water was added as needed to each

container to keep the soil moist. All dishes were incubated at 20°C ±2 and 70% relative humidity in a light/dark cycle of 16 hours of light and 8 hours of darkness. At the end of 7 and 14 days, the number of germinated seeds was recorded for each dish. After two weeks emergence, the emerged plants were harvested and washed to remove any soil that might be clinging to them. The samples were then placed in the oven at 103°C overnight and dry weights recorded after cooling. Three replicates of each sample were performed. The seed germination data for each composted soil sample was measured and compared to controls. Statistical analysis was performed on the results using Tukey Test for multiple mean comparisons. Differences were considered significant at $p \leq 0.05$.

5.5.3 Earthworm Toxicity Assay

The earthworm toxicity assay was used to assess potential soil toxicity before and after the composting process. One species of mature earthworms *(Lumbricus terrestris*; nightcrawlers) were purchased locally and stored at 15°C. *L. terrestris* was selected because it is a soil inhabiting worm native to Canada and is comparable to *Eisenia fetida* which recommended by standardized toxicity tests available (Cook et al. 2002). Ten days before using earthworms in the experiment, earthworm digestive tracts were cleaned of previously ingested content by incubating them in potting mix soil at 15°C.

Adult earthworms (3 to 5 g) were washed, blotted dry with a paper towel and weighed. Five adult earthworms were added to each composted soil sample in a mesh covered container. The experimental containers, 1.5 L glass jars, were prepared by adding 500 g of moist composted soil sample to each jar. Soil with fresh feedstock (untreated) and potting mix soil were used as controls. The earthworms were incubated in environmental

chamber at 15°C and re-weighed at either 14 or 28 days. Three replicates were performed. Differences between initial and final *L. terrestris* weights were used to assess earthworm growth rates.

5.5.4 Statistical Analysis

The analysis of Variance Test (ANOVA) was used to determine the overall significance of treatment from the control. Tukey test for multiple mean comparisons was performed to test for differences between the treatments. Differences were considered significant at $p \leq 0.05$.

5.6 Experimental Results & Discussion for Phase 2A

The experimental objective of this phase was to observe the performance of the soil composting process by measuring the reduction in target diesel fuel concentrations (phenanthrene and extractable diesel range organics). The data collected during the experiment is presented in complete form in Appendix D. The results are summarized in the following sections.

5.6.1 Reactor Temperature Pattern

The reactor and chamber temperature data are included in Appendix D-1. The temperature profiles observed in the reactors are presented in Figure 5-1. The temperature profiles for the control reactors containing contaminated soil with no co-substrate (not shown in Figure 5-1) were constant at 50°C for the first eight weeks, then constant at 35°C for the final ten weeks. For the fresh feedstock reactors (CFT), two temperature peaks were observed during the thermophilic phase. The initial reactor temperature peak reached a maximum value of 58°C after one week. After this initial

temperature peak, the reactor temperature fell gradually to 54°C and did not recover until the reactor was mixed for the sampling event on the second week where the reactor temperature gradually increased to reach its second temperature peak value of 62°C on day 17. This second peak of thermophilic temperatures lasted for 3 days and started to fall gradually to level out at 50°C within 4 weeks. As composting progressed, the reactor temperature did not peak again during the subsequent mixing events. Mixing events are indicated by the temperature valleys in Figure 5-1. The same temperature pattern was observed with finished compost reactors (CCT). However, as shown in Figure 5-1, the fresh feedstock reactor temperatures were higher than the finished compost reactor temperatures during the first 4 weeks. This was due to the higher biodegradable organic matter content of the fresh feedstock. For the finished compost reactors operated at a constant mesophilic temperature (CCM) for the entire period of the study, the reactor temperature climbed to the upper mesophilic range (45°C) during the first 3 days and subsequently dropped and stayed constant at 35°C for the remaining period. In general, the temperature profiles indicated all reactors contained active aerobic compost mixtures.

5.6.2 ^{14}C Phenanthrene in Soil Reactors

Phenanthrene mineralization and extractable data are presented in Appendix D-2 and D-3. Time profiles of the phenanthrene mineralization, extractable and non-extractable phenanthrene for each individual treatment is presented in Figures 5-2 to 5-5. Since volatilization of ^{14}C phenanthrene was detected not in the reactors with or without co-substrates, the volatilization of phenanthrene was not considered further in the following discussion.

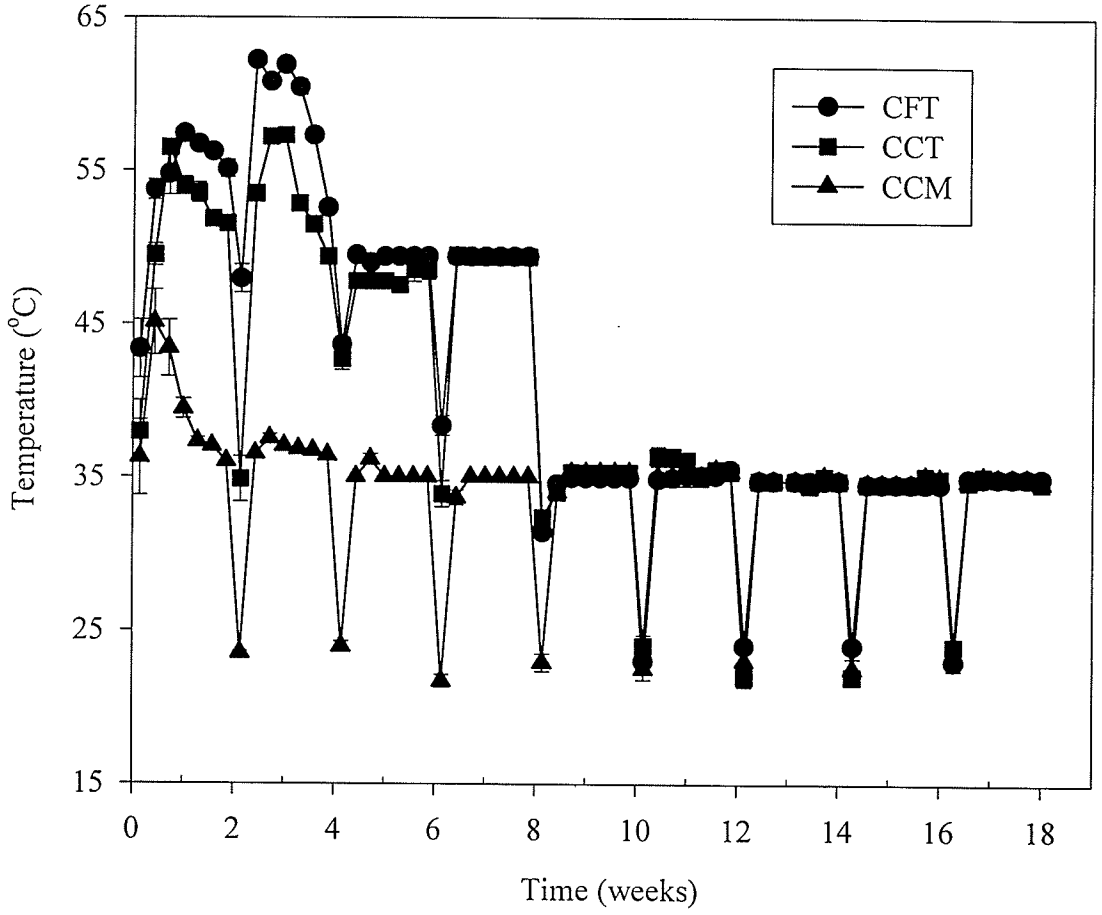


Figure 5-1. Composting temperature profiles for both thermophilic and mesopphilic operated systems. Temperature valleys represent the mixing events.

5.6.2.1 Contaminated Soil with no Co-substrate

In the contaminated soil reactor with no co-substrate addition (CNT) more than 67% of initial extractable phenanthrene was found in the methanol phase, while less than 1% was found in the methylene chloride phase. Water extractable ^{14}C was negligible. Over the 18-week experiment, the extractable phenanthrene remained unchanged (Figure 5-2) and no release of phenanthrene as $^{14}CO_2$ occurred during the same period.

5.6.2.2 Contaminated Soil with Co-substrate

In the contaminated soil with fresh feedstock operated at thermophilic temperature (CFT), the initial extractable concentration in the methanol phase was about 55% of the total added ^{14}C phenanthrene, while 10% of the total added was extracted in the methylene chloride phase (Figure 5-3). No ^{14}C phenanthrene was detected in the water phase. Over the 18-week experiment, the ^{14}C phenanthrene in the soil extract decreased considerably, without any lag phase, resulting in an extractable concentration (methanol extractable) of 1%. The greatest reduction of extractable fraction of phenanthrene occurred within the first 7 weeks. Within this period, the greatest portion of phenanthrene had also been mineralized. The total phenanthrene mineralization amounted to 23.2% of the initially added ^{14}C phenanthrene at the end of experiment. After week 7, the non-extractable bound residue became a stable sink for phenanthrene and its metabolites. This fraction was not readily bioavailable and hence the extent of ^{14}C mineralization peaked at around 23%.

In the contaminated soil with finished compost operated at thermophilic temperature (CCT), the initial extractable amount in the methanol phase was 51% of the total added

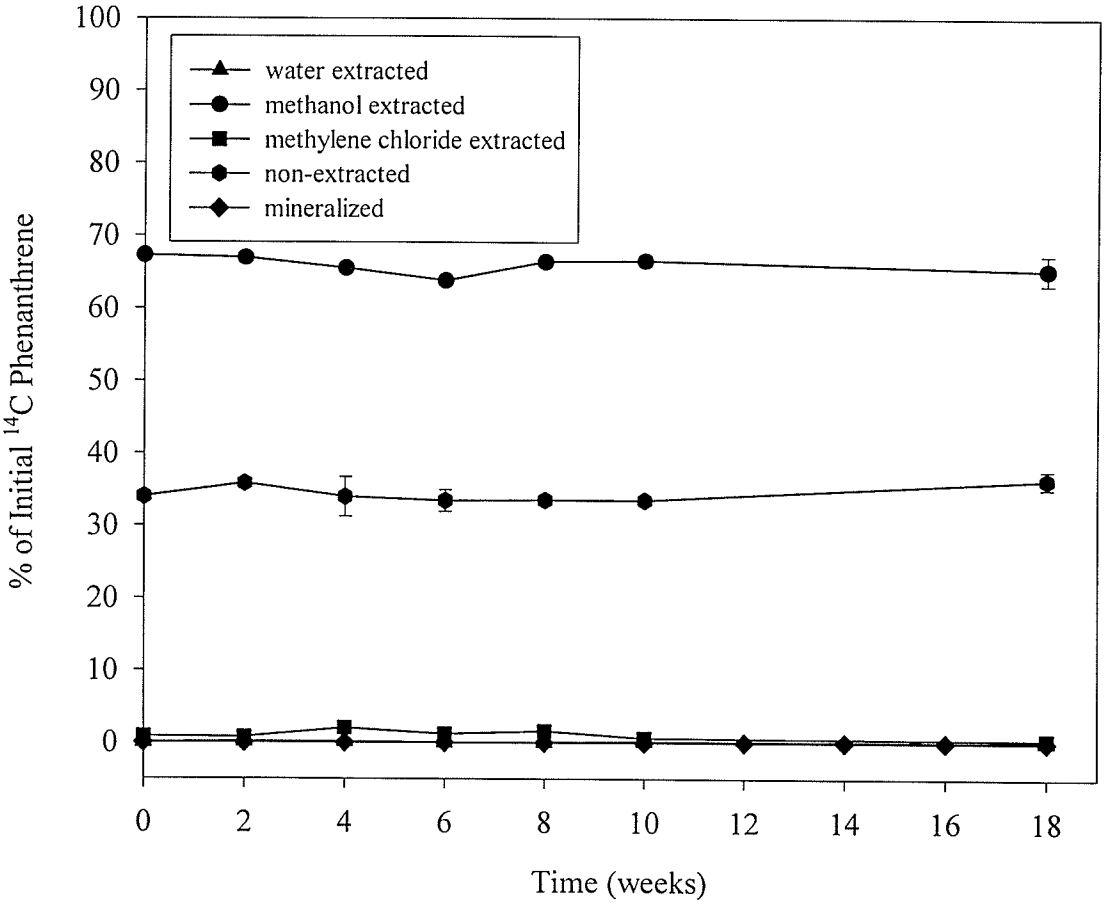


Figure 5-2. ^{14}C phenanthrene recovery in CNT. Each point represents the mean of two replicates. The error bars show standard error.

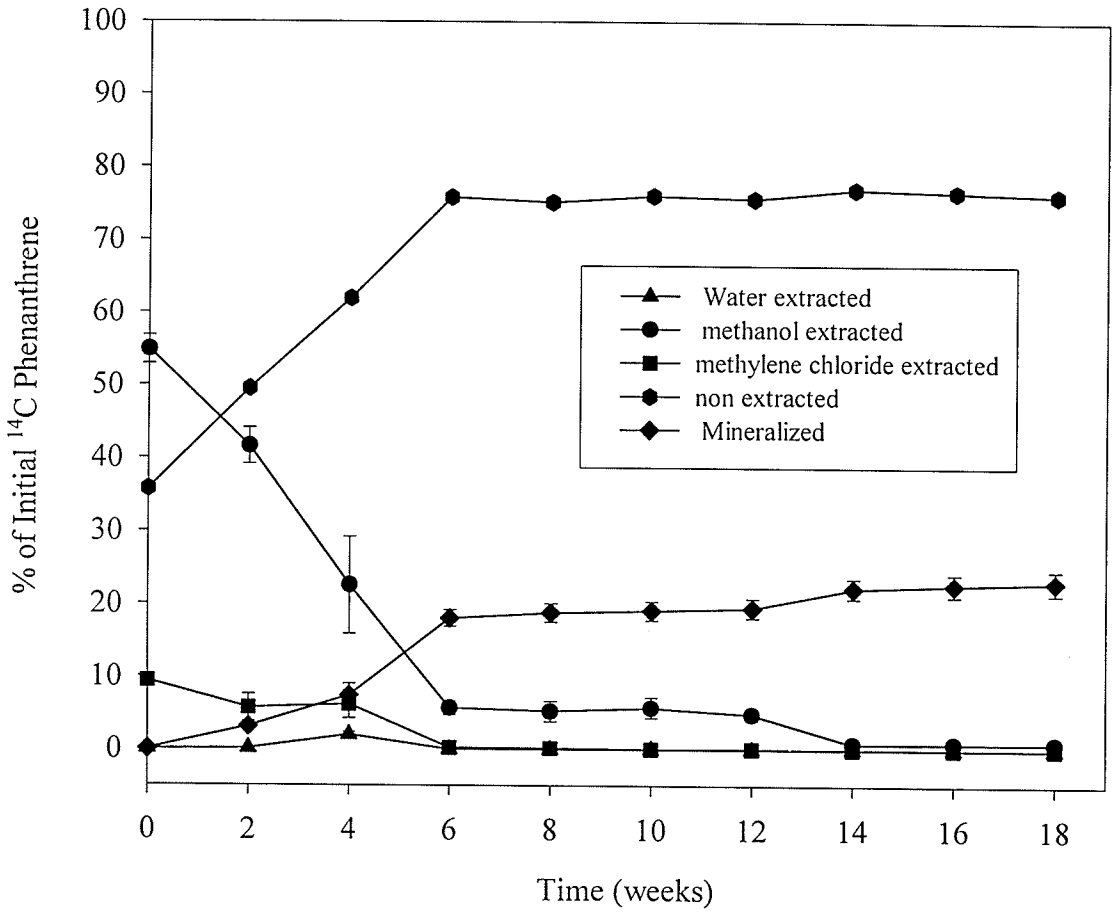


Figure 5-3. ^{14}C phenanthrene recovery in CFT. Each point represents the mean of two replicates. The error bars show standard error.

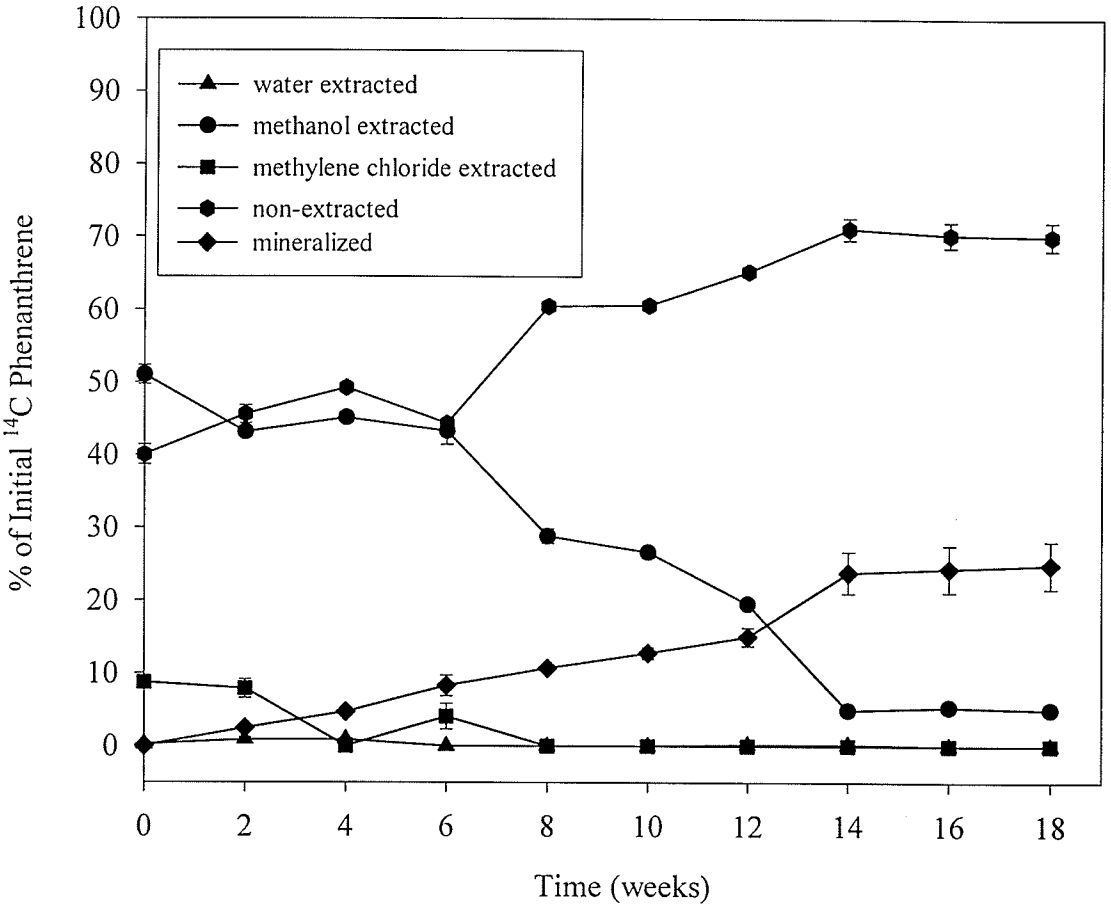


Figure 5-4. ^{14}C phenanthrene recovery in CCT. Each point represents the mean of two replicates. The error bars show standard error.

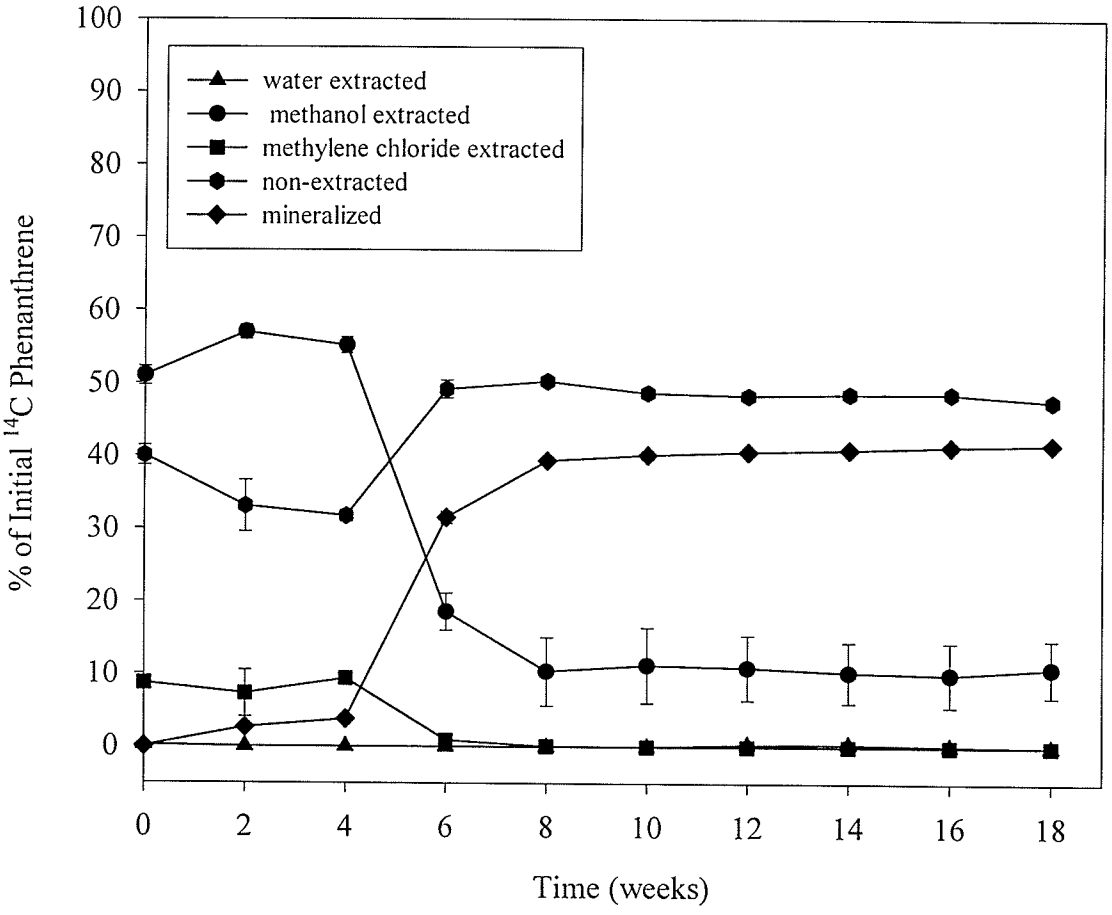


Figure 5-5. ^{14}C phenanthrene recovery in CCM. Each point represents the mean of two replicates. The error bars show standard error.

^{14}C phenanthrene, while 9% of the total added was extracted in the methylene chloride phase (Figure 5-4). No ^{14}C phenanthrene was detected in the water phase. The ^{14}C phenanthrene in the methanol extract dropped slightly during the first week and then remained relatively constant up to week 7. After this lag period, the extractable fraction decreased gradually to a final concentration of about 5% (methanol extractable) of initially added ^{14}C phenanthrene. The greatest reduction of extractable fraction of phenanthrene occurred between week 7 and 14. Within this period, the greatest portion of phenanthrene had also been mineralized. The total phenanthrene mineralization amounted to 24.9% of the initially added ^{14}C phenanthrene at the end of experimental run. After week 14, the non-extractable bound residue became a stable sink for phenanthrene and its metabolites. This fraction was not readily bioavailable and hence the extent of ^{14}C mineralization remained relatively low after week 14.

In the contaminated soil with finished compost at the mesophilic temperatures (CCM), the concentration of extractable phenanthrene remained relatively constant during the first 4 weeks (Figure 5-5). This probably represents the lag phase when the microbial community was acclimating to the phenanthrene. After this lag period, the next four weeks showed significant phenanthrene mineralization and decrease in extractability. The extractable fraction decreased to a concentration of about 11% (methanol extractable) of initially added ^{14}C phenanthrene at week 8. This decrease correlated well with the mineralization profile of phenanthrene (Figure 5-5). While less than 5% of phenanthrene had been mineralized by week 4, by week 8 that had increased to about 38% of the initially added ^{14}C phenanthrene. The greatest portion of phenanthrene was mineralized between week 4 and 8. After week 8, the non-extractable bound residue was

the most important sink for phenanthrene or its metabolites in the CCT. This fraction was not readily bioavailable and hence the extent of ^{14}C mineralization remained relatively low after week 8.

For comparison, the mineralization profiles for individual reactors are shown in Figure 5-6. The total ^{14}C evolved as $^{14}CO_2$ and the maximum mineralization rate for each treatment is summarized in Table 5-3. The total phenanthrene mineralization rate was similar for both CFT and CCT reactors after 18 weeks of composting (Figure 5-6), while the CCM reactors achieved approximately 70% higher mineralization. This result was interesting because suggests that the temperature was a more important factor than the type of co-substrate.

A significant difference in the initial rate of mineralization was observed between the thermophilic reactors. For example, after 8 weeks, the treatment with fresh feed stock (CFT) achieved a total conversion rate of approximately 18%, while the compost treatment (CCT) achieved only 10%. The difference in the initial mineralization rate may be attributed to the type of biomass in the co-substrates. The fresh feedstock would contain more readily degradable biomass as compared to the finished compost and it may have sustained a higher microbial population at the beginning of the composting treatment than did the reactors with the finished compost.

For comparison, the total extractable phenanthrene profiles for individual reactors are shown in Figure 5-7. The extractable phenanthrene for each treatment is summarized in Table 5-4. For the treatments with co-substrates, the total extractable phenanthrene concentrations remaining in the contaminated soil regardless of co-substrate type and

operating temperature ranged from 1% to 10% at the end of the 18-week experimental run. This was remarkable compared to the 68% remaining in treatment with no co-substrate addition. These results demonstrated that both fresh feedstock and finished compost effectively removed the phenanthrene from the extractable phase (Table 5-4). Statistical analysis, however, showed there was a significant difference in the total extractable phenanthrene between fresh feedstock and finished compost treatments. The fresh feedstock was the best material for removal of phenanthrene from the extractable phase.

The mass balances of ^{14}C phenanthrene added to the soil with or without co-substrates addition are listed in Table 5-5. Volatilization of ^{14}C phenanthrene was not involved in the fate of phenanthrene in the soil with or without co-substrate. This was expected as phenanthrene has a low vapor pressure (0.113 Pa at 25°C) and high octanol/water coefficient (K_{ow} 4.53 at 25°C) (Piatt et al. 1996). This data indicated, however, that bound residue formation was the major mechanism for phenanthrene removal and its metabolites. Residual ^{14}C phenanthrene remaining in the soil was 47% in the CCM, 70% in the CCT and 76% in CFT. In general, the finished compost at the mesophilic temperatures (CCM) showed a lower tendency for bound residue formation and a higher mineralization rate compared to other treatments at thermophilic temperature. This indicated that phenanthrene degrading microorganisms were likely more active at the mesophilic temperatures compared to their thermophilic counterparts.

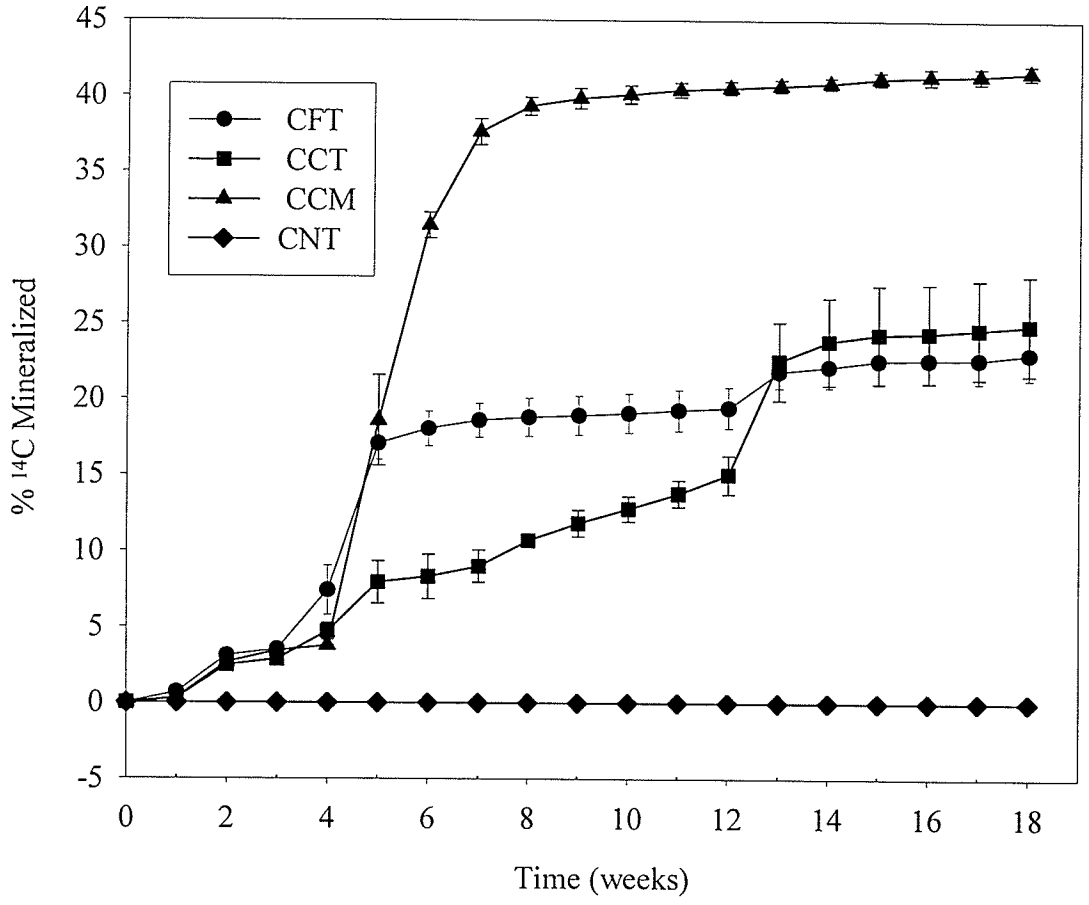


Figure 5-6. Cumulative mineralization of phenanthrene at thermophilic and mesophilic temperature patterns. Each data point represents the mean of two replicates. The error bars show standard errors.

