BIOREMEDIATION OF CREOSOTE-TREATED WOOD WASTE

by

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DEDICATION

TO:

My parents

Xin Zhang and Lin Shen

My fiancée

Yan Gu

TABLE OF CONTENTS

Page

LIST OF TABLES

Table **Page**

LIST OF FIGURES

Figure **Page**

ABSTRACT

Currently, creosote-treated wood waste is mainly landfilled but the Province of Nova Scotia would prefer to see the waste converted to a valuable product. Bioremediation provides a treatment option for creosote-treated wood waste and the production of a value-added product that would have economic and environmental benefits. Composting technique can be used to degrade the contaminants and convert wood waste into humus and nutrients rich product and reduces the waste volume. The aim of this study was, therefore, to test the efficiency of the composting process in degrading the creosote in the contaminated wood waste.

A laboratory scale bioremediation process was carried out in three specially designed in-vessel bioreactors equipped with thermo-insulating outer layer, mixing units, controlled aeration units, thermocouples a data logger and a computer. The three bioreactors were used as replicates. The ability of three thermophilic and cellulolytic microorganisms (*T. curvata*, *T. aurantiacus* and *T. fusca*) to degrade lignocellulose substrate was tested. The bioremediation process was conducted for 15 days during which used cooking oil was added into the system as bio-available carbon at a rate of 36 ml every 12 h. The environmental parameters (temperature, volatile solids, ash content, pH, total carbon and total Kjeldahl nitrogen) were monitored throughout the process. The concentration of phenolic compounds and cellulose and lignin contents were monitored during the experiment.

The temperature reached the thermophilic phase (above 45˚C) in all trials because of the heat generated from the degradation of organic matter. The psychrophilic, mesophilic and thermophilic lag phases usually encountered in the composting process were clearly identified and the kinetic parameters were determined. The highest peak temperature (54.5˚C) was reached in Trial 4 (all organisms). The pH in all trials increased during the first week of the bioremediation (7.4-8.5) and then decreased back to weakly acidic (5.5-6.8) at the end of the process. Reductions in moisture content, volatile solids, total carbon and total Kjeldahl nitrogen (TKN) were observed in all trials but Trial 4 (all organisms) achieved the highest reductions. The C:N ratio increased in all trials because the initial nitrogen content was high which resulted in a faster reduction of organic nitrogen than the reduction in total carbon. For cellulosic material, C:N ratio should be calculated on the basis of bio-available carbon and nitrogen as the degradation process of cellulosic compounds is very slow. About 68.0-77.0% of phenolic compounds were degraded after 15 days of bioremediation. The highest PC reduction was observed in Trial 4 (all organisms). The cellulose and lignin contents were reduced in all trials (20.2-32.3% for cellulose and 13.1-30.8% for lignin). The highest degradation of both cellulose and lignin was achieved in Trial 4 (all organisms). The product of Trial 4 (all organisms) achieved both maturity and stability. Inoculating the contaminated wood waste with individual species of microorganisms did not seem to have significant effect on the bioremediation process but when these microorganisms were added together to the contaminated mixture improvements were observed as indicated by the reductions in total carbon, TKN, total solids, PC, cellulose and lignin contents.

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ADF	Acid Detergent Fiber
ADL	Acid Detergent Lignin
ANOVA	Analysis of Variance
AOAC	American Organization of Analytical Chemists
ATCC	American Type Culture Collection
BTEX	Acronym that stands for Benzene, Toluene, Ethylbenzene, and Xylenes
C&D	Construction and Demolition
CCA	Chromium Copper Arsenate
CEC	Cation Exchange Capacity
CFU	Colony Forming Units
CI	Confidence Interval
DM	Dry Mass
DPA	2,2'-Diphenic Acid
GI	Germination Index
HR	Humification Ratio
Lac	Laccase
LiPs	Lignin Peroxidases
MC	Moisture Content
MnPs	Manganese Peroxidases
MSW	Municipal Solid Waste
PAHs	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyls
PCP	Pentachlorophenol
PDA	Potato Dextrose Agar
PHE	Phenanthrene
PQ	Phenanthrene-9,10-quinone
PVC	Polyvinyl Chloride
RO/DI	Reverse Osmosis / De-Ionisation
SAR	Sodium Adsorption Ratio

SD	Standard Deviation
TCE	Trichloroethylene
TKN	Total Kjeldahl Nitrogen
TNT	Trinitrotoluene
VOCs	Volatile Organic Compounds
VS	Volatile Solids

Symbols:

C-ext	Extractable Humic Carbon
C:N	Carbon-Nitrogen Ratio
C-org	Total Organic Carbon
C_w	Water Soluble Carbon
Ec	Electrical Conductivity
N_{org}	Organic Nitrogen

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1. INTRODUCTION

During new construction, renovation and demolition projects, wood waste is collected by various means and is mixed with other construction and demolition (C&D) materials (such as drywall) prior to being sent to waste resource management facilities (CH2M HILL, 2007; USEPA, 2008). Currently, C&D waste has little use and is predominantly landfilled. Some municipal solid waste (MSW) is ground and utilized as daily landfill cover (SWANA, 2002). However, because of the contaminants it contains, it is generally unsuitable as a hog fuel. On the other hand, the manufacturing processes or coatings which made it esthetically unpleasant limit its use as a mulch material. Furthermore, the Province of Nova Scotia would prefer to see the C&D waste be utilized as a value added marketable product as opposed to being landfilled (ENS, 2008). Hence, the bioremediation of creosote-treated wood waste (as a representative of C&D wood waste) and the production of value-added product would have economic and environmental benefits.

Creosote (or "coal tar creosote") is a kind of wood preservative and water-proofing agent. Creosote-treated wood has been widely used in railway sleepers, utility poles, and in the foundations of buildings, bridges, building fences, stakes for agricultural and fruit production, garden furniture and outdoor recreational facilities in parks (CICAD, 2004; Ikarashi et al., 2005).

Creosote is distilled from crude coke oven tar and consists of around 75% of polycyclic aromatic hydrocarbons (PAHs), 2-17% phenolic compounds, and 10-18% heterocyclic organic compounds and minor aromatic amines. It is widely used as preservatives of wood product (Bedient et al., 1984; CEPA, 1993; ATSDR, 2002). However, because of different sources and preparation procedures in manufacturing procedures, the components of creosote may vary in concentration as well as type (ATSDR, 2002). Creosote is toxic to human being and has carcinogenic and genotoxic potentials due to the presence of PAHs as the main component of creosote (ATSDR, 2002; CICAD, 2004). Phenolic compounds contained in creosote are possible carcinogens and are toxic to aquatic living creatures (CICAD, 2004).

Because of its toxicity of creosote, creosote-treated wood does not degrade easily in the environment and requires special disposal methods. A possible disposal option is incineration which is problematic because components such as PAHs and furans could be produced (CICAD, 2004). The pollutants contained in the creosote-treated wood waste are, also, barriers to the use of the waste as landfill cover due to potential migration of contaminants into ground water. Therefore, a proper disposal solution should include an effective degradation of all the pollutants in creosote-treated wood waste.

Composting (as a bioremediation technique) has the advantage of degrading wood waste. Through mineralization and humification, the wood waste can be converted into a substance rich in humus and nutrients for plants while at the same time result in reducing the volume of the final product. If the contaminants in the waste have been degraded during the composting, the final product could be used as a soil amendment (Löser et al., 1999; Borazjani et al,. 2000; McMahon et al., 2008).

Even though the temperature during thermophilic phase of composting could be higher than 70˚C which indicates a fast degradation of organic matter inside the composting pile, the phase typically lasts no longer than 2-3 days. A maturing phase of at least 3 weeks (and even 1-2 years) is required to result in a stable and mature compost product (Haug, 1993; CCME, 1996; Gajalakshmi and Abbasi, 2008). In order to accelerate the whole process, a controlled prolonged thermophilic phase is a solution. The inoculation of cellulolytic microorganisms would, also, help to achieve a fast bioremediation process. Given that mesophilic cellulolytic microorganisms would be severely deactivated under temperature higher than 37˚C, thermophilic fungi or bacteria would serve as better decomposers under elevated temperature environment (Cooney and Golueke, 1964).

The aim of this study was to evaluate the possibility of using composting approach as a bioremediation option to effectively decrease the concentration of phenolic compounds in the creosote-treated wood waste, while at the same time produce a marketable value-added soil conditioner or mulch products. Phenolic compounds were chosen as the target contaminants because extensive studies have been conducted on PAHs, but few reports

were focusing on phenolic compounds and the phenolic compounds are water soluble which makes them easy to be monitored.

2. OBJECTIVES

The aim of this study was to evaluate the possibility of using the composting approach as a bioremediation option to effectively decrease the concentration of phenolic compounds in creosote-treated wood waste.

A pilot scale (laboratory) study was conducted to meet the following specific objectives:

1. Evaluate the changes in bioremediation operation parameters.
2. Determine the kinetic parameters of the bioremediation process.
3. Determine the capacity of the bioremediation process for degradation of phenolic compounds.
4. Assess the stability and maturity of the final product.

3. LITERATURE REVIEW

3.1 Creosote-treated Wood Waste

3.1.1 Sources and Characteristic

Creosote is the name used for a variety of products that are mixtures of many chemicals. The creosote used as a wood preservative is actually coal tars creosote (ATSDR, 2002). Creosote (coal tar creosote), is distilled from crude coke oven tar, which consists of PAHs, phenolic compounds and other organic compounds. It is widely used as preservatives of wood product (ATSDR, 2002). The components of creosote include around 75% of polycyclic aromatic hydrocarbons (PAHs), 2-17% phenolic compounds, 10-18% heterocyclic organic compounds and minor aromatic amines (ATSDR, 2002; Bedient et al., 1984). However, because of different sources and preparation procedures in manufacturing procedures, the components of creosote vary in their concentrations as well as their types (ATSDR, 2002; CICAD, 2004). The most common compounds in creosote are listed in Figure 3.1.

Creosote-treated wood was mainly used for railway sleepers and utility poles, but nowadays is used in the foundations of buildings, building fences, stakes for agricultural and fruit production, garden furniture and outdoor recreational facilities in parks (Ikarashi et al., 2005). The application of creosote will significantly elongate the service time of wood products. According to Bestari et al. (1998), the wood life expectancy of marine pilings was extended from typical 10 years to 40 years while 75% of creosote was still left in the treated wood.

In Canada, there are five creosote pressure-treating facilities in operation which collectively use 21 000 tonnes of creosote per year. Preservation of railway ties uses 54% of the creosote, marine pilings use 37%, and bridge deckings, timers, and utility poles use the remaining 9% (CICAD, 2004; CEPA, 1993).