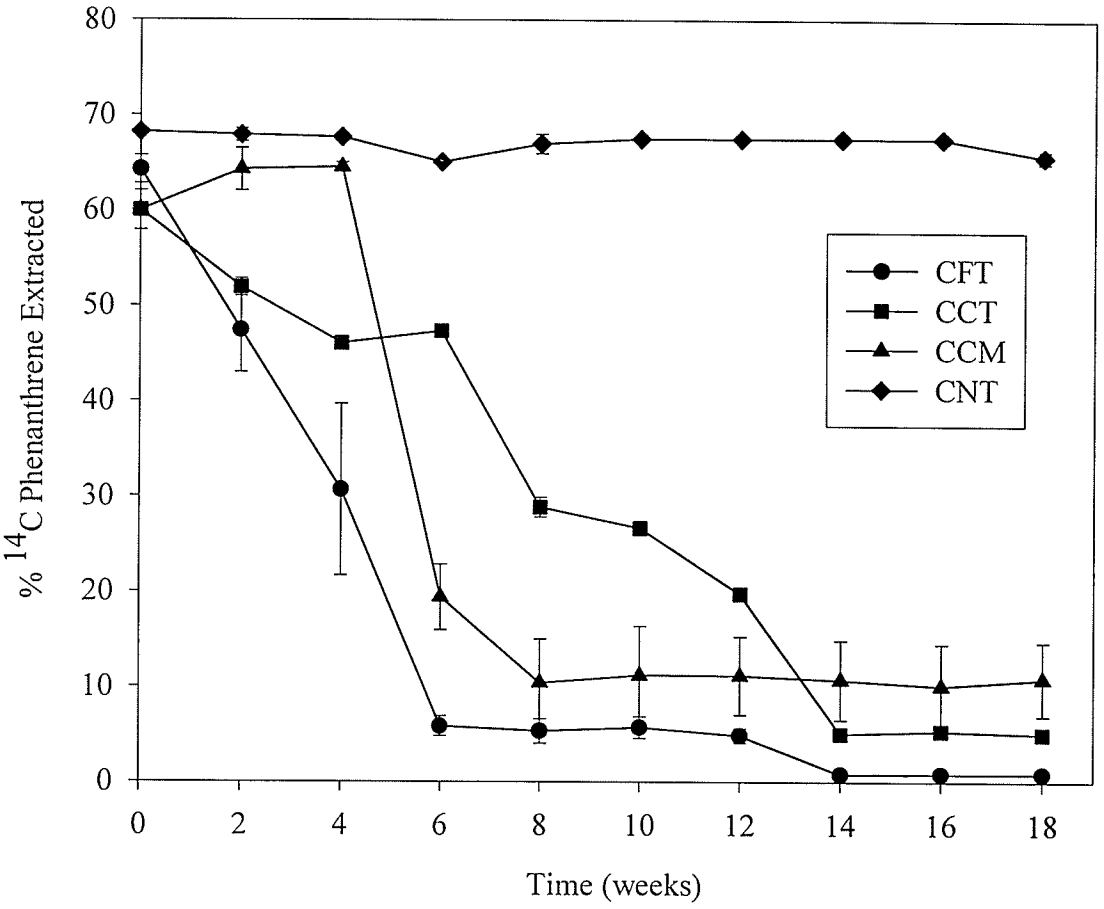


Figure 5-7. Total extractable phenanthrene in soil with or without co-substrates. Each point represents the mean of two replicates. The error bars show standard error.

Table 5-3. Total percent phenanthrene recovered as $^{14}CO_2$ over the 18-week experimental period and maximum mineralization rate. Means followed by common letter are not significantly different ($p \geq 0.05$).

Treatment Designation	Total $^{14}CO2$ Recovered (%) Mean ±SE[3]	Maximum Mineralization Rate (% / day)[2]
CCM	41.62b ± 0.46	2.05
CCT	24.91a ± 3.27	0.71
CFT	23.15a ± 1.65	~1
CNT	ND[1]	

Notes: [1] not detected, [2] calculated from three consecutive points on the steepest slope of the mineralization curves; [3] standard error.

Table 5-4. Mean value of sequential extraction of ^{14}C from soil compost mixtures after 18 weeks of experiment run. Means followed by common letter are not significantly different ($p \geq 0.05$).

Treatment Designation	% ^{14}C Water Extracted Mean ±SE[1]	% ^{14}C Methanol Extracted Mean ±SE	% ^{14}C Methylene Extracted Mean ±SE	% ^{14}C Total Extracted Mean ±SE
CFT	ND[2]	0.83a ± 0.3	ND	0.83a ± 0.3
CCT	ND	4.99b ± 0.6	ND	4.99b ± 0.6
CCM	ND	10.8c ± 3.9	ND	10.8c ± 3.9
CNT	0.11 ± 0.01	65.26d ± 2.0	0.38 ± 0.2	65.75d ± 0.2

Notes: [1] standard error; [2] not detected.

Table 5-5. Mass balance of ^{14}C phenanthrene at the end of the experiment (18 weeks).

Parameters	Treatment Designation			
	CCM	*CFT*	*CCT*	*CNT*
^{14}C mineralizaed (%)	41.62	23.16	24.91	ND[1]
^{14}C extracted (%)	10.8	0.83	4.99	63.60
^{14}C non-extracted (%)	47.58	76.10	70.1	36.34
^{14}C volatilized (%)	ND	ND	ND	ND

Notes: [1] not detected

5.6.3 Diesel Range Organics in Soil Reactors

Examples of typical chromatographic response charts and the results of extractable diesel range organics (EDRO) for individual reactors are presented in Appendix D-4. The time profiles of the EDRO for each individual treatment are presented in Figures 5-8. Table 5-6 summarizes the overall reduction of EDRO from initial condition. The results are discussed in the following sections.

5.6.3.1 Contaminated Soil with no Co-substrate

In the contaminated soil reactor with no co-substrate addition (CNT), the concentrations of EDRO decreased from 18,516 to 8,508 mg kg^{-1} ash (54% removal) over the 18-week period (Figure 5-8). Most EDRO were removed within the first 8 weeks. After week 8 no noticeable removal of EDRO was observed. The total volatilization loss by the end of experiment amounted to about 5% of the original mass of diesel fuel.

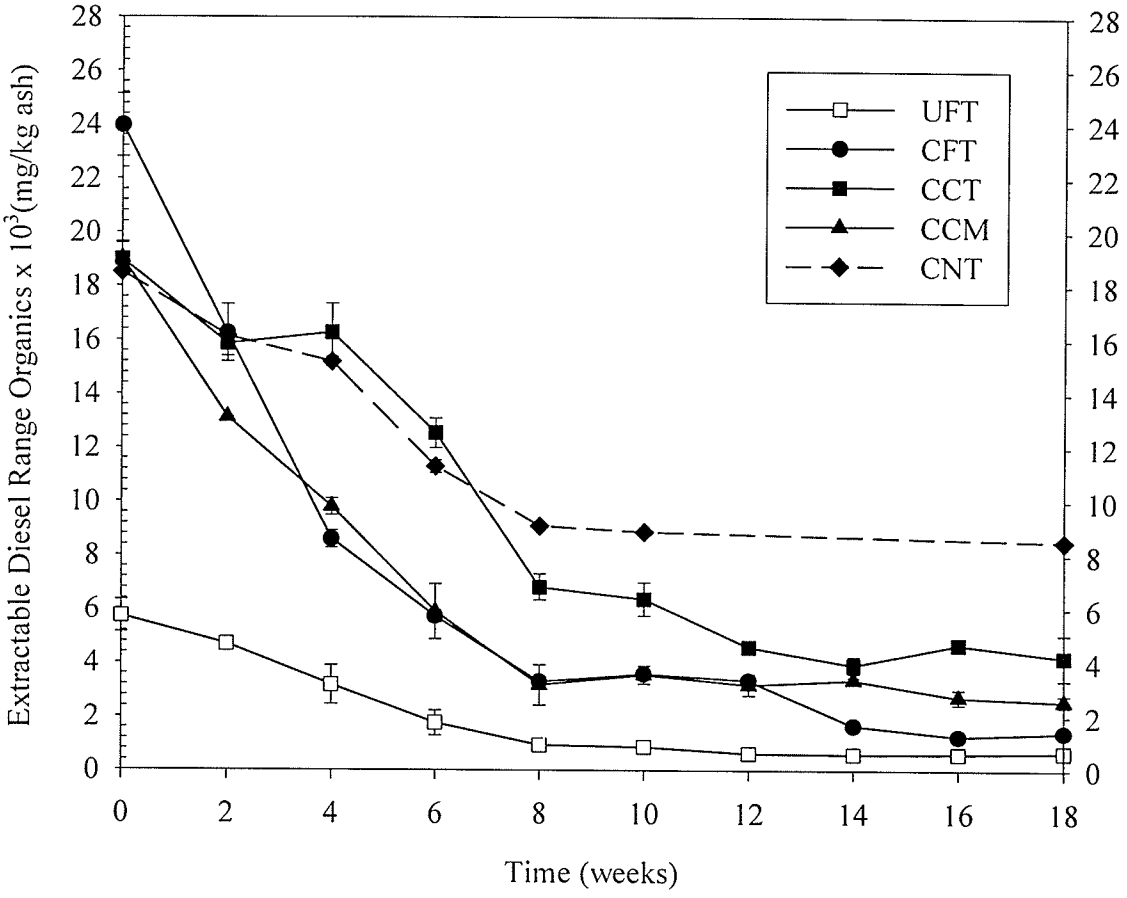


Figure 5-8. Extractable diesel range organics based on ash weight of samples. Each point represents the mean of two replicates. The error bars show standard error.

5.6.3.2 Contaminated Soil with Co-substrate

The concentration of EDRO in the reactors with co-substrate decreased substantially compared to the reactor with no co-substrate (Table 5-6, Figure 5-8). Sources of EDRO originated from both the hydrocarbons (diesel fuel) added to the soil and the fresh compost feedstock. This was highlighted by the results found on day 0 (Figure 5-8), where the UFT (no hydrocarbon addition) had an initial EDRO concentration of 5,734 mg kg^{-1} ash, while the treatments with contaminated soil and finished compost had initial EDRO concentrations of around 19,000 mg kg^{-1} ash. The combination of fresh co-substrate and contaminated soil (CFT reactors) resulted in the highest initial EDRO concentration of 23,967 mg kg^{-1} ash. The EDRO originating from fresh co-substrate was reduced by 88.5% (5,734 mg kg^{-1} to 662 mg kg^{-1} ash) over the 18-week experimental period. Most EDRO originated from fresh feedstock were removed within the first 8 weeks. Within the same period, rapid removal of EDRO in the CFT treatment was also observed. Following the rapid removal of EDRO, residual EDRO was removed slowly compared with the early stage (i.e. within 8 weeks). The residual EDRO (originated from both the diesel fuel added and the fresh compost feedstock) concentration reduced by 94% (23, 967 mg kg^{-1} ash to 1,418 mg kg^{-1} ash) at the end of 18 weeks experimental period. As stated previously that the addition of fresh feedstock resulted in an increase of initial EDRO in CFT. Assuming that the same quantity of EDRO of co-substrate was removed in the fresh feedstock with contaminated soil (CFT) as in the one with clean soil (UFT), then approximately 47% of the total residual EDRO in CFT was contributed from co-substrate origin. Although the CFT reactors started with higher EDRO due to EDRO available in fresh feedstock co-substrate, it ended with lower EDRO when compared to

CCT reactors (Figure 5-8). Higher rate removal of EDRO in CFT was observed compared to CCT over the 18-week period. This may be attributed to biomass type in both co-substrates. The fresh feedstock had relatively high degradable biomass compared to the finished compost and that may sustained a higher microbial population compared to the finished compost.

In the CCM reactor, EDRO decreased from 18,995 to 2,572 mg kg^{-1} ash (86% removal) over the same period (Figure 5-8). EDRO in the CCM reactors decreased more rapidly than that in the CCT reactors. A clear difference in the EDRO removal was observed up to week 10, after which, both CCM and CCT treatments started to approach similar levels of removal. Statistical analysis, however, showed there was a significant difference ($p \leq 0.05$) in the final EDRO removal percentages between the treatments.

The EDRO concentration remaining in the contaminated soils with co-substrate ranged from 1,418 to 4,208 mg kg^{-1} ash over the 18-week period. Since no volatile loss of diesel range organic was detected by the end of experiment, the EDRO could have preferentially mineralized and/or strongly bind to the humus fraction of compost. In general, these results demonstrated that both fresh feedstock and finished compost removed the EDRO effectively from the contaminated soil in all treatments.

The acceptable levels of EDRO as mandated by the Manitoba clean-up guidelines are below 500 mg kg^{-1} total solids for agriculture usage and 2000 mg kg^{-1} total solids for residential land usage. The results in Figure 5-9 and Table 5-6 showed the EDRO remaining in the soil after treatment with fresh feedstock at thermophilic temperature (CFT) or finished compost at mesophilic temperature (CCM) were below the acceptable

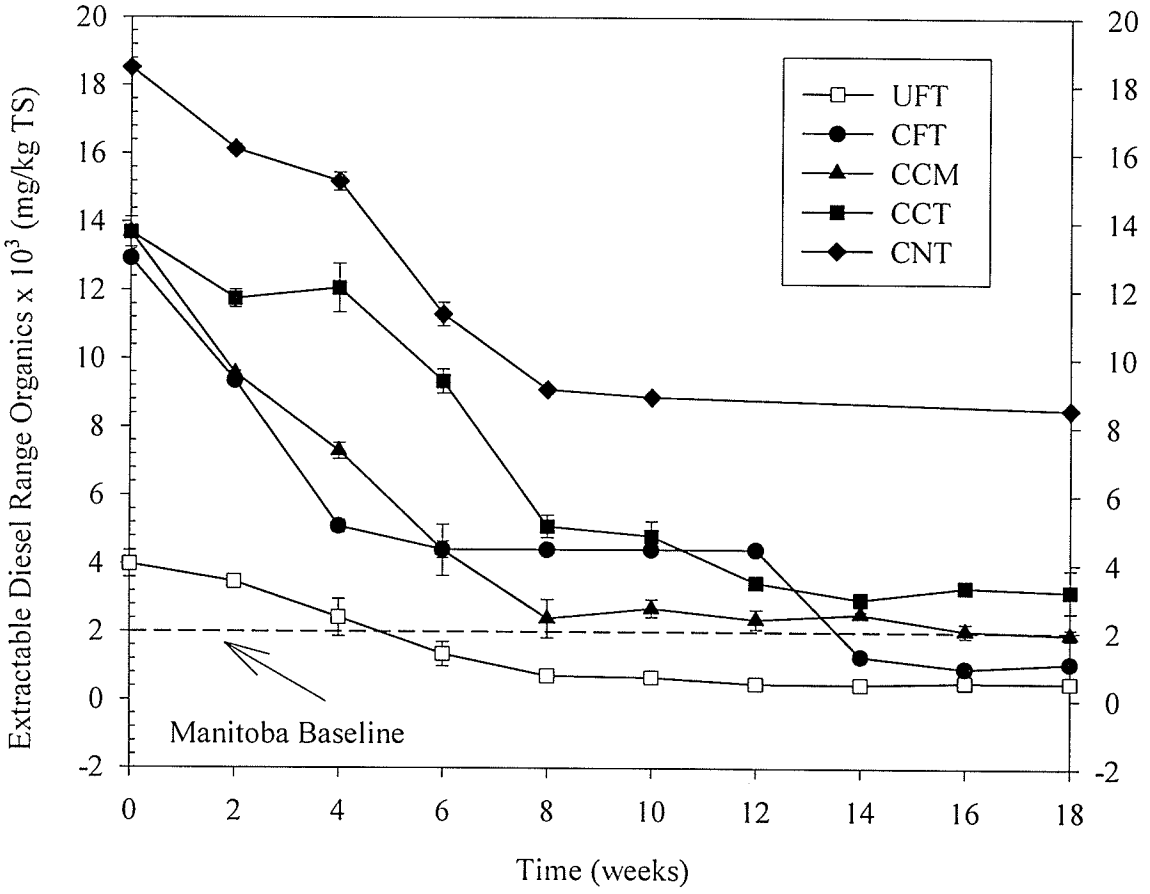


Figure 5-9. Extractable Diesel Range Organics based on total solid of samples. Each point represents the mean of two replicates. The error bars show standard error.

levels of 2000 mg kg^{-1} for residential land usage. While both CFT and CCM treatments achieved the guidelines, the EDRO removal in CFT was approximately two times greater than CCM. This result indicates that the addition of fresh feedstock co-substrate enhances to a greater extent the removal of EDRO when compared to finished compost.

Table 5-6. EDRO reduction in the compost mixtures at the end of experiment runs. Means followed by common letter are not significantly different ($p \geq 0.05$).

Treatment Designation	EDRO (mg/kg TS) Mean ± SE[1]	EDRO (mg/kg ash) Mean ± SE	Reduction[2] (%)
CFT	1092a ±49	1418a ±64	94
CCM	1938b ±169	2574b ±221	86
CCT	3213c ±633	4208c ±845	78
CNT	8507d ±42	NA[3]	54
UFT	514 ±95	662 ±122	88.5

Notes: [1] standard error, [2]% reduction based on ash weight, except with CNT, [3] not applicable

5.6.4 Carbon Sources Resupplement & Phenanthrene Mineralization

Time profiles of volatile solids removal, phenanthrene mineralization and EDRO for each soil treatment are presented in Figures 5-10 to 5-12. The maximum VS reduction obtained was 43% in the CFT treatment and 28 to 32% in the CCT and CCM treatments. This was expected since the finished compost had less available biodegradable VS. The VS reduction rate decreased over time for all the treatments, indicating the depletion of biodegradable VS. In the CFT treatment, most of biodegradable VS were depleted in approximately 7 weeks (Figure 5-10), after which, the VS reduction rate remained

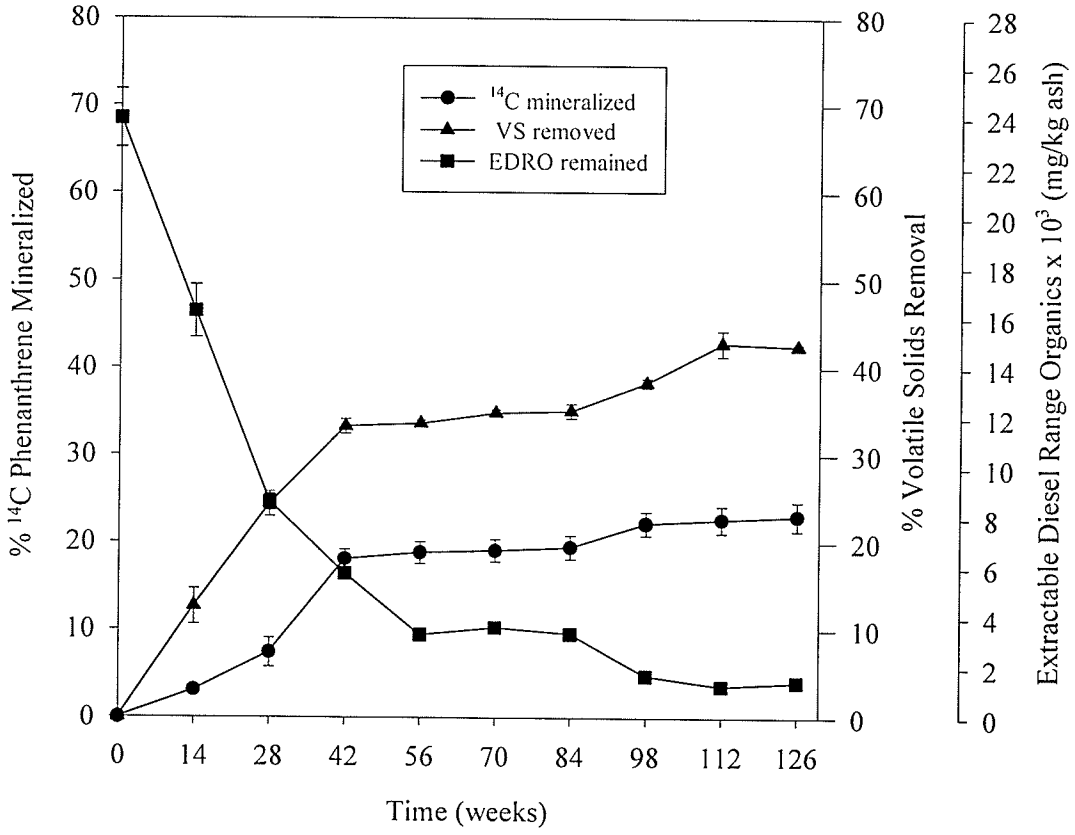


Figure 5-10. Volatile solids removal, phenanthrene mineralization and extractable diesel range organics for CFT reactors. Each data point represents the mean of two replicates. The error bars show standard error.

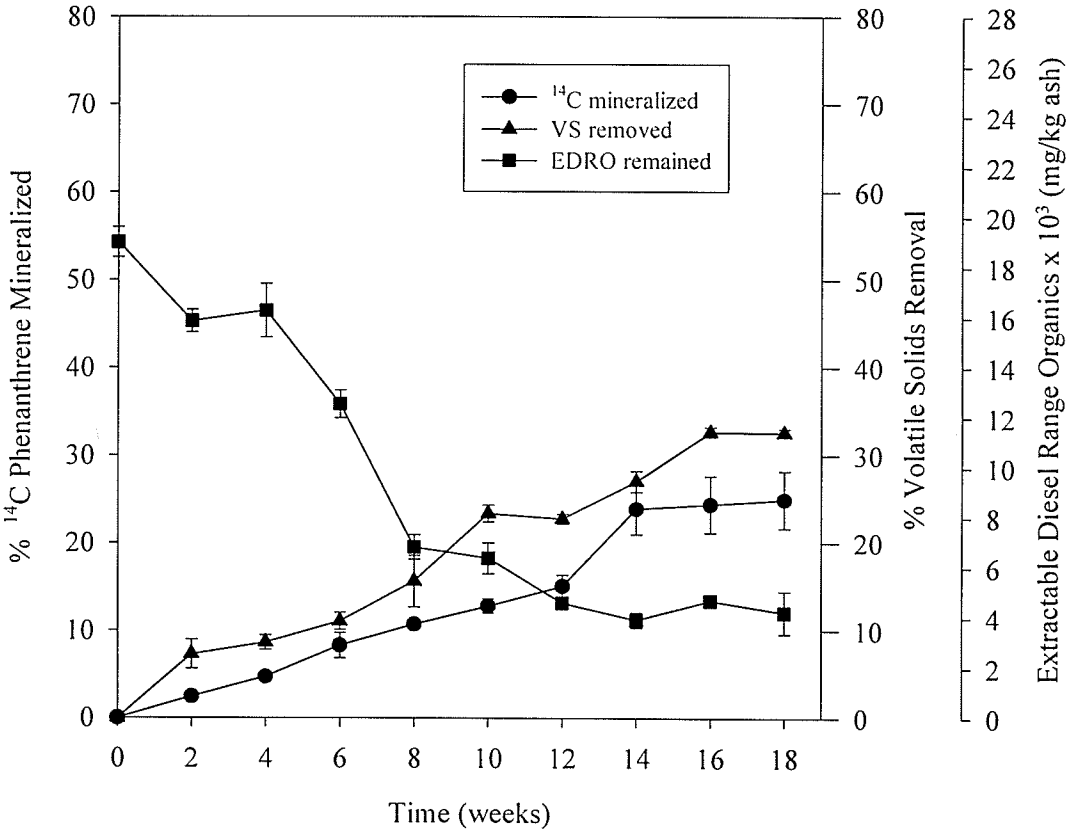


Figure 5-11. Volatile solids removal, phenanthrene mineralization and extractable diesel range organics profiles for CCT reactors. Each data point represents the mean of two replicates. The error bars show standard error.

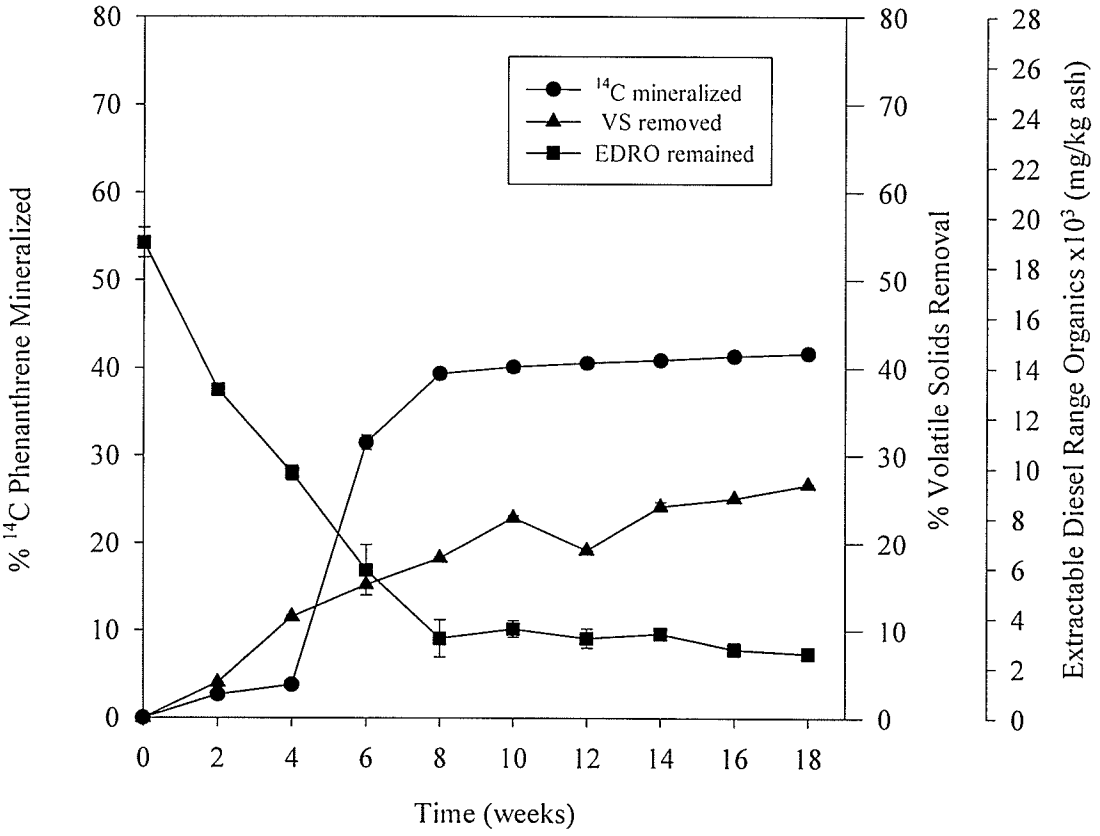


Figure 5-12. Volatile solids removal, phenanthrene mineralization and extractable diesel range organics profiles for CCM reactors. Each point represents the mean of two replicates. The error bars show standard error.

relatively low over the 18-week experiment. Within the same period, phenanthrene mineralization and EDRO removal rates also remained relatively low, indicating a plateau concentration was reached. Similar phenomenon was observed with the CCT and CCM treatments (Figure 5-11 and 5-12).

It was thought that depletion of bioavailable VS during the composting process was limiting to further phenanthrene mineralization and EDRO removal. Under conditions of limited nutrients, microorganisms shift their outer membranes to a less permeable state and thus they become less capable of consuming organic contaminants from their surroundings (Potter et al. 1999). To determine whether excess amounts of available carbon, such as a fresh feedstock or glucose, could eventually trigger the release of ^{14}C activity as $^{14}CO_2$ during the plateau stage of compost treatment, a subsequent experiment was conducted in a 500 ml microcosms apparatus. The results of phenanthrene mineralization after resupplement of a carbon source are presented in Table 5-7. For composted samples with no additional excess carbon, the amount of phenanthrene mineralized at either mesophilic (35°C) or thermophilic (50°C) temperature was less than 8% over the 10-week period. Over the same time period, similar amount of phenanthrene was mineralized at either mesophilic (35°C) or thermophilic (50°C) temperature for composted samples with additional excess of carbon. These results indicated the addition of available carbon source would not lead to further removal of ^{14}C from the composted soil (based on the mineralization activity). Thus, biodegradable volatile solids depletion did not play a factor in the plateau of ^{14}C mineralization curves. It is therefore unlikely that resupplementing of carbon source would result in a more pronounced release of ^{14}C phenanthrene as $^{14}CO_2$. This suggests that at the plateau stage, the phenanthrene

becomes poorly available to the microorganisms. Therefore, one expects the residual remaining might not be available to induce toxicity. To evaluate the effect of compost treatment on soil toxicity, toxicity tests were performed on earthworms and plants. The results of toxicity tests are presented in the following section.

Table 5-7. Additional phenanthrene mineralized after resupplement of available carbon source. Means followed by common letter are not significantly different ($p \geq 0.05$).

Treatment Description	Phenanthrene Mineralization (%)	
	35°C Mean ±SE	50°C Mean ±SE
Composted sample plus fresh feedstock	6.98a ±0.16	7.64a ±0.11
Composted samples plus glucose	7.07a ±0.13	7.70a ±0.25
Composted sample alone	7.35a ±0.36	7.65a ±0.21

5.7 Experimental Results and Discussion for Phase 2B

Treatment of soil contaminated with diesel fuel by composting either at mesophilic or thermophilic temperature pattern produced a significant reduction in extractable diesel range organic and phenanthrene. However, whether the change in extractable levels and formation of bound residue due to composting treatment significantly reduced toxicity associated with soil, needs to be evaluated. To evaluate the efficacy of composting treatment to reduce toxicity associated with contaminated diesel soil, plant and earthworm toxicity assays were used. The results are summarized in the following sections.

5.7.1 Seed Germination Assay

The average percentages and plant growth in untreated and treated compost soil samples are shown in Table 5-8 and Figure 5-13, respectively. The percentages of germination for radish, cress and lettuce in the potting soil (PS, control) were 100%, 93% and 100%, respectively. All were greater than 90% as required by CCME (1996) protocol. From these results all three species appear to be suitable seeds for seed germination tests.

Table 5-8. Germination rate (%) of radish, cress and lettuce after 7 days. Means followed by common letter are not significantly different ($p \geq 0.05$).

Designation	Radish	Cress	Lettuce
	Treated Samples		
CCM	73a	26b	24c
CCT	27c	0	53b
CFT	7d	0	21c
UFT	99a	75a	58b
	Untreated Sample (Controls)		
PS	100a	93a	100a
UF	73a	0	50b
CF	23c	0	17c

The uncontaminated soil with fresh feedstock before treatment (UF) exhibited inhibition to seed germination compared to the PS control. The percentages of germination for radish, cress and lettuce in the UF were 73%, 0% and 50%, respectively (Table 5-8). However, the percentages of germination in the uncontaminated soil with fresh feedstock after treatment (UFT) for radish, cress and lettuce were 99%, 75% and 58%, respectively.

The difference in the germination percentages between treated and untreated samples were not significant using radish and lettuce. A significant difference was only detected in cress seeds. Cress appeared to be very sensitive to background levels of diesel range organics in the UF samples before treatment.

The contaminated soil with fresh feedstock before treatment (CF) showed inhibition to seed germination. The percentages of germination for radish, cress and lettuce were 23%, 0% and 17%, respectively. However, the percentages of germination in the contaminated soil after treatment at thermophilic temperature were 7% and 21% in the fresh feedstock treatment (CFT), and 27% and 53% in the finished compost treatment CCT), for radish and lettuce, respectively. No germination occurred in the cress seeds for both treated fresh feedstock and finished compost mixtures. This indicates that cress is the most sensitive to the diesel fuel contamination. In general, the percentages of germination for all the seeds did not differ significantly from the untreated CF samples. The results of radish plant weight, however, showed significant differences between untreated and treated samples (Figure 5-13).

The contaminated soil with finished compost mixture treated at the mesophilic temperatures (CCM) showed 73%, 26% and 24% percentages germination for radish, cress and lettuce, respectively. These data suggested that mesophilic treatment significantly reduced the toxicity of diesel fuel compared to thermophilic treatment. A significant difference between the treatments was only detected in radish and cress seeds. The results of radish plant weight, however, showed significant differences between all treatments (Figure 5-13). The accuracy of the seed germination was confirmed by testing

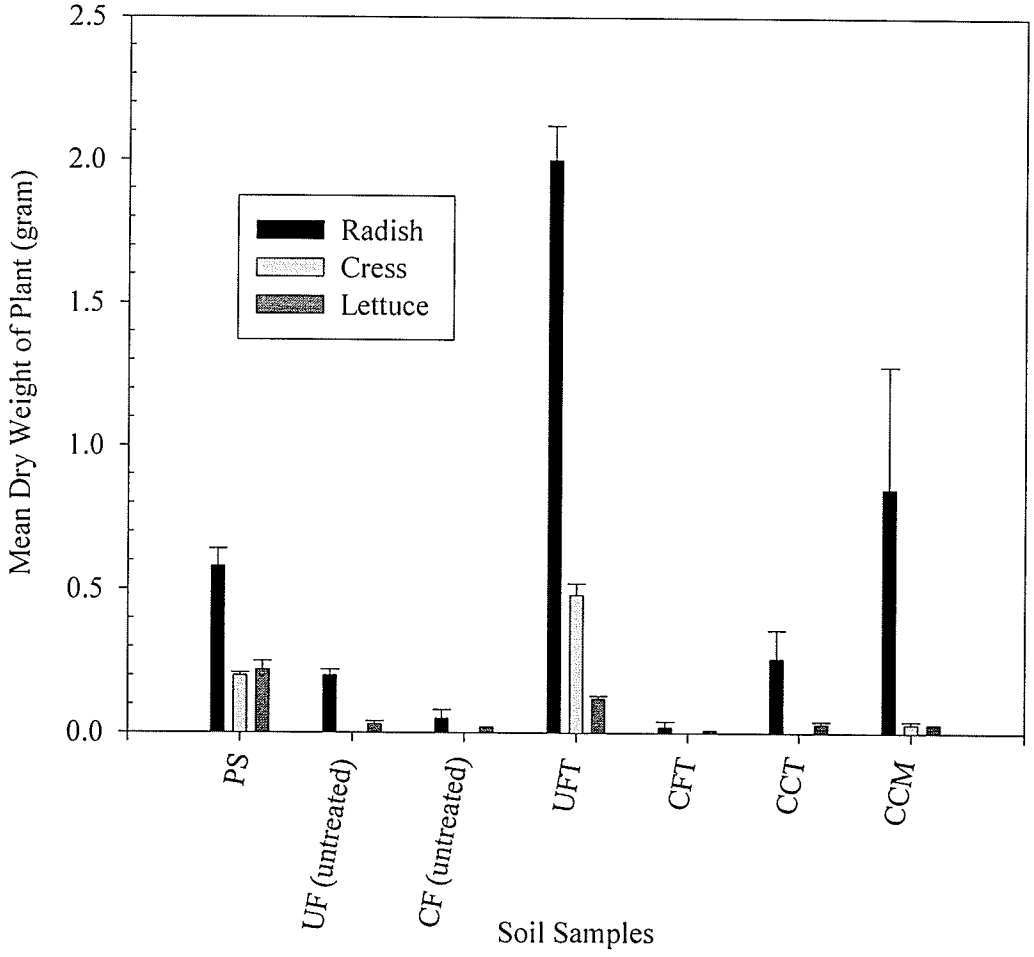


Figure 5-13. Mean dry weight of seeds grown in untreated and treated composted soil samples. Each point represents the mean of two replicates. The error bars show standard error.

radish seed again in the treatment and control samples.

Seed germination assays revealed mesophilic and thermophilic treatments did not eliminate the toxicity of diesel fuel even with more than 85% of target diesel fuel components (phenanthrene and EDRO) removed. Since the UFT control showed an indication of compost toxicity to plant species, particularly cress and lettuce seeds compared to the PS control, this may be evidence that other compost constituents besides the spiked diesel compound and its intermediate products were involved in plant toxicity. Further studies on the toxicity effect of compost constituents, such as heavy metals, are required to finally assess the composting of contaminated diesel fuel soil.

5.7.2 Earthworm Toxicity Assay.

No earthworms (*Lumbricus terrestris* species) survived in the contaminated or non-contaminated soil with either fresh feedstock or finished compost mixture. However, 100% survived in the potting soil control. Potter et al. (1999) observed that exposure of earthworms *L. terrestris and E. fetida* to 100% composted PAHs contaminated soil resulted in 50% mortality to both earthworms. Other studies by Sayles et al. (1999), Phillips et al. (2000) and Cook et al. (2002) found no mortality when *L. terrestris* and *E. fetida* were exposed to 100% composted PAHs and petroleum hydrocarbons contaminated soils. However, Fordham and Wilber (1992), in assessing the effect of composted sewage sludge on *L. terrestris*, observed a dose response relationship between mortality and sludge content in soil. They found all *L. terrestris* were dead within the first six days of their study when the sludge content in the soil was above 50%. Since the test samples from the uncontaminated soil with fresh feedstock (UFT) resulted in 100%

mortality of earthworms, this may be evidence that other compost constituents besides the spiked diesel compound and its intermediate products were involved in the toxicity. Further research needs to be carried out to better interpret the results.

5.8 Summary & Conclusions

The purpose of Phase 2 was to investigate the effect of co-substrates and operating temperatures on the performance of composting diesel fuel contaminated soil. The performance was determined by measuring the reduction in phenanthrene and extractable diesel range organics concentration (Phase 2A) and by assessing the treatment's ability to lower soil toxicity to plant and earthworm species (Phase 2B).

5.8.1 Phase 2A

The mineralization of phenanthrene was significantly enhanced by the addition of either fresh feedstock or finished compost. However, finished compost at the mesophilic temperatures resulted in 42% mineralization rates of phenanthrene, which was approximately 70% higher than the thermophilic temperatures.

The amount of extractable phenanthrene in contaminated soil with no co-substrate addition remained unchanged (68%) during the 18-week experimental run. However, the amount of extractable phenanthrene in contaminated soil with co-substrate addition decreased to less than 11% at the end of the experiment. The fresh feedstock was the best material for removal of phenanthrene from the extractable phase compared to the finished compost material.

The concentration of EDRO remaining in the contaminated soil with no co-substrate addition was 8,200 mg kg^{-1} ash over the 18-week period. Over the same time period, the EDRO concentration remaining in the contaminated soils with co-substrate ranged from 1,418 to 4,208 mg kg^{-1} ash. The EDRO remaining in soil fresh feedstock at thermophilic temperature and finished compost at mesophilic temperature were below the acceptable level mandated by the Manitoba clean-up guidelines for residential land usage.

Since the finished compost at the mesophilic temperature showed higher mineralization rate of phenanthrene, and residual EDRO below the clean-up guidelines for residential land usage, this study suggested the use of finished compost and mesophilic temperature for diesel fuel bioremediation.

5.8.2 Phase 2B

The seed germination assay found that the soil composted at the mesophilic temperatures decreased the toxicity effect on radish species. However, the seed germination assays found that the soil composted at the thermophilic temperatures did not decrease the toxicity effect on any of the species tested. The earthworm assay showed no survival of *L. terrestris* in both contaminated and uncontaminated composts. This may be evidence that other compost constituents besides the spiked diesel compound and its intermediate products have caused the toxicity.

6. Overall Summary and Conclusions

The main objective of the study was to evaluate the potential of utilizing composting, a substrate dense, self-heating waste treatment method, as a technique for the bioremediation of diesel fuel contaminated soil. The study was divided into two major phases.

The first phase investigated the effect of soil loading and texture on the microbial performance during the active (thermophilic) phase of composting of diesel fuel contaminated soil. The thermophilic microbial activity was simulated using bench scale (28 L) reactors under controlled temperature and aeration conditions. As a measure of composting microbial activity, the relative heat generation and volatile solids removal were measured. The major findings of the phase one were as follows:

1. The results of the relative heat generation values indicated that a change in microbial activity may have occurred at sand and silt loads above 40% and 20%, respectively.
2. The volatile solids reductions observed for feedstock mixed with sand and silt were 18% and 16%, respectively. However, the reduction in volatile solids was poorly correlated to soil loads. Also, the volatile solids reduction observed did not correspond with the relative heat generation values observed. Sample variability may have played an important role in these observations.
3. Microbial activity, as measured by the relative heat generation and volatile solids removal, was not affected by sand loads tested and low clay load. However, microbial activity was affected at higher clay soil load (40%).

In the second phase, the effect of co-substrates and operating temperatures on composting of diesel fuel contaminated soil was determined by measuring the reduction in target concentrations of phenanthrene and extractable diesel range organics and by assessing the treatment's ability to lower soil toxicity. The mineralized and extractable fractions of ^{14}C labeled phenanthrene, and the extractable diesel range organics were determined over the 126-day experimental period. Volatilization of phenanthrene and diesel range organics were determined at the end of experiment. Two toxicity bioassays, plant and earthworm, were used to evaluate the efficacy of compost treatment to reduce toxicity associated with contaminated diesel soil. The major findings of this phase were as follows:

1. While no mineralization of phenanthrene was detected in the soil with no co-substrate, phenanthrene mineralization was enhanced by 100% with the addition of either fresh feedstock or finished compost co-substrates.
2. While fresh feedstock and finished compost at the thermophilic temperature resulted in similar mineralization rates of phenanthrene (25%), finished compost at the mesophilic temperatures resulted in 42% mineralization rate of phenanthrene, which is 70% higher than with the thermophilic temperature.
3. Extraction of ^{14}C was 1% for fresh feedstock and 5% for finished compost at the thermophilic temperature. For finished compost at the mesophilic temperature, extraction of ^{14}C was 11% of the total added at the end of experiment.
4. The finished compost at the mesophilic temperature (CCM) showed a lower tendency for bound residue (47%) formation and a higher mineralization rate

(42%) compared to other treatments at the thermophilic temperature. This indicated that phenanthrene degraders were likely more active at mesophilic temperatures compared to their thermophilic counterparts.

5. The reduction of extractable diesel fuel concentration was significantly enhanced regardless of the kind of co-substrates and operating temperatures. The concentration of extractable diesel range organic at the end of the 126-day experiment was 8,200 mg kg^{-1} ash for the soil with no co-substrate while for the soil with co-substrate it ranged from 1418 mg kg^{-1} ash to 4208 mg kg^{-1} ash. In general, when the results were reported based on the total solids rather than ash content, the soil mixed with fresh feedstock at the thermophilic temperature or finished compost at the mesophilic temperature showed extractable diesel range organics below the acceptable levels of 2000 mg kg^{-1} mandated by the Manitoba clean-up guidelines for residential land usage.
6. The seed germination assay found that the soil composted at the mesophilic temperature decreased the toxicity effect on radish species. However, the seed germination assays found that the soil composted at the thermophilic temperature did not decrease the toxicity effect on any of the species tested.
7. The earthworm assay showed no survival of *L. terrestris* in both contaminated and uncontaminated composts. This may be evidence that other compost constituents besides the spiked diesel compound and its intermediate products have caused the toxicity.

The following conclusions can be made from the above studies:

1. Thermophilic microbial activity affected differently by different soil load and texture. As such, soil load and texture must be considered in any compost study to maintain a maximum level of microbial activity during the active phase of composting of diesel fuel contaminated soils.
2. While further work is required to perfect finished compost as a novel co-substrate for other diesel fuel components, this study showed that finished compost and mesophilic temperature were a superior combination for phenanthrene mineralization.
3. Fresh feedstock proved to be a good co-substrate for removal of diesel fuel organics from an extractable fraction to a level below the clean up standard required by the Manitoba government for residential land use. As such, fresh feedstock could be used as an alternative to finished compost where cost or availability is a constraint.

7. Recommendations for Future Work

This study demonstrated that both fresh feedstock and finished compost played a role in composting bioremediation of diesel fuel contaminated soil. However, the finished compost and mesophilic temperature would be a superior combination for mineralization of phenanthrene contained in diesel fuel. Is it possible that trend could be noticed with other contaminants in diesel fuel as well as from addition of mature compost from other sources? Future investigation is needed to extend the mineralization potential to other compost types and diesel fuel compounds as well.

Since only phenanthrene compound contained in diesel fuel was examined within this work and there are hundreds of other compounds in diesel fuel that could require characterization in regards to bioremediation, it should not be assumed that one treatment temperature is the most appropriate for all bioremediation scenarios. Also, because elevated temperatures are the hallmark of composting, the role of thermophilic temperature with other diesel fuel target compounds should receive further investigation.

As seen within this work, composting treatment was highly effective in eliminating diesel fuel target compounds. However, the toxicity measurements by earthworm and seed germination assays indicated that there was still toxicity associated with compost matrix. Further research is needed to establish the following:

Although evidences of the ability of plants and earthworms to uptake heavy metals are numerous in the literature (Barrera et al. 2001; Cook et al. 2002), some heavy metal concentrations in the municipal biosolids used in this work may have

been in much higher concentrations than necessary to induce toxic effect. Therefore, one should consider the use of other substrates to better interpret the toxicity of diesel fuel after composting. Also, because the differences of gut morphology and functionality, metabolic patterns and specific detoxifying systems may influence bioaccumulation efficiency for a given metal between earthworm species (Morgan and Morgan, 1992; Eijsackers, 1998), one should consider the use of more than one species.

As highlighted within this work, composting treatment was able to mineralize phenanthrene at mesophilic and thermophilic temperatures. It is unknown as to whether degradation is performed entirely by a single organism, or if a consortium of many organisms is responsible. Degradation pathways of phenanthrene by some organisms accumulated considerable amounts of metabolites without further degradation. Some of the phenanthrene metabolites, such as 1-hydroxy-2-naphthoic acids, have been reported to be toxic to the phenanthrene degraders themselves and to the environmental microflora in general (Balashova al. 1999). Certainly, comprehensive studies should be made to identify phenanthrene metabolites and their toxicity under both mesophilic and thermophilic conditions.

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Appendices (A through D)

Appendix A

Effect of Compaction on Free Air Space (FAS)

A. Effect of Compaction on FAS

Composting systems need to keep enough porosity and FAS in the co-substrate materials for aeration. Municipal biosolids, used in this study as co-substrate material, has a high moisture content (greater than 70%). The use of this material as substrate in composting becomes impractical since sufficient air can not penetrate the compost matrix, either by diffusion or forced aeration, and that may impact microbial activity. To provide aerobic conditions, woodchips, as a bulking agent, was added to the municipal biosolids to provide structural support for wet co-substrate and create free air space within the voids between the particles.

In compost pile, vertical loads increases with depth due to self-loading caused by the weight of the compost material. The vertical loads results in the commonly observed phenomenon of settlement of the compost material. This settlement or compaction is the result of the loss of free air space (FAS). To estimate the effect of compaction on FAS of co-substrate material, a bench-scale apparatus was used to determine the FAS at pressure of 5.6 kPa, which simulated the compressive load at a vertical depth of 1.05m in a compost pile. The compressive load was applied to the co-substrate mixture (biosolids and woodchips) in incremental mass loads of 0, 9.7, 19.4, and 29 kg. The change in FAS with loading was measured by observing the height change of co-substrate mixture. The change in FAS with time is shown in Figure A-1. From Figure A-1, it can be seen that most of the maximum compressive effect occurred in the first one hour. The change of FAS of co-substrate mixture with loading is presented in Figure A-2. The resulting final FAS value was above the minimum FAS (20 %) suggested by Haug (1993) for windrow composting systems. Detailed steps followed for compressive settlement test and the

calculations used to determine the compressive load discussion can be found in McCartney and Chen (2001).

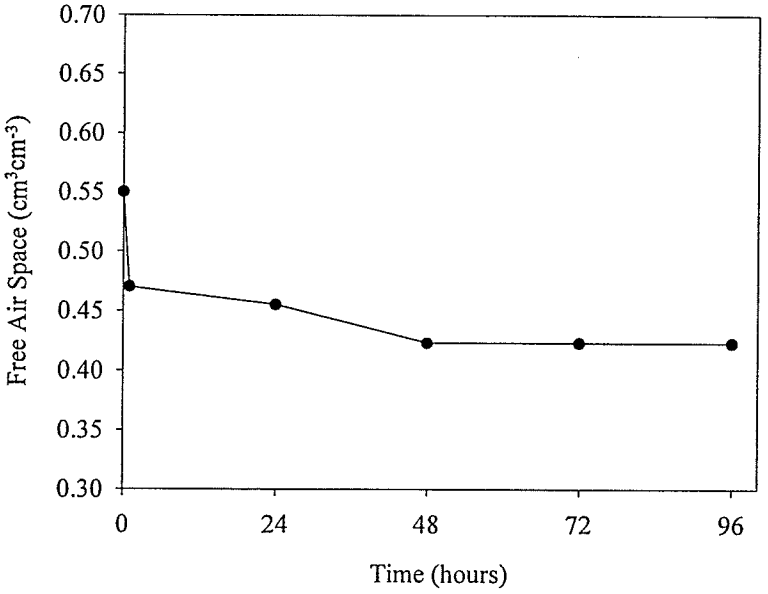

Figure A-1. Change of free air space within feedstock mixture with time.

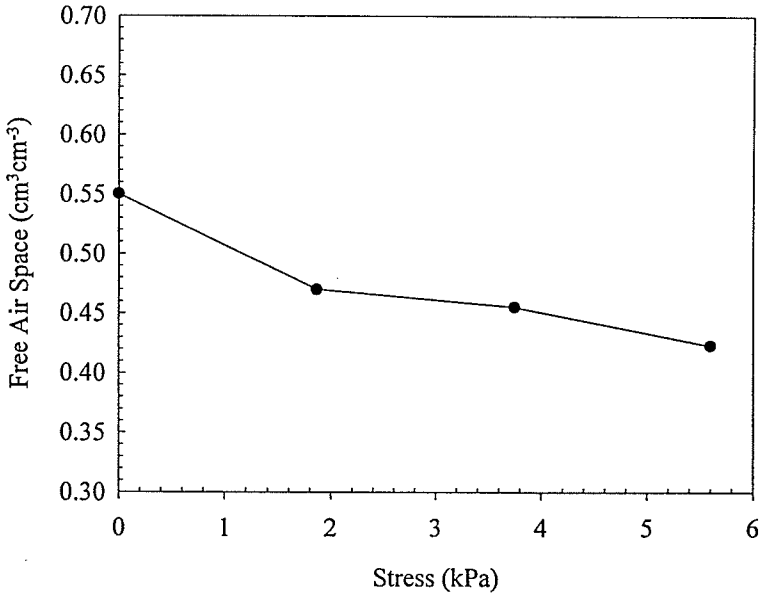

Figure A-2. Effect compressive stress on free air space within feedstock mixture.

Appendix B

Method for Compost Sampling

B. Method for Compost Sampling

B-1 Problem Statement

The material sampling and analysis used for VS determination from Phase 1A was reviewed. In most cases, the variability was high (see Figure 4-7 & 4-8). The lack of trend may have resulted from the sample variability. The spatial VS variability of the material needs to be determined. Discrete samples with a large enough volume may eliminate this problem. If the discrete samples are used to form one composite sample, then quartering must be used to obtain a volume that is small enough to be analyzed. Thus, the same problem may occur again. For these reasons, a random sampling method with discrete samples will be employed for initial and final VS determination.

B-2 Objective

The objective of this study was to determine the volatile solids variability in feedstock materials.

B-3 Sampling Theory

The most critical element in a program designed to evaluate the physical and chemical properties of a solid waste is the plan for sampling the waste. An appropriate sampling plan can be developed once the objectives have been clearly identified. Sampling objectives will assist in development of proper sample collection, in identifying sampling interval and sample size, and the end use of sample data (U.S. EPA, 1986; U.S. Department of Agriculture and U.S. Composting Council, 2000). If the objective of sampling of waste is for process design and monitoring, specific sampling procedures for

the parameters of interest should be designed. For example, if the mass of VS is considered a very important parameter, then very low variations in sample VS determination become a major consideration and a sample collection plan must be designed to support to this requirement.

A sound sampling plan requires that representative samples of waste to be collected from appropriate sampling locations which exhibit average properties of the whole waste and enough samples be collected o represent the variability of the waste. These two factors are responsible for ensuring the sampling accuracy. If the measurements are sufficiently accurate and precise, they will be considered reliable estimates of the properties of the waste (U.S. EPA, 1986; U.S. Composting Council, 2000).

Sample collection techniques and variability of compost also affect the relative accuracy of sampling and the reliability of laboratory analytical determinations. Failure to adjust sampling plan according to the nature and source of variations may invalidate test results. The potential key sources that may result in significant error or variability in the test results includes: 1) inaccurate sample collection is due to systematic or intentionally selective sampling introduced by the sampler, and 2) sample heterogeneity (U.S. Composting Council, 2000). The key sources of heterogeneity that give rise to significant sampling error are as following:

1. Sample size affects sampling accuracy. Large samples produce results with less variability.

2. Poor initial mixing effects the variability of the parameters throughout the composting process. Complete and thorough mixing throughout the composting process improve the quality and ease of sampling.
3. Soil and stones are frequently picked up during routine compost production operations. In some cases, sampler may introduce error by deliberately excluding gravel and stones present in compost. Sampler should make note of how sample manipulated.
4. In municipal solid waste where large and variable amount of foreign and non-compostable matter are present always poses a problem to the sampler, and the laboratory. The best approach is to take large samples and blend frequently before testing.
5. Varying particle size is one of the most common sources of sample variability.
6. Layering, compaction and gradients of composts arise as a result of inadequate initial mixing, infrequent or excessive turning/mixing during feedstock preparation, or during composting process because of inappropriate selection and use of bulking materials.

Sampling accuracy is usually achieved by some form of random sampling and by taking an appropriate number of samples from the population. Maximizing the physical size of the samples (weight or volume) also increases sampling accuracy (U.S. EPA, 1986; U.S. Composting Council, 2000).

Simple random sampling can be used if the waste is heterogeneous with regard to its chemical characteristics and random heterogeneity remains constant from batch to batch.

In simple random sampling, all locations or points in all batches of waste has a theoretically equal chance of being sampled and measured. Consequently, statistics generated by the sample (e.g. sample mean and standard deviation) are unbiased estimators of true population parameters (U.S. Composting Council, 2000). The following section identified the general procedures for simple random sampling adapted from U.S. EPA (1986).

B-4 Simple Random Sampling General Procedures

1. Obtain preliminary estimate of mean of volatile solids measurements from the previous data.

$$x^{-} = \frac{\sum x_i}{n} \qquad (1)$$

$\bar{x}$ = mean of volatile solids measurements
n = total number of volatile solids samples
x_i = volatile solids value for individual samples.

2. Obtain preliminary estimate of variance for volatile solids measurements from the previous data.

$$s^2 = \frac{\sum x^2 - \left(\sum x_i\right)^{2/n}}{n-1} \qquad (2)$$

where:
s^2 = variance of simple random sample of volatile solids,
n = total number of volatile solids samples,
$\sum x_i$ = sum of all volatile solids results.

3. Estimate the appropriate number of samples to be collected from the compost for volatile

   solids measurements

$$n = \frac{s^2 t^2}{\Delta^2} \qquad (3)$$

Where:

n = total number of volatile solids samples,

s^2 = variance of simple random sample of volatile solids,

t^2 = tabulated "t" value for confidence level chosen,

Δ = variability from the mean that can be tolerated for the chosen confidence level.

4. Randomly collect the estimated number of samples from the compost. Be sure to maximize the physical size of all samples that are collected.

5. Analyze the samples for volatile solids.

6. Calculate the mean ($\bar{x}$), sample variance (s^2), standard deviation (s), and standard error (s_x) for new volatile solids results by equation 1, 2, 4, and 5.

$$s = \sqrt{s^2} \qquad (4)$$

$$s_x = \frac{s}{\sqrt{n}} \qquad (5)$$

7. Determine the variability from the mean (Δ) for the particular confidence limit chosen.

$$\Delta = \sqrt{\frac{s^2 \times t^2}{n}} \qquad (6)$$

8. Re-estimate the total number of samples (n) to be collected from the compost using the newly calculated values of $\bar{x}$ and s^2.

B-5 Sampling Methodology

For this study, we chose to sample two categories of materials: fresh feedstock and finished compost. The fresh feedstock consists of two raw materials, municipal biosolids and woodshavings. Finished compost consists of the two raw materials and clay soil that had been thermophilically composted for 14 days. Since we are dealing with heterogeneous material, substantial error of compost analytical data may occur. To minimize the potential sampling error:

1. Simple random sampling" was used to collect appropriate number of samples from both the fresh feedstock and finished compost. The number of samples were collected from the both mixtures was estimated using the existing data on the physical parameter measured previously that has the greatest standard deviation. The parameters measured are total solids (TS) and volatile solids (VS). Volatile solids data was selected because standard deviation observed from analysis of compost produced was higher than that observed with TS analysis. Table B-1 shows a set of volatile solids data analysis from Phase 1A that had the highest standard deviation. This data was chosen to calculate the appropriate number of samples to be employed in VS analysis of fresh feedstock and finished compost.

Table B-1. The Final volatile solids analysis of fresh feedstock mixed with silt

Representative Sample No.	*Percent Volatile Solids*
1	47.899
2	44.068
3	49.921
4	54.579
5	54.614

The method used to determine the minimum number of samples required achieving a certain level of precision at a desired confidence level is described in section B-4. For the purpose of this study, a confidence level of 95% and variability of 5% from the mean was considered. The following table (Table B-2) illustrates the preliminary estimates of mean ($\bar{x}$) and sample variance (s^2) for VS analysis. These preliminary statistical analyses were used to calculate the appropriate number of samples to be employed in VS analysis of compost. The resulting minimum number of samples was 20.

Table B-2. Preliminary statistical values used to estimate the minimum number of samples

Property	$\bar{x}$	S	s^2	s_x	N
Volatile Solids (%)	50.216	4.041	16.33	1.807	20

2. Once the number of the sample required be estimated, the soil mixed with fresh feedstock or finished compost was thoroughly mixed. The completely mixed material (28L-reactor volume) was spread onto clean plastic sheet, approximately square, to a depth of approximately 2cm (Figure B-1a).

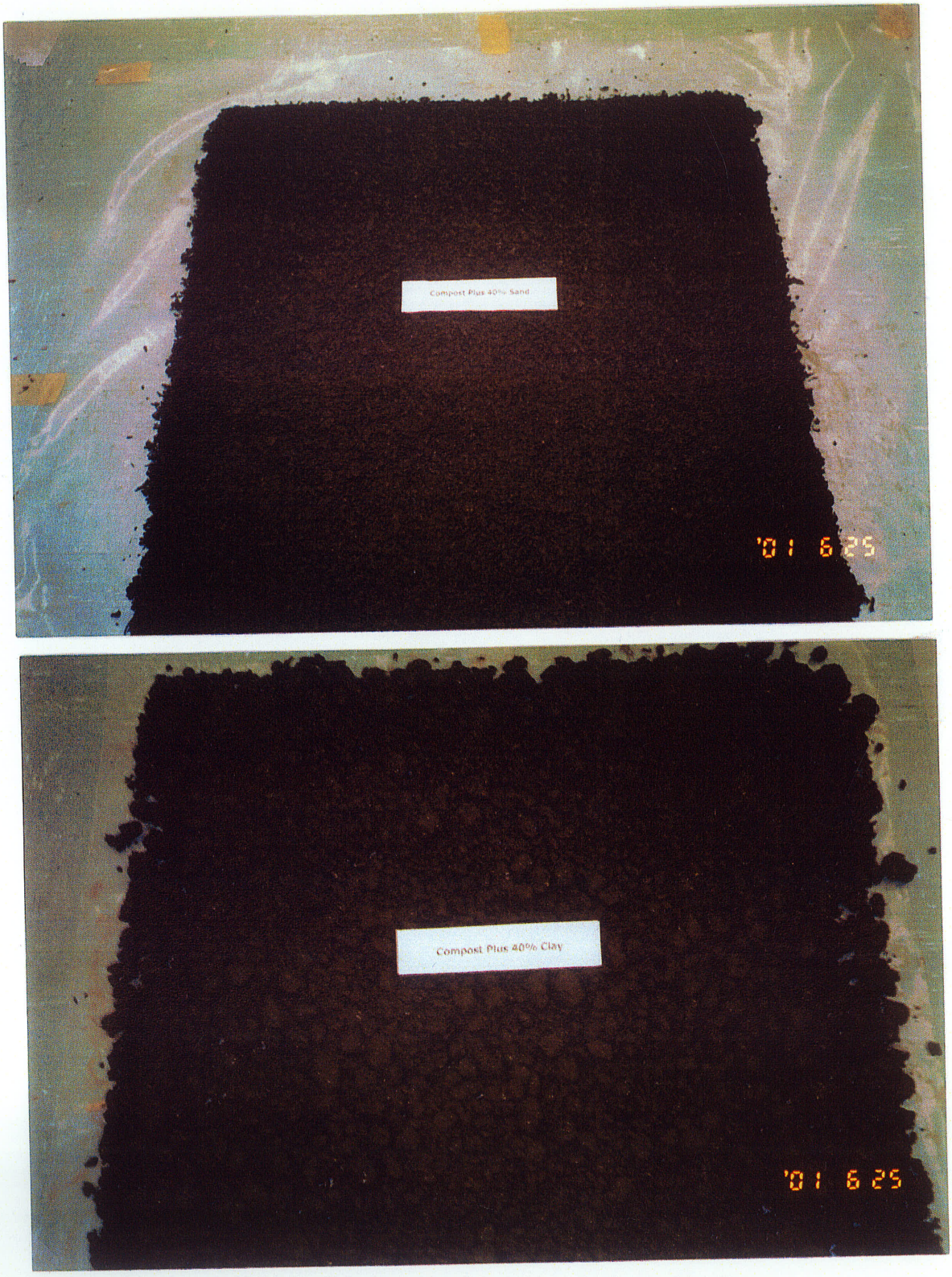

Figure B-1a. Preparation of composted soil for sampling

Figure B-1b

3. A plastic frame, divided into a grid with each grid size equal to size 7.5cm x 7.5cm, was placed on top of the entire compost (Figure B-1b). The grid sections were assigned numbers from 1 to100.
4. The sampling points (20 samples) were randomly selected using the random number generator in Corel Quattro Pro.
5. The representative samples collected in the grid chosen were placed in plastic bags and stored at 4°C until are ready for further analysis.
6. The samples were then analyzed for TS and VS.
7. The new value of mean ($\bar{x}$) and sample variance (s^2) were calculated for the new set of data.
8. The variability from the mean for chosen confidence level was determined.
9. The number of samples required for future testing were determined again using the newly calculated value of $\bar{x}$, s^2 and Δ.

B-6 Results and Discussion

Tables B-3 and B-4 present the statistical values of fresh feedstock and finished compost from Phase 1A.

Table B-3. Statistical values of compost feedstock

Run No.	Parameters	$\bar{x}$	s	s^2	s_x	Δ
1	TS (%)	44.949	0.166	0.027	0.037	0.06
	VS (%)	66.493	0.347	0.120	0.077	0.13
2	TS	44.981	0.378	0.143	0.084	0.15
	VS (%)	66.252	0.622	0.387	0.139	0.24

Table B-4. Statistical values of finished compost

Run No.	Parameters	$\bar{x}$	s	s^2	s_x	Δ
1	TS (%)	52.984	0.917	0.840	0.205	0.35
	VS (%)	35.508	0.360	0.129	0.080	0.14
2	TS (%)	52.790	0.416	0.173	0.093	0.16
	VS (%)	35.777	0.543	0.295	0.121	0.21

The analysis of randomly selected samples for TS and VS at a 95% confidence level resulted in values within 0.06 to 0.35 of the mean. This sampling technique showed a greater degree of accuracy relative to the quartering sampling performed in the previous phase of the research.

Based on the preliminary statistical values, the required minimum number of samples at 95% confidence was 20. Using the newly existing statistical data the number of samples required for analyses was estimated. The number of samples is dependent on the variability we are willing to accept in the mean. The following table (Table B-5) illustrates the number of samples required for further testing of volatile solids at a 95% confidence interval using the newly calculated value of mean and sample variance.

Table B-5. Number of samples required for further Testing of Compost

Parameter	Δ= 1%	Δ=2%	Δ=3%
TS	3	1	-
VS	9	3	1

Choosing a value of 3 representative samples to be used for further testing of compost

will result in a 95% confident that our value of total and volatile solids will be within 2% of the mean.