3.1.2 Potential Pollutants

There are over 300 chemicals in creosote, but the most important and highly toxic ones are PAHs and phenolic compounds (ATSDR, 2002; Smith, 2008). It was reported

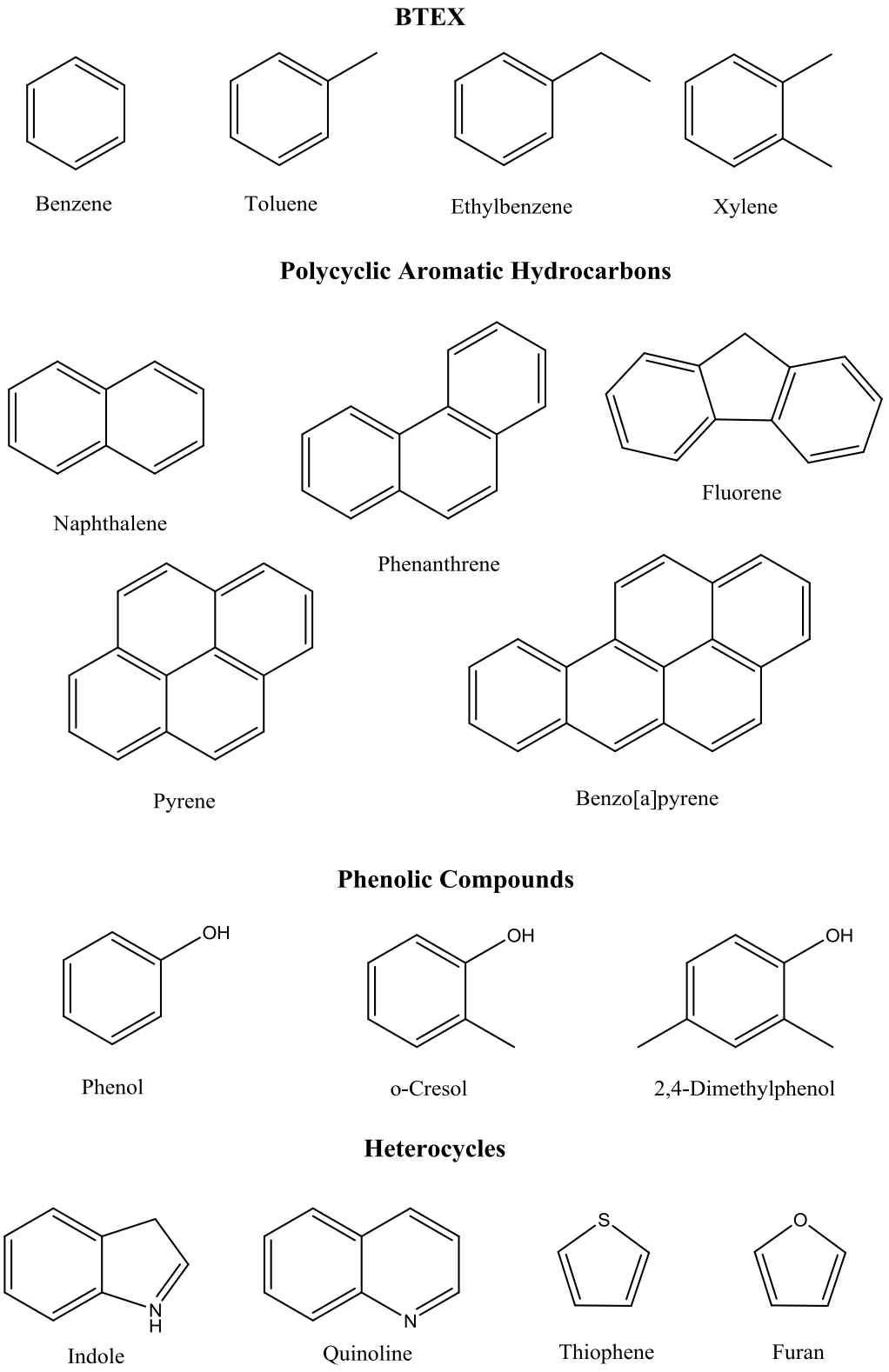


Figure 3.1 Major components of creosote (CICAD, 2004)

that acute exposure to large amounts of creosote may result in a rash or an irritation of the skin, chemical burns of the eye surfaces, convulsions and mental confusion, kidney and liver problems, unconsciousness and even death (ATSDR, 2002). Longer exposures to lower levels of creosote may result in increased sensitivity to sunlight, damage to the cornea and skin damage or skin cancer (ATSDR, 2002). Creosote is, also, toxic to other forms of life. The toxicity of creosote to photosynthesis has been reported and as a result, plant and algae in soil and aquatic environment are affected (Marwood et al., 2003).

Wood treatment facilities using creosote may cause soil, groundwater and air pollutions. As creosote-containing water leaves the source, some volatile components will evaporate to the air; some soluble compounds in water move though the soil to reach groundwater and insoluble compounds are filtered and left in soil with little migration (ATSDR, 2002).

According to ATSDR (2002) report, potential domestic human exposure to creosote include: (a) dermal contact with creosote-treated wood products such as landscaping poles using creosote-treated wood, (b) inhale of gases after the combustion of creosote-treated wood products and (c) contact with contaminated sites in the soil or groundwater.

PAHs are a class of organic chemicals consisting of two or more benzene rings fused in a linear, angular or cluster arrangement. PAHs are major constituents of creosote making up at least 75% of all creosote components (ATSDR, 2002; Smith, 2008; Grant et al., 2007). PAHs are pollutants of special concern because of their toxic, carcinogenic and genotoxic potentials. Several PAHs (including benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene, and indeno [*1,2,3,c,d*]pyrene) have been found to cause tumors when exposed to laboratory animal and are known as animal carcinogens while benz[*a*]anthracene and benzo[*a*]pyrene are carcinogenic to humans (ATSDR, 1995). Moreover, due to their low water solubility and association with organic matter in soils and sediments, PAHs are persistent in the environment (Byss et al., 2008). US EPA identified 16 species of PAHs (naphthalene, acenaphthylene, acenaphthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indono[*1,2,3,c,d*]pyrene, dibenz[*a,h*]anthracene,

benzo[*g,h,i*]perylene) as Priority Pollutants. Benzo[*a*]pyrene and dibenzo[*a,h*]anthracene are the most potent carcinogens (Antizar-Ladislao et al., 2004; Smith, 2008).

Phenol compounds are also components in creosote. Unlike most PAHs, phenol compounds are all water-soluble and easily leach through soil to groundwater. Phenol itself is quickly broken down in air, soil and water. Respiratory irritation may be caused by inhalation exposure to phenol. Death from cancer and other diseases due to exposure to phenol were, also, reported (ATSDR, 2008a).

Besides phenol itself, three kinds of creosols can, also, be classified into phenolic compounds which are harmful to the environmental and human health. Inhalation and dermal contact with cresols may cause nose and throat irritation or skin damage. USEPA has classified cresols as possible human carcinogens (ATSDR, 2008b).

3.1.3 Utilization/Disposal Options for Creosote-treated Wood Waste

Current utilization options for wood waste include: reuse, recycling and incineration as fuel (Solo-Gabriele et al., 1998). When reuse and recycling are not available for the treated wood waste and the potential contamination from the ashes limits the treatment by incineration, construction and demolition (C&D) landfill disposal is considered to be the last option. As a result, the main stream of creosote-treated wood waste is disposed in C&D landfill. However, potential contamination of soil and groundwater exists from a C&D landfill and there is no recovery of any value from the wood waste this way (Gomes, 2004).

3.1.3.1 Reuse: Wood waste as utility poles can be reused for fence posts, landscaping, land pilings and parking lots. Construction off-cuts of lumbers and timbers can be used for smaller applications including composting bins, planter boxes, shipping crates, picnic tables, and walking edging (Solo-Gabriele et al., 1998). If the wood poles are undamaged, regenerating new utility poles is, also, optional (Solo-Gabriele et al., 1998). Products like creosote-treated poles are usually well preserved and in good condition for reuse (ATSDR, 2002). However, potential exposure of creosote to humans is a problem.

3.1.3.2 Recycling: Being different from reuse, recycling always includes re-manufacture or re-build processes which change the properties of the original materials. For example, the making of composite wood panel is considered as a recycling process (Solo-Gabriele et al., 1998). The untreated portion of treated wood products, such as the heartwood portion of treated poles, can be recovered as clean wood and used for further purposes because they are always not penetrated by preservatives (Solo-Gabriele et al., 1998). Processed or chipped wood in small pieces can, also, be used as a composting bulk agent and feedstock of engineered wood products (Felton and de Groot, 1996; USDA, 2002; USEPA, 2008). Wood waste can, also, be used as daily landfill cover, animal bedding and wood flour filler for plastic products. Generally, wood waste from construction sites would be more proper because of its relatively clean and homogeneous nature (USDA, 2002). However, sorting of treated wood from clean wood is not an easy job (Jacobi et al., 2007).

3.1.3.3 Combustion: Energy recovery from wood waste by combustion is used in certain industrial facilities such as in cement kilns and cogeneration plants (Gomes, 2004; Solo-Gabriele et al., 1998). The oven-dried wood produces about 2.1×10^7 Joule per kilogram in the process of combustion (USDA, 2002). The main advantages of incinerating wood waste are reduction of fossil fuel requirement and alleviation of landfill burden through volume reduction (Solo-Gabriele et al., 1998). Moreover, C&D wood waste is preferred because of its low moisture content (USEPA, 2008).

However, only industrially used creosote-treated wood can be burned in an industrial incinerator or boiler. Other wood waste from home or farm sources should not be incinerated because the combustion process will result in PAHs, halogenated dioxins and furans emissions (ATSDR, 2002; CICAD, 2004). Also, Cu, Cr and As cannot be eliminated through the combustion process that takes place in these facilities which results in arsenic emissions (Gomes, 2004). Specifically, some arsenic are volatilized into air when the temperature is greater than 300˚C while high concentration of copper, chromium and arsenic remain in the ash which should be classified as hazardous waste (Solo-Gabriele et al., 1998).

3.1.3.4 Disposal in Landfill: Landfill disposal of wood waste should be considered a last alternative when all other options have been exhausted because it has high potential to contaminate soil and groundwater. As creosote-treated wood waste is sometimes a stream of C&D waste that is always disposed to C&D landfills which could be unlined (Jambeck et al., 2008; Solo-Gabriele et al., 1998). Besides, landfill disposal provides no recovery of any value from the wood waste. Nevertheless, it is still one of the most common waste management options of treated wood waste (Gomes, 2004).

3.2 Bioremediation of Creosote-treated Wood Waste

Bioremediation as a treatment option of wood waste has been studied recently. Compared with other treatment or disposal options of creosote-treated wood waste such as combustion and landfill, bioremediation is a less expensive and more environmentally friendly method. A successful bioremediation process of creosote-treated wood waste should consider the elimination of contaminants in creosote components as the most important goal, while the decomposition of lignocellulose contents should be considered as a secondary goal as well. In order to deal with creosote as contaminant in the creosote-treated wood waste, the microbial population in compost must be augmented with microorganisms that are capable of breaking down the compounds in creosote as well as the lignocelluloses (John et al., 1995).

3.2.1 Bioremediation of Creosote Components

Fungal degradation of creosote treated wood or creosote contaminated soil has been reported (Polcaro et al., 2008; Atagana et al., 2006). White rot fungi (Basidiomycetes) are among the most studied group because of their ability to produce non-specific extracellular enzymes referred to as lignin-modifying enzymes which have the potential to detoxify a broad range of environmental pollutants such as polychlorinated biphenyls (PCBs), dioxins, 2,4,6-trinitrotoluene (TNT), pesticides, PCP, phenolic resin and creosote, as well as to reduce the bioavailability of heavy metals (Yadav and Reddy, 1993; Paszczynski and Crawford, 1995; Reddy, 1995; Pointing, 2001; Zheng and Obbard, 2002; Gusse et al., 2006; Jiang et al., 2006; Zeng et al., 2007).