B-7 Summary & Conclusions

The objective of this study was to determine the volatile solids variability in compost. To do this, a simple random sampling plan was designed to collect representative samples from both fresh feedstock and finished compost.

The analysis of randomly selected samples for TS and VS resulted in values of low variability from the mean ($\leq 1\%$). Using the new statistical data, the required minimum number of samples to be used for further testing of compost at a 95% confidence level was 3. However, the number of samples required for analyses are dependent on the variability we are willing to accept in the mean.

In conclusion, 10 randomly collected samples should be used for all future analyses for these specific feedstock materials. These randomly collected samples should be used to form one composite sample, then quartering must be used to obtain one sample from each composite sample.

B-8 References

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U.S. EPA 1986. Test methods for evaluating solid waste: physical and chemical methods, Vol. 2, EPA SW-846, Chapter 9.

Appendix C

Experimental Results of Phase 1

Phase 1A

Appendix C-1: Reactor temperature & relative heat generation data

Appendix C-2: Volatile solids and percent removal data

Appendix C-3: Determination of variability in VS results

Phase 1B

Appendix C-4: Reactor temperature & relative heat generation

Appendix C-5: Volatile solids and percent removal data

Appendix C-1: Reactor temperature & relative heat generation data

Table C-1. Reactor temperatures durig the composting of fresh feedstock with various sand loads.

Sand load	Time (day)													
%	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Temperature (oC)														
0	20.1	50.5	57	57.3	55.4	52.4	52.5	52	51	50.7	50.6	50.4	50.2	50.1	49.9
4	19.3	51.1	52.4	60.3	59.2	57	55.5	53.6	52.2	51.7	51.6	51.5	51.4	51.3	50.4
6	20.1	47.7	60.7	61	59.2	55.1	54.4	54.1	53.1	52.4	52.1	51.7	51.2	50.9	49.9
8	20.4	49.5	56.9	57.8	56.6	53.6	52.9	52.5	51.4	51.1	51	50.8	50.5	50.3	49.5
chamber		46.5	46.5	46.5	46.5	46.8	47	46.7	46.7	46.7	46.7	46.7	46.9	46.9	46.9
10	21.1	50	60.3	60.2	58.6	53.5	51.4	50.9	50.8	51.2	50.4	50.6	50.6	50.3	50.1
12	21.2	49.6	56	57.9	57.3	53.5	52.7	51.8	51.1	51	50.3	50.3	50.3	50.1	50
14	21.2	50.4	62.7	60.2	55.8	52	50.9	50.8	50.8	51.2	50.7	50.6	50.5	50.3	49.9
16	21.2	50.2	57.1	57.1	55.6	52.5	51.3	50.6	50.3	50.5	49.7	49.5	49.5	49.4	49.4
chamber		46.8	46.8	46.8	46.8	46.8	46.8	47	46.5	46.7	46.7	46.7	46.9	46.9	46.9
18	19	50	58.2	57.7	57.6	56.1	54	52.2	51.5	50.9	50.7	50.7	50.6	49.9	49.5
20	18.2	49.6	58.2	57.6	58.1	57.2	55.5	53.3	52.2	51.4	51	50.5	50.5	49.9	49.6
22	18	50.4	57.6	57.3	57	55.7	53.8	51.9	51	50.3	50	49.9	49.9	49.5	49.3
24	18.3	50.2	58.5	56.8	56.5	54.9	52.7	51	50.6	49.9	49.9	49.9	49.6	49.8	49.6
chamber		46.6	46.6	46.4	46.6	46.8	46.7	46.8	46.9	46.6	46.8	46.7	47	46.9	46.9
26	18.8	51.2	58.8	58	55.6	53.7	51.8	51.4	50.5	50.4	50.4	50.2	50.3	50.3	50.2
28	18	51.6	59.8	58.3	56.4	54	52.6	52	51.7	51.5	51	50.9	50.4	49.8	49.6
30	18.2	51.2	58.4	56.9	55	53.2	52.2	51.6	51.4	51	50.4	50.1	49.6	49.4	49.3
chamber		46.5	46.5	46.6	46.7	46.8	46.8	46.8	46.8	46.6	46.6	46.7	46.6	46.9	46.9
36	19.5	48.3	55.6	57	55.6	54.5	52.5	52.4	52	51	50.9	50.8	50.4	50.3	50.1
40	19.4	47.9	56.2	58.5	56.8	54.8	52.7	52.5	52.1	51.2	51	50.9	50.5	50.3	50
44	19.4	47.4	55.5	57.5	55.8	54	52.3	52.1	51.8	50.9	50.7	50.6	50.3	50	49.7
48	20.2	47	53.7	55.6	54.4	53	51.9	51.7	51.3	50.4	50.2	50.1	49.7	49.5	49.4
chamber		45.6	45.6	46.4	46.6	46.5	46.9	46.9	46.9	46.6	46.7	46.6	46.6	46.6	46.7
52	19	44.2	53.5	54.1	53.5	53.1	52.4	51.7	50.8	50.4	50.3	50.2	50.2	49.9	49.9
56	18.2	44	53.5	52.8	53.7	53.5	52.8	52	51.2	50.5	50.4	50.2	50.1	49.8	49.8
60	18.8	44.1	52.8	52.3	53.2	53	52.4	51.7	50.9	50.4	50.2	50	49.9	49.7	49.7
64	18	44.2	52.3	52.7	52.4	52.3	51.4	50.7	50	49.7	49.6	49.6	49.5	49.4	49.1
chamber		47	47.4	47	46.2	46.5	46.5	46.6	46.6	46.7	46.8	46.7	46.7	46.6	46.5

Table C-2. Relative heat generation values during composting of fresh feedstock with various sand loads.

Sand load	Time (day)														
%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	Relative Heat Generation, RHG (Degree Celcius-Day)														T. RHG
0	4	10.5	10.8	8.9	5.6	5.5	5.3	4.3	4	3.9	3.7	3.3	3.2	3	76
4	4.6	5.9	13.8	12.7	10.2	8.5	6.9	5.5	5	4.9	4.8	4.5	4.4	3.5	95.2
6	1.2	14.2	14.5	12.7	8.3	7.4	7.4	6.4	5.7	5.4	5	4.3	4	3	99.5
8	3	10.4	11.3	10.1	6.8	5.9	5.8	4.7	4.4	4.3	4.1	3.6	3.4	2.6	80.4
10	3.2	13.5	13.4	11.8	6.7	4.6	3.9	4.3	4.5	3.7	3.9	3.7	3.4	3.2	83.8
12	2.8	9.2	11.1	10.5	6.7	5.9	4.8	4.6	4.3	3.6	3.6	3.4	3.2	3.1	76.8
14	3.6	15.9	13.4	9	5.2	4.1	3.8	4.3	4.5	4	3.9	3.6	3.4	3	81.7
16	3.4	10.3	10.3	8.8	5.7	4.5	3.6	3.8	3.8	3	2.8	2.6	2.5	2.5	67.6
18	3.4	11.6	11.3	11	9.3	7.3	5.4	4.6	4.3	3.9	4	3.6	3	2.6	85.3
20	3	11.6	11.2	11.5	10.4	8.8	6.5	5.3	4.8	4.2	3.8	3.5	3	2.7	90.3
22	3.8	11	10.9	10.4	8.9	7.1	5.1	4.1	3.7	3.2	3.2	2.9	2.6	2.4	79.3
24	3.6	11.9	10.4	9.9	8.1	6	4.2	3.7	3.3	3.1	3.2	2.6	2.9	2.7	75.6
26	4.7	12.3	11.4	8.9	6.9	5	4.6	3.7	3.8	3.8	3.5	3.7	3.4	3.3	79
28	5.1	13.3	11.7	9.7	7.2	5.8	5.2	4.9	4.9	4.4	4.2	3.8	2.9	2.7	85.8
30	4.7	11.9	10.3	8.3	6.4	5.4	4.8	4.6	4.4	3.8	3.4	3	2.5	2.4	75.9
36	2.7	10	10.6	9	8	5.6	5.5	5.1	4.4	4.2	4.2	3.8	3.7	3.4	80.2
40	2.3	10.6	12.1	10.2	8.3	5.8	5.6	5.2	4.6	4.3	4.3	3.9	3.7	3.3	84.2
44	1.8	9.9	11.1	9.2	7.5	5.4	5.2	4.9	4.3	4	4	3.7	3.4	3	77.4
48	1.4	8.1	9.2	7.8	6.5	5	4.8	4.4	3.8	3.5	3.5	3.1	2.9	2.7	66.7
52	-2.8	6.1	7.1	7.3	6.6	5.9	5.1	4.2	3.7	3.5	3.5	3.5	3.3	3.4	60.4
56	-3	6.1	5.8	7.5	7	6.3	5.4	4.6	3.8	3.6	3.5	3.4	3.2	3.3	60.5
60	-2.9	5.4	5.3	7	6.5	5.9	5.1	4.3	3.7	3.4	3.3	3.2	3.1	3.2	56.5
64	-2.8	4.9	5.7	6.2	5.8	4.9	4.1	3.4	3	2.8	2.9	2.8	2.8	2.6	49.1

Table C-3. Reactors temperatures durig composting of fresh feedstock with various silt loads.

Silt load	Time (day)														
(%)	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	Temperature (oC)														
0	22.7	53.4	59.5	56.8	53.9	51.4	50.7	50.9	50.9	50.6	50.6	50.1	49.9	49.9	49.5
4	23.3	53.5	59.5	56.8	54.3	51.7	51.3	51	50.3	50.1	50	49.8	49.6	49.5	49.5
8	25.2	55	60.5	57.7	54.6	52.4	51.5	51.1	50.9	50.9	50.7	50.3	50	49.6	49.5
Chamber	27.5	46.7	46.7	46.5	46.7	46.7	46.7	46.7	46.8	46.7	46.8	46.8	46.6	46.8	46.9
12	22.7	48.1	59.4	58.4	56.5	53.5	52.3	51.5	51.1	50.9	50.7	50.4	50.3	50	49.7
16	24	49.3	59.1	57.7	55.6	52.9	51.8	51.3	51	50.7	50.4	50.1	49.8	49.5	49.3
Chamber	27.5	46.5	46.8	46.6	46.8	46.7	46.7	46.7	46.5	46.7	46.7	46.7	47	46.6	46.8
20	18.9	48.3	55.2	58	57.4	56.5	53.9	51.1	50.5	50	49.8	49.8	49.6	49.6	49.6
24	19.1	48.6	55.7	58	57.2	55.4	53.1	51.9	51.4	51	50.8	50.5	50	49.9	49.9
28	18.8	48	54.7	57.2	55.6	53.3	51.1	50.3	50.3	50	49.8	49.6	49.6	49.6	49.6
32	19.3	47.9	53.5	57	55.4	53	51.3	50.5	50.1	50	49.7	49.6	49.6	49.6	49.7
Chamber	22.6	46.5	46.5	46.4	46.7	46.8	46.8	46.8	46.8	46.8	46.6	46.6	46.6	49.6	46.5
36	21	48.8	53.5	57.7	54.5	53.4	50.6	50.6	50.5	50.5	50.7	50.7	50.4	50.3	49.9
40	20	48.6	55.4	57.4	56.3	54.1	50.8	50.8	50.6	50.5	50.4	50.5	50.4	50	49.8
44	20	48.5	55	57.2	56.4	54.5	50.8	51.1	50.8	50.7	50.8	50.7	50.5	50.3	49.8
48	21.2	48.7	55.8	56.7	55.2	53.1	50.8	50.2	50.2	50.2	50.2	50.3	50.1	49.8	49.5
Chamber	25.9	47.1	47.1	47	46.9	46.9	46.9	47.1	47.1	47.1	47.4	47.3	47.3	46.8	47.2

Table C-4. Relative heat generation values during composting of fresh feedstock with various silt loads.

Silt load	Time (day)													
%	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
	Relative Heat Generation, RHG (Degree Celcius,Day)														Total RH
0	6.7	12.8	10.3	7.2	4.7	4	4.2	4.1	3.9	3.8	3.3	3.3	3.1	2.6	74
4	6.8	12.8	10.3	7.6	5	4.6	4.3	3.5	3.4	3.2	3	3	2.7	2.6	72.8
8	8.3	13.8	11.2	7.9	5.7	4.8	4.4	4.1	4.2	3.9	3.5	3.4	2.8	2.6	80.6
12	1.6	12.6	11.8	9.7	6.8	5.6	4.8	4.6	4.2	4	3.7	3.3	3.4	2.9	79
16	2.8	12.3	11.1	8.8	6.2	5.1	4.6	4.5	4	3.7	3.4	2.8	2.9	2.5	74.7
20	1.8	8.7	11.6	10.7	9.7	7.1	4.3	3.7	3.2	3.2	3.2	3	0	3.1	73.3
24	2.1	9.2	11.6	10.5	8.6	6.3	5.1	4.6	4.2	4.2	3.9	3.4	0.3	3.4	77.4
28	1.5	8.2	10.8	8.9	6.5	4.3	3.5	3.5	3.2	3.2	3	3	0	3.1	62.7
32	1.4	7	10.6	8.7	6.2	4.5	3.7	3.3	3.2	3.1	3	3	0	3.2	60.9
36	1.7	6.4	10.7	7.6	6.5	3.7	3.5	3.4	3.4	3.3	3.4	3.1	3.5	2.7	62.9
40	1.5	8.3	10.4	9.4	7.2	3.9	3.7	3.5	3.4	3	3.2	3.1	3.2	2.6	66.4
44	1.4	7.9	10.2	9.5	7.6	3.9	4	3.7	3.6	3.4	3.4	3.2	3.5	2.6	67.9
48	1.6	8.7	9.7	8.3	6.2	3.9	3.1	3.1	3.1	2.8	3	2.8	3	2.3	61.6

Appendix C-2: Volatile solids and percent removal data

Table C-5. Initial volatile solids of fresh feedstock mixed with various sand loads

	Cr. Wt. (g)	Cr &S (g)	Dried (g)	Ignited (g)	TS %	MC %	VS %	F.dry.wt (kg)	Total VS (kgdb)
Experimetal run #1:sand load: 0% - 8%									
	27.220	30.800	28.860	27.470	45.810	54.190	84.756		
	31.130	37.950	34.140	31.630	44.135	55.865	83.389		
	28.370	33.140	30.540	28.710	45.493	54.507	84.332		
	28.03	33.09	30.34	28.43	45.652	54.348	82.684		
	28.32	32.84	30.2	28.68	41.593	58.407	80.851		
Mean					**44.537**	**55.463**	**83.202**	2.895	2.409
STDEVP					**1.588**	**1.588**	**1.380**		
%RELSTD					**3.565**	**2.863**	**1.659**		
Experimetal run #2: sand load 10% - 16 % sand									
	32.040	38.700	35.010	32.880	44.595	55.405	71.717		
	31.960	38.620	35.190	32.760	48.498	51.502	75.232		
	32.370	38.670	35.140	33.250	43.968	56.032	68.231		
	29.160	35.030	31.750	30.040	44.123	55.877	66.023		
	29.680	36.780	32.870	30.620	44.930	55.070	70.533		
Mean					**45.223**	**54.777**	**70.347**	2.939	2.067
STDEVP					**1.673**	**1.673**	**3.129**		
%RELSTD					**3.700**	**3.054**	**4.448**		
Experimetal run #3: sand load 18% - 24%									
	32.380	39.580	35.610	33.300	44.861	55.139	71.517		
	26.950	36.770	31.190	28.260	43.177	56.823	69.104		
	28.320	34.130	30.770	29.150	42.169	57.831	66.122		
	26.190	30.280	28.210	26.660	49.389	50.611	76.733		
	33.390	40.190	36.490	34.310	45.588	54.412	70.323		
Mean					**45.037**	**54.963**	**70.760**	2.927	2.071
STDEVP					**2.488**	**2.488**	**3.484**		
%RELSTD					**5.525**	**4.527**	**4.924**		
Experimetal run #4: sand load 26% - 30%									
	30.120	37.480	33.380	31.040	44.293	55.707	71.779		
	32.380	40.220	36.090	33.440	47.321	52.679	71.429		
	29.500	36.440	32.720	30.300	46.398	53.602	75.155		
	33.380	40.230	36.600	34.240	47.007	52.993	73.292		
	29.170	36.240	32.370	30.060	45.262	54.738	72.188		
Mean					**46.056**	**53.944**	**72.768**	2.994	2.179
STDEVP					**1.128**	**1.128**	**1.348**		
%RELSTD					**2.449**	**2.091**	**1.852**		
Experimetal run #5: sand load 36% - 48%									
	25.97	35.98	30.67	27.31	46.953	53.047	71.489		
	29.86	39.87	34.35	31.21	44.855	55.145	69.933		
	30.13	39.72	34.29	31.4	43.379	56.621	69.471		
	29.51	39.31	33.94	30.82	45.204	54.796	70.429		
	27.97	37.93	32.45	29.27	44.980	55.020	70.982		
Mean					**45.074**	**54.926**	**70.461**	2.930	2.065
STDEVP					**1.138**	**1.138**	**0.720**		
%RELSTD					**2.525**	**2.073**	**1.021**		
Experimetal run #6: sand load 52% - 64%									
	31.980	41.020	35.990	33.250	44.358	55.642	68.329		
	24.430	32.860	28.300	25.530	45.907	54.093	71.576		
	28.370	35.680	31.900	29.160	48.290	51.710	77.620		
	25.960	36.910	30.840	27.470	44.566	55.434	69.057		
	25.960	38.730	32.150	28.590	48.473	51.527	57.512		
Mean					**46.319**	**53.681**	**68.819**	3.011	2.072
STDEVP					**1.767**	**1.767**	**6.530**		
%RELSTD					**3.815**	**3.292**	**9.489**		

Table C-6. Final volatile solids of fresh feedstock mixed with various sand loads.

	Crucible (g)	Cr. + Sa (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	% VS Red
Soil content: 0%										
	25.660	35.450	29.700	26.340	41.267	58.733	83.168			
	28.240	36.240	31.720	29.024	43.500	56.500	77.471			
	29.370	36.670	32.450	29.940	42.192	57.808	81.494			
	26.950	38.090	31.520	27.730	41.023	58.977	82.932			
	28.270	35.390	31.030	28.750	38.764	61.236	82.609			
Mean					**41.349**	**58.651**	**81.535**	2.506	2.043	15.193
STDEVP					**1.558**	**1.558**	**2.111**			
%RELSTD					**3.767**	**2.656**	**2.589**			
Soil content: 4%										
4	27.220	35.880	30.910	28.260	42.610	57.390	71.816			
4	29.510	38.480	33.350	30.630	42.809	57.191	70.833			
4	27.520	36.680	31.620	28.680	44.760	55.240	71.707			
4	28.320	34.130	30.830	29.040	43.201	56.799	71.315			
	27.960	36.710	32.050	28.930	46.743	53.257	76.284			
Mean					**44.025**	**55.975**	**72.391**	2.804	2.030	15.733
STDEVP					**1.555**	**1.555**	**1.977**			
%RELSTD					**3.531**	**2.778**	**2.731**			
Soil content: 6%										
6	26.540	31.700	28.890	27.290	45.543	54.457	68.085			
6	32.250	37.060	34.440	32.920	45.530	54.470	69.406			
6	32.800	38.570	35.720	33.570	50.607	49.393	73.630			
6	30.130	36.060	32.790	31.030	44.857	55.143	66.165			
6	31.130	36.560	33.880	31.990	50.645	49.355	68.727			
Mean					**47.436**	**52.564**	**69.203**	3.048	2.109	12.453
STDEVP					**2.616**	**2.616**	**2.463**			
%RELSTD					**5.515**	**4.977**	**3.560**			
Soil content: 8%										
8	26.200	31.320	28.660	27.080	48.047	51.953	64.228			
8	33.390	38.470	35.860	34.290	48.622	51.378	63.563			
8	28.370	33.580	30.820	29.270	47.025	52.975	63.265			
8	28.660	34.580	31.860	29.970	54.054	45.946	59.063			
	30.130	34.720	32.350	30.870	48.366	51.634	66.667			
Mean					**49.223**	**50.777**	**63.357**	3.330	2.110	12.412
STDEVP					**2.476**	**2.476**	**2.458**			
%RELSTD					**5.030**	**4.876**	**3.879**			
Soil content: 10%										
	32.800	39.900	36.410	34.630	50.845	49.155	49.307			
	29.370	35.990	32.550	30.950	48.036	51.964	50.314			
	28.370	35.900	31.880	30.030	46.614	53.386	52.707			
	28.240	38.120	32.890	30.380	47.065	52.935	53.978			
	29.700	37.330	33.640	31.440	51.638	48.362	55.838			
Mean					**48.840**	**51.160**	**52.429**	3.425	1.796	13.111
STDEVP					**2.030**	**2.030**	**2.380**			
%RELSTD					**4.157**	**3.968**	**4.539**			
Soil content: 12%										
	30.120	40.460	35.390	32.380	50.967	49.033	57.116			
	27.220	35.780	31.440	29.490	49.299	50.701	46.209			
	25.660	36.270	30.580	28.070	46.371	53.629	51.016			
	31.970	40.410	36.010	33.970	47.867	52.133	50.495			
	30.130	37.490	33.820	31.950	50.136	49.864	50.678			
Mean					**48.928**	**51.072**	**51.103**	3.487	1.782	13.788
STDEVP					**1.638**	**1.638**	**3.483**			
%RELSTD					**3.348**	**3.207**	**6.816**			
Soil content: 14%										
	28.640	37.350	32.960	30.910	49.598	50.402	47.454			
	28.260	37.670	33.040	30.560	50.797	49.203	51.883			

	27.960	36.830	32.390	30.160	49.944	50.056	50.339			
	31.120	40.930	35.750	33.210	47.197	52.803	54.860			
	26.540	35.740	31.210	28.940	50.761	49.239	48.608			
Mean					**49.659**	**50.341**	**50.629**	3.629	1.837	11.127
STDEVP					**1.316**	**1.316**	**2.597**			
%RELSTD					**2.650**	**2.614**	**5.129**			
Soil content: 16%										
	29.170	39.200	34.330	31.890	51.446	48.554	47.287			
	32.050	41.170	36.880	34.460	52.961	47.039	50.104			
	32.240	41.140	36.830	34.560	51.573	48.427	49.455			
	29.510	38.800	34.440	32.370	53.068	46.932	41.988			
	27.530	37.610	32.690	30.290	51.190	48.810	46.512			
Mean					**52.048**	**47.952**	**47.069**	3.897	1.834	11.272
STDEVP					**0.800**	**0.800**	**2.866**			
%RELSTD					**1.536**	**1.667**	**6.090**			
Soil content: 18%										
	32.800	42.460	37.600	35.380	49.689	50.311	46.250			
	32.250	41.910	37.230	34.990	51.553	48.447	44.980			
	29.690	39.350	34.660	32.370	51.449	48.551	46.076			
	25.660	35.890	30.710	28.500	49.365	50.635	43.762			
	28.640	40.520	34.680	31.950	50.842	49.158	45.199			
Mean					**50.580**	**49.420**	**45.253**	3.878	1.755	15.258
STDEVP					**0.899**	**0.899**	**0.891**			
%RELSTD					**1.777**	**1.819**	**1.969**			
Soil content: 20%										
20	28.360	38.630	33.930	31.510	54.236	45.764	43.447			
	26.540	37.150	32.250	29.780	53.817	46.183	43.257			
	27.520	36.960	32.510	30.250	52.860	47.140	45.291			
	28.260	39.810	34.560	31.650	54.545	45.455	46.190			
	30.130	41.610	36.420	34.070	54.791	45.209	37.361			
Mean					**54.050**	**45.950**	**43.109**	4.251	1.833	11.492
STDEVP					**0.678**	**0.678**	**3.080**			
%RELSTD					**1.255**	**1.476**	**7.145**			
Soil content: 22%										
22	26.960	38.780	33.450	31.060	54.907	45.093	36.826			
	31.130	40.000	35.930	33.900	54.115	45.885	42.292			
	28.310	37.630	33.310	31.260	53.648	46.352	41.000			
	26.200	35.080	31.130	29.330	55.518	44.482	36.511			
	27.960	38.700	33.490	31.150	51.490	48.510	42.315			
Mean					**53.936**	**46.064**	**39.789**	4.387	1.746	15.693
STDEVP					**1.382**	**1.382**	**2.594**			
%RELSTD					**2.562**	**2.999**	**6.518**			
Soil content 24%										
24	29.370	37.010	33.600	32.200	55.366	44.634	33.097			
	31.970	43.060	38.190	35.680	56.087	43.913	40.354			
	32.040	42.540	37.940	35.910	56.190	43.810	34.407			
	28.240	35.320	32.060	30.470	53.955	46.045	41.623			
	27.220	34.540	31.190	29.710	54.235	45.765	37.280			
Mean					**55.167**	**44.833**	**37.352**	4.586	1.713	17.286
STDEVP					**0.924**	**0.924**	**3.287**			
%RELSTD					**1.675**	**2.061**	**8.801**			
Soil content: 26%										
	26.960	35.040	31.710	30.180	58.787	41.213	32.211			
	24.410	32.120	28.860	27.200	57.717	42.283	37.303			
	28.370	36.450	33.010	31.300	57.426	42.574	36.853			
	24.930	32.790	29.460	27.830	57.634	42.366	35.982			
	25.440	32.830	29.640	27.910	56.834	43.166	41.190			
Mean					**57.679**	**42.321**	**36.708**	4.923	1.807	17.072
STDEVP					**0.634**	**0.634**	**2.871**			
%RELSTD					**1.099**	**1.498**	**7.822**			

Soil content: 28%										
	29.850	35.950	33.290	32.010	56.393	43.607	37.209			
	30.710	37.860	34.600	33.200	54.406	45.594	35.990			
	29.980	38.080	34.660	32.970	57.778	42.222	36.111			
	26.690	33.400	30.620	29.250	58.569	41.431	34.860			
	25.610	35.430	31.370	29.310	58.656	41.344	35.764			
Mean					**57.160**	**42.840**	**35.987**	4.996	1.798	17.485
STDEVP					**1.599**	**1.599**	**0.752**			
%RELSTD					**2.797**	**3.732**	**2.090**			
Soil content: 30%										
	29.120	37.920	34.440	32.840	60.455	39.545	30.075			
	25.370	34.300	30.580	28.670	58.343	41.657	36.660			
	29.310	38.080	34.370	32.540	57.697	42.303	36.166			
	29.550	34.910	32.740	31.740	59.515	40.485	31.348			
	25.980	35.010	31.320	29.420	59.136	40.864	35.581			
Mean					**59.029**	**40.971**	**33.966**	5.354	1.819	16.521
STDEVP					**0.951**	**0.951**	**2.709**			
%RELSTD					**1.612**	**2.322**	**7.976**			
Soil content: 36%										
	27.53	39.25	34.92	33.07	63.055	36.945	25.034			
	25.62	36.28	32.17	29.92	61.445	38.555	34.351			
	28.24	40.95	35.9	33.72	60.268	39.732	28.460			
	27.31	40.49	35.57	33.42	62.671	37.329	26.029			
	24.42	35.34	30.84	28.92	58.791	41.209	29.907			
Mean					**61.246**	**38.754**	**28.756**	6.076	1.747	15.400
STDEVP					**1.570**	**1.570**	**3.287**			
%RELSTD					**2.563**	**4.051**	**11.429**			
Soil content: 40%										
	30.13	40.61	36.65	35.07	62.214	37.786	24.233			
	26.19	38.26	33.78	31.93	62.883	37.117	24.374			
	25.04	36.04	32.05	30.27	63.727	36.273	25.392			
	29.31	43.62	37.99	35.63	60.657	39.343	27.189			
	25.71	39.97	34.9	32.39	64.446	35.554	27.312			
Mean					**62.785**	**37.215**	**25.700**	6.618	1.701	17.627
STDEVP					**1.305**	**1.305**	**1.328**			
%RELSTD					**2.078**	**3.506**	**5.168**			
Soil content: 44%										
	25.99	38.81	34.51	32.45	66.459	33.541	24.178			
	26.95	40.01	35.51	33.42	65.544	34.456	24.416			
	28.37	38.35	35.05	33.59	66.934	33.066	21.856			
	29.17	39.34	35.82	34.19	65.388	34.612	24.511			
	25.38	36.13	32.62	31.07	67.349	32.651	21.409			
Mean					**66.335**	**33.665**	**23.274**	7.542	1.755	15.012
STDEVP					**0.765**	**0.765**	**1.352**			
%RELSTD					**1.153**	**2.272**	**5.810**			
Soil content: 48%										
	29.7	42.44	38.26	36.35	67.190	32.810	22.313			
	31.98	47.18	42.36	40.16	68.289	31.711	21.195			
	32.06	45.84	41.23	39.34	66.546	33.454	20.611			
	28.38	41.13	36.81	34.96	66.118	33.882	21.945			
	32.38	42.12	39.06	37.66	68.583	31.417	20.958			
Mean					**67.345**	**32.655**	**21.404**	8.256	1.767	14.431
STDEVP					**0.959**	**0.959**	**0.631**			
%RELSTD					**1.423**	**2.935**	**2.949**			
Soil content: 52%										
	26.540	38.320	34.700	33.230	69.270	30.730	18.015			
	29.370	39.600	36.540	35.150	70.088	29.912	19.386			
	27.970	42.760	38.480	36.230	71.062	28.938	21.408			
	26.000	38.950	35.310	33.860	71.892	28.108	15.575			
	28.380	42.830	38.620	36.780	70.865	29.135	17.969			

Mean					**70.635**	**29.365**	**18.471**	9.444	1.744	15.830
STDEVP					**0.892**	**0.892**	**1.914**			
%RELSTD					**1.263**	**3.037**	**10.362**			
Soil content: 56%										
	32.390	42.820	39.980	38.750	72.771	27.229	16.206			
	30.650	41.440	38.690	37.430	74.513	25.487	15.672			
	25.390	34.790	32.030	30.690	70.638	29.362	20.181			
	29.180	43.290	39.340	37.660	72.006	27.994	16.535			
	27.320	38.950	35.770	34.590	72.657	27.343	13.964			
Mean					**72.517**	**27.483**	**16.512**	10.515	1.736	16.216
STDEVP					**1.254**	**1.254**	**2.037**			
%RELSTD					**1.729**	**4.562**	**12.338**			
Soil content: 60%										
	25.450	38.520	35.300	33.990	75.363	24.637	13.299			
	29.990	42.790	39.320	37.850	72.891	27.109	15.756			
	28.270	42.410	38.880	37.280	75.035	24.965	15.080			
	28.650	43.770	39.870	38.180	74.206	25.794	15.062			
	25.610	40.360	36.690	35.170	75.119	24.881	13.718			
Mean					**74.523**	**25.477**	**14.583**	11.968	1.745	15.782
STDEVP					**0.904**	**0.904**	**0.922**			
%RELSTD					**1.213**	**3.549**	**6.320**			
Soil content: 64%										
	32.050	42.650	39.870	38.750	73.774	26.226	14.322			
	29.860	43.770	40.460	39.140	76.204	23.796	12.453			
	26.900	43.390	39.320	37.650	75.318	24.682	13.446			
	28.300	44.490	40.280	38.700	73.996	26.004	13.189			
	26.700	41.710	38.070	36.650	75.750	24.250	12.489			
Mean					**75.008**	**24.992**	**13.180**	13.381	1.764	14.865
STDEVP					**0.962**	**0.962**	**0.690**			
%RELSTD					**1.282**	**3.848**	**5.237**			

Table C-7. Initial volatile solids of fresh feedstock mixed with various silt loads

	Cr Wt. (g)	Cr.&S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)
Experimental run#1: soil content 0% - 8%									
	25.62	31.68	28.34	26.39	44.884	55.116	71.691		
	30.13	35.29	32.34	30.84	42.829	57.171	67.873		
	27.52	37.17	32.27	28.51	49.223	50.777	79.158		
	24.93	29.95	27.2	25.68	45.219	54.781	66.960		
	26.54	31.64	28.8	27.2	44.314	55.686	70.796		
MEAN					**45.294**	**54.706**	**71.296**	**2.944**	**2.099**
STDEVP					**2.128**	**2.128**	**4.306**		
%STD					**4.698**	**3.890**	**6.040**		
Experimental run#2: soil content: 12% - 16%									
	32.81	42	36.79	33.97	43.308	56.692	70.854		
	26.94	34.61	30.44	27.98	45.632	54.368	70.286		
	29.51	38.23	33.31	30.73	43.578	56.422	67.895		
	26.94	33.36	29.8	27.92	44.548	55.452	65.734		
	29.31	39.51	34.16	30.44	47.549	52.451	76.701		
MEAN					**44.923**	**55.077**	**70.294**	**2.920**	**2.053**
STDEVP					**1.546**	**1.546**	**3.685**		
%STD					**3.442**	**2.808**	**5.243**		
Experimental run#3: soil content 20% - 32%									
	28.27	38	33.03	29.44	48.921	51.079	75.420		
	26.89	35.13	30.72	28.4	46.481	53.519	60.574		
	25.99	35.66	30.46	27.29	46.225	53.775	70.917		
	31.12	38.51	34.44	32.1	44.926	55.074	70.482		
	31.97	39.4	35.35	32.96	45.491	54.509	70.710		
MEAN					**46.409**	**53.591**	**69.621**	**3.017**	**2.100**
STDEVP					**1.370**	**1.370**	**4.880**		
%STD					**2.953**	**2.557**	**7.010**		
Experimental run#4: soil content: 36% - 48%									
	26.95	33.38	29.92	27.71	46.190	53.810	74.411		
	27.96	35.79	31.62	28.95	46.743	53.257	72.951		
	26.54	34.75	30.23	27.65	44.945	55.055	69.919		
	32.05	37.3	34.73	32.55	51.048	48.952	81.343		
	26.7	33.25	29.94	27.48	49.466	50.534	75.926		
MEAN					**47.678**	**52.322**	**74.910**	**3.099**	**2.321**
STDEVP					**2.241**	**2.241**	**3.779**		
%STD					**4.700**	**4.283**	**5.045**		

Table C-8. Final volatile solids of fresh feedstock mixed with variou silt loads.

	Cr.Wt (g)	Cr&S (g)	Dried (g)	Ignited (g)	TS %	MC %	VS %	F.dry.wt (kg)	Total VS (kgdb)	%VS removal
Soil content: 0%										
	26.950	32.130	29.130	27.630	42.085	57.915	68.807			
	28.380	33.490	30.680	29.220	45.010	54.990	63.478			
	28.240	34.110	30.810	28.980	43.782	56.218	71.206			
	24.420	29.120	26.460	25.130	43.404	56.596	65.196			
	25.990	31.610	28.510	26.800	44.840	55.160	67.857			
Mean					**43.824**	**56.176**	**67.309**	**2.717**	**1.829**	**12.863**
STDEVP					**1.062**	**1.062**	**2.716**			
%RELSTD					**2.424**	**1.891**	**4.035**			
Soil content: 4%										
4.000	29.850	36.790	33.090	30.940	46.686	53.314	66.358			
	29.690	34.590	31.910	30.550	45.306	54.694	61.261			
	32.250	37.820	34.760	33.310	45.063	54.937	57.769			
	30.710	36.460	33.380	31.740	46.435	53.565	61.423			
	32.800	36.990	34.630	33.530	43.675	56.325	60.109			
Mean					**45.433**	**54.567**	**61.384**	**2.908**	**1.785**	**14.960**
STDEVP					**1.078**	**1.078**	**2.809**			
%RELSTD					**2.374**	**1.976**	**4.576**			
Soil content: 8%										
	27.300	32.370	29.750	28.500	48.323	51.677	51.020			
	25.380	31.000	27.990	26.450	46.441	53.559	59.004			
	29.130	35.360	32.230	30.490	49.759	50.241	56.129			
	29.990	35.140	32.460	31.160	47.961	52.039	52.632			
	29.370	34.410	31.960	30.450	51.389	48.611	58.301			
Mean					**48.775**	**51.225**	**55.417**	**3.297**	**1.827**	**12.959**
STDEVP					**1.680**	**1.680**	**3.123**			
%RELSTD					**3.445**	**3.280**	**5.636**			
Soil content: 12%										
	25.040	36.820	30.720	27.980	48.217	51.783	48.239			
	28.290	40.170	34.410	30.750	51.515	48.485	59.804			
	29.130	39.020	34.050	31.490	49.747	50.253	52.033			
	27.530	36.980	32.290	29.900	50.370	49.630	50.210			
	25.780	35.620	30.810	28.120	51.118	48.882	53.479			
Mean					**50.194**	**49.806**	**52.753**	**3.584**	**1.891**	**7.891**
STDEVP					**1.161**	**1.161**	**3.940**			
%RELSTD					**2.313**	**2.331**	**7.468**			
Soil content: 16%										
	24.670	35.610	30.620	27.770	54.388	45.612	47.899			
	30.640	40.620	35.950	33.610	53.206	46.794	44.068			
	28.370	40.590	34.660	31.520	51.473	48.527	49.921			
	26.950	38.110	32.410	29.430	48.925	51.075	54.579			
	24.800	35.050	30.110	27.210	51.805	48.195	54.614			
Mean					**51.959**	**48.041**	**50.216**	**3.892**	**1.954**	**4.822**
STDEVP					**1.840**	**1.840**	**4.041**			
%RELSTD					**3.540**	**3.829**	**8.046**			
Soil content: 20%										
	26.550	36.950	32.010	29.240	52.500	47.500	50.733			
	30.720	40.850	35.860	33.460	50.740	49.260	46.693			
	31.130	42.330	37.010	34.460	52.500	47.500	43.367			
	26.700	37.810	32.270	29.610	50.135	49.865	47.756			
	28.650	39.400	34.360	31.870	53.116	46.884	43.608			
Mean					**51.798**	**48.202**	**46.431**	**4.139**	**1.922**	**8.476**
STDEVP					**1.150**	**1.150**	**2.745**			
%RELSTD					**2.219**	**2.385**	**5.913**			
Soil content: 24%										
	33.390	45.520	39.860	36.970	53.339	46.661	44.668			
	27.520	38.170	33.150	30.900	52.864	47.136	39.964			

	29.510	41.750	36.110	33.720	53.922	46.078	36.212			
	26.950	36.190	31.970	29.800	54.329	45.671	43.227			
	27.300	37.420	32.770	30.550	54.051	45.949	40.585			
Mean					**53.701**	**46.299**	**40.931**	**4.484**	**1.835**	**12.619**
STDEVP					**0.529**	**0.529**	**2.917**			
%RELSTD					**0.985**	**1.142**	**7.128**			
Soil content: 28%										
	25.620	35.850	31.050	28.810	53.079	46.921	41.252			
	29.130	39.320	34.850	32.740	56.133	43.867	36.888			
	28.250	41.010	35.280	32.870	55.094	44.906	34.282			
	24.420	36.000	30.750	28.160	54.663	45.337	40.916			
	29.560	42.520	36.780	33.740	55.710	44.290	42.105			
Mean					**54.936**	**45.064**	**39.089**	**4.900**	**1.914**	**8.857**
STDEVP					**1.056**	**1.056**	**3.002**			
%RELSTD					**1.923**	**2.344**	**7.681**			
Soil content: 32%										
	26.890	38.390	33.700	31.530	59.217	40.783	31.865			
	27.960	40.200	34.970	32.660	57.271	42.729	32.953			
	29.170	42.470	37.030	34.500	59.098	40.902	32.188			
	25.660	37.280	32.050	29.650	54.991	45.009	37.559			
	32.250	45.010	39.610	37.090	57.680	42.320	34.239			
Mean					**57.652**	**42.348**	**33.761**	**5.390**	**1.821**	**13.286**
STDEVP					**1.534**	**1.534**	**2.067**			
%RELSTD					**2.661**	**3.622**	**6.124**			
Soil content: 36%										
	29.51	47.42	39.32	35.23	54.774	45.226	41.692			
	31.13	44.28	38.67	36.06	57.338	42.662	34.615			
	28.32	45.49	38.15	34.59	57.251	42.749	36.216			
	33.39	50.99	43.55	40.08	57.727	42.273	34.154			
	29.32	44.76	38.24	35	57.772	42.228	36.323			
Mean					**56.973**	**43.027**	**36.600**	**5.692**	**2.084**	**10.288**
STDEVP					**1.118**	**1.118**	**2.686**			
%RELSTD					**1.963**	**2.599**	**7.339**			
Soil content: 40%										
40	28.38	36.88	33.51	31.79	60.353	39.647	33.528			
	24.43	37.88	32.63	30.04	60.967	39.033	31.585			
	29.37	41.9	36.88	34.08	59.936	40.064	37.284			
	26.89	45.3	38.09	34.33	60.837	39.163	33.571			
	25.62	44.15	36.98	33.21	61.306	38.694	33.187			
Mean					**60.680**	**39.320**	**33.831**	**6.420**	**2.170**	**6.543**
STDEVP					**0.481**	**0.481**	**1.873**			
%RELSTD					**0.793**	**1.224**	**5.536**			
Soil content: 44%										
	28.65	50.23	42.35	38.65	63.485	36.515	27.007			
	25.45	47.98	39.81	35.92	63.737	36.263	27.089			
	25.38	47.23	39.3	35.68	63.707	36.293	26.006			
	28.27	41.61	36.72	34.38	63.343	36.657	27.692			
	27.3	45.09	38.58	35.52	63.406	36.594	27.128			
Mean					**63.536**	**36.464**	**26.984**	**7.237**	**1.952**	**15.971**
STDEVP					**0.159**	**0.159**	**0.546**			
%RELSTD					**0.250**	**0.436**	**2.024**			
Soil content: 48%										
	28.37	46.6	40.07	36.83	64.180	35.820	27.692			
	25.99	36.03	32.37	30.58	63.546	36.454	28.056			
	31.98	53.32	45.5	42.29	63.355	36.645	23.743			
	32.38	51.5	44.29	40.89	62.291	37.709	28.547			
	30.64	46.2	40.55	38.06	63.689	36.311	25.126			
Mean					**63.412**	**36.588**	**26.633**	**7.781**	**2.072**	**10.805**
STDEVP					**0.624**	**0.624**	**1.868**			
%RELSTD					**0.983**	**1.705**	**7.012**			

Appendix C-3: Determination of variability in VS results

C-3 Determination of Variability in VS Results

An attempt was made to add the variability in data resulted from initial and final analysis of TS and VS into one variable with the help of derived statistical equations 1 through 3 presented below:

$$E\left[\frac{x^2y^2}{u^2v^2}\right]=\frac{\bar{x}^2\bar{y}^2}{\bar{u}^2\bar{v}^2}+\frac{1}{2!}\left[\begin{array}{l}\sigma_x^2*\frac{2\bar{y}^2}{\bar{u}^2\bar{v}^2}+\sigma_y^2*\frac{2\bar{x}^2}{\bar{u}^2\bar{v}^2}+2COV(x,y)*\frac{4\bar{x}^2\bar{y}^2}{\bar{u}^2\bar{v}^2}+\sigma_u^2*\frac{12\bar{x}^2\bar{y}^2}{\bar{u}^4\bar{v}^2}+\\ \sigma_v^2*\frac{12\bar{x}^2\bar{y}^2}{\bar{u}^2\bar{v}^4}+2COV(u,v)*\frac{12\bar{x}^2\bar{y}^2}{\bar{u}^3\bar{v}^3}\end{array}\right] \quad (1)$$

$$E\left[\frac{xy}{uv}\right]=\frac{\bar{x}\bar{y}}{\bar{u}\bar{v}}+\frac{1}{2!}\left[\begin{array}{l}\sigma_x^2*0+\sigma_y^2*0+2COV(x,y)\frac{1}{\bar{u}\bar{v}}+\sigma_u^2*\frac{2\bar{x}\bar{y}}{\bar{u}^3\bar{v}}+\sigma_v^2*\frac{2\bar{x}\bar{y}}{\bar{u}\bar{v}^3}+2COV(u,v)\\ *\frac{\bar{x}\bar{y}}{\bar{u}^2\bar{v}^2}\end{array}\right] \quad (2)$$

$$VAR\left(\frac{xy}{uv}\right)=E\left[\frac{x^2y^2}{u^2v^2}\right]-E\left[\frac{xy}{uv}\right]^2 \quad (3)$$

Where x and y are the initial TS and VS, respectively, u and v are the final TS and VS, respectively, σ is the standard deviation, $\bar{x}$, $\bar{y}$, $\bar{u}$, and $\bar{v}$ are the means of TS and VS, and COV is the coefficient of variation.

Using these equations the variation was estimated. The variability (VAR) resulted from VS analysis of compost feedstock is presented in the following below:

Table C-9. The Variabilty in volatile solids analysis of fresh feedstock mixed with various sand loads.

Sand content (%)	e(xy/uv)	e(x2y2/u2v2)	VAR(x,y)
0	1.102	1.307	0.094
4	1.166	1.669	0.308
6	1.135	1.848	0.559
8	1.193	1.603	0.181
10	1.249	2.000	0.439
12	1.283	2.449	0.804
14	1.271	1.859	0.243
16	1.306	2.138	0.433
18	1.398	2.096	0.142
20	1.379	2.528	0.626
22	1.496	2.731	0.493
24	1.563	3.673	1.228
26	1.592	3.425	0.890
28	1.631	2.819	0.158
30	1.682	3.808	0.980
36	1.827	6.020	2.682
40	1.975	4.743	0.843
44	2.064	5.048	0.787
48	2.206	5.175	0.309
52	2.468	10.480	4.390
56	2.699	13.984	6.697
60	2.942	10.692	2.034
64	3.231	12.078	1.640

Table C-10. The Variabilty in volatile solids analysis of fresh feedstock mixed with various silt

Silt content (%)	e(xy/uv)	e(x2y2/u2v2)	VAR(x,y)
0.000	0.999	1.121	0.122
4.000	1.165	1.718	0.361
8.000	1.204	1.946	0.497
12.000	1.203	2.268	0.821
16.000	1.220	1.986	0.498
20.000	1.350	2.261	0.439
24.000	1.479	2.981	0.794
28.000	1.515	3.187	0.892
32.000	1.667	3.230	0.450
36.000	1.726	3.801	0.822
40.000	1.750	3.650	0.589
44.000	2.090	4.523	0.156
48.000	2.131	6.083	1.541

Appendix C-4: Reactor temperature & relative heat generation

Table C-11. Reactor temperatures during composting of fresh feedstock mixed with sand.

Time (day)	Percent sand load: 20 % Sand Temperature (oC) Run-1	Run-2	Mean	SE	40% Sand Temperature (oC) Run-1	Run-2	Mean	SE	Chamber
0	23.1	22.3	22.7	0.28	23.5	23.5	23.5	0.00	31.20
1	46.3	48.2	47.25	0.67	45.3	45.2	45.25	0.04	44.80
2	52.2	56.2	54.2	1.42	51.5	54.3	52.9	0.99	44.80
3	53.3	52.1	52.7	0.43	55.3	53.8	54.55	0.53	45.00
4	49.8	48.6	49.2	0.43	51	49.7	50.35	0.46	45.00
5	47.3	47	47.15	0.11	47.5	47.5	47.5	0.00	45.20
6	46.4	46.1	46.25	0.11	46.5	46.5	46.5	0.00	45.10
7	45.9	45.8	45.85	0.04	45.9	45.9	45.9	0.00	45.10
8	45.6	45.7	45.65	0.04	45.6	45.7	45.65	0.04	45.10
9	45.5	45.7	45.6	0.07	45.8	45.5	45.65	0.11	45.00
10	45.8	45.6	45.7	0.07	45.9	45.3	45.6	0.21	45.00
11	45.9	46	45.95	0.04	46.1	45.4	45.75	0.25	45.00
12	45.9	45.7	45.8	0.07	46	45.2	45.6	0.28	45.00
13	46	45.3	45.65	0.25	45.8	45	45.4	0.28	45.00
14	46.2	45	45.6	0.43	45.8	44.8	45.3	0.35	44.80

Table C-12. Reactors temperatures during composting of fresh feedstock mixed with clay.

Time (day)	Percent clay load: 20% Clay Run-1	Run-2	Mean	SE	40% Clay EXP # 1	EXP # 2	Mean	SE	Chamber
	23.40	20.90	22.15	0.89	25.10	21.10	23.10	1.42	31.20
0	46.30	46.50	46.40	0.07	45.30	43.30	44.30	0.71	44.80
1	50.50	54.50	52.50	1.42	48.20	44.00	46.10	1.49	44.80
2	51.60	50.10	50.85	0.53	48.60	44.50	46.55	1.45	45.00
3	48.50	47.70	48.10	0.28	48.50	45.30	46.90	1.13	45.00
4	46.70	46.30	46.50	0.14	47.70	49.10	48.40	0.50	45.20
5	46.10	45.80	45.95	0.11	47.20	50.10	48.65	1.03	45.10
6	46.40	46.10	46.25	0.11	47.10	48.50	47.80	0.50	45.10
7	47.90	46.70	47.30	0.43	47.00	47.40	47.20	0.14	45.10
8	47.50	46.70	47.10	0.28	46.80	47.00	46.90	0.07	45.00
9	46.70	46.70	46.70	0.00	46.40	46.90	46.65	0.18	45.00
10	46.10	46.30	46.20	0.07	46.20	46.80	46.50	0.21	45.00
11	45.70	45.70	45.70	0.00	45.70	45.70	45.70	0.00	45.00
12	45.40	45.40	45.40	0.00	45.40	45.30	45.35	0.04	45.00
13	45.10	45.10	45.10	0.00	45.10	45.00	45.05	0.04	44.80

Table C-13. Relative heat generation value during composting of fresh feedstock mixed with sand and clay soils

	Time (day)															
Sand load	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total	
%	Relative Heat Generation, RHG (Degree Celcius,Day)															SE
20	2.35	9.3	7.9	4.3	2.05	1.35	0.85	0.55	0.6	0.7	0.85	0.8	0.65	0.7	32.95	0.43
40	0.35	8	9.75	5.45	2.4	1.6	0.9	0.55	0.65	0.6	0.65	0.6	0.4	0	31.9	1.36
Clay load																
(%)																
20	1.5	7.6	6.05	3.2	1.4	1.05	1.25	2.2	2.1	1.7	1.1	0.7	0.4	0.2	30.45	0.3
40	0	0	0	2	3.3	3.75	2.8	2.1	1.9	1.65	1.4	0.7	0.35	0.15	20.1	1.2

Appendix C-5: Volatile solids and percent removal data

Table C-14. The initial volatile solids of fresh feedstock used with sand and clay soils

Experimental Run # 1

Sample No.	Crucibl (g)	Cr + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)
1	71.640	124.510	95.490	79.750	45.111	54.889	65.996		
2	85.200	152.220	115.350	95.320	44.987	55.013	66.434		
3	94.170	156.700	122.260	103.740	44.922	55.078	65.931		
4	103.920	159.720	129.010	112.390	44.964	55.036	66.242		
5	90.960	140.880	113.390	98.380	44.932	55.068	66.919		
6	84.11	142.94	110.63	92.81	45.079	54.921	67.195		
7	90.87	144.25	114.87	98.83	44.961	55.039	66.833		
8	81.63	140.64	108.23	90.61	45.077	54.923	66.241		
9	89.850	146.720	115.400	98.430	44.927	55.073	66.419		
10	90.39	141.59	113.43	98.06	45.000	55.000	66.710		
11	90.95	139.51	112.7	98.09	44.790	55.210	67.172		
12	90.47	142.16	113.51	98.19	44.573	55.427	66.493		
13	91.61	145.64	115.88	99.79	44.919	55.081	66.296		
14	92.98	144.9	116.19	100.79	44.703	55.297	66.351		
15	89.81	142.39	113.3	97.78	44.675	55.325	66.071		
16	90.01	148.89	116.54	98.89	45.058	54.942	66.528		
17	90.16	141.61	113.42	97.92	45.209	54.791	66.638		
18	91.65	140.61	113.81	99.06	45.261	54.739	66.561		
19	93.5	151.85	119.72	102.24	44.936	55.064	66.667		
20	100.58	150.48	122.98	108.16	44.890	55.110	66.161		
Mean					**44.949**	**55.051**			
STDEVP					**0.166**	**0.166**	**66.493**	2.922	1.943
STERR					**0.037**	**0.037**	**0.347**		
							0.078		

Table C-15. The final volatile solids results of fresh feedstock mixed with sand

Sample No.	Crucibl (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	%Red
sand load: 20%										
1	88.82	145.84	119.72	106.47	54.192	45.808	42.880			
2	88.16	156.18	125.08	109.47	54.278	45.722	42.281			
3	90.01	141.56	118.02	106.09	54.336	45.664	42.592			
4	90	162.74	129.6	112.82	54.440	45.560	42.374			
5	71.64	130.75	103.59	89.82	54.052	45.948	43.099			
6	93.51	173.21	136.57	118.06	54.028	45.972	42.987			
7	90.73	153.88	124.81	110.28	53.967	46.033	42.635			
8	88.65	164.34	129.52	112.05	53.997	46.003	42.745			
9	91.88	159.81	128.81	113.06	54.365	45.635	42.648			
10	87.12	154.82	124.07	108.35	54.579	45.421	42.544			
11	89.85	161.28	128.51	111.83	54.123	45.877	43.145			
12	91.33	160.87	129	112.82	54.170	45.830	42.952			
13	89.21	145.62	119.84	106.88	54.299	45.701	42.311			
14	89.19	150.93	122.66	108.52	54.211	45.789	42.247			
15	91.07	156	126.27	111.3	54.212	45.788	42.528			
16	85.57	144.49	117.6	103.93	54.362	45.638	42.679			
17	89.82	142.11	118.15	106.09	54.179	45.821	42.570			
18	90.96	170.4	133.93	115.63	54.091	45.909	42.588			
19	87.34	141.72	116.92	104.33	54.395	45.605	42.563			
20	90.33	155.38	125.78	110.71	54.497	45.503	42.511			
Mean					**54.239**	**45.761**	**42.644**			
STDEVP					**0.166**	**0.166**	**0.252**	4.334	1.848	4.873
STERR					**0.037**	**0.037**	**0.056**			

Sample No.	Crucibl (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	%Red
Sand load: 40%										
1	88.71	168.63	140.39	126.31	64.665	35.335	27.245			
2	88.87	167.17	138.69	125.14	63.627	36.373	27.198			
3	87.4	174.63	142.82	127.63	63.533	36.467	27.409			
4	91.02	185.17	150.82	134.49	63.516	36.484	27.308			
5	89.87	162.54	136.28	123.75	63.864	36.136	26.998			
6	89.9	187.77	152.35	135.18	63.809	36.191	27.494			
7	88.21	169.81	140.23	126.02	63.750	36.250	27.316			
8	90.07	186.37	151.28	134.59	63.562	36.438	27.267			
9	91.93	176.67	147.38	132.31	65.435	34.565	27.178			
10	89.23	173.76	143.01	128.37	63.622	36.378	27.222			
11	87.17	186.55	150.46	133.24	63.685	36.315	27.208			
12	85.61	174.01	141.95	126.95	63.733	36.267	26.624			
13	89.26	176.59	144.92	129.84	63.735	36.265	27.093			
14	71.69	144.88	118.17	105.61	63.506	36.494	27.022			
15	90.05	186.6	151.71	134.8	63.863	36.137	27.425			
16	84.16	169.22	138.64	123.73	64.049	35.951	27.368			
17	93.56	191.49	155.84	138.94	63.596	36.404	27.136			
18	90.79	183.36	149.41	133.59	63.325	36.675	26.987			
19	91.13	169.99	141.28	127.78	63.594	36.406	26.919			
20	91.39	174.51	144.61	130.06	64.028	35.972	27.339			
Mean					**63.825**	**36.175**	**27.188**			
STDEVP					**0.459**	**0.459**	**0.200**	6.816	1.853	4.604
STERR					**0.103**	**0.103**	**0.045**			

Table C-16. The final volatile solids of fresh feedstock mixed with clay

Sample No.	Crucibl (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	Clay VS (kgdb)	TVS-CVS	% Red
Clay load: 20%												
1	104.03	164.9	133.66	118.9	48.678	51.322	49.814					
2	90.96	162.74	126.09	108.81	48.941	51.059	49.189					
3	103.93	168.69	135.68	120.93	49.027	50.973	46.457					
4	81.63	139.66	109.89	95.96	48.699	51.301	49.292					
5	89.77	154	120.56	105.27	47.937	52.063	49.659					
6	90.87	160.17	124.45	107.89	48.456	51.544	49.315					
7	89.32	148.7	117.88	103.67	48.097	51.903	49.755					
8	92.18	153.09	122.01	107.21	48.974	51.026	49.614					
9	94.18	163.4	127.93	111.09	48.758	51.242	49.896					
10	90.82	160.83	124.96	108.24	48.764	51.236	48.975					
11	86.02	154.38	119.32	102.83	48.713	51.287	49.520					
12	88.63	166.98	126.22	107.91	47.977	52.023	48.710					
13	88.48	162.31	125.84	108.31	50.603	49.397	46.922					
14	91.65	148.43	119.22	105.68	48.556	51.444	49.111					
15	91.61	160.72	124.8	108.5	48.025	51.975	49.111					
16	96.84	159.99	127.32	112.25	48.266	51.734	49.442					
17	90.4	154.6	122.08	106.54	49.346	50.654	49.053					
18	70.99	129.74	99.67	85.57	48.817	51.183	49.163					
19	88.05	152.13	119.32	103.78	48.798	51.202	49.696					
20	90.17	155.03	122.02	106.13	49.106	50.894	49.890					
Mean					**48.727**	**51.273**	**49.129**					
STDEVP					**0.577**	**0.577**	**0.878**	3.879	1.906	0.04901	1.857	4.435
STERR					**0.129**	**0.129**	**0.196**					

Sample No.	Crucibl (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	Clay VS kgdb	TVS-CVS	% Red
Clay load: 40%												
1	90.93	194.94	145.6	126.38	52.562	47.438	35.156					
2	92.25	187.54	143.13	125.26	53.395	46.605	35.122					
3	104.1	214.16	161.75	141.45	52.381	47.619	35.212					
4	90.44	184.71	139.87	122.31	52.434	47.566	35.525					
5	91.67	175.97	138.18	121.69	55.172	44.828	35.455					
6	90.22	194.89	144.79	125.38	52.135	47.865	35.569					
7	96.9	202.48	152.85	132.73	52.993	47.007	35.961					
8	91.7	175.37	138.34	121.84	55.743	44.257	35.377					
9	81.68	207.76	149.21	125.05	53.561	46.439	35.777					
10	103.99	200.75	155.34	137	53.069	46.931	35.716					
11	90.53	197.32	146.65	126.87	52.552	47.448	35.246					
12	91.01	192.39	144.18	125.03	52.446	47.554	36.017					
13	90.87	176.63	136.23	120.02	52.892	47.108	35.736					
14	94.24	173.75	135.99	121.09	52.509	47.491	35.689					
15	89.37	182.14	138.56	120.67	53.024	46.976	36.369					
16	89.8	174.83	134.48	118.55	52.546	47.454	35.654					
17	88.1	200.94	147.36	126.41	52.517	47.483	35.353					
18	90.38	185.4	140.83	122.99	53.094	46.906	35.362					
19	72.46	161.98	119.67	103.17	52.737	47.263	34.950					
20	71.03	155.43	114.85	99.55	51.919	48.081	34.916					
Mean					**52.984**	**47.016**	**35.508**					
STDEVP					**0.917**	**0.917**	**0.360**	5.638	2.002	0.13398	1.868	3.856
STERR					**0.205**	**0.205**	**0.080**					

Table C-17. The initial volatile solids results of fresh feedstock used with sand and clay soils

Experimental Run # 2

Sample No.	Crucible (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)
	94.17	146.79	117.94	102.22	45.173	54.827	66.134		
	91.94	143.79	115.63	99.85	45.689	54.311	66.610		
	91.09	136.47	111.42	97.95	44.799	55.201	66.257		
	103.93	150.98	125.18	111.08	45.165	54.835	66.353		
	94.11	143.08	116.08	101.49	44.864	55.136	66.409		
	89.78	151.18	117.52	99.06	45.179	54.821	66.547		
	88.67	146.65	114.67	97.46	44.843	55.157	66.192		
	71	123.19	94.54	78.79	45.104	54.896	66.907		
	92.99	162.77	124.74	103.45	45.500	54.500	67.055		
	90.4	139.63	112.64	97.92	45.176	54.824	66.187		
	88.64	138.15	111.15	96.09	45.466	54.534	66.904		
	88.17	143.76	112.75	96.96	44.217	55.783	64.239		
	89.86	160.64	121.66	100.71	44.928	55.072	65.881		
	90.17	144.88	114.98	98.36	45.348	54.652	66.989		
	87.35	146.58	113.84	96.24	44.724	55.276	66.440		
	90.74	145.01	114.82	99.07	44.371	55.629	65.407		
	86.04	149.05	114.52	95.56	45.199	54.801	66.573		
	90.01	158.5	120.49	100.48	44.503	55.497	65.650		
	71.65	121.55	93.99	79.22	44.770	55.230	66.115		
	90.02	155.22	119.1	99.85	44.601	55.399	66.197		
Mean					**44.981**	**55.019**	**66.252**	2.924	1.937
STDEVP					**0.378**	**0.378**	**0.622**		
STERR					**0.085**	**0.085**	**0.139**		

Table C-18. The final volatile solids results of fresh feedstock mixed with sand

Sample No.	Crucible (g)	Cr +S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS (kgdb)	% VS Red
Sand load: 20%										
1	103.93	167.11	138.14	123.51	54.147	45.853	42.765			
2	92.99	149.19	123.67	110.51	54.591	45.409	42.894			
3	70.99	138.14	107.27	91.82	54.028	45.972	42.585			
4	90.97	144.67	120.1	107.62	54.246	45.754	42.842			

5	89.21	166.39	130.99	113.54	54.133	45.867	41.766			
6	91.33	148.16	121.91	108.88	53.810	46.190	42.610			
7	90.02	158.3	126.92	111.16	54.042	45.958	42.710			
8	90.33	158.7	127.29	111.46	54.059	45.941	42.830			
9	90.01	159.27	127.42	111.37	54.014	45.986	42.903			
10	88.82	158.36	126.38	110.35	54.012	45.988	42.678			
11	88.66	161.56	127.92	111.64	53.855	46.145	41.467			
12	90.74	154.25	124.97	110.93	53.897	46.103	41.017			
13	87.13	167.19	130.54	112	54.222	45.778	42.709			
14	89.86	164.53	130.08	112.71	53.864	46.136	43.187			
15	91.08	153.97	124.74	110.3	53.522	46.478	42.900			
16	93.51	153.95	126.11	112.13	53.938	46.062	42.883			
17	91.88	161.35	129.3	113.38	53.865	46.135	42.544			
18	90.82	147.87	121.72	108.54	54.163	45.837	42.654			
19	88.64	149.02	121.32	107.31	54.124	45.876	42.870			
20	90.17	157.85	126.76	111.03	54.063	45.937	42.990			
Mean					**54.030**	**45.970**	**42.590**	4.322	1.841	4.963
STDEVP					**0.209**	**0.209**	**0.528**			
STERR					**0.047**	**0.047**	**0.118**			