Polcaro et al. (2008) used *Pleurotus ostreatus*, a selected white-rot fungal mycelium, to degrade contaminated wood and wheat straw (1:2) for 44 days after which a complete degradation of creosote components was attained. Galli et al. (2006) used white-rot fungi *Pleusotus ostreatus* mycelium in the bioremediation of creosote-treated wood and found that PAHs, phenolic compounds, and heterocyclic compounds were all degraded significantly after 30 days of inoculation.

Fungi other than white-rot fungi were reported in degradation of creosote as well. Atagana et al. (2006) achieved 94.1% removal of creosote in a contaminated soil under the concentration of 250 $g \cdot kg^{-1}$. A mixed population of fungi species (*Cladosporium*, *Fusarium*, *Penicillium*, *Aspergillus* and *Pleusotus*) were, also, used with nutrient supplements in the bioremediation process of creosote-treated wood waste.

Given the fact that PAHs are the main components in creosote, bioremediation of PAHs could provide useful information of bioremediation of creosote treated wood. Even though other treatments of PAHs contaminants exist (like soil washing, ozonation and other chemical oxidation methods), bioremediation is always considered to be one of the safest, most cost-effective and environmentally friendly technology to treat PAHs, except for its low reduction of high-ring PAHs (Haapea and Tuhkanen, 2006; Rivas, 2006; Grant et al., 2007).

Bioremediation of PAHs using different technologies has been studied (Antizar-Ladislao et al., 2006). Different PAH compounds have different physical and chemical properties which may affect their biodegradability. The recalcitrance of PAHs to biodegradation generally increases with their molecular weight and their octanol-water partitioning coefficient (Cookson, 1995; Antizar-Ladislao et al., 2004). High-molecular-weight PAHs (like four or five ring PAHs) are always difficult to degrade due to their low water solubility, high resonance energy and toxicity (Antizar-Ladislao et al., 2004; Atagana et al., 2006). Fortunately, creosote contains approximately 50% two-ring, 39% three ring, 9% four-ring and 2% five-ring PAHs which means that high-molecular-weight PAHs are much less than the biodegradable ones (Grant et al., 2007).

Various kinds of bacteria, fungi and algae have been used for bioremediation of PAH-contaminated waste and water (Antizar-Ladislao et al., 2004; Grant et al., 2007). However, different pathways are used by bacteria, fungi and algae in the metabolism of PAHs. Bacteria oxidize PAHs to form cis-dihydrodiols as the first step, while fungi results in trans-dihydrodiols due to the differences in their enzyme systems from those of bacteria as shown in Figure 3.2 (Antizar-Ladislao et al., 2004). Further steps include: formation of catechol, the cleavage of aromatic ring by oxidation and finally formation of compound of either an aldehyde or a carboxyl acid (Cookson, 1995; Löser et al., 1999; Antizar-Ladislao et al., 2004).

Lignocellulose-degrading fungi (especially white rot fungi) have been reported to have the potential to oxidize PAHs by their extra-cellular enzymes because of the similarity of lignin and PAH compounds (Loick et al., 2009). Even though the white rot fungi have the ability to oxidize lignin by these enzymes, lignin is not the source of energy and the degradation only happens during secondary metabolism in order to access wood polysaccharides locked in lignin-carbohydrate complexes (Jeffries, 1990; Reddy and Mathew, 2001). These extra-cellular enzymes (lignin-modifying enzymes) are capable of mineralizing a variety of environmental organic pollutants because the structural nature of these contaminants is similar to the key chemical character of these enzymes in lignin degradation in most cases. These extracellular enzymes have two advantages in bioremediation application: (a) they have the potential to oxidize substrates of low solubility and (b) the microorganisms themselves can tolerate relatively higher concentration of toxic environment than would otherwise be possible (Reddy, 1995; Reddy and Mathew, 2001).

The key components of these enzymes include two glycosylated heme-containing peroxidases (lignin peroxidase (LiP) and Mn dependant peroxidase (MnP)) as well as a copper-containing phenoloxidase (laccase (Lac)) (Pointing, 2001). A free radical mechanism explaining the degradation of a variety of xenobiotic chemicals by these enzymes is described by Reddy (1995) and Pointing (2001). Free radicals generated from enzymatic substrate oxidation in a one-electron oxidation that can carry out several reactions including: benzylic alcohol oxidation, carbon-carbon bond cleavage, hydroxylation,

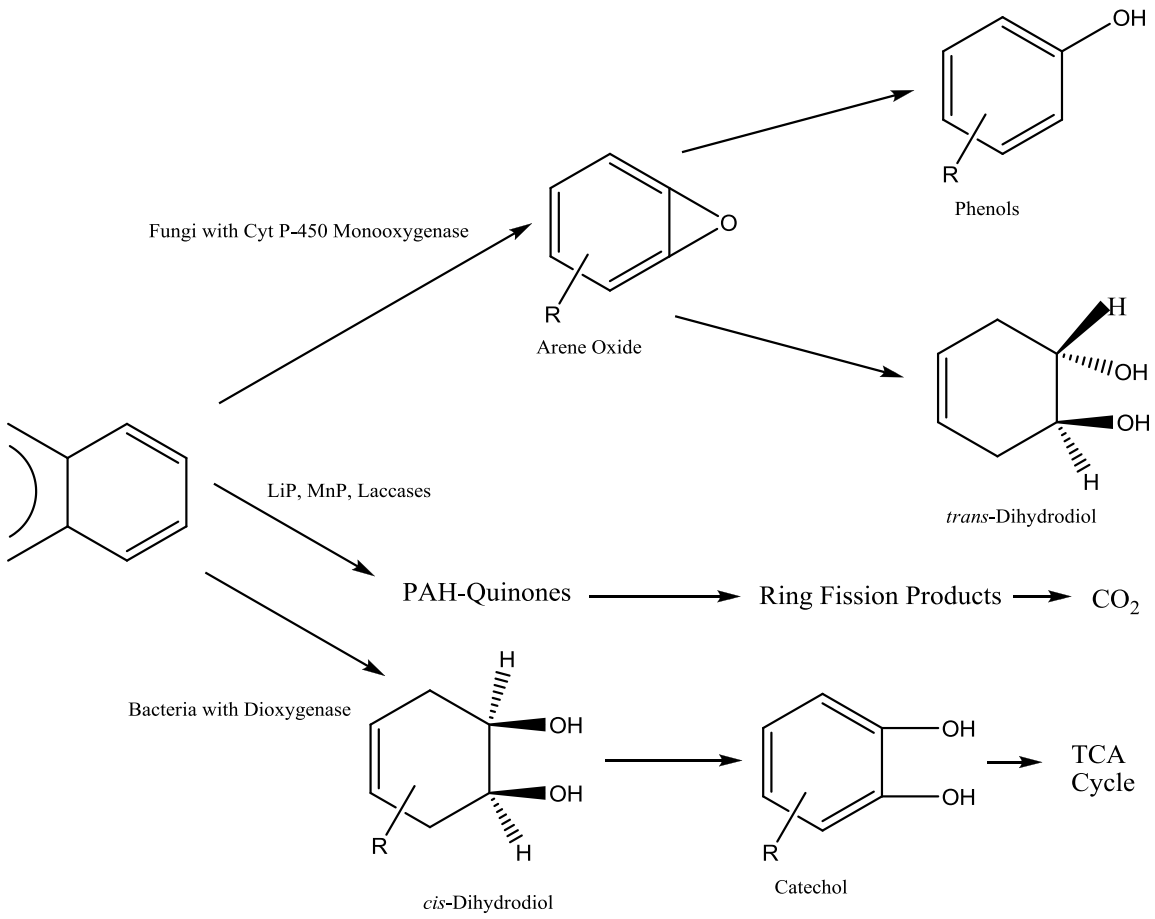


Figure 3.2 Pathways for the fungal and microbial metabolism of PAHs (Cerniglia, 1997; Loick, 2009)

phenol dimerization or polymerization and demethylation. Further steps include: formation of catechol, the cleavage of aromatic ring by oxidation and finally form molecule of either an aldehyde or a carboxyl acid (Antizar-Ladislao et al., 2004; Cookson, 1995; Löser et al., 1999).

The basidiomycete *Phanerochaete chrysosporium* is well-studied fungi species for their functions as ligninolytic organisms as well as PAHs degraders (Bumpus, 1989; Pointing, 2001; Zheng and Obbard, 2002; Lopez et al., 2006) which effectively produces LiP and MnP during the secondary metabolism (Tuomela et al., 2000).

Bumpus (1989) reported that at least 22 kinds of the PAHs, including the more abundant PAHs in anthracene oil, underwent 70-100% breakdown in 27 days in nitrogen-limited cultures of *P. chrysosporium*. Zheng and Obbard (2002) used *P. chrysosporium* to oxidize PAH in soil and found that the fungi acted synergistically with indigenous soil microorganisms in the oxidation of low molecular weight PAH (i.e. acenaphthene, fluorene, phenanthrene, fluoranthene and pyrene) in a soil-slurry, where oxidation was enhanced by up to 43% in the presence of fungus. However, the oxidation of high molecular weight PAH (chrysene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and benzo[*g,h,i*]perylene) was limited.

Phenanthrene (PHE) is one of the 16 PAHs on the USEPA's priority list with three aromatic rings and is found in relatively high concentration in PAH contaminated, creosote-treated wood preserving sites (Bezalel et al., 1996; Laor et al., 1999; Antizar-Ladislao et al., 2004; Puglisi et al., 2007). A lot of research has been conducted on the degradation of PHE by *P. chrysosporium*. A possible metabolite of phenanthrene-9,10-quinone (PQ) is detected in the first step. Then, the aromatic ring breaks and 2,2'-diphenic acid (DPA) is formed. The PQ to DPA step is much faster than the first step of PHE to PQ (Hammel et al., 1992; Moen et al., 1994). It has been reported that the degradation of PHE by *P. chrysosporium* is the consequence of lipid peroxidation by MnP instead of LiP (Sutherland et al., 1991; Hammel et al., 1992; Moen et al., 1994). Most metabolites of PHE degradated by *P. chrysosporium* can be considered detoxified (Sutherland et al., 1991; Löser et al., 1999).

Other white-rot fungi were, also, reported to have the ability to degrade PAHs. Kotterman et al. (1998) reported the degradation of benzo[*a*]pyrene by *Bjerkandera* sp. Strain BOS55. Andersson et al. (2003) compared the effects of white-rot fungi *Pleurotus ostreatus* and brown-rot fungi *Antrodia vaillantii* in remediation of PAH-contaminated soil. Their results indicated that *A. vaillantii* had a better degradation effect on PAHs compared with *P. ostreatus* because the former had a positive effect on indigenous microflora which helped in mineralization of PAHs. der Wiesche et al. (1996) used two white-rot fungi, *D. squalens* and *Pleurotus* sp. in the degradation of pyrene. Their results showed a higher mineralization of pyrene by *D. squalens* with the help of soil microorganisms. Kotterman et al. (1998) tested the ability of white-rot fungus *Bjerkandera* sp. Strain BOS55 to degrade benzo[*a*]pyrene in a laboratory incubation study. Even though the mineralization was not achieved by the fungus only, the mineralization was attained with the help of indigenous microbes. Also, the mutagenic potential of benzo[*a*]pyrene was significantly reduced by the fungus because its metabolites were much less mutagenic.