Sample No.	**Crucible (g)**	**Cr. + S (g)**	**Dried (g)**	**ignited (g)**	**TS (%)**	**MC (%)**	**VS (%)**	**F.dry.wt (kg)**	**Total VS (kgdb)**	**% VS Red**
Sand load: 40%										
1	91.65	173.33	143.54	129.39	63.528	36.472	27.269			
2	89.77	181.91	147.87	132.46	63.056	36.944	26.523			
3	89.32	169.21	139.86	126.21	63.262	36.738	27.008			
4	104.04	183.49	154.56	140.81	63.587	36.413	27.217			
5	94.18	193.31	156.95	139.8	63.321	36.679	27.322			
6	91.94	174.3	144.16	130	63.405	36.595	27.116			
7	86.04	177.18	143.8	127.9	63.375	36.625	27.528			
8	81.64	169.58	137.35	122.15	63.350	36.650	27.284			
9	88	182.31	147.66	131.27	63.259	36.741	27.472			
10	89.18	171.75	141.39	126.89	63.231	36.769	27.772			
11	89.83	184.67	149.89	133.91	63.328	36.672	26.607			
12	90.97	170.82	141.5	127.55	63.281	36.719	27.607			
13	91.62	197.2	158.83	140.56	63.658	36.342	27.183			
14	90.4	164.48	137.34	124.56	63.364	36.636	27.226			
15	92.19	208.49	165.99	145.88	63.457	36.543	27.249			
16	90.88	175.4	144.64	130.04	63.606	36.394	27.158			

17	88.16	154.84	130.37	118.93	63.302	36.698	27.103				
18	87.35	169.91	139.84	125.54	63.578	36.422	27.243				
19	71.65	142.68	116.86	104.69	63.649	36.351	26.919				
20	88.06	165.47	137.5	124.23	63.868	36.132	26.841				
Mean					**63.423**	**36.577**	**27.182**	6.767	1.840	5.036	
STDEVP					**0.186**	**0.186**	**0.298**				
STERR					**0.042**	**0.042**	**0.067**				

Table C-19. The final volatile solids results of fresh feedstock used with clay

Sample No.	Crucible (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.dry.wt (kg)	Total VS	Clay VS kgdb	TVS-CVS	%VS Red
clay load: 20%												
1	89.33	148.8	118.28	104.18	48.680	51.320	48.705					
2	91.62	188	138.9	115.93	49.056	50.944	48.583					
3	92.19	164.74	127.41	110.08	48.546	51.454	49.205					
4	88.01	152.12	119.17	103.8	48.604	51.396	49.326					
5	89.19	155.2	121.04	105.46	48.250	51.750	48.917					
6	91.65	169.7	129.01	110.77	47.867	52.133	48.822					
7	104.03	172.97	137.71	121.24	48.854	51.146	48.901					
8	90.97	164.12	126.79	109.45	48.968	51.032	48.409					
9	90.88	161.61	124.96	108.29	48.183	51.817	48.914					
10	81.64	134.76	107.25	94.73	48.212	51.788	48.887					
11	88.05	155.7	120.91	104.75	48.574	51.426	49.178					
12	89.83	153.06	120.43	105.49	48.395	51.605	48.824					
13	91.88	151.05	120.47	106.58	48.318	51.682	48.583					
14	93.52	163.8	128.13	111.43	49.246	50.754	48.252					
15	90.82	152.39	120.53	106.02	48.254	51.746	48.839					
16	89.22	156.47	122.09	106.01	48.877	51.123	48.920					
17	90.97	147.82	117.77	104.7	47.142	52.858	48.769					
18	90.33	156.1	122.06	106.53	48.244	51.756	48.944					
19	87.13	159.4	122.26	105.11	48.609	51.391	48.819					
20	91.33	159.38	124.23	108.24	48.347	51.653	48.602					
Mean					**48.461**	**51.539**	**48.820**	3.867	1.888	0.04932	1.839	5.080
STDEVP					**0.451**	**0.451**	**0.251**					
STERR					**0.101**	**0.101**	**0.056**					

Sample No.	Crucible (g)	Cr. + S (g)	Dried (g)	ignited (g)	TS (%)	MC (%)	VS (%)	F.wt (kgdb)	TVS (kgdb)	CVS kgdb	TVS-CVS	%VS Red
Clay load: 40%												
	90.4	181.04	138.76	121.21	53.354	46.646	36.290					
	87.35	151.35	121.06	108.85	52.672	47.328	36.221					
	89.78	185.45	140.46	122.89	52.974	47.026	34.669					
	71.65	163.91	120.36	103.18	52.796	47.204	35.270					
	86.04	179.24	134.65	117.58	52.157	47.843	35.116					
	91.93	181	139.14	121.93	53.003	46.997	36.454					
	94.17	195.35	147.74	128.29	52.945	47.055	36.308					
	88.16	171.19	131.74	116.17	52.487	47.513	35.727					
	90.17	172.76	133.19	117.62	52.089	47.911	36.192					
	88.64	160.64	127.13	112.97	53.458	46.542	36.789					
	103.94	222.05	167.03	144.36	53.416	46.584	35.933					
	88.82	221.78	158.46	133.34	52.377	47.623	36.071					
	90.74	198.01	147.41	127.37	52.829	47.171	35.363					
	91.09	212.12	154.58	131.84	52.458	47.542	35.817					
	71	176.94	127.51	107.45	53.342	46.658	35.498					
	88.66	166.85	129.71	114.93	52.500	47.500	36.005					
	90.02	191.96	143.31	124.47	52.276	47.724	35.354					
	92.99	191.8	145.64	126.9	53.284	46.716	35.594					
	90.01	204.18	150.15	128.48	52.676	47.324	36.033					
	89.86	186.71	140.9	123.12	52.700	47.300	34.835					
Mean					**52.790**	**47.210**	**35.777**	5.606	2.006	0.13923	1.867	3.642
STDEVP					**0.416**	**0.416**	**0.543**					
STERR					**0.093**	**0.093**	**0.122**					

Appendix D

Experimental Results of Phase 2

Phase 2A

Appendix D-1: Reactor temperature data

Appendix D-2: Mineralization data for ^{14}C phenanthrene

Appendix D-3: Sequential extraction data for ^{14}C phenanthrene

Appendix D-4: Extractable diesel range organics data

Phase 2B

Appendix D-4: Seed germination data

Appendix D-1: Reactor temperature data

Table D-1. Temperature of reactors subjected to thermophilic temperature pattern.

CFT Reactor Temperatures

Time day	Rep-1 T°C	Rep-2 T°C	Mean T°C	STDEV	STERR	Chamber T°C
1	42	44.7	43.35	1.91	1.35	35
2	53.8	52.2	53	1.13	0.80	43
3	54.2	53.3	53.75	0.64	0.45	43
4	54	53.8	53.9	0.14	0.10	43
5	53.8	55.7	54.75	1.34	0.95	52
6	57.9	57.6	57.75	0.21	0.15	56
7	57.6	57.2	57.4	0.28	0.20	56
8	57.3	56.9	57.1	0.28	0.20	56
9	57	56.4	56.7	0.42	0.30	56
10	56.8	56	56.4	0.57	0.40	55
11	56.3	56.1	56.2	0.14	0.10	55
12	55.3	55	55.15	0.21	0.15	54
13	55.5	54.7	55.1	0.57	0.40	54
14	54.9	54.5	54.7	0.28	0.20	54
15	48.6	47.3	47.95	0.92	0.65	54
16	60	56.7	58.35	2.33	1.65	57
17	62	62.4	62.2	0.28	0.20	57
18	61.8	62.4	62.1	0.42	0.30	61
19	61	60.6	60.8	0.28	0.20	60
20	61	60.6	60.8	0.28	0.20	60
21	61.8	62	61.9	0.14	0.10	60
22	61.8	60.7	61.25	0.78	0.55	60
23	60.1	60.8	60.45	0.49	0.35	58
24	58	58.5	58.25	0.35	0.25	58
25	57	57.6	57.3	0.42	0.30	56
26	55.7	56.2	55.95	0.35	0.25	55
27	52.7	52.5	52.6	0.14	0.10	54
28	50.6	51.1	50.85	0.35	0.25	52
29	49	49.5	49.25	0.35	0.25	50
30	43.7	43.6	43.65	0.07	0.05	50
31	49.5	49.5	49.5	0.00	0.00	50
32	49.4	49.4	49.4	0.00	0.00	50
33	49	49	49	0.00	0.00	50
34	49.4	49.4	49.4	0.00	0.00	50
35	49.4	49.4	49.4	0.00	0.00	50
36	49.4	49.5	49.45	0.07	0.05	50
37	49.4	49.4	49.4	0.00	0.00	50
38	49.4	49.4	49.4	0.00	0.00	50
39	49.4	49.4	49.4	0.00	0.00	50
40	49.4	49.4	49.4	0.00	0.00	50
41	49.4	49.4	49.4	0.00	0.00	50
42	49.4	49.4	49.4	0.00	0.00	50
43	37.9	38.8	38.35	0.64	0.45	50
44	49.4	49.6	49.5	0.14	0.10	50
45	49.4	49.4	49.4	0.00	0.00	50
46	49.4	49.4	49.4	0.00	0.00	50
47	49.4	49.4	49.4	0.00	0.00	50
48	49.4	49.4	49.4	0.00	0.00	50
49	49.4	49.4	49.4	0.00	0.00	50
50	49.4	49.4	49.4	0.00	0.00	50
51	49.4	49.4	49.4	0.00	0.00	50
52	49.4	49.4	49.4	0.00	0.00	50
53	49.4	49.4	49.4	0.00	0.00	50
54	49.4	49.4	49.4	0.00	0.00	50
55	49.4	49.4	49.4	0.00	0.00	50
56	49.4	49.4	49.4	0.00	0.00	50
57	31.4	31.4	31.4	0.00	0.00	35
58	32.4	32.4	32.4	0.00	0.00	35
59	34.7	34.4	34.55	0.21	0.15	35
60	34.8	34.9	34.85	0.07	0.05	35
61	35	34.9	34.95	0.07	0.05	35
62	35	34.9	34.95	0.07	0.05	35
63	35	34.9	34.95	0.07	0.05	35
64	35	34.9	34.95	0.07	0.05	35
65	35	34.9	34.95	0.07	0.05	35

Cont.

Time day	Rep-1 T°C	Rep-2 T°C	Mean T°C	STDEV	STERR	Chamber T°C
66	35	34.9	34.95	0.07	0.05	35
67	35	34.9	34.95	0.07	0.05	35
68	35	34.9	34.95	0.07	0.05	35
69	35	34.9	34.95	0.07	0.05	35
70	35	34.9	34.95	0.07	0.05	35
71	34	34	34	0.00	0.00	35
72	34.4	34.9	34.65	0.35	0.25	35
73	34.8	34.9	34.85	0.07	0.05	35
74	34.8	34.9	34.85	0.07	0.05	35
75	35	34.9	34.95	0.07	0.05	35
76	34.8	34.9	34.85	0.07	0.05	35
77	35	35.2	35.1	0.14	0.10	35
78	35	35.2	35.1	0.14	0.10	35
79	35	35.2	35.1	0.14	0.10	35
80	35	35.2	35.1	0.14	0.10	35
81	35	35.2	35.1	0.14	0.10	35
82	35.4	35.6	35.5	0.14	0.10	35
83	35.4	35.6	35.5	0.14	0.10	35
84	32.7	32.5	32.6	0.14	0.10	35
85	34.3	34.5	34.4	0.14	0.10	35
86	34.7	34.8	34.75	0.07	0.05	35
87	34.7	34.8	34.75	0.07	0.05	35
88	34.7	34.8	34.75	0.07	0.05	35
89	34.7	34.8	34.75	0.07	0.05	35
91	34.7	34.8	34.75	0.07	0.05	35
92	34.7	34.8	34.75	0.07	0.05	35
93	34.7	34.8	34.75	0.07	0.05	35
94	34.7	34.8	34.75	0.07	0.05	35
95	34.7	34.8	34.75	0.07	0.05	35
96	34.7	34.8	34.75	0.07	0.05	35
97	34.7	34.8	34.75	0.07	0.05	35
98	34.7	34.8	34.75	0.07	0.05	35
99	34.1	34.7	34.4	0.42	0.30	35
100	32.1	32.4	32.25	0.21	0.15	35
101	34.7	34.1	34.4	0.42	0.30	35
102	34.7	34.4	34.55	0.21	0.15	35
103	34.7	34.4	34.55	0.21	0.15	35
104	34.7	34.4	34.55	0.21	0.15	35
105	34.7	34.4	34.55	0.21	0.15	35
106	34.7	34.4	34.55	0.21	0.15	35
107	34.7	34.4	34.55	0.21	0.15	35
108	34.7	34.4	34.55	0.21	0.15	35
109	34.7	34.4	34.55	0.21	0.15	35
110	34.7	34.4	34.55	0.21	0.15	35
111	34.7	34.4	34.55	0.21	0.15	35
112	34.7	34.4	34.55	0.21	0.15	35
113	34.1	34	34.05	0.07	0.05	35
114	32.5	33.3	32.9	0.57	0.40	35
115	34.9	35	34.95	0.07	0.05	35
116	34.9	35	34.95	0.07	0.05	35
117	34.9	35	34.95	0.07	0.05	35
118	34.9	35	34.95	0.07	0.05	35
119	34.9	35	34.95	0.07	0.05	35
120	34.9	35	34.95	0.07	0.05	35
121	34.9	35	34.95	0.07	0.05	35
122	34.9	35	34.95	0.07	0.05	35
123	34.9	35	34.95	0.07	0.05	35
124	34.9	35	34.95	0.07	0.05	35
125	34.9	35	34.95	0.07	0.05	35
126	34.9	35	34.95	0.07	0.05	35

UFT Reactor Temperatures

Time day	Rep-1 T°C	Rep-2 T°C	Mean T°C	STDEV	STERR	Chamber T°C
1	45	44	44.5	0.71	0.50	35
2	58.6	57.3	57.95	0.92	0.65	43
3	55.6	55.9	55.75	0.21	0.15	43
4	53.3	53.4	53.35	0.07	0.05	43
5	59.2	59.5	59.35	0.21	0.15	52
6	59.9	59.6	59.75	0.21	0.15	56
7	56.8	57	56.9	0.14	0.10	56
8	56.5	56.8	56.65	0.21	0.15	56
9	56.7	57	56.85	0.21	0.15	56
10	56.8	57	56.9	0.14	0.10	55
11	56.1	56.4	56.25	0.21	0.15	55
12	54.1	54.8	54.45	0.49	0.35	54
13	54.1	54.8	54.45	0.49	0.35	54
14	53.2	54	53.6	0.57	0.40	54
15	42.3	40.7	41.5	1.13	0.80	54
16	53.9	53.3	53.6	0.42	0.30	57
17	58.4	56.9	57.65	1.06	0.75	57
18	60.4	61.2	60.8	0.57	0.40	61
19	60	58.7	59.35	0.92	0.65	60
20	60	58.7	59.35	0.92	0.65	60
21	58.6	58.4	58.5	0.14	0.10	60
22	57.2	56.4	56.8	0.57	0.40	60
23	57	56.6	56.8	0.28	0.20	58
24	56.5	56.6	56.55	0.07	0.05	58
25	54.3	54.1	54.2	0.14	0.10	56
26	53.3	53.4	53.35	0.07	0.05	55
27	51	51	51	0.00	0.00	54
28	48.8	48.7	48.75	0.07	0.05	52
29	48.8	47	47.9	1.27	0.90	50
30	43.7	41.3	42.5	1.70	1.20	50
31	49.5	49.5	49.5	0.00	0.00	50
32	49.4	49.4	49.4	0.00	0.00	50
33	49	49	49	0.00	0.00	50
34	49.3	49.5	49.4	0.14	0.10	50
35	49.3	49.3	49.3	0.00	0.00	50
36	49.5	49.6	49.55	0.07	0.05	50
37	49.3	49.4	49.35	0.07	0.05	50
38	49.5	49.5	49.5	0.00	0.00	50
39	49.4	49.4	49.4	0.00	0.00	50
40	49.4	49.3	49.35	0.07	0.05	50
41	49.6	49.5	49.55	0.07	0.05	50
42	49.4	49.5	49.45	0.07	0.05	50
43	37.9	38.8	38.35	0.64	0.45	50
44	49.5	49.7	49.6	0.14	0.10	50
45	49.5	49.5	49.5	0.00	0.00	50
46	49.3	49.5	49.4	0.14	0.10	50
47	49.4	49.4	49.4	0.00	0.00	50
48	49.4	49.4	49.4	0.00	0.00	50
49	49.4	49.4	49.4	0.00	0.00	50
50	49.4	49.4	49.4	0.00	0.00	50
51	49.4	49.4	49.4	0.00	0.00	50
52	49.3	49.5	49.4	0.14	0.10	50
53	49.4	49.4	49.4	0.00	0.00	50
54	49.4	49.4	49.4	0.00	0.00	50
55	49.4	49.4	49.4	0.00	0.00	50
56	49.4	49.4	49.4	0.00	0.00	50
57	31.4	31.4	31.4	0.00	0.00	35
58	32.4	32.4	32.4	0.00	0.00	35
59	34.7	34.4	34.55	0.21	0.15	35
60	34.8	34.9	34.85	0.07	0.05	35
61	35	34.9	34.95	0.07	0.05	35
62	35	34.9	34.95	0.07	0.05	35
63	35	34.9	34.95	0.07	0.05	35
64	35	34.9	34.95	0.07	0.05	35
65	35	34.9	34.95	0.07	0.05	35

Cont.

Time	Rep-1	Rep-2	Mean	STDEV	STERR	Chamber
day	T°C	T°C	T°C			T°C
66	35	34.9	34.95	0.07	0.05	35
67	35	34.9	34.95	0.07	0.05	35
68	35	34.9	34.95	0.07	0.05	35
69	35	34.9	34.95	0.07	0.05	35
70	35	34.9	34.95	0.07	0.05	35
71	34	34	34	0.00	0.00	35
72	34.4	34.9	34.65	0.35	0.25	35
73	34.8	34.9	34.85	0.07	0.05	35
74	34.8	34.9	34.85	0.07	0.05	35
75	35	34.9	34.95	0.07	0.05	35
76	34.8	34.9	34.85	0.07	0.05	35
77	35	35.2	35.1	0.14	0.10	35
78	35	35.2	35.1	0.14	0.10	35
79	35	35.2	35.1	0.14	0.10	35
80	35	35.2	35.1	0.14	0.10	35
81	35	35.2	35.1	0.14	0.10	35
82	35.4	35.6	35.5	0.14	0.10	35
83	35.4	35.6	35.5	0.14	0.10	35
84	32.7	32.5	32.6	0.14	0.10	35
85	34.3	34.5	34.4	0.14	0.10	35
86	34.7	34.8	34.75	0.07	0.05	35
87	34.7	34.8	34.75	0.07	0.05	35
88	34.7	34.8	34.75	0.07	0.05	35
89	34.7	34.8	34.75	0.07	0.05	35
91	34.7	34.8	34.75	0.07	0.05	35
92	34.7	34.8	34.75	0.07	0.05	35
93	34.7	34.8	34.75	0.07	0.05	35
94	34.7	34.8	34.75	0.07	0.05	35
95	34.7	34.8	34.75	0.07	0.05	35
96	34.7	34.8	34.75	0.07	0.05	35
97	34.7	34.8	34.75	0.07	0.05	35
98	34.7	34.8	34.75	0.07	0.05	35
99	34.1	34.7	34.4	0.42	0.30	35
100	32.1	32.4	32.25	0.21	0.15	35
101	34.7	34.1	34.4	0.42	0.30	35
102	34.7	34.4	34.55	0.21	0.15	35
103	34.7	34.4	34.55	0.21	0.15	35
104	34.7	34.4	34.55	0.21	0.15	35
105	34.7	34.4	34.55	0.21	0.15	35
106	34.7	34.4	34.55	0.21	0.15	35
107	34.7	34.4	34.55	0.21	0.15	35
108	34.7	34.4	34.55	0.21	0.15	35
109	34.7	34.4	34.55	0.21	0.15	35
110	34.7	34.4	34.55	0.21	0.15	35
111	34.7	34.4	34.55	0.21	0.15	35
112	34.7	34.4	34.55	0.21	0.15	35
113	34.1	34	34.05	0.07	0.05	35
114	32.5	33.3	32.9	0.57	0.40	35
115	34.9	35	34.95	0.07	0.05	35
116	34.9	35	34.95	0.07	0.05	35
117	34.9	35	34.95	0.07	0.05	35
118	34.9	35	34.95	0.07	0.05	35
119	34.9	35	34.95	0.07	0.05	35
120	34.9	35	34.95	0.07	0.05	35
121	34.9	35	34.95	0.07	0.05	35
122	34.9	35	34.95	0.07	0.05	35
123	34.9	35	34.95	0.07	0.05	35
124	34.9	35	34.95	0.07	0.05	35
125	34.9	35	34.95	0.07	0.05	35
126	34.9	35	34.95	0.07	0.05	35

CCT Reactor Temperatures

Time day	Rep-1 T°C	Rep-2 T°C	Mean T°C	STDEV	STERR	Chamber T°C
1	49.00	50.00	49.50	0.71	0.50	43
2	54.20	54.60	54.40	0.28	0.20	43
3	56.70	56.30	56.50	0.28	0.20	52
4	54.50	55.30	54.90	0.57	0.40	56
5	53.60	54.40	54.00	0.57	0.40	56
6	53.10	54.00	53.55	0.64	0.45	56
7	53.10	54.00	53.55	0.64	0.45	56
8	54.30	53.40	53.85	0.64	0.45	55
9	51.70	52.00	51.85	0.21	0.15	55
10	51.50	51.60	51.55	0.07	0.05	54
11	51.50	51.60	51.55	0.07	0.05	54
12	51.50	51.30	51.40	0.14	0.10	54
13	33.80	35.90	34.85	1.48	1.05	54
14	53.20	53.40	53.30	0.14	0.10	57
15	53.50	53.50	53.50	0.00	0.00	57
16	57.50	57.20	57.35	0.21	0.15	61
17	57.20	57.20	57.20	0.00	0.00	60
18	57.30	57.30	57.30	0.00	0.00	60
19	57.30	57.30	57.30	0.00	0.00	60
20	57.30	57.50	57.40	0.14	0.10	60
21	52.60	53.10	52.85	0.35	0.25	58
22	52.60	53.10	52.85	0.35	0.25	58
23	51.40	51.60	51.50	0.14	0.10	56
24	51.20	51.00	51.10	0.14	0.10	55
25	49.30	49.50	49.40	0.14	0.10	54
26	47.80	47.40	47.60	0.28	0.20	52
27	42.20	43.20	42.70	0.71	0.50	50
28	46.50	46.60	46.55	0.07	0.05	50
29	47.80	47.80	47.80	0.00	0.00	50
30	47.80	47.80	47.80	0.00	0.00	50
31	47.80	47.80	47.80	0.00	0.00	50
32	47.80	47.80	47.80	0.00	0.00	50
33	47.80	47.80	47.80	0.00	0.00	50
34	47.80	47.80	47.80	0.00	0.00	50
35	47.70	47.40	47.55	0.21	0.15	50
36	48.20	48.00	48.10	0.14	0.10	50
37	49.10	48.00	48.55	0.78	0.55	50
38	49.20	48.10	48.65	0.78	0.55	50
39	49.00	48.10	48.55	0.64	0.45	50
40	49.00	48.30	48.65	0.49	0.35	50
41	33.30	34.50	33.90	0.85	0.60	50
42	49.00	49.00	49.00	0.00	0.00	50
43	49.40	49.60	49.50	0.14	0.10	50
44	49.40	49.40	49.40	0.00	0.00	50
45	49.40	49.40	49.40	0.00	0.00	50
46	49.40	49.40	49.40	0.00	0.00	50
47	49.40	49.40	49.40	0.00	0.00	50
48	49.40	49.40	49.40	0.00	0.00	50
49	49.40	49.40	49.40	0.00	0.00	50
50	49.40	49.40	49.40	0.00	0.00	50
51	49.40	49.40	49.40	0.00	0.00	50
52	49.40	49.40	49.40	0.00	0.00	50
53	49.40	49.40	49.40	0.00	0.00	50
54	49.40	49.40	49.40	0.00	0.00	50
55	32.70	32.00	32.35	0.49	0.35	35
56	33.30	33.60	33.45	0.21	0.15	35
57	34.00	34.00	34.00	0.00	0.00	35
58	34.80	35.20	35.00	0.28	0.20	35
59	34.90	35.70	35.30	0.57	0.40	35
60	35.00	35.70	35.35	0.49	0.35	35
61	35.00	35.60	35.30	0.42	0.30	35

Cont.

Time	Rep-1	Rep-2	Mean	STDEV	STERR	Chamber
day	T°C	T°C	T°C			T°C
62	35.00	35.60	35.30	0.42	0.30	35
63	35.00	35.60	35.30	0.42	0.30	35
64	35.00	35.60	35.30	0.42	0.30	35
65	35.00	35.60	35.30	0.42	0.30	35
66	35.00	35.60	35.30	0.42	0.30	35
67	35.00	35.60	35.30	0.42	0.30	35
68	35.00	35.60	35.30	0.42	0.30	35
69	32.10	31.10	31.60	0.71	0.50	35
70	34.60	35.80	35.20	0.85	0.60	35
71	35.90	36.80	36.35	0.64	0.45	35
72	35.90	36.80	36.35	0.64	0.45	35
73	36.00	36.70	36.35	0.49	0.35	35
74	35.70	36.60	36.15	0.64	0.45	35
75	36.00	36.20	36.10	0.14	0.10	35
76	32.60	32.60	32.60	0.00	0.00	35
77	34.60	35.50	35.05	0.64	0.45	35
78	35.50	35.40	35.45	0.07	0.05	35
79	35.50	35.50	35.50	0.00	0.00	35
80	35.40	35.30	35.35	0.07	0.05	35
81	35.40	35.30	35.35	0.07	0.05	35
82	34.80	34.70	34.75	0.07	0.05	35
83	32.10	33.00	32.55	0.64	0.45	35
84	34.70	34.80	34.75	0.07	0.05	35
85	34.70	34.80	34.75	0.07	0.05	35
86	34.70	34.80	34.75	0.07	0.05	35
87	34.70	34.80	34.75	0.07	0.05	35
88	34.70	34.80	34.75	0.07	0.05	35
89	34.70	34.80	34.75	0.07	0.05	35
91	32.30	32.30	32.30	0.00	0.00	35
92	34.10	34.80	34.45	0.49	0.35	35
93	34.30	35.90	35.10	1.13	0.80	35
94	34.70	35.40	35.05	0.49	0.35	35
95	34.70	35.40	35.05	0.49	0.35	35
96	34.70	34.80	34.75	0.07	0.05	35
97	34.70	34.70	34.70	0.00	0.00	35
98	32.80	32.90	32.85	0.07	0.05	35
99	34.70	34.10	34.40	0.42	0.30	35
100	34.70	34.40	34.55	0.21	0.15	35
101	34.70	34.40	34.55	0.21	0.15	35
102	34.70	34.40	34.55	0.21	0.15	35
103	34.70	34.40	34.55	0.21	0.15	35
104	34.70	34.40	34.55	0.21	0.15	35
105	34.70	34.50	34.60	0.14	0.10	35
106	34.70	34.40	34.55	0.21	0.15	35
107	34.90	35.80	35.35	0.64	0.45	35
108	34.70	35.50	35.10	0.57	0.40	35
109	34.70	35.60	35.15	0.64	0.45	35
110	34.70	35.20	34.95	0.35	0.25	35
111	34.70	35.00	34.85	0.21	0.15	35
112	33.30	33.20	33.25	0.07	0.05	35
113	34.90	33.90	34.40	0.71	0.50	35
114	34.90	34.60	34.75	0.21	0.15	35
115	34.90	35.50	35.20	0.42	0.30	35
116	34.90	35.30	35.10	0.28	0.20	35
117	34.90	34.90	34.90	0.00	0.00	35
118	34.90	35.00	34.95	0.07	0.05	35
119	34.90	35.00	34.95	0.07	0.05	35
120	34.90	35.00	34.95	0.07	0.05	35
121	34.90	34.90	34.90	0.00	0.00	35
122	34.90	35.00	34.95	0.07	0.05	35
123	34.90	34.90	34.90	0.00	0.00	35
124	35.00	34.30	34.65	0.49	0.35	35
125	34.90	35.00	34.95	0.07	0.05	35
126	34.90	35.00	34.95	0.07	0.05	35

Table D-2. Temperature of reactors subjected to mesophilic temperature pattern.

CCM Reactor Temperatures

Time day	Rep-1 T°C	Rep-2 T°C	Mean T°C	STDEV	STERR	Chamber T°C
1	38	34.5	36.25	2.47	1.76	35
2	43.2	38.6	40.9	3.25	2.31	35
3	46.6	43.6	45.1	2.12	1.50	35
4	44.1	43.7	43.9	0.28	0.20	35
5	42.1	44.7	43.4	1.84	1.30	35
6	40.3	39.4	39.85	0.64	0.45	35
7	39.9	39	39.45	0.64	0.45	35
8	38.6	38.5	38.55	0.07	0.05	35
9	37.5	37.1	37.3	0.28	0.20	35
10	37.8	37.2	37.5	0.42	0.30	35
11	37	37	37	0.00	0.00	35
12	36.5	36.5	36.5	0.00	0.00	35
13	36	36	36	0.00	0.00	35
14	36	36	36	0.00	0.00	35
15	23.4	23.6	23.5	0.14	0.10	35
16	35	35	35	0.00	0.00	35
17	36.5	36.5	36.5	0.00	0.00	35
18	37	37	37	0.00	0.00	35
19	37.3	37.7	37.5	0.28	0.20	35
20	37.3	37.5	37.4	0.14	0.10	35
21	37	37	37	0.00	0.00	35
22	37	37	37	0.00	0.00	35
23	36.8	36.8	36.8	0.00	0.00	35
24	36.7	36.7	36.7	0.00	0.00	35
25	36.7	36.7	36.7	0.00	0.00	35
26	36.5	36.5	36.5	0.00	0.00	35
27	36.4	36.4	36.4	0.00	0.00	35
28	24.2	23.7	23.95	0.35	0.25	35
29	35	35	23.95	0.35	0.25	35
30	34.9	35.2	35.05	0.21	0.15	35
31	35	35	35	0.00	0.00	35
32	35.6	35.5	35.55	0.07	0.05	35
33	35.9	36.4	36.15	0.35	0.25	35
34	35.3	36	35.65	0.49	0.35	35
35	35	35	35	0.00	0.00	35
36	35	35	35	0.00	0.00	35
37	35	35	35	0.00	0.00	35
38	35	35	35	0.00	0.00	35
39	35	35	35	0.00	0.00	35
40	35	35	35	0.00	0.00	35
41	35	35	35	0.00	0.00	35
42	35	35	35	0.00	0.00	35
43	21	22	22	0.42	0.30	35
44	33	33	33	0.14	0.10	35
45	34	33	34	0.49	0.35	35
46	35	35	35	0.00	0.00	35
47	35	35	35	0.00	0.00	35
48	35	35	35	0.00	0.00	35
49	35	35	35	0.00	0.00	35
50	35	35	35	0.00	0.00	35
51	35	35	35	0.00	0.00	35
52	35	35	35	0.00	0.00	35
53	35	35	35	0.00	0.00	35
54	35	35	35	0.00	0.00	35
55	35	35	35	0.00	0.00	35
56	35	35	35	0.00	0.00	35
57	23	23	23	0.57	0.40	35
58	33	34	33	0.21	0.15	35
59	34	34	34	0.00	0.00	35
60	35	35	35	0.28	0.20	35

Cont.

Time	Rep-1	Rep-2	Mean	STDEV	STERR	Chamber
day	T°C	T°C	T°C			T°C
61	34.9	35.7	35.3	0.57	0.40	35
62	35	35.7	35.35	0.49	0.35	35
63	35	35.6	35.3	0.42	0.30	35
64	35	35.6	35.3	0.42	0.30	35
65	35	35.6	35.3	0.42	0.30	35
66	35	35.6	35.3	0.42	0.30	35
67	35	35.6	35.3	0.42	0.30	35
68	35	35.6	35.3	0.42	0.30	35
69	35	35.6	35.3	0.42	0.30	35
70	35	35.6	35.3	0.42	0.30	35
71	23	22	22.5	0.71	0.50	35
72	34.6	35.5	35.05	0.64	0.45	35
73	34.6	35.5	35.05	0.64	0.45	35
74	34.6	35.5	35.05	0.64	0.45	35
75	34.6	35.5	35.05	0.64	0.45	35
76	34.6	35.5	35.05	0.64	0.45	35
77	34.6	35.5	35.05	0.64	0.45	35
78	34.6	35.5	35.05	0.64	0.45	35
79	34.6	35.5	35.05	0.64	0.45	35
80	35.5	35.4	35.45	0.07	0.05	35
81	35.5	35.5	35.5	0.00	0.00	35
82	35.4	35.3	35.35	0.07	0.05	35
83	35.4	35.3	35.35	0.07	0.05	35
84	34.8	34.7	34.75	0.07	0.05	35
85	22	24	23	1.41	1.00	35
86	34.7	34.8	34.75	0.07	0.05	35
87	34.7	34.8	34.75	0.07	0.05	35
88	34.7	34.8	34.75	0.07	0.05	35
89	34.7	34.8	34.75	0.07	0.05	35
91	34.7	34.8	34.75	0.07	0.05	35
92	34.7	34.8	34.75	0.07	0.05	35
93	32.3	32.3	32.3	0.00	0.00	35
94	34.1	34.8	34.45	0.49	0.35	35
95	34.3	35.9	35.1	1.13	0.80	35
96	34.7	35.4	35.05	0.49	0.35	35
97	34.7	35.4	35.05	0.49	0.35	35
98	34.7	34.8	34.75	0.07	0.05	35
99	34.7	34.7	34.7	0.00	0.00	35
100	22	23	22.5	0.71	0.50	35
101	34.7	34.1	34.4	0.42	0.30	35
102	34.7	34.4	34.55	0.21	0.15	35
103	34.7	34.4	34.55	0.21	0.15	35
104	34.7	34.4	34.55	0.21	0.15	35
105	34.7	34.4	34.55	0.21	0.15	35
106	34.7	34.4	34.55	0.21	0.15	35
107	34.7	34.5	34.6	0.14	0.10	35
108	34.7	34.4	34.55	0.21	0.15	35
109	34.9	35.8	35.35	0.64	0.45	35
110	34.7	35.5	35.1	0.57	0.40	35
111	34.7	35.6	35.15	0.64	0.45	35
112	34.7	35.2	34.95	0.35	0.25	35
113	34.7	35	34.85	0.21	0.15	35
114	23	23.3	23.15	0.21	0.15	35
115	34.9	33.9	34.4	0.71	0.50	35
116	34.9	34.6	34.75	0.21	0.15	35
117	34.9	35.5	35.2	0.42	0.30	35
118	34.9	35.3	35.1	0.28	0.20	35
119	34.9	34.9	34.9	0.00	0.00	35
120	34.9	35	34.95	0.07	0.05	35
121	34.9	35	34.95	0.07	0.05	35
122	34.9	35	34.95	0.07	0.05	35
123	34.9	34.9	34.9	0.00	0.00	35
124	34.9	35	34.95	0.07	0.05	35
125	34.9	34.9	34.9	0.00	0.00	35
126	35	34.3	34.65	0.49	0.35	35

Appendix D-2: Mineralization data for ^{14}C phenanthrene

Table D-3. Mineralization of ^{14}C phenanthrene in reactors subjected to thermophilic temperatures.

CFT Reactor-1

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	130,834	81,376		212,210	0.096	0.804	0.804
2	474,718	169,015		643,733	0.290	2.439	3.244
3	79,076	25,079		104,155	0.047	0.395	3.638
4	880,963	236,740	304,350	1,422,053	0.641	5.389	9.027
5	1,066,387	944,493	401,125	2,412,005	1.086	9.140	18.167
6	177,000	66,000	23,250	266,250	0.120	1.009	19.176
7	87,240	44,132	10,624	141,996	0.064	0.538	19.714
8	0	64,134	27,920	92,054	0.041	0.349	20.063
9	40,320	448	0	40,768	0.018	0.154	20.217
10	23,400	11,956	6,400	41,756	0.019	0.158	20.376
11	58,830	6,810	0	65,640	0.030	0.249	20.624
12	21,497	12,660	8,655	42,812	0.019	0.162	20.787
13	448,280	85,320	19,635	553,235	0.249	2.096	22.883
14	82,670	65,130	21,435	169,235	0.076	0.641	23.524
15	111,685	18,536	4,554	134,775	0.061	0.511	24.035
16	1,350	7,448	2,656	11,454	0.005	0.043	24.079
17	23,660	0	0	23,660	0.011	0.090	24.168
18	54,300	38,500	37,760	130,560	0.059	0.495	24.663

CFT Reactor-2

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	88,200	55,611		143,811	0.065	0.545	0.545
2	446,895	194,180		641,075	0.289	2.429	2.974
3	85,145	27,624		112,769	0.051	0.427	3.402
4	246,975	243,338	136,554	626,867	0.282	2.375	5.777
5	1,823,200	866,950	0	2,690,150	1.212	10.194	15.971
6	174,000	66,250	7,392	247,642	0.112	0.938	16.910
7	97,867	49,197	0	147,064	0.066	0.557	17.467
8	0	14,074	5,565	19,639	0.009	0.074	17.541
9	26,151	0	0	26,151	0.012	0.099	17.640
10	36,191	0	0	36,191	0.016	0.137	17.778
11	40,656	174	0	40,830	0.018	0.155	17.932
12	31,350	15,892	149	47,391	0.021	0.180	18.112
13	701,835	1,566	0	703,401	0.317	2.665	20.777
14	1,420	3,944	0	5,364	0.002	0.020	20.798
15	36,043	1,568	0	37,611	0.017	0.143	20.940
16	20,860	56	0	20,916	0.009	0.079	21.020
17	27,008	868	0	27,876	0.013	0.106	21.125
18	33,845	29,960	0	63,805	0.029	0.242	21.367

CCT Reactor-1

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	50,412	17,157		67,569	0.030	0.256	0.256
2	467,820	55,052		522,872	0.236	1.981	2.237
3	96,936	34,815		131,751	0.059	0.499	2.737
4	441,693	173,578	55,890	671,161	0.302	2.543	5.280
5	420,240	276,160	367,304	1,063,704	0.479	4.031	9.311
6	36,482	64,368	14,307	115,157	0.052	0.436	9.747
7	53,157	23,115	4,080	80,352	0.036	0.304	10.052
8	178,408	108,360	0	286,768	0.129	1.087	11.138
9	339,845	56,220	17,310	413,375	0.186	1.566	12.705
10	113,812	90,300	32,550	236,662	0.107	0.897	13.602
11	153,368	92,887	32,004	278,259	0.125	1.054	14.656
12	190,017	171,013	70,098	431,128	0.194	1.634	16.290
13	1,325,340	800,310	197,428	2,323,078	1.046	8.803	25.093
14	77,784	195,990	153,020	426,794	0.192	1.617	26.710
15	159,222	44,485	11,808	215,515	0.097	0.817	27.527
16	2,400	15,686	3,664	21,750	0.010	0.082	27.609
17	35,564	19,840	14,240	69,644	0.031	0.264	27.873
18	20,960	28,365	28,640	77,965	0.035	0.295	28.169

CCT Reactor-2

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	64,372	18,410		82,782	0.037	0.314	0.314
2	423,381	196,765		620,146	0.279	2.350	2.664
3	53,380	29,768		83,148	0.037	0.315	2.979
4	195,960	96,908	12,292	305,159	0.137	1.156	4.135
5	342,732	272,428	23,128	638,288	0.288	2.419	6.554
6	80,135	0	0	80,135	0.036	0.304	6.858
7	175,700	86,335	24,780	286,815	0.129	1.087	7.944
8	399,384	180,482	25,680	605,546	0.273	2.295	10.239
9	189,584	145	0	189,729	0.085	0.719	10.958
10	172,733	79,141	9,810	261,684	0.118	0.992	11.950
11	202,125	47,328	4,298	253,751	0.114	0.962	12.911
12	166,355	55,796	2,786	224,937	0.101	0.852	13.764
13	990,760	585,510	60,578	1,636,848	0.737	6.203	19.966
14	117,000	107,400	43,848	268,248	0.121	1.017	20.983
15	26,235	0	0	26,235	0.012	0.099	21.082
16	14,355	4,526	0	18,881	0.009	0.072	21.154
17	67,089	0	0	67,089	0.030	0.254	21.408
18	4,488	41,850	19,040	65,378	0.029	0.248	21.656

Table D-4. Mean accumulation percentages of ^{14}C phenanthrene as $^{14}CO2$ at thermophilic temperature pattern.

CFT Reactors

Time week	Replicate-1 %	eplicate- %	Mean %	STDEV	STERR
1	0.804	0.545	0.675	0.183	0.130
2	3.244	2.974	3.109	0.190	0.135
3	3.638	3.402	3.520	0.167	0.119
4	9.027	5.777	7.402	2.298	1.630
5	18.167	15.971	17.069	1.553	1.101
6	19.176	16.910	18.043	1.603	1.137
7	19.714	17.467	18.591	1.589	1.127
8	20.063	17.541	18.802	1.783	1.265
9	20.217	17.640	18.929	1.822	1.292
10	20.376	17.778	19.077	1.837	1.303
11	20.624	17.932	19.278	1.904	1.350
12	20.787	18.112	19.449	1.891	1.341
13	22.883	20.777	21.830	1.489	1.056
14	23.524	20.798	22.161	1.928	1.367
15	24.035	20.940	22.488	2.188	1.552
16	24.079	21.019	22.549	2.164	1.535
17	24.168	21.125	22.647	2.152	1.526
18	24.663	21.367	23.015	2.331	1.653

CCT Reactors

Time Week	Replicate-1 %	eplicate- %	Mean %	STDEV	STERR
0	0	0			
1	0.256	0.314	0.285	0.041	0.029
2	2.237	2.664	2.451	0.301	0.214
3	2.737	2.979	2.858	0.171	0.121
4	5.280	4.135	4.708	0.810	0.574
5	9.311	6.554	7.932	1.949	1.383
6	9.747	6.858	8.302	2.043	1.449
7	10.052	7.944	8.998	1.490	1.057
8	11.138	10.239	10.689	0.636	0.451
9	12.705	10.958	11.831	1.235	0.876
10	13.602	11.950	12.776	1.168	0.828
11	14.656	12.911	13.784	1.234	0.875
12	16.290	13.764	15.027	1.786	1.267
13	25.093	19.966	22.530	3.625	2.571
14	26.710	20.983	23.847	4.050	2.872
15	27.527	21.082	24.305	4.557	3.232
16	27.609	21.154	24.382	4.564	3.237
17	27.873	21.408	24.641	4.572	3.242
18	28.169	21.656	24.912	4.605	3.266

Table D-5. Mineralization of ^{14}C phenanthrene in reactors subjected to mesophilic temperatur

CCM Reactor-1

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	49,893	41,282		91,174	0.041	0.345	0.345
2	164,808	308,767	177,537	651,112	0.293	2.467	2.813
3	109,857	12,905	1,089	123,851	0.056	0.469	3.282
4	33,000	83,781	53,444	170,225	0.077	0.645	3.927
5	1,781,532	1,065,635	265,344	3,112,511	1.402	11.795	15.722
6	680,580	2,461,748	1,271,184	4,413,512	1.988	16.725	32.447
7	1,557,948	55,320	15,920	1,629,188	0.734	6.174	38.620
8	34,000	50,340	14,800	99,140	0.045	0.376	38.996
9	97,681	16,089	0	113,770	0.051	0.431	39.427
10	65,410	21,576	65	87,051	0.039	0.330	39.757
11	45,756	0	0	45,756	0.021	0.173	39.930
12	39,742	9,600	544	49,886	0.022	0.189	40.119
13	43,800	3,131	0	46,931	0.021	0.178	40.297
14	32,550	10,633	5,145	48,328	0.022	0.183	40.480
15	47,010	24,645	0	71,655	0.032	0.272	40.752
16	7,410	0	0	7,410	0.003	0.028	40.780
17	22,860	0	0	22,860	0.010	0.087	40.867
18	21,750	37,448	18,120	77,318	0.035	0.293	41.160

CCM Reactor-2

Time Week	Trap-1 DPM	Trap-2 DPM	Trap-3 DPM	Total DPM	Total uci	Mine %	Accum %
1	28,473	19,843		48,316	0.022	0.183	0.183
2	152,008	245,647	205,282	602,937	0.272	2.285	2.468
3	134,046	110,635	51,447	296,128	0.133	1.122	3.590
4	0	16,269	22,836	39,105	0.018	0.148	3.738
5	2,190,744	1,963,335	548,080	4,702,159	2.118	17.819	21.557
6	196,956	1,243,587	950,064	2,390,607	1.077	9.059	30.616
7	1,416,576	159,600	47,968	1,624,144	0.732	6.155	36.770
8	288,728	377,910	159,424	826,062	0.372	3.130	39.901
9	129,704	26,412	7,709	163,825	0.074	0.621	40.522
10	43,059	5,766	780	49,605	0.022	0.188	40.710
11	38,924	9,715	0	48,639	0.022	0.184	40.894
12	0	17,429	3,886	21,315	0.010	0.081	40.975
13	27,480	1,530	0	29,010	0.013	0.110	41.085
14	32,220	9,210	0	41,430	0.019	0.157	41.242
15	53,820	34,230	0	88,050	0.040	0.334	41.575
16	68,820	0	0	68,820	0.031	0.261	41.836
17	14,370	0	0	14,370	0.006	0.054	41.890
18	22,500	25,200	0	47,700	0.021	0.181	42.071

Table D-6. Mean accumulation percentages of ^{14}C phenanthrene as $^{14}CO2$ at mesophilic temper

CCM Reactors

Time week	Replicate-1 %	Replicate-2 %	Mean %	STDEV	STERR
1	0.345	0.183	0.264	0.115	0.081
2	2.813	2.468	2.640	0.244	0.173
3	3.282	3.590	3.436	0.218	0.154
4	3.802	3.738	3.770	0.045	0.032
5	15.597	21.557	18.577	4.214	2.989
6	32.322	30.616	31.469	1.206	0.855
7	38.495	36.770	37.633	1.220	0.865
8	38.742	39.901	39.321	0.819	0.581
9	39.173	40.522	39.847	0.953	0.676
10	39.503	40.710	40.106	0.853	0.605
11	39.930	40.894	40.412	0.681	0.483
12	40.119	40.975	40.547	0.605	0.429
13	40.297	41.085	40.691	0.557	0.395
14	40.480	41.242	40.861	0.538	0.382
15	40.752	41.575	41.164	0.582	0.413
16	40.780	41.836	41.308	0.747	0.530
17	40.867	41.890	41.379	0.724	0.513
18	41.160	42.071	41.615	0.645	0.457

Appendix D-3: Sequential extraction data for ^{14}C phenanthrene

Table D-7. Mean extraction values of phenanthrene from reactors operated at thermophilic temperature patterns.

CFT Reactors

Time	% Water Extracted			% Methanol Extracted			% Methylene Extracted			% Total Extracted		
Week	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR
0	0.00	0.00	0.00	54.85	2.76	1.95	9.40	0.67	0.47	64.25	2.09	1.48
2	0.09	0.13	0.09	41.64	3.50	2.48	5.71	2.66	1.89	47.44	6.29	4.46
4	1.95	0.60	0.42	22.55	9.37	6.65	6.16	2.74	1.94	30.66	12.71	9.01
6	0.00	0.00	0.00	5.66	1.38	0.98	0.18	0.12	0.09	5.84	1.50	1.06
8	0.00	0.00	0.00	5.22	1.98	1.40	0.11	0.16	0.11	5.33	1.82	1.29
10	0.00	0.00	0.00	5.70	1.99	1.41	0.00	0.00	0.00	5.70	1.99	1.41
12	0.00	0.00	0.00	4.80	1.17	0.83	0.06	0.09	0.06	4.87	1.08	0.77
14	0.01	0.02	0.01	0.78	0.24	0.17	0.00	0.00	0.00	0.80	0.22	0.15
16	0.00	0.00	0.00	0.83	0.30	0.21	0.00	0.00	0.00	0.83	0.30	0.21
18	0.00	0.00	0.00	0.83	0.38	0.27	0.00	0.00	0.00	0.83	0.38	0.27

CCT Reactors

Time	% Water Extracted			% Methanol Extracted			% Methylene Extracted			% Total Extracted		
Week	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR
0	0.21	0.25	0.18	51.02	1.83	1.30	8.73	1.35	0.96	59.96	2.93	2.08
2	0.91	0.37	0.26	43.15	0.13	0.10	7.87	1.82	1.29	51.92	1.32	0.93
4	0.89	0.26	0.19	45.15	0.21	0.15	0.00	0.00	0.00	46.04	0.05	0.03
6	0.00	0.00	0.00	43.31	2.56	1.82	4.04	2.53	1.79	47.35	0.03	0.02
8	0.00	0.00	0.00	28.86	1.47	1.04	0.00	0.00	0.00	28.86	1.47	1.04
10	0.00	0.00	0.00	26.64	1.09	0.77	0.00	0.00	0.00	26.64	1.09	0.77
12	0.17	0.23	0.17	19.57	0.21	0.15	0.00	0.00	0.00	19.73	0.45	0.32
14	0.15	0.07	0.05	4.90	0.20	0.14	0.00	0.00	0.00	5.05	0.27	0.19
16	0.00	0.00	0.00	5.34	0.63	0.45	0.00	0.00	0.00	5.34	0.63	0.45
18	0.00	0.00	0.00	4.99	0.79	0.56	0.00	0.00	0.00	4.99	0.79	0.56

CNT Reactors

Time	% Water Extracted			% Methanol Extracted			% Methylene Extracted			% Total Extracted		
Week	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR
0	0.00	0.00	0.00	67.31	0.64	0.45	0.88	0.81	0.58	68.19	0.18	0.13
2	0.17	0.10	0.07	66.97	0.79	0.56	0.76	0.06	0.04	67.89	0.95	0.67
4	0.12	0.01	0.01	65.51	0.55	0.39	2.03	0.02	0.01	67.67	0.59	0.42
6	0.00	0.00	0.00	63.83	0.41	0.29	1.21	0.22	0.15	65.05	0.19	0.14
8	0.00	0.00	0.00	66.45	0.35	0.25	1.60	0.30	0.21	66.98	1.45	1.03
10	0.16	0.00	0.00	66.58	1.04	0.74	0.64	1.05	0.74	67.54	0.01	0.01
12												
14												
16												
18	0.11	0.00	0.00	65.26	2.85	2.02	0.38	0.22	0.16	65.65	0.92	0.65

Table D-8. Mean extraction values of phenanthrene from reactors operated at mesophilic temperature pattern.

CCM Reactors

	% Water Extracted			% Methanol Extracted			% methylene Extracted			% Total Extracted		
Time Week	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR	Mean	STDEV	STERR
0	0.21	0.25	0.18	51.02	1.83	1.30	8.73	1.35	0.96	59.96	2.93	2.08
2	0.00	0.00	0.00	56.98	1.37	0.97	7.30	4.52	3.20	64.28	3.15	2.24
4	0.00	0.00	0.00	55.16	1.56	1.11	9.36	0.84	0.60	64.52	0.72	0.51
6	0.00	0.00	0.00	18.50	3.60	2.55	0.88	1.25	0.88	19.39	4.85	3.44
8	0.00	0.00	0.00	10.33	6.61	4.69	0.07	0.11	0.07	10.40	6.50	4.61
10	0.00	0.00	0.00	11.19	7.28	5.17	0.00	0.00	0.00	11.19	7.28	5.17
12	0.31	0.43	0.31	10.86	6.25	4.44	0.00	0.00	0.00	11.16	5.82	4.13
14	0.37	0.13	0.09	10.21	5.90	4.18	0.00	0.00	0.00	10.58	5.77	4.09
16	0.13	0.06	0.04	9.92	6.20	4.40	0.00	0.00	0.00	10.06	6.26	4.44
18	0.00	0.00	0.00	10.80	5.49	3.89	0.00	0.00	0.00	10.80	5.49	3.89

Appendix D-4: Extractable diesel range organics data

Table D-9. Extractable diesel range organics from reactors operated at thermophilic temperature

CNT Reactor-1

Time Week	Sample No	Wet Wt g	Dry Wt g	GC mg/25ml	EDRO mg/kgdb
0	1	10.02	9.17	168	18,321
	2	10.02	9.17	171	18,648
Mean				170	18,484
STDEVP					164
STERR					116
2	1	10.01	9.20	137	14,910
	2	10.01	9.20	158	17,168
Mean				148	16,039
STDEVP					1,129
STERR					801
4	1	10.01	9.27	144	15,521
	2	10.04	9.29	141	15,225
Mean				143	15,373
STDEVP					148
STERR					105
6	1	10.00	9.31	100	10,760
	2	10.00	9.26	105	11,349
Mean				103	11,054
STDEVP					294
STERR					209
8	1	10.02	9.38	86	9,156
	2	10.03	9.39	84	8,923
Mean				85	9,039
STDEVP					116
STERR					82
10	1	10.07	9.42	83	8,797
	2	10.06	9.41	84	8,925
Mean				83	8,861
STDEVP					64
STERR					45
18					
	1	10.01	9.47	85	8,976
	2	10.04	9.54	76	7,956
Mean				80	8,466
STDEVP					510
STERR					362

CNT Reactor-2

Time Week	Sample No	Wet Wt g	Dry Wt g	GC mg/25ml	EDRO mg/kgdb

0	1	10.01	9.16	167	18,231
	2	10.02	9.17	173	18,866
Mean				170	18,549
STDEVP					317
STERR					225
2	1	10.08	9.27	153	16,551
	2	10.08	9.27	147	15,916
Mean				150	16,234
STDEVP					317
STERR					225
4	1	10.05	9.27	144	15,556
	2	10.01	9.23	133	14,438
Mean				139	14,997
STDEVP					559
STERR					397
6	1	10.05	9.31	94	10,116
	2	10.00	9.26	120	12,968
Mean				107	11,542
STDEVP					1,426
STERR					1,012
8	1	10.03	9.39	89	9,459
	2	10.03	9.39	83	8,817
Mean				86	9,138
STDEVP					321
STERR					228
10	1	10.07	9.42	82	8,755
	2	10.06	9.41	85	8,984
Mean				84	8,869
STDEVP					114
STERR					81
18	1	10.03	9.63	85	8,827
	2	10.07	9.67	80	8,273
Mean				83	8,550
STDEVP					277
STERR					196

UFT Reactor-1

Time Week	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	10.62	6.54	4.57	25.19	3,854	5,514
	2	10.81	6.65	4.65	22.15	3,329	4,763
Mean					23.67	3,592	5,138
STDEVP					1.52	262	375
STERR					1.08	186	266
2	1	10.01	6.27	4.62	24.00	3,831	5,197
	2	10.01	6.27	4.62	20.17	3,220	4,369
Mean					22.09	3,525	4,783
STDEVP					1.91	305	414
STERR					1.36	217	294
4	1	10.02	6.29	4.78	18.07	2,874	3,777
	2	10.03	6.29	4.79	19.31	3,068	4,031
Mean					18.69	2,971	3,904
STDEVP					0.62	97	127
STERR					0.44	69	90
6	1	10.03	6.32	4.84	5.96	943	1,231
	2	10.03	6.32	4.84	6.64	1,052	1,373
Mean					6.30	997	1,302
STDEVP					0.34	54	71
STERR					0.24	39	50
			3.704				
8	1	10.03	6.27	4.84	3.70	590	764
	2	10.03	6.27	4.84	4.90	781	1,012
Mean					4.30	686	888
STDEVP					0.60	96	124
STERR					0.43	68	88
10	1	10.03	6.11	4.71	4.04	661	858
	2	10.03	6.12	4.72	3.62	592	767
Mean					3.83	626	813
STDEVP					0.21	35	45
STERR					0.15	25	32
12	1	10.03	6.09	4.63	3.67	602	792
	2	10.03	6.08	4.62	2.57	423	557
Mean					3.12	513	675
STDEVP					0.55	90	118
STERR					0.39	64	84
14	1	10.03	6.20	4.76	4.11	663	863

	2	10.03	6.20	4.76	2.43	392	510
Mean					3.27	527	687
STDEVP					0.84	135	176
STERR					0.60	96	125
16	1	10.06	5.91	4.58	3.44	582	751
	2	10.17	5.98	4.63	2.47	413	533
Mean					2.96	498	642
STDEVP					0.49	85	109
STERR					0.34	60	77
18	1	10.08	6.08	4.71	2.33	383	494
	2	10.03	6.05	4.69	2.28	377	486
Mean					2.31	380	490
STDEVP					0.03	3	4
STERR					0.02	2	3

UFT Reactor-2

Time	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
Week	1	10.63	6.43	4.45	26.71	4,153	6,001
0	2	10.6	6.41	4.44	29.56	4,610	6,661
					28.13	4,381	6,331
Mean					1.43	228	330
STDEVP					1.01	162	234
STERR							
	1	10.03	6.07	4.50	23.57	3,882	5,239
2	2	10.02	6.07	4.49	17.92	2,955	3,987
					20.75	3,418	4,613
Mean					2.82	464	626
STDEVP					2.00	329	444
STERR							
	1	10.08	6.29	4.79	10.15	1,613	2,117
4	2	10.01	6.25	4.76	13.34	2,135	2,802
					11.74	1,874	2,460
Mean					1.60	261	343
STDEVP					1.13	185	243
STERR							
	1	10.04	6.24	4.77	10.18	1,631	2,135
6	2	10	6.22	4.75	11.03	1,774	2,322
					10.61	1,703	2,229
Mean					0.43	72	94
STDEVP					0.30	51	67
STERR							
	1	10.04	6.14	4.70	4.50	733	957
8	2	10	6.21	4.76	4.72	760	992
					4.61	746	975
Mean					0.11	14	18

STDEVP					0.08	10	13
STERR							
	1	10.04	6.12	4.69	4.06	663	865
10	2	10	6.08	4.66	4.66	766	999
					4.36	715	932
Mean					0.30	52	67
STDEVP					0.21	37	48
STERR							
	1	10.04	6.29	4.82	3.43	545	711
12	2	10	6.29	4.82	2.31	367	479
					2.87	456	595
Mean					0.56	89	116
STDEVP					0.40	63	82
STERR							
	1	10.04	6.25	4.81	2.21	354	459
14	2	10	6.18	4.76	2.67	432	561
					2.44	393	510
Mean					0.23	39	51
STDEVP					0.16	28	36
STERR							
	1	10.05	5.96	4.62	3.03	508	655
16	2	10.12	6.00	4.65	2.41	403	519
					2.72	455	587
Mean					0.31	53	68
STDEVP					0.22	37	48
STERR							
	1	10.11	6.13	4.76	4.13	674	868
18	2	10.04	6.08	4.72	3.78	622	801
					3.96	648	835
Mean					0.17	26	34
STDEVP					0.12	18	24
STERR							

CFT Reactor-1

Time Week	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	10.02	6.35	4.2545	111	17,480	26,090
	2	10.06	6.375	4.27125	103.316	16,270	24,189
Mean					107.158	16,875	25,139
STDEVP					3.842	605	951
STERR					2.72482	429	674
2	1	10.02	6.331	4.58364	76.34	12,058	16,655
	2	10.05	6.35	4.5974	63.123	9,941	13,730
Mean					69.7315	10,999	15,193
STDEVP					6.6085	1,059	1,462
STERR					4.68688	751	1,037

4	1	10.08	6.307	4.74286	43.573	6,909	9,187
	2	10.02	6.269	4.71429	40.843	6,515	8,664
Mean					42.208	6,712	8,925
STDEVP					1.365	197	262
STERR					0.96809	140	186
6	1	10.01	6.28	4.8356	24.832	3,954	5,135
	2	10.03	6.29	4.8433	28.335	4,505	5,850
Mean					26.5835	4,229	5,493
STDEVP					1.7515	275	358
STERR					1.2422	195	254
8	1	10.01	6.27	4.7652	18.425	2,939	3,867
	2	10.03	6.26	4.7576	14.925	2,384	3,137
Mean					16.675	2,661	3,502
STDEVP					1.75	277	365
STERR					1.24113	197	259
10	1	10.01	6.21	4.74444	15.71	2,530	3,311
	2	10.03	6.21	4.74444	19.49	3,138	4,108
Mean					17.6	2,834	3,710
STDEVP					1.89	304	398
STERR					1.34043	216	283
12	1	10.01	6.31	4.83346	14	2,219	2,896
	2	10.03	6.32	4.84112	20.65	3,267	4,266
Mean					17.325	2,743	3,581
STDEVP					3.325	524	685
STERR					2.35816	372	485
14	1	10.01	6.15	4.797	9.25	1,504	1,928
	2	10.03	6.16	4.8048	6.32	1,026	1,315
Mean					7.785	1,265	1,622
STDEVP					1.465	239	306
STERR					1.03901	170	217
16	1	10.06	5.935	4.56995	5.69	959	1,245
	2	10.05	5.93	4.5661	6.425	1,083	1,407
Mean					6.0575	1,021	1,326
STDEVP					0.3675	62	81
STERR					0.26064	44	57
18	1	10.09	6.05	4.6585	7.05	1,165	1,513
	2	10.06	6.02	4.6354	5.53	919	1,193
Mean					6.29	1,042	1,353
STDEVP					0.76	123	160
STERR					0.53901	87	114

CFT Reactor-2

Time Week	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	7.04	4.412	3.08399	68.155	15,448	22,100
	2	7.03	4.406	3.07979	72.37	16,425	23,498
Mean					70.2625	15,936	22,799
STDEVP					2.1075	489	699
STERR					1.49468	347	496
2	1	10.01	6.315	4.57585	73.225	11,595	16,002
	2	10.01	6.315	4.57585	85.195	13,491	18,618
Mean					79.21	12,543	17,310
STDEVP					5.985	948	1,308
STERR					4.24468	672	928
4	1	10	6.206	4.62347	34.876	5,620	7,543
	2	10.07	6.249	4.65551	41.981	6,718	9,017
Mean					38.4285	6,169	8,280
STDEVP					3.5525	549	737
STERR					2.5195	389	523
6	1	10.01	6.209	4.78093	26.36	4,245	5,514
	2	10.06	6.24	4.8048	30.928	4,956	6,437
Mean					28.644	4,601	5,975
STDEVP					2.284	355	462
STERR					1.61986	252	327
8	1	10.01	6.35	4.85775	15.48	2,438	3,187
	2	10.06	6.22	4.7583	14.38	2,312	3,022
Mean					14.93	2,375	3,104
STDEVP					0.55	63	82
STERR					0.39007	45	58
10	1	10.01	6.17	4.71388	15.094	2,446	3,202
	2	10.06	6.16	4.70624	17.5	2,841	3,718
Mean					16.297	2,644	3,460
STDEVP					1.203	197	258
STERR					0.85319	140	183
12	1	10.01	6.09	4.6893	14.06	2,309	2,998
	2	10.06	6.11	4.7047	15.09	2,470	3,207
Mean					14.575	2,389	3,103
STDEVP					0.515	81	105
STERR					0.36525	57	74