Steffen et al. (2003) found the litter-decomposing basidiomycete *Stropharia coronilla* to have the ability to degrade and mineralize benzo[*a*]pyrene. This kind of fungi prefers to colonize grasslands and its ability to bioremediate benzo[*a*]pyrene is enhanced with the present of Mn^{2+} because of MnP degradation mechanism involvement.

The LiP and MnP enzyme systems were reported to be able to effectively degrade p-cresol and phenol by Kennes and Lema (1994). After the lag time of five days, the compounds were rapidly degraded in a few hours and the biodegradation rates were observed between p-cresol and phenol, even though an additional carbon source such as glucose was required for the growth of the fungus.

3.2.2 Bioremediation of Lignocellulose

Lignocellulose accounts for the major component of biomass, especially for plant materials. Wood constituents are a mixture of cellulose (40%), hemicellulose (20-30%), and lignin (20-30%) (Tuomela et al., 2000; Yu et al., 2007). Both cellulose and lignin are the main components of cell wall and they are two of the most abundant biopolymers on

this planet. Synthesized in the cell walls of all higher plants, lignin forms a quarter to a third of the dry mass of wood.

All of brown rot fungi, white rot fungi and soft-rot fungi have the ability to biodegrade lignocellulose but white rot basidiomycetous fungi are the most efficient ones (Dutton et al., 1993; Tuomela et al., 2000; Makela et al., 2002). Extracellular ligninolytic enzymes are essential in the biochemical reactions involved in lignin biodegradation process.

Brown-rot fungi have the ability to degrade cellulose and hemicelluloses in wood, but its ability to degrade lignin is limited. Soft-rot fungi (*Ascomycotina* or *Deuteromycotina*) can degrade all wood components but at a lower rate compared with other kinds of fungi. Bacterial strains (especially actinomycetes) can solubilize and modify the lignin structure extensively, even though little mineralization of lignin can be attained (Tuomela et al., 2000).

Some organic acids produced by these fungi (like oxalic acid) have the ability to attack cellulose and hemicellulose of wood cells to achieve the degradation process (Kartal et al., 2004). They, also, provide proper chemical and physical conditions such as chelating unstable Mn^{3+} ions, generating H_2O_2 and providing low pH environment outside of the fungal hyphae, for the effective performance of lignin peroxidases (Makela et al., 2002). However, production of oxalic acid by wood-decaying fungi shows physiological variation between species. In brown rot fungi secretion of oxalic acid continues throughout their life cycle while white rot fungi secrete oxalic acid upon secondary metabolism (Dutton et al., 1993).

Microbial degradation of cellulosic materials is the result of synergistic action of enzymes such as endo-β-1,4- glucanase, exo-β-1,4-glucanase and β-glucosidase, all of which attack β-1,4-glycosidic bonds (Beguin and Aubert, 1994; Brienzo et al., 2008). Endo-β-1,4-glucanase and exo-β-1,4-glucanase both act upon cellulose to produce cellobiose as final product. Endo-β-1,4-glucanase cleaves randomly β-glycosidic bonds in β-1,4-glucan chains to produce free chain ends and exo-β-1,4-glucanases acts at chain ends by removing cellobiose units from the free chain (De Palma-Fernandez et al., 2002;

Brienzo et al., 2008). On the other hand, β-glucosidase hydrolyses cellobiose to glucose, reducing the inhibition effect of cellobiose on endoglucanase and exo-cellobiohydrolase (Gomes et al., 2004; De Palma-Fernandez et al., 2002).

The thermophilic ascomycetous fungus *Thermoascus aurantiacus* can produce all cellulolytic enzymes required for complete degradation of cellulose to glucose (Brienzo et al., 2008); its ability to produce xylanase has been reported (Yu et al., 1987). Cellulolytic enzymes produced by *T. aurantiacus* (especially endo-glucanase) have been applied in industrial production because of their superior thermostability, high rates of substrate hydrolysis and stability over a wide range of pH values (Mamma et al., 2009).

Actinomycetes (bacteria which grow as branching hyphae) have an important role in carbon cycle because of they are well adapted to the penetration and degradation of organics such as lignocellulose (Tuncer et al., 1999). The group of *Thermomonosporas* is of particular interest because they have the ability to produce thermostable cellulolytic enzymes (Ball and McCarthy, 1989).

One of the thermophilic actinomycetes (*Thermomonospora fusca*) was tested for its ability to break down carboxymethyl-cellulose by Crawford and McCoy (1972). Cellulases produced by *T. fusca* hydrolyse the cellulose chain into cellobiose, glucose and intermediate length oligosaccharides. Tuncer et al. (1999) conducted a study on the extracellular lignocellulolytic enzyme produced by *T. fusca* BD25. The optimum temperature for these enzymes was at 50˚C at a pH of 7.0-8.0. The production of xylanases by *T. fusca* has, also, been reported by Ball and McCarthy (1989). The biodegradation of aliphatic-aromatic copolyesters by *T. fusca* has been reported by Kleeberg et al. (1998). The two strains which exhibited about 20-fold higher degradation of 1,4-butanediol, adipic acid and terephthalic acid were identified as *T. fusca* strains.

The thermophilic actinomycetes (*Thermomonospora curvata*) is a dominant bacterial population in a variety of aerated composts which secretes a variety of thermostable extracellular enzymes during growth on either complex plant materials or on purified polymeric substrates (Stutzenberger 1971; Bernier et al. 1988; Stutzenberger 1994; Lin and Stutzenberger, 1995). The abundance of *T. curvata* in municipal solid waste compost

was tested by Stutzenberger (1971). This cellulytic and thermophilic actinomycete can produce cellulase at a pH of 6.0 and a temperature of 65˚C. Stutzenberger (1972) achieved 75% breakdown of the cellulose during a 10-day period using *T. curvata* at a temperature of 55˚C and a pH of 8.0 on the substrate of cotton fiber. The use of bagasse as sole carbon source for extracellular enzyme production by T. curvata at 55˚C has been reported by Stutzenberger, 1994. The combined activity of extracellular enzymes include cellulose and xylanase from *T. curvata* caused a 27% solubilization of the fiber and yielded a mixture of cellooligosaccharides, cellobiose, xylobiose, glucose, xylos, fructose, arabinose and mannitol.

3.3 Parameters Affecting Bioremediation Process

To some extent, the job of an environmental engineer is to find proper environmental conditions that are optimum for the microorganisms used in degrading contaminants. These parameters can be cataloged as physical parameters and chemical parameters. The physical parameters include: moisture content, oxygen (aeration) and temperature. The chemical parameters include: chemical properties of pollutants, pH value, carbon-nitrogen ratio and nutrients.

3.3.1 Moisture Content

Moisture content (MC) is indispensable for the decomposition process, because in the decomposition process which takes place in the thin liquid layers on the surfaces of particles, moisture essentially affects microbial activities. The importance of water is not only that it is necessary media for metabolic reactions for microorganisms and the transportation of nutrients, but it establishes the necessary connection between microbial cells and contaminants as well (Gajalakshmi and Abbasi, 2008).

The voids or pores in the soil could be filled with air, water or a mixture of air and water which result in a certain amount of MC (Haug, 1993). Hence, water content and air space in the medium have very close relationship to each other, i.e. given other phases are stable except water phase and gas phase, if too much water fill in the voids, which mean

the MC is high, the pore space that allows air to diffuse through is limited. As a result, aerobic microbial activity is inhibited due to anaerobic condition. On the other hand, when MC is low, it leads to dehydration of microbes. Therefore, the metabolic and physiological activites of microbes are defined by MC (Epstein, 1997; Tiquia et al., 2002; Gajalakshmi and Abbasi, 2008).

Typically, MC in a range of 150 to 250 g of water per kg of dry soil is optimal for bioremediation of unsaturated soils (Eweis et al., 1998). For specific bioremediation approach such as creosote-treated wood waste, MC of 60–70% is generally considered ideal to start with, and at later stages of decomposition, the ideal MC may be 50–60% (Epstein, 1997; Gajalakshmi and Abbasi, 2008). However, not only the absolute water content is considered in operation, different materials may have different water-holding capacities which should, also, be taken into consideration (Finstein and Hogan, 1993). As a result, different feedstock requires different starting MC. Maximum recommended MCs for various biodegradable materials are given in Table 3.1.

3.3.2 Aeration

Presence of oxygen as terminal electron acceptor indicates that the reaction is aerobic. This is the common situation in unsaturated soils, composting and bioremediation systems, and most hydrocarbons can be metabolized by microorganisms in this way. For example, in the process of composting which is typically an aerobic process, adequate supply of air to the compost heap is key parameter engineers must draw attention to. Small but sufficient oxygen exists at the beginning of composting will trigger a temperature raise which leads to natural convection known as “chimney effect” (Gajalakshmi and Abbasi, 2008). However, when it comes to groundwater, conducting aerobic remediation is a major problem and is essential in bioremediation strategies like biosparging (Cookson, 1995).

Other alternatives other than oxygen as the electron acceptor exist in anaerobic processes including NO_3^-, NO_2^-, SO_4^{2-}, Fe^{3+}, CO_2 and organic matters. Nevertheless, anaerobic metabolism pathway exists in limited kinds of microbes. Anaerobic metabolism is not as effective as aerobic metabolism and the rate is lower than aerobic metabolism as well, but they are very effective in remediation of haloids hydrocarbons. However, to

Table 3.1 Maximum recommended moisture contents for various biodegradable materials (Haug, 1993)

Types of Waste	**MC (% of total weight)**
Theoretical	100
Straw	75-85
Wood (sawdust, small chips)	75-90
Rice hulls	75-85
Municipal refuse	55-65
Manures	55-65
Digested or raw sludge	55-60
“Wet” wastes (grass clippings, garbage, etc.)	55-65

some degrading recalcitrant contaminants such as polychlorinated biphenyls (PCBs), nitroaromatics such as trinitrotoluene (TNT), trichloroethylene (TCE), inorganic contaminants such as toxic metals, radionuclides and arsenic, anaerobic microbes have the irreplaceable functions and probably provide the only effective approach of bioremediation (Cookson, 1995; Eweis et al., 1998; Vidali, 2001; Coates and Chakraborty, 2003)

3.3.3 Temperature

Temperature affects all kinds of chemical and biochemical reaction rates, and the rates of many of them approximately double for each 10˚C rise in temperature. The rate of degradation is important in bioremediation practices. Natural attenuation is happening at a limited speed if the environment parameters are not optimum for decomposers of contaminants. If the bioremediation process is conducted at higher temperature (within the operating range) while other parameters are properly controlled, the bioremediation process will be faster than under lower temperature.

A perfect example of elevated temperature would be the composting process, because temperature determines many rates of the biological processes and plays a selective role in the development and the succession of the microbiological communities (Tang et al., 2007). Metabolic activities of microorganisms release energy which heat up the compost materials. Temperature increases in composting piles to typically 50–60˚C in just a few days in an aerobic system and can even go up to 70˚C in some cases. Mesophilic microorganisms which are dominant at room temperature give way to thermophilic microorganisms, which may originally exist in compost heap under lower temperature but were not active (Epstein, 1997; Tang et al., 2007; Gajalakshmi and Abbasi, 2008). Thermophilic microorganisms, within an optimum temperature range, have the highest respiration rate; they can decompose substrates more rapidly making the thermophilic phase an important decomposition phase in the composting process (Finstein et al., 1987).

However, since the main goal of bioremediation is to degrade or eliminate pollutants in a contaminated site, it is more important to have efficient remediation of pollutants instead of a rapid decomposition of substrate. Unfortunately, many Proteobacteria and fungi which have abilities to degrade certain contaminants are mesophilic (Tang et al., 2007).

As a result, effective bioremediation projects require long period of time in terms of weeks, months or even years.

3.3.4 pH

The pH value of a bioremediation substrate will affect the activity of microorganisms in most bioremediation sites. A diverse group of organisms is typically involved, so the tolerance of pH values is broad except at extremes of acidity or alkalinity (Alexander, 1999). The range of pH values suitable for bacterial development is 6.0–7.5, while fungi prefer an environment in the range of pH 5.5–8.0 (Nakasaki et al., 1993).

Although the effect of pH on the bioremediation of contaminants is not intensively studied, caution should always be taken if the properties of pollutants in the composting material such as volatile acids will change if pH changes. Common practice to adjust soil pH using limestone or other material has been conducted for a long time (Epstein, 1997; Alexander, 1999; Vidali, 2001).

3.3.5 Carbon:Nitrogen Ratio

The proportion of carbon and nitrogen (C:N) is a major controlling factor in the bioremediation and composting processes. Carbon serves primarily as an energy source for the microorganisms. While a small fraction of the carbon is incorporated to the microbial cells; nitrogen is critical for microbial population growth, as it is a constituent of protein that forms over 50% of dry bacterial cell mass (Cookson, 1995; Gajalakshmi and Abbasi, 2008). Furthermore, bacteria may contain 7-11% N of dry bacterial cell, and this amount ranges from 4% to 6% to fungi (Epstein, 1997).

A balance between carbon amount and nitrogen amount is critical in bioremediaiton process. If nitrogen is limiting, microbial populations will cease to grow and it will take longer to decompose the available carbon. However, excess nitrogen, beyond the microbial requirements, is often lost from the system as ammonia gas (Epstein, 1997; Gajalakshmi and Abbasi, 2008). Cambardella et al. (2003) reported that the rate of inorganic N release to the soil from composted manure depends on the rate of decomposition of the organic matter and on subsequent turnover of the decomposed C and N in soil. Release of plant available N from manure in the soil is controlled by the

balance of N immobilization and mineralization, which in turn is controlled, to a large extent, by the C:N ratio of the decomposing organic material. According to several researchers, initial C:N ratio between 25 and 30 is optimum (Sharma et al., 1997; Zhu, 2007; Gajalakshmi and Abbasi, 2008).

3.3.6 Chemical Properties of Pollutants

Chemical properties, along with physical properties of contaminants, decide their fate and transport at the contaminated sites, such as in the soil and groundwater. Several phases such as soil particle, soil water, groundwater and soil air space are related to the transfer of contaminants through the processes of adsorption, desorption, solubilization, dissolution and evaporation.

Microorganisms are always accessible to limited phases of contaminants and certain range of concentration of these contaminants due to the process of "aging". This indicates that the compound has not changed but became hidden and inaccessible in the matrix such as soil. Contaminants may also accumulate in micro-pores that are too small for bacteria to colonize (Eweis et al., 1998). As the bioavailability of pollutants declines, the degradation activities of microorganisms become limited.

On the other hand, the chemical property, especially molecular structure, of contaminants is an important factor to their biodegradability. For example, *n*-alkanes are more easily degraded than isomers. Typically, biodegradability of hydrocarbons can be ranked as follows: *n*-alkanes > *iso*-alkanes > cyclocalkanes > aromatics (Eweis et al., 1998; Wentzel et al., 2007). Additional atoms and functional groups like chlorine, nitrate, nitrite, sulfate and phosphate tend to make these molecules more stable to microbial attacks. Amino compounds are the exception, because amino acids are basic biological molecules and they are easily used by many microorganisms (Eweis et al., 1998; Alexander, 1999). For PAHs, a similar rule is also true. The higher the ring number, the more persistent it will be. So the biodegradability will decrease as 3-ring > 4-ring > 5-ring PAHs (Alexander, 1999). Table 3.2 summarizes the susceptibility of some organic compounds to mineralization.

3.3.7 Nutrients

Nutrients are chemicals required for microbial growth but do not provide microorganism with energy or carbon source. Swannell (2003) found that indigenous microorganisms that exist in most contaminated sites have the ability to break down most contaminants. However, without sufficient nutrients, potential pollutants-degrading microorganisms cannot accumulate to necessary numbers required for bioremediation of the site. As a result, biostimulation has been developed as a process of addition of nutrients to stimulate microbial activities (Vidali, 2001).

The most commonly required nutrients are nitrogen and phosphorus for most organisms. For example, nitrogen is a critical element for microorganisms because it is an important component of proteins, nucleic acids, amino acids, enzymes and co-enzymes that are essential for cell growth and functioning. The main elements in microorganisms and their physiological function are listed in Table 3.3.

However, due to biochemical properties of nutrients, some are usually below what is required for optimum microbial activities. On the other hand, too much of nitrogen may result in nitrogen losses from the bioremediation systems as ammonia gas which causes odor problems (Epstein, 1997; Gajalakshmi and Abbasi, 2008). As a result, addition of proper amount of nitrogen and phosphorus to stimulate microbial activities is paramount for successful bioremediation projects.

3.3.8 Bulking Agent

The bioremediation substrate structure decides the effective delivery of air, water, and nutrients through them. To improve the structure, materials such as gypsum, wood straw or organic matter can be applied as bulking agents. Low soil permeability is always a barrier for *in situ* remediation strategies as well large-scale remediation techniques like composting. Therefore, improvements of substrates or soil structure are essential for a successful remediation application.

Table 3.2 Susceptibility of some organic compounds to mineralization (Epstein, 1997; Alexander, 1999)

Organic Compound	**Susceptibility to Mineralization**
Sugars	Very susceptible
Starches, glycogen, pectin	
Fatty acids, glycerol, lipids, fats, phospholipids	
Amino acids	
Nucleic acids	
Protein	Usually susceptible
Hemicellulose	
Cellulose	
Chitin	
Low molecular weight aromatics and aliphatic compounds	Resistant
Lignin	
High molecular weight polyaromatics	Very resistant

Table 3.3 Composition of typical microbial cell on a dry-weight basis (Cookson, 1995; Eweis et al., 1998; Vidali, 2001)

Element	Percent of dry weight	General physiological function
Carbon	50	Constituent of organic cell materials
Oxygen	20	Constituent of organic cell materials and cellular water
Nitrogen	14	Constituent of proteins, nucleic acids, coenzymes
Hydrogen	8	Constituent of cellular water and organic cell materials
Phosphorus	3	Constituent of nucleic acids, phospholipids, coenzymes
Sulfer	1	Constituent of proteins and coenzymes
Potassium	1	Major cation in cell process
Sodium	1	Major cation in cell processes
Calcium	0.5	Major cation in cell processes and enzyme cofactor
Magnesium	0.5	Major cation in cell processes, cofactor in ATP reactions
Chlorine	0.5	Major anion in cell processes
Iron	0.2	Constituent of cytochromes and other proteins, enzyme
All trace elements	0.3	Inorganic contituents of special enzymes

3.3.9 Microbial Population

Microorganisms have the central role in the bioremediation; because they are the living creatures that metabolize different kinds of pollutants. Other environmental factors would affect the bioremediation process, but their effects are always exerted on the growth of microorganisms. Different bioremediation-related microorganisms always function together in bioremediation sites (Epstein, 1997; Eweis et al., 1998; Vidali, 2001). Not only do the species involved in specific bioremediation sites vary, but the categories, numbers and roles of species will change significantly during different phases of a bioremediation process (Finstein and Morris, 1975). This is especially true when composting is used as a bioremediation system as shown in Tables 3.4 and 3.5.

3.3.9.1 Aerobic Bacteria: Presence of oxygen is required as electron acceptor for aerobic bacteria. They are diverse in the environment, which makes them reasonable to be the most active and effective degraders of many contaminants (Cooksen, 1995; Allard and Neilson, 1997). Furthermore, the substrates in many bioremediation sites are aerobically degradable organic matter. For example, in the composting process, aerobic bacteria as well as aerobic actimycetes and fungi are active and dominant in the matrix (Haug, 1993).

Examples of aerobic bacteria recognized for their degradation abilities are genera *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons including: alkanes, PAH compounds and phenolic compounds. Many of these bacteria use contaminants as the sole sources of carbon and energy (Allard and Neilson, 1997; Eweis et al., 1998; Vidali, 2001).

3.3.9.2 Anaerobic Bacteria: Instead of using oxygen as the electron acceptors, anaerobic bacteria can use other compounds such as carbon dioxide, sulphate, nitrate, chlorate, Fe^{3+}, or Cr^{6+} etc as electron acceptors. Facultative aerobic bacteria can use alternative terminal electron acceptors and grow in the presence or absence of oxygen.

Table 3.4 Microorganism population during aerobic composting (Haug, 1993)

Microbe		No./Wet g Compost			No. of Species Identified
		Mesophilic (Initial – 40°C)	Thermophilic (40 – 70°C)	Mesohpilic (70°C – final)	
Bacteria	Mesophilic	10^8	10^6	10^{11}	6
	Thermophilic	10^4	10^9	10^7	1
Actinomyces	Thermophilic	10^4	10^6	10^5	14
Fungi	Mesophilic	10^6	10^3	10^5	18
	Thermophilic	10^3	10^7	10^6	16

Table 3.5 Microbial species in composting systems (Epstein, 1997; Gajalakshmi and Abbasi, 2008)

Microorganism	Mesophilic	Thermophilic
Bacteria	*Bacillus* spp. *Azotobacter* spp., *Pseudomonas, Flavobacterium, Micrococcus*	*Bacillus subtilis, Bacillus licheniformis; Bacillus circulans; Thermus* spp.
Fungi	*Aspergillus, Penicillium, Rhizopus, Fusarium, Chaetomonium, Trichoderma, Alternaria, Cladiosporium; Phanerochaete chrysosporium; Coriolus versicolor; Phanerochaete flavidoalba*	*Aspergillus fumigatus; Chaetomium thermophil;, Humicola lanuginos;, Humicola insolens; Malbranchae pulechella; Taloromyces emersonii; T. thermophilus; Thermoascus auranticus; Thermomyces lanuginosus; Thermoascus aurantiacus*
Actinomycetes	*Actinomycetes thermophilus, Streptomyces; Micromonospora* spp.	*Nocardia; Streptomyces; Thermoactinomyce;, Micromonospora*

Anaerobic bacteria are not as commonly encountered as aerobic bacteria in bioremediation projects (Eweis et al., 1998; Vidali, 2001). However, to some degrading recalcitrant contaminants such as PCBs, nitroaromatics such as TNT, TCE, inorganic contaminants such as toxic metals, radionuclides and arsenic, anaerobic microbes have the irreplaceable functions that are drawing more attention from engineers (Eweis et al., 1998; Vidali, 2001; Coates and Chakraborty, 2003). Anaerobic degradation of pollutants such as PAHs and phenolic compounds were also reported, although through different metabolic pathways compared to aerobic degradation (Allard and Neilson, 1997; Coates and Chakraborty, 2003).