14	1	10.01	6.09	4.60404	7.58	1,245	1,646
	2	10.06	6.07	4.58892	8.2	1,351	1,787
Mean					7.89	1,298	1,717
STDEVP					0.31	53	70
STERR					0.21986	38	50
16	1	10.01	5.886	4.4969	5.397	917	1,200
	2	10.12	5.947	4.54351	5.504	926	1,211
Mean					5.4505	921	1,206
STDEVP					0.0535	4	6
STERR					0.03794	3	4
18	1	10.12	5.97	4.5969	7.19	1,204	1,564
	2	10.09	5.947	4.57919	6.42	1,080	1,402
Mean					6.805	1,142	1,483
STDEVP					0.385	62	81
STERR					0.27305	44	57

CCT Reactor-1

Time Week	Sample No		Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	10.01	6.01	4.34	84.74	14,105	19,536	
	2	10.03	6.02	4.35	85.40	14,187	19,650	
Mean					85.07	14,146	19,593	
STDEVP					0.33	41	57	
STERR					0.23	29	40	
2	1	10.00	6.11	4.50	70.03	11,469	15,562	
	2	10.00	6.11	4.50	76.77	12,574	17,060	
Mean					73.40	12,021	16,311	
STDEVP					3.37	552	749	
STERR					2.39	392	531	
4	1	10.13	5.42	3.99	68.44	12,627	17,133	
	2	10.19	5.45	4.02	70.48	12,927	17,540	
Mean					69.46	12,777	17,337	
STDEVP					1.02	150	203	
STERR					0.72	106	144	
6	1	10.03	6.54	4.84	65.24	9,974	13,478	
	2	10.04	6.55	4.85	61.60	9,407	12,712	
Mean					63.42	9,690	13,095	
STDEVP					1.82	284	383	
STERR					1.29	201	272	
8	1	10.03	6.10	4.53	32.39	5,310	7,157	
	2	10.04	6.10	4.53	33.71	5,526	7,448	
Mean					33.05	5,418	7,302	

STDEVP					0.66	108	146
STERR					0.47	77	103
10	1	10.03	5.79	4.34	29.82	5,150	6,867
	2	10.04	5.80	4.35	30.95	5,336	7,115
Mean					30.39	5,243	6,991
STDEVP					0.57	93	124
STERR					0.40	66	88
12	1	10.03	5.93	4.44	20.61	3,476	4,646
	2	10.04	5.96	4.46	22.45	3,767	5,036
Mean					21.53	3,621	4,841
STDEVP					0.92	146	195
STERR					0.65	103	138
14	1	10.03	5.99	4.50	20.32	3,393	4,512
	2	10.04	5.98	4.50	17.78	2,973	3,953
Mean					19.05	3,183	4,232
STDEVP					1.27	210	279
STERR					0.90	149	198
16	1	10.04	5.95	4.57	19.99	3,357	4,372
	2	10.03	5.95	4.57	24.57	4,129	5,377
Mean					22.28	3,743	4,874
STDEVP					2.29	386	503
STERR					1.62	274	356
18	1	10.05	5.95	4.53	21.73	3,650	4,796
	2	10.03	5.94	4.52	23.99	4,039	5,307
Mean					22.86	3,844	5,051
STDEVP					1.13	195	256
STERR					0.80	138	181

CCT Reactor-2

Time Week	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	10.03	5.99	4.31	84.23	14,064	19,533
	2	10.05	6.00	4.32	74.59	12,429	17,263
Mean					79.41	13,247	18,398
STDEVP					4.82	817	1,135
STERR					3.42	580	805
2	1	10.00	6.18	4.61	71.71	11,610	15,562
	2	10.00	6.20	4.62	70.51	11,380	15,255
Mean					71.11	11,495	15,409
STDEVP					0.60	115	154
STERR					0.43	81	109

4	1	10.06	5.39	4.03	60.04	11,141	14,915
	2	10.01	5.36	4.01	62.05	11,572	15,491
Mean					61.04	11,357	15,203
STDEVP					1.00	215	288
STERR					0.71	153	204
6	1	10.08	6.60	4.95	61.11	9,255	12,356
	2	10.00	6.55	4.91	57.00	8,701	11,617
Mean					59.06	8,978	11,987
STDEVP					2.05	277	370
STERR					1.46	196	262
8	1	10.08	6.00	4.51	29.92	4,987	6,631
	2	10.00	6.00	4.51	27.20	4,533	6,028
Mean					28.56	4,760	6,330
STDEVP					1.36	227	301
STERR					0.96	161	214
10	1	10.08	6.07	4.61	29.84	4,916	6,477
	2	10.00	6.03	4.58	22.99	3,813	5,024
Mean					26.42	4,365	5,751
STDEVP					3.42	551	727
STERR					2.43	391	515
12	1	10.08	6.17	4.65	19.48	3,157	4,187
	2	10.00	6.14	4.63	20.93	3,409	4,521
Mean					20.21	3,283	4,354
STDEVP					0.73	126	167
STERR					0.51	89	118
14	1	10.08	6.19	4.70	17.77	2,870	3,782
	2	10.00	6.17	4.68	16.07	2,605	3,432
Mean					16.92	2,738	3,607
STDEVP					0.85	133	175
STERR					0.60	94	124
16	1	10.09	5.98	4.60	21.72	3,633	4,718
	2	10.06	5.96	4.59	19.63	3,293	4,277
Mean					20.68	3,463	4,497
STDEVP					1.05	170	221
STERR					0.74	120	156
18	1	10.00	6.00	4.60	16.51	2,752	3,588
	2	10.15	6.14	4.71	14.80	2,410	3,143
Mean					15.66	2,581	3,365
STDEVP					0.86	171	222
STERR					0.61	121	158

Table D-10. Extractable diesel range organics from reactors operated at mesophilic temperature

CCM Reactor-1

Time Week	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
0	1	10.03	5.99	4.31	84.23	14,064	19,533
	2	10.05	6.00	4.32	74.59	12,429	17,263
Mean					79.41	13,247	18,398
STDEVP					4.82	817	1,135
STERR					3.42	580	805
2	1	10.02	6.03	4.42	59.38	9,851	13,439
	2	10.00	6.03	4.42	56.74	9,412	12,840
Mean					58.06	9,631	13,140
STDEVP					1.32	219	299
STERR					0.94	156	212
4	1	10.16	6.12	4.55	43.67	7,132	9,600
	2	10.07	6.07	4.51	42.38	6,984	9,399
Mean					43.03	7,058	9,499
STDEVP					0.64	74	100
STERR					0.46	53	71
6	1	10.02	6.09	4.53	28.32	4,648	6,247
	2	10.06	6.12	4.55	34.55	5,649	7,593
Mean					31.43	5,149	6,920
STDEVP					3.12	501	673
STERR					2.21	355	477
8	1	10.02	6.12	4.61	17.94	2,931	3,888
	2	10.06	6.20	4.67	18.48	2,981	3,954
Mean					18.21	2,956	3,921
STDEVP					0.27	25	33
STERR					0.19	18	23
10	1	10.02	6.08	4.61	19.51	3,209	4,228
	2	10.06	6.09	4.62	16.46	2,703	3,561
Mean					17.99	2,956	3,894
STDEVP					1.53	253	333
STERR					1.08	179	236
12	1	10.02	6.02	4.48	10.12	1,681	2,259
	2	10.06	6.03	4.49	14.95	2,479	3,332
Mean					12.54	2,080	2,796
STDEVP					2.41	399	536
STERR					1.71	283	380

14	1	10.02	6.00	4.51	12.34	2,057	2,735
	2	10.06	6.00	4.51	17.09	2,848	3,788
Mean					14.72	2,453	3,261
STDEVP					2.38	396	526
STERR					1.68	281	373
16	1	10.09	5.94	4.45	12.44	2,094	2,796
	2	10.03	5.85	4.38	14.14	2,417	3,227
Mean					13.29	2,256	3,011
STDEVP					0.85	162	216
STERR					0.60	115	153
18	1	10.04	5.89	4.44	11.49	1,951	2,588
	2	10.08	5.92	4.46	13.40	2,263	3,001
Mean					12.44	2,107	2,794
STDEVP					0.95	156	206
STERR					0.67	110	146

CCM Reactor-2

Time	Sample No	Wet Wt g	Dry Wt g	Ash Wt g	GC mg/25ml	EDRO mg/kgdb	EDRO mg/kgash
Week	1	10.01	6.01	4.33	84.74	14,105	19,590
0	2	10.03	6.02	4.33	85.40	14,187	19,704
					85.07	14,146	19,647
Mean					0.33	41	57
STDEVP					0.23	29	40
STERR							
	1	10.00	6.01	4.37	59.13	9,833	13,543
2	2	10.01	6.02	4.37	55.30	9,186	12,653
					57.22	9,509	13,098
Mean					1.92	323	445
STDEVP					1.36	229	316
STERR							
	1	10.03	6.12	4.56	40.72	6,658	8,937
4	2	10.07	6.14	4.57	51.59	8,403	11,279
					46.16	7,530	10,108
Mean					5.44	872	1,171
STDEVP					3.86	619	831
STERR							
	1	10.09	6.04	4.51	23.50	3,888	5,212
6	2	10.03	6.01	4.48	20.44	3,403	4,562
					21.97	3,646	4,887
Mean					1.53	243	325
STDEVP					1.08	172	231
STERR							
	1	10.09	6.12	4.59	11.55	1,886	2,515
8	2	10.03	6.07	4.55	10.68	1,760	2,346

					11.11	1,823	2,431
Mean					0.43	63	85
STDEVP					0.31	45	60
STERR							
	1	10.09	6.02	4.56	14.14	2,349	3,103
10	2	10.03	6.05	4.58	15.26	2,522	3,332
					14.70	2,436	3,217
Mean					0.56	87	115
STDEVP					0.40	62	81
STERR							
	1	10.09	6.05	4.50	15.45	2,554	3,432
12	2	10.03	6.07	4.52	16.73	2,757	3,705
					16.09	2,655	3,569
Mean					0.64	101	136
STDEVP					0.45	72	97
STERR							
	1	10.09	5.94	4.46	16.34	2,750	3,667
14	2	10.03	6.01	4.51	14.75	2,456	3,274
					15.54	2,603	3,470
Mean					0.79	147	196
STDEVP					0.56	104	139
STERR							
	1	10.02	5.89	4.41	11.19	1,900	2,537
16	2	10.00	5.89	4.41	10.52	1,786	2,385
					10.86	1,843	2,461
Mean					0.34	57	76
STDEVP					0.24	40	54
STERR							
	1	10.01	5.87	4.41	9.95	1,696	2,255
18	2	10.05	5.89	4.43	10.86	1,844	2,452
					10.41	1,770	2,353
Mean					0.45	74	98
STDEVP					0.32	53	70
STERR							

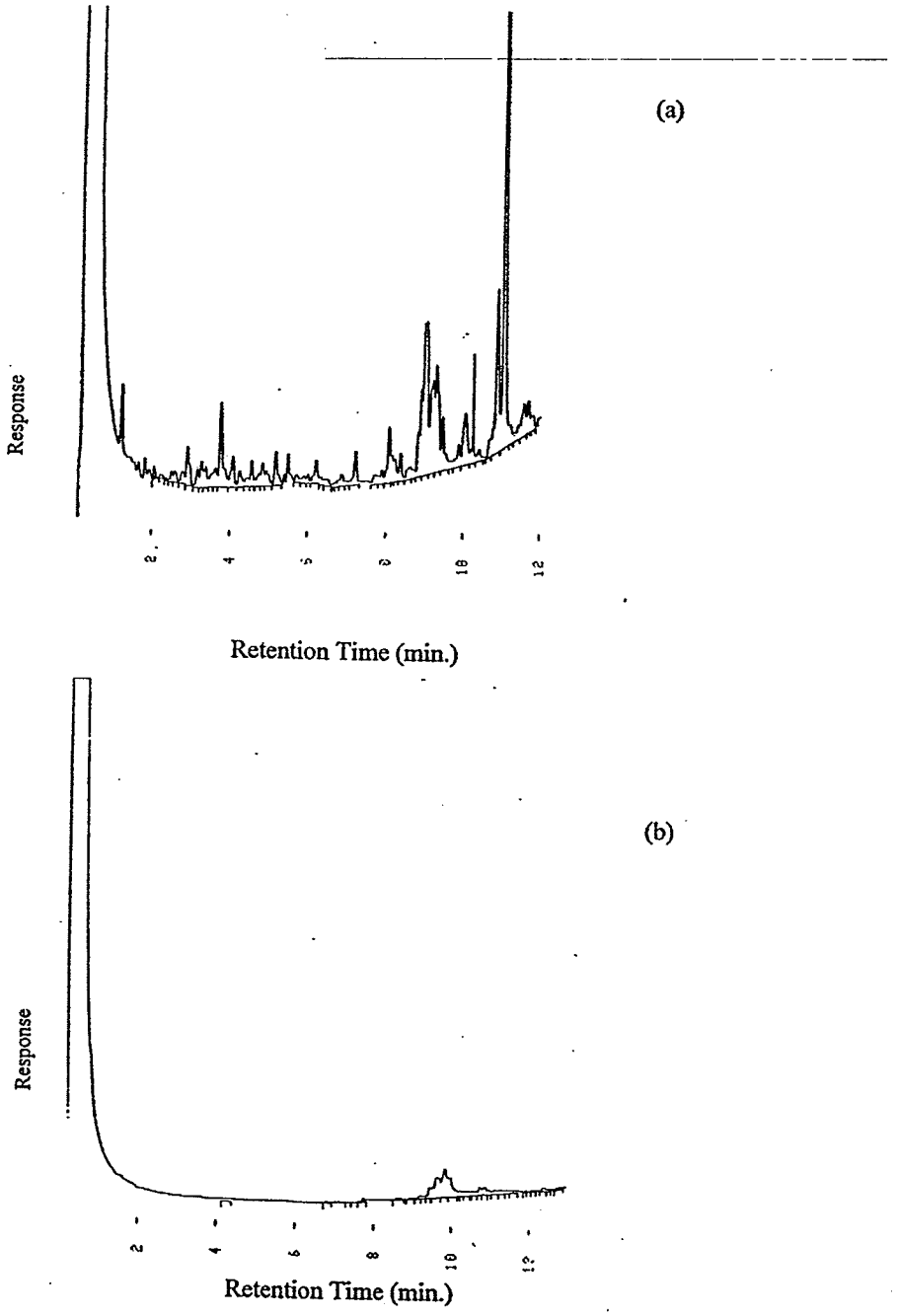

Figure D-1. Gas chromatography with flame ionization chromatograms of UFT extracts at day 0 (a) and 126 (b).

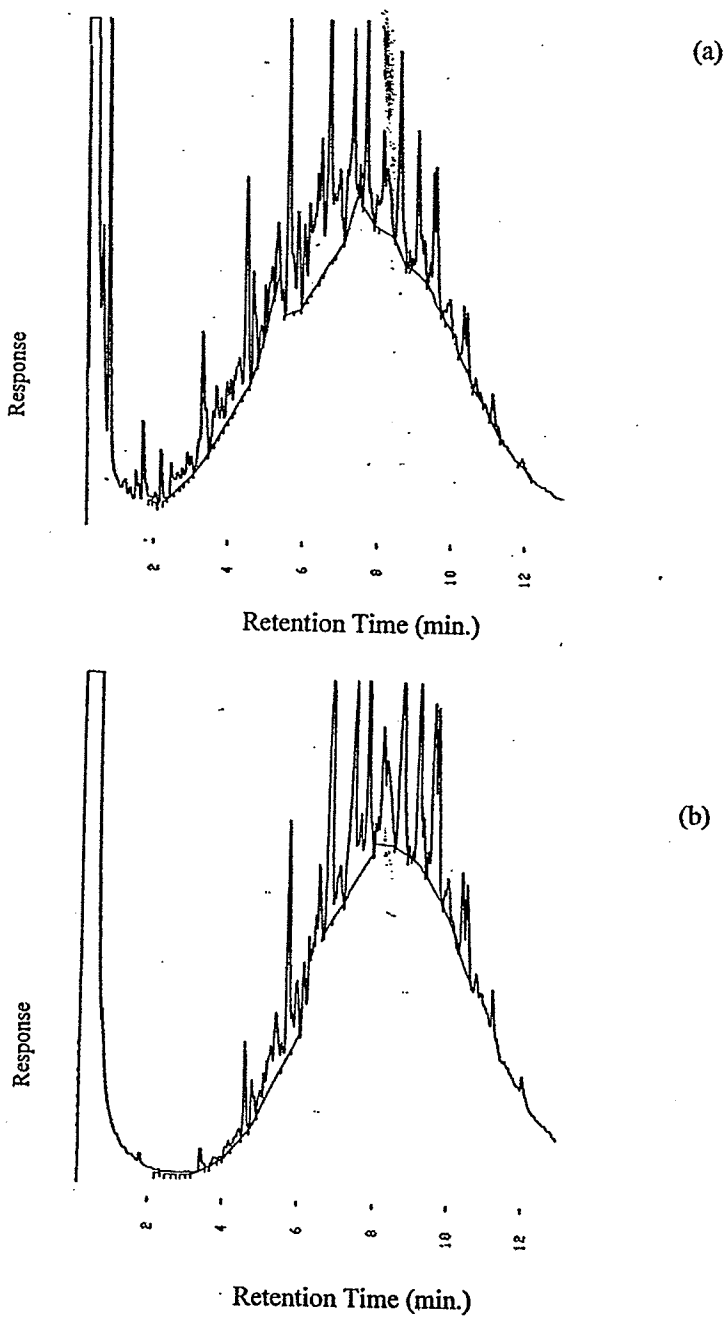

Figure D-2. Gas chromatography with flame ionization chromatograms of CNT extracts at day 0 (a) and 126 (b).

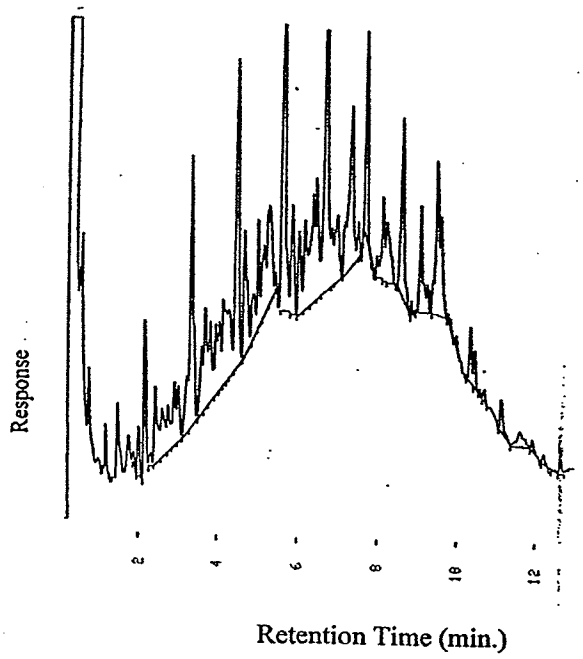

Figure D-3. Gas chromatography with flame ionization chromatograms of CFT extracts at day 0 (a) and 126 (b).

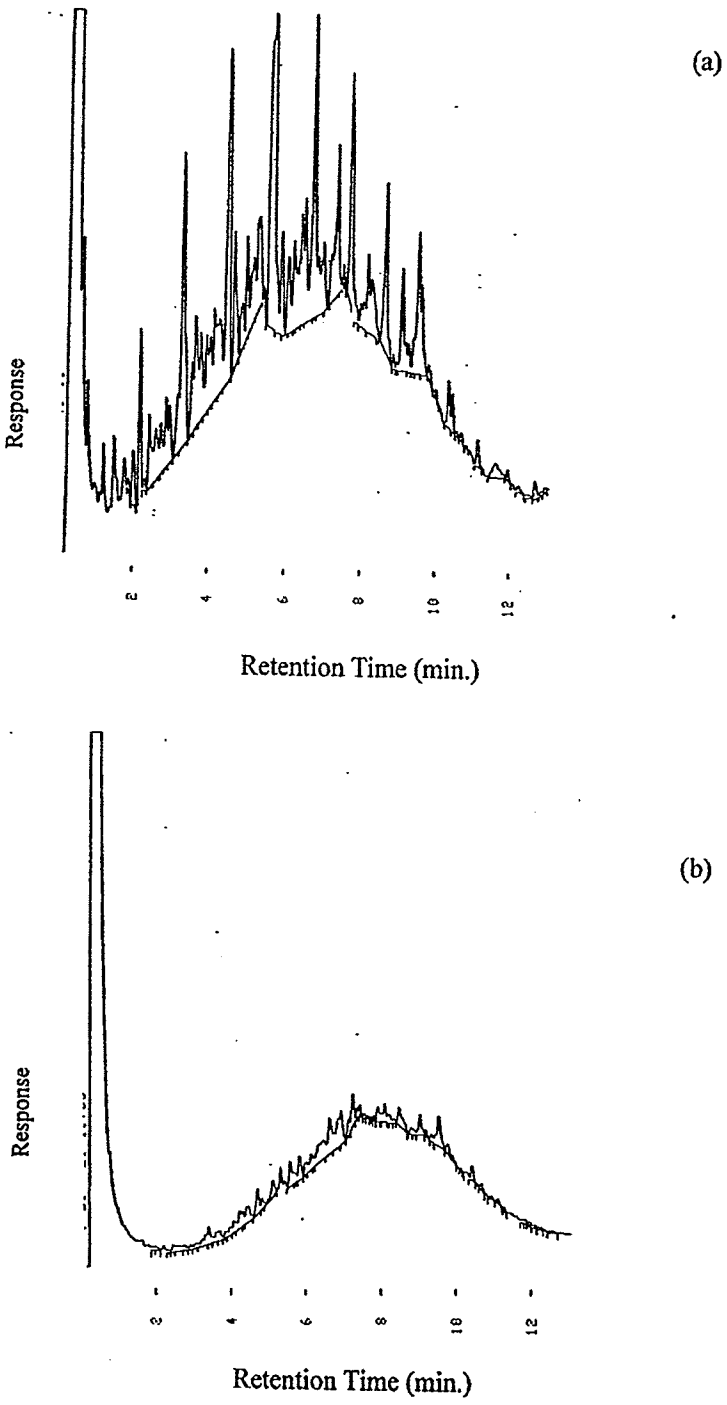

Figure D-4. Gas chromatography with flame ionization chromatograms of CCT extracts at day 0 (a) and 126 (b).

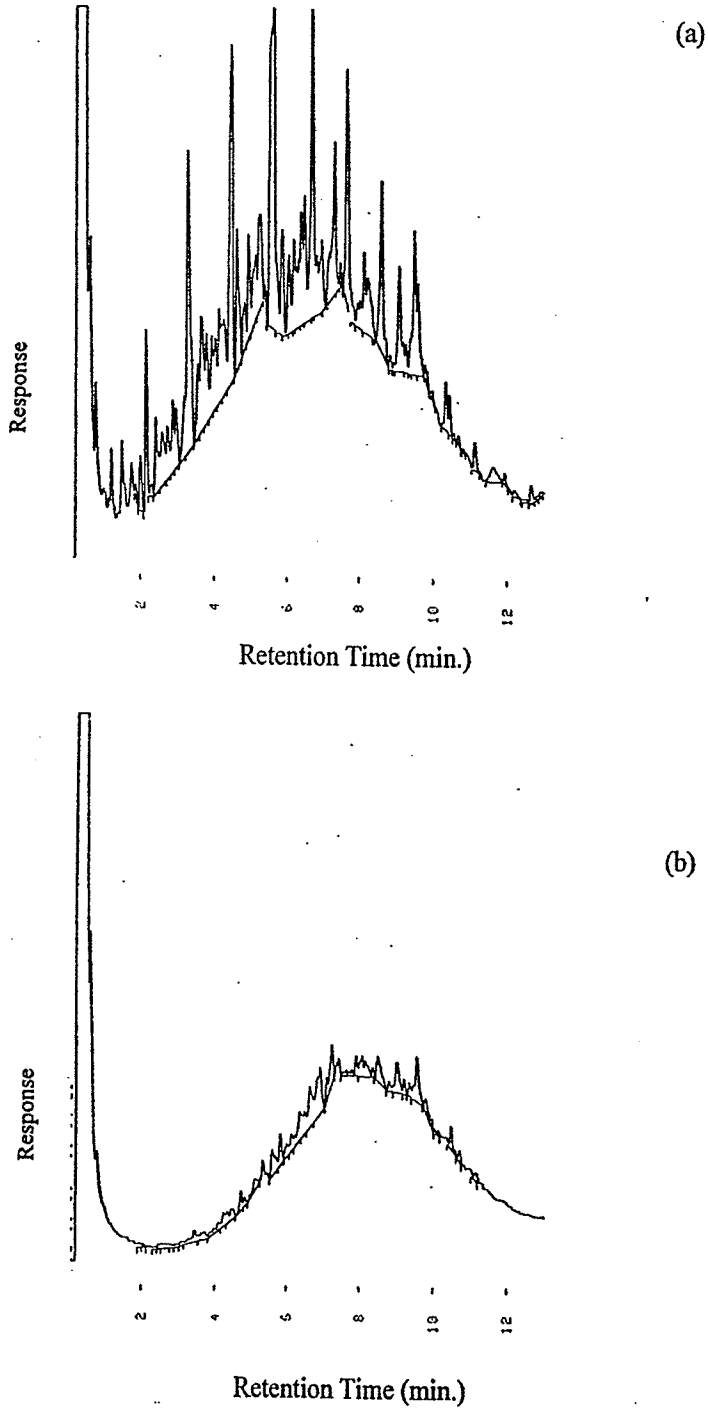

Figure D-5. Gas chromatography with flame ionization chromatograms of CCM extracts at day 0 (a) and 126 (b).

Appendix D-4: Seed germination data

Table D-11. Germination rate for soil and soil compst mixtures

Untreated Samples (Controls):

PS Samples

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	100	100	60	80	50	100
2	100	100	90	100	80	100
3	80	100	60	100	30	100
Mean	93.33	100.00	70.00	93.33	53.33	100.00
Sterr	6.67	0.00	10.01	6.67	14.55	0.00

UF Samples

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	20	60	0	0	0	30
2	40	80	0	0	20	80
3	20	80	0	0	10	40
Mean	26.67	73.33	0.00	0.00	10.00	50.00
Sterr	6.67	6.79	0.00	0.00	5.78	15.29

CF Samples

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	20	20	0	0	0	20
2	0	20	0	0	0	10
3	20	30	0	0	0	20
Mean	13.33	23.33	0.00	0.00	0.00	16.67
Sterr	6.67	3.40	0.00	0.00	0.00	3.34

Treated samples:

UFT Reactor-1

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	100	100	60	70	20	40
2	90	90	70	80	10	40
3	100	100	80	90	10	80
Mean	96.67	96.67	70.00	80.00	13.33	53.33
Sterr	3.34	3.40	5.78	5.78	3.34	13.35

UFT Reactor-2

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	100	100	50	70	20	70
2	100	100	60	60	20	70
3	100	100	70	80	20	50
Mean	100.00	100.00	60.00	70.00	20.00	63.33
Sterr	0.00	0.00	5.78	5.78	0.00	6.67

CFT Reactor-1

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	10	10	0	0	0	10
2	10	10	0	0	0	20
3	10	10	0	0	0	0
Mean	10	10	0	0	0	10.00
Sterr	0.00	0.00	0.00	0.00	0.00	5.78

CFT Reactor-2

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	10	10	0	0	0	30
2	0	0	0	0	0	30
3	0	0	0	0	0	40
Mean	3.33	3.33	0.00	0.00	0.00	33.33
Sterr	3.34	3.40	0.00	0.00	0.00	3.34

CCT Reactor-1

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	0	30	0	0	0	30
2	0	20	0	0	0	50
3	0	20	0	0	0	30
Mean	0.00	23.33	0.00	0.00	0.00	36.67
Sterr	0.00	3.40	0.00	0.00	0.00	6.67

CCT Reactor-2

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	1	30	0	0	0	70
2	1	40	0	0	0	70
3	3	20	0	0	0	70
Mean	1.67	30.00	0.00	0.00	0.00	70.00
Sterr	0.67	5.88	0.00	0.00	0.00	0.00

CCM Reactor-1

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	80	80	0	30	20	50
2	90	90	10	40	0	40
3	100	100	0	40	10	50
Mean	90.00	90.00	3.33	36.67	10.00	46.67
Sterr	5.78	5.88	3.34	3.34	5.78	3.34

CCM Reactor-2

Replicates	Germination Rate, %					
	Radish		Cress		Lettuce	
	7-days	14-days	7-days	14-days	7-days	14-days
1	40	40	0	10	0	30
2	70	70	0	20	0	20
3	60	60	0	20	0	30
Mean	56.67	56.67	0.00	16.67	0.00	26.67
Sterr	8.83	8.99	0.00	3.34	0.00	3.34

Table D-12. Reassessing of Germination rate of radish for soils and soil-compost mixtures.

Untreated Samples (Controls):

PS Samples

Replicates	Germination Rate, %	
	7-days	14-days
1	30	100
2	20	90
3	10	100
Mean	20.00	96.67
Sterr	5.78	3.40

UF Samples

Replicates	Germination Rate, %

		7-days	14-days
	1	30	30
	2	20	40
	3	10	50
Mean		20.00	40.00
Sterr		5.78	5.88

Treated Samples:

UFT Reactor-1

Replicates		Germination Rate, %	
		7-days	14-days
	1	100	100
	2	90	90
	3	100	100
Mean		96.67	96.67
Sterr		3.34	3.40

UFT Reactor-2

Replicates		Germination Rate, %	
		7-days	14-days
	1	90	90
	2	100	100
	3	90	90
Mean		93.33	93.33
Sterr		3.34	3.40

CFT Reactor-1

Replicates		Germination Rate, %	
		7-days	14-days
	1	10	10
	2	10	30
	3	10	10
Mean		10.00	16.67
Sterr		0.00	6.79

CFT Reactor-2

Replicates		Germination Rate, %	
		7-days	14-days
	1	10	10
	2	0	0
	3	10	10
Mean		6.67	6.67
Sterr		3.34	3.40

CCT Reactor-1

Replicates	Germination Rate, %

		7-days	14-days
	1	40	50
	2	60	60
	3	60	60
Mean		53.33	56.67
Sterr		6.67	3.40

CCT Reactor-2

Replicates		Germination Rate, %	
		7-days	14-days
	1	30	50
	2	70	70
	3	70	70
Mean		56.67	63.33
Sterr		13.35	6.79

CCM Reactor-1

Replicates		Germination Rate, %	
		7-days	14-days
	1	60	80
	2	80	100
	3	90	100
Mean		76.67	93.33
Sterr		8.83	6.79

CCM Reactor-2

Replicates		Germination Rate, %	
		7-days	14-days
	1	40	40
	2	70	70
	3	60	60
Mean		56.67	56.67
Sterr		8.83	8.99