3.4 Composting as a Bioremediation Option of Contaminated Wood Waste

Composting as a bioremediation technique of contaminated wood waste has many advantages: (a) it reduces the waste volume, (b) it detoxifies the waste and (c) it transforms the waste into a product that can be used as a soil amendment (Borazjani et al,. 2000; Löser et al., 1999; McMahon et al., 2008). Using compost as a soil amendment increases the organic matter content and improves the water-holding capacity and texture of the soil. These advantages enable the product to have good usage in horticulture and agriculture (Borazjani et al., 2000). A composting operation can be implemented at a plant site and requires limited knowledge, equipment and space.

Wood wastes like wood chips have been primarily used as structure improvement additives in composting MSW or sewage sludge (Löser et al., 1999). Reports in the literature concerning composting of wood waste are really limited. However, several kinds of wood wastes have been successfully degraded by composting including: furniture wood wastes, hardwood sawmills wastes and plywood wastes (Borazjani et al., 2000). But composting of C&D wood waste is still an area that needs more attention, because of its contamination with many toxic and environmentally harmful substances such as chromium copper arsenate (CCA), creosote, pentachlorophenol (PCP) and heavy metals.

The addition of amendments such as chicken manure, cow manure, horse manure, gin trash and inorganic fertilizer allow wood to decay faster and increases the nitrogen content of the end product (Borazjani et al., 2000). Poultry manure was found to be the best nitrogen additive by McMahon et al. (2008). Optimum C:N ratio values between 15:1 and 30:1 and MC of 50% have been suggested (Borazjani et al., 2000; McMahon et al., 2008).

In the research conducted by McMahon et al. (2008), three compost mixes prepared by mixing shredded chip board, medium density fiber, hardboard and melamine were amended with poultry manure, Eco-Bio mixture and green waste. Poultry manure and green waste served as nutrient supplements and showed improvement in plant growth. Toxicity, phytotoxicity and bioaerosal emission tests showed a prospective usage of compost product.

Antizar-Ladislao et al. (2004) conducted a detailed review of bioremediation of PAH-contaminated waste using the composting approach. Since the beginning of 1990s, pilot studies had been conducted to prove the feasibility of composting PAH-contaminants. Later research focused on finding suitable or optimum environmental factors such as oxygen supply, nutrients supply, pH, temperature, and moisture for the microbial activity. Fungi had been found to be able to degrade lignin to obtain the cellulose while at the same time have the potential to degrade PAHs. In-vessel composting, which has the advantage of strongly controlling of environmental conditions within an enclosed container to achieve excellent degradation process, has also been developed in recent years. Adding mature compost product to improve bioremediation was also reported.

Tuomela et al. (2000) reviewed the bioremediation of lignin and other lignincellulose using compost techniques. Thermophilic fungi are probably the most important lignin degraders while white-rot fungi do not survive the thermophilic phase of composting. However, mesophilic fungi can still be effective during the cooling and maturation phase. Mineralization of lignin may be attained but not in a large amount; most of degraded lignin fragments are left as building units for humic substances. This process is affected by temperature, the original lignin content and the thickness of the material.

Antizar-Ladislao et al. (2006) conducted an experiment for bioremediation of PAHs in coal-tar-contaminated soil using in-vessel composting methods. Amended with green waste, 75% PAHs concentrations was reduced after 56 days of continuous treatment under 38˚C. However, the constant low temperature was not effective to eliminate pathogens. As a result, using a variable temperature profile during in-vessel composting was required to promote contaminant degradation, microbial activity, mature final compost and deactivation of pathogen.

Löser et al. (1999) used PAHs contaminated wood as a composting feedstock. After 61 days, phenanthrene was reduced from 1000 mg/kg to 26 mg/kg and pyrene was reduced from 1000 mg/kg to 83 mg/kg.

Yu et al. (2007) studied microbial community succession during composting of agricultural waste which contained lignocellulose. A gradual degradation of lignocellulose was observed. Hemicellulose resembled cellulose in decomposing trend but lignin showed a difference. Hemicellulose was partially degraded during the initial stage of composting, remained stable during the thermophilic phase and attained large decomposition during the maturing phase, because many mesophile fungi are responsible for cellulose and hemicellulose metabolization. Lignin showed little degradation until the maturing phase and temperature began to fall. Actinobacteria was believed to be responsible for lignin degradation during the composting process.

3.5 Bioremediation Systems

Several kinds of bioremediation strategies have been developed. They are all based on the microorganism activities to eliminate contaminants, but their principles, techniques, economical applicability and limitations are different from each other. They can be categorized as *in situ* methods and *ex situ* methods, according to whether the method is applied on-site or off-site. *In situ* techniques are those that are applied to soil and groundwater at the site with minimal disturbance. *Ex situ* techniques are those that are

applied to soil and groundwater which has been removed from the site via excavation (soil) or pumping (water).

3.5.1 *In situ* Techniques

These techniques are generally the most desirable options due to lower cost and fewer disturbances because they provide the treatment in place avoiding excavation and transport of contaminants. As a result, many of the risks and costs associated with materials handling are reduced or eliminated. The techniques involve always enhancing natural biodegradation processes by adding nutrients, oxygen (if the treatment is aerobic) and microorganisms as inoculation to stimulation degradation process (USEPA, 1993a). Typical *in situ* treatments are effective to a limited depth of soil or groundwater which affects the wide application of these treatments. Bioventing and biosparging are two important *in situ* bioremediation strategies.

3.5.1.1 Bioventing: If contaminants are less volatile (typically a Henry's law coefficient < 0.1) or simple hydrocarbons, bioventing is commonly used in *in situ* treatment of soil (Eweis et al., 1998; Vidali, 2001). Transformation and degradation of the contaminants take place at the contamination site in the soil which largely minimizes contaminant migration. Installation of a number of wells is required at the site. Through these wells air and nutrients are provided to stimulate the indigenous bacteria (Vidali, 2001). The aeration can be either passive or impassive (Eweis et al., 1998). A schematic of a bioventing system is shown in Figure 3.3.

Typically, low air flow rates are employed in order to minimize volatilization and release of contaminants to the atmosphere and provide only necessary amount of oxygen for the bioremediation (Vidali, 2001). Regular soil sampling is required and daily monitoring of off-gas, oxygen and carbon dioxide concentrations can provide information of microbial activity. However, in cases of the presence of volatile compounds, off-gases must be collected and treated, which will increase operation costs (Eweis et al., 1998; Rushton et al., 2007).

Application of bioventing technology is limited, and most reported researches were conducted on bioremediation of petroleum oil-contaminated soil (Hoeppel et al., 1991).

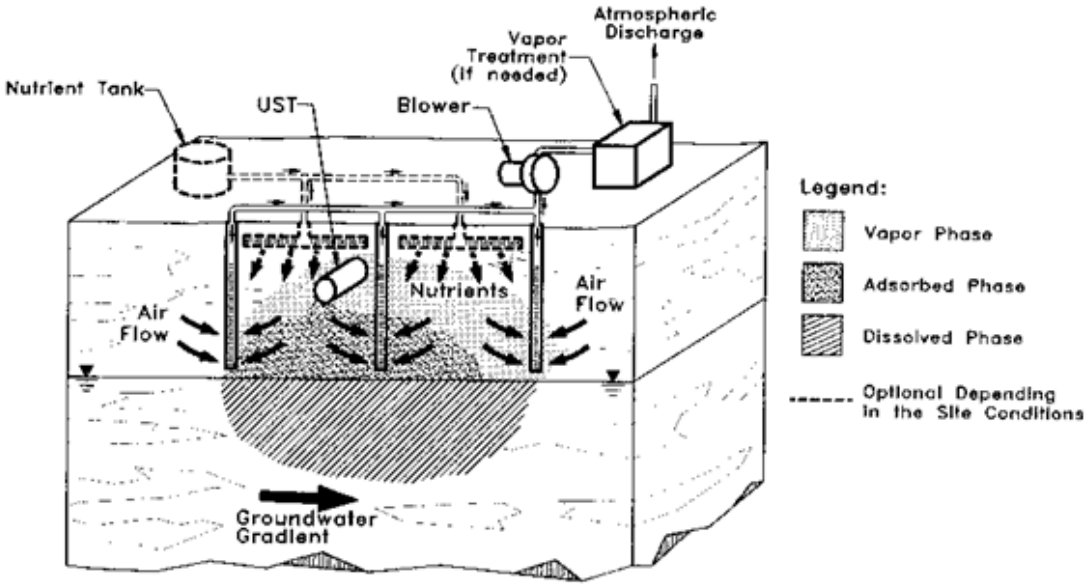


Figure 3.3 A typical bioventing system using vapor extraction (NMED, 2010)

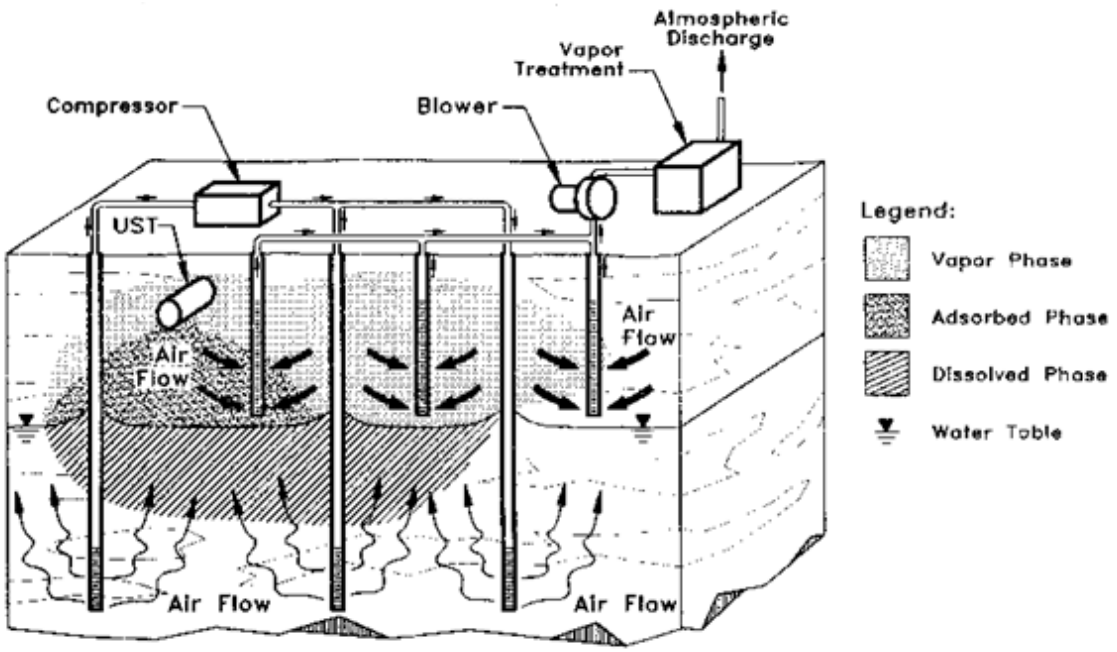


Figure 3.4 Biosparging system with soil vapour extraction (NMED, 2010)

For example, Hinchee et al. (1991) used bioventing technology to remediate JP-4 jet fuel contaminated soil in Utah State. The degradation rate of up to 10 mg/(kg·day) was achieved.

3.5.1.2 Biosparging: Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria (Vidali, 2001). Trapped immiscible contaminants or dissolved contaminant plumes are both applicable as biosparging increases the mixing in the saturated zone and thereby increase the contact between the soil and groundwater (Johnson et al., 2001). A schematic set up of biosparging system is shown in Figure 3.4.

Because of the ease and low cost of installing small-diameter air injection points and the considerable flexibility in the design and construction of the system, bioventing is now likely to be the most practiced engineered *in situ* remediation option when targeting the treatment of hydrocarbon-impacted aquifers (Johnson et al., 2001; Vidali, 2001). BTEX contaminated groundwater can be remediated using biosparging attaining a decrease of more than 70% in concentration (Kao et al., 2008).

3.5.2 *Ex situ* Techniques

Ex situ techniques involve the excavation or removal of contaminated soil from original sites. These *ex situ* methods include land farming, composting, biopiles, and using a bioreactor.

3.5.2.1 Land Farming: Land farming is an *ex situ* bioremediation process performed in the upper soil zone. The soil is used as inoculum and support medium for aerobic biological process which can be controlled to optimize degradation. In order to aerate and provide nutrient to the soil, periodical tilling is needed. Tilling also makes the soil homogeneous and increases the porosity of the soil. Bulk agents can be added to adjust soil porosity while gypsum can be used to reduce MC of soils with higher clay content. The pH of the soil pH can be controlled by lime, alum or phosphoric acid (Cookson, 1995). A schematic set up of land farming system is shown in Figure 3.5.

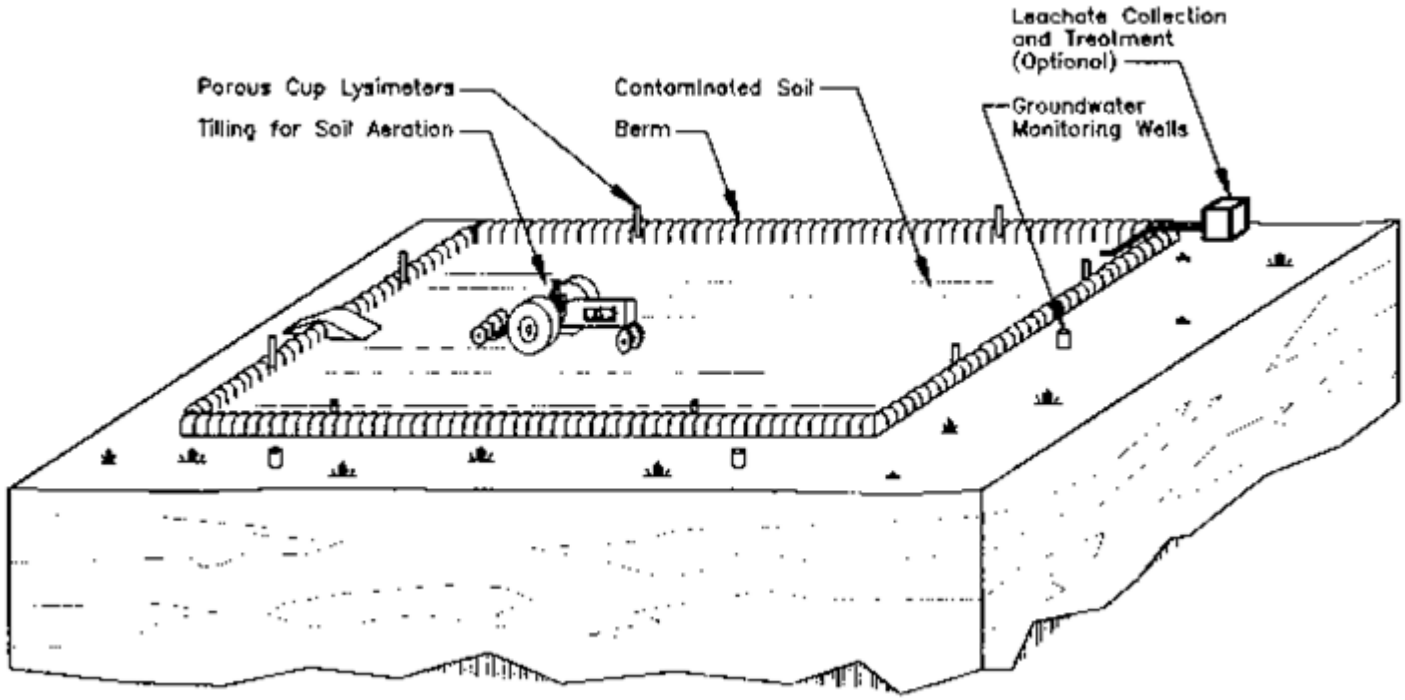


Figure 3.5 Schematic of land farming bioremediation system (NMED, 2010)

Land farming is widely applied to degrade many waste types, though it has many disadvantages including: (a) vast land usage, (b) long time process, (c) low bioavailability to contaminants due to strong adsorption, (d) release of volatile organic compounds (VOCs) to the atmosphere and (e) the potential to contaminate groundwater. The reasons for its application are the economical advantages such as low capital and operating costs, and the effectiveness in treating various kinds of contaminants (Cookson, 1995; Eweis et al., 1998; Rushton et al., 2007).

3.5.2.2 Biopiles: Biopiles are a hybrid of land farming and composting. Engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons they are a refined version of land farming that tend to control physical losses of the contaminants by leaching and volatilization. Moreover, biopiles are very effective in nutrient supplementation and consequently provides a favorable environment for indigenous aerobic and anaerobic microorganisms (Rushton et al., 2007). A schematic of biopile bioremediation system is shown in Figure 3.6.

3.5.2.3 Bioreactors: Bioreactors can be solid-phase, liquid-phase, or gas-phase operations with different sizes (Eweis et al., 1998). Bioreactors include static bed reactors, continuous mix reactors, horizontal drum reactors, fungal compost reactors, slurry-phase reactors, dual injection turbulent suspension reactors and packed bed reactors (Rushton et al., 2007).

In a bioreactor environment, greater and more uniform process control can be attained than other bioremediation methods. Especially in a slurry-phase bioreactor (Figure 3.7), solubility of contaminants can be tremendously enhanced by applying surfactant, which results in an increase in microorganism and contaminant contact (Cookson, 1995). Distribution of nutrients is, also, improved. Oxygen can be added as required and off-gas controls are often used to prevent loss of volatile organic compounds through stripping (Eweis et al., 1998). Bioreactors can be cheaper than other treatment systems and take less space (Rushton et al., 2007).

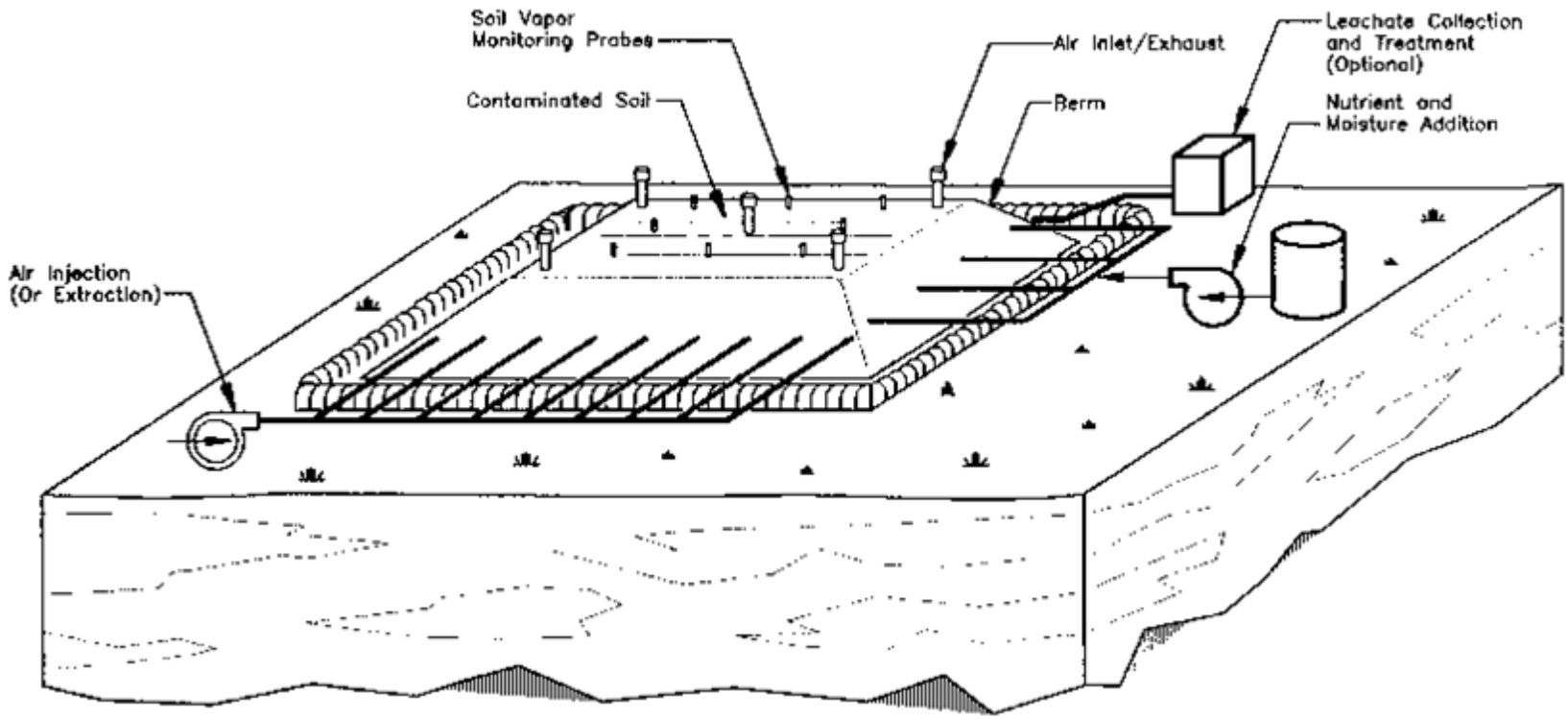


Figure 3.6 Schematic of biopile system (NMED, 2010)

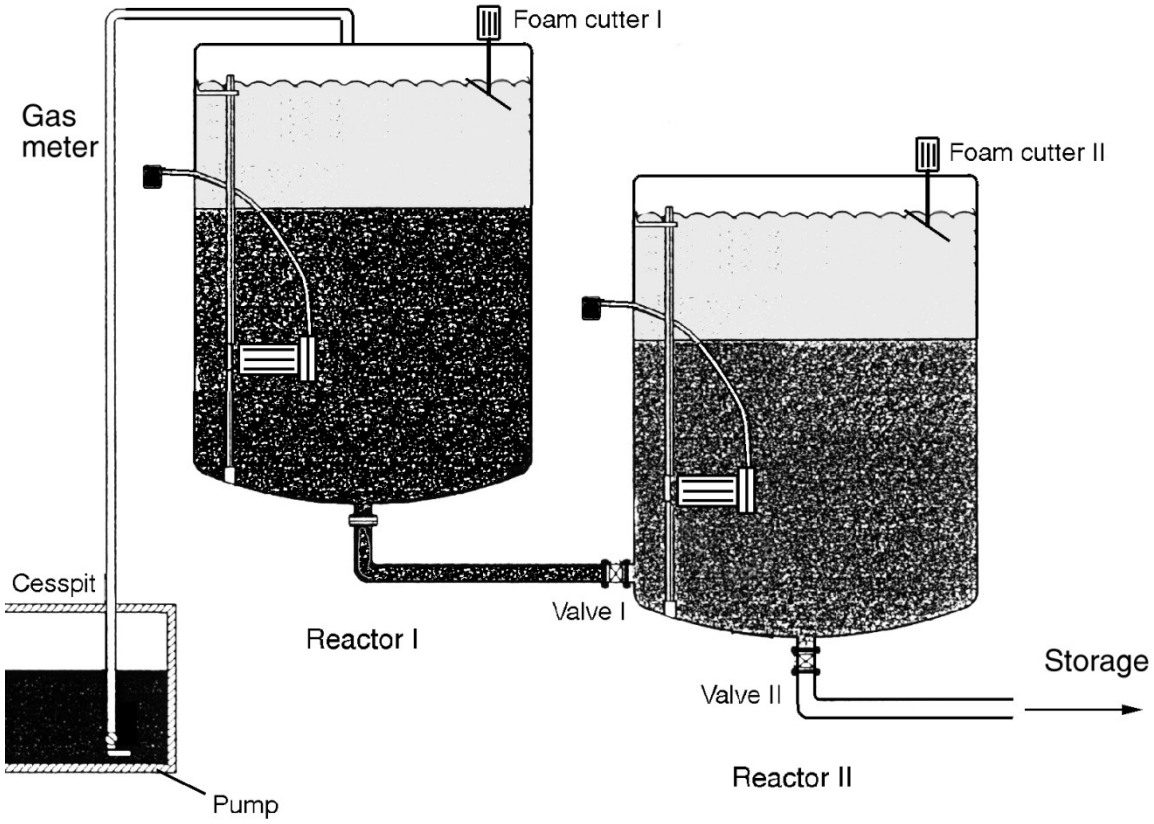


Figure 3.7 Slurry-phase bioreactor used in cattle slurry fermentation (Paluszak, 1998)

3.5.2.4 Biofilters: Biofilters are actually closed packed-bed reactors through which contaminated air is either blown or drawn. Microbial communities growing on the packing surface are used to treat organic vapors. They are attached on extracellular polysaccharides and bound water. Oxygen concentrations, temperature, nutrient concentrations, moisture levels, pH and carbon level are adjusted to optimize contaminant degradation, resulting in significant vapor phase contaminant reductions (Eweis et al., 1998).

The primary components of biofilters are: (a) an air blower, (b) an air distribution system, (c) a humidification system, (d) filter media and (e) a drainage system as shown in Figure 3.8 (USEPA, 1993a; Eweis et al., 1998; LIT, 2010). Chlorinated aliphatic, aromatic compounds and light aliphatic compounds can be treated by biofilter, while light aliphatic compounds like propane and isobutane can be effectively eliminated by biofilter with a removal range of 95 to 99% (Eweis et al., 1998). The biofilters have been used for odor control at wastewater-treatment plants, composting plants, and industrial processes for VOCs or other exhaust contamination elimination.

3.5.2.5 Composting: Composting is an *ex situ* process of biological decomposition and stabilization of organic substrates at higher temperature that enable faster rate of degradation. Thermophilic decomposition and the usage of the final product of composting make composting more favourable than other bioremediation methods (Haug, 1993). Moisture, nutrient, oxygen and pH can be controlled through the process. Bulking agents, like sand, bark chips and wood straw are important as additives to improve aeration (Jorgensen et al., 2000; Nano et al., 2003; Gajalakshmi and Abbasi, 2008). The capital and operating costs of composting are relatively low and the operation and design are relatively simple and result in relatively high treatment efficiency (Namkoong et al., 2002). Composting technologies include: windrow system, static solid bed system, agitated solids bed system, composting bins system and rotary drums system. These composting systems can be catalogued briefly into “In-vessel” and “open systems”.

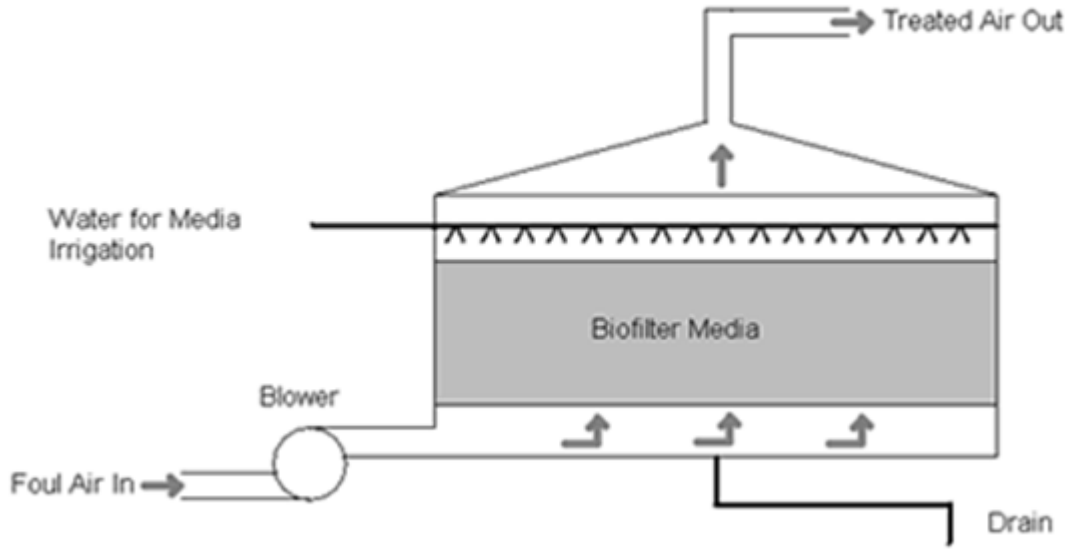


Figure 3.8 Schematic of biofilter system (LIT, 2010)

In-vessel composting systems are more suitable for strictly designed engineering projects than conventional systems because of their better process efficiency, control and optimization as the undergoing decomposition process takes place within an enclosed space (Petoit et al., 2004). Another advantage of in-vessel systems is being able to operate with large mass burden of waste while using less land spaces than conventional composting options. However, the use of machinery and power to force aeration and mechanical turning in the systems makes them more expensive than conventional systems (Gajalakshmi and Abbasi, 2008). These systems may not give sufficient retention time to produce mature compost because the design aims at maximizing microorganism activity during the initial composting period (Gouin, 1998). Thus, intensive management is required to maximize their efficiency.

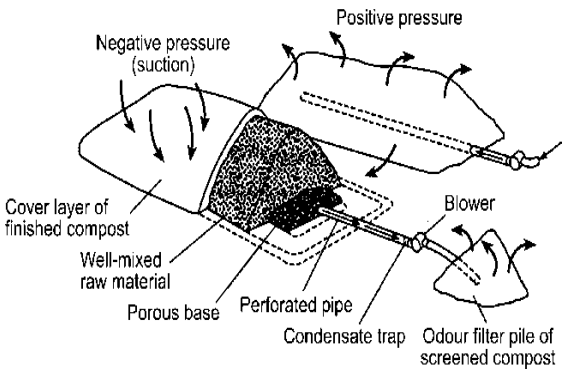
Bin composting (Figure 3.9a) is the most primitive of in-vessel composting methods that is used now. It represents a simple technology that can be accomplished with very ordinary and common devices and operations. Effective hand sorting of the feedstock is possible, especially in household composting bins (Haug, 1993). This prevents undesirable materials from going into the composting bins and results in a good quality product. Household composting bins provide potential resolution of vast amount of household biodegradable waste. Passive or natural aeration is the main aeration method of passively-aerated bins. The passive aeration can be attained through oxygen diffusion driven by concentration gradient, “chimney effect” deriving from heat production or wind blowing air through the materials (Rynk, 2000). On the other hand, agitated bins provide containment and controlled aeration plus the ability to agitate or turn materials within the unit. Both forced aeration and mechanical agitation of solids can be applied (Haug, 1993).

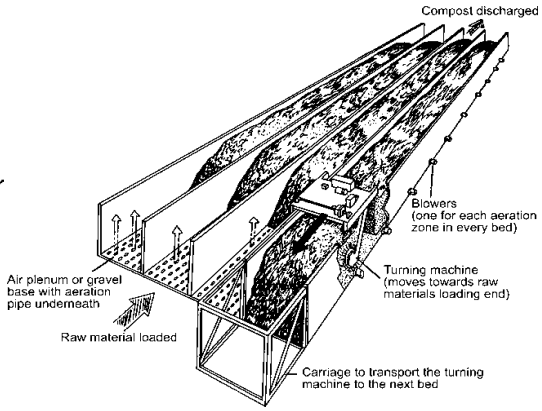
The rotary drum is another kind of in-vessel composting system. Typically, feedstocks are transferred to static pile, agitated windrow, or aerated static pile systems to produce marketable compost (Gouin, 1998). No matter what details and process management of various drums, they share the basic idea of promoting decomposition by tumbling material in an enclosed reactor as shown in Figure 3.9c (Rynk, 2000). Typically, feedstock inlet is located on the opposite ends of outlet of compost. Inside the drum, tumbling action slowly mixes material while moves them through the drum. The essential idea of rotary drum is to expose the composting mass to air, add oxygen and release heat

(a) Composting bins

(b) Windrow composting system

(c) Rotary drum composting system





(d) Aerated static pile composting system (e) Agitated static pile composting system

Figure 3.9 Different composting systems (Misra et al., 2003; GECF, 2008; Laporte and Hawkins, 2009; City of Roseville, 2010)

and gaseous products during decomposition (Rynk, 2000). These systems have been used with various feedstock including MSW, agricultural wastes, and sewage sludge (Haug, 1993). However, due to the complexities of feed and withdrawal and limited mixing result, this reactor style is not commonly used in bioremediation.

Windrow composting is used in conjunction with in-vessel systems when composting highly variable feedstock (Gajalakshmi and Abbasi, 2008). In windrow composting system (Figure 3.9b), feedstock are placed in parallel rows and turned periodically by mechanical equipment into a natural shape of trapezoid. The height and width of trapezoids vary depending on the nature of the feedstock and the equipment used for turning. A balance is required in order to ensure windrows are neither too large to be anaerobic in the centre, nor too small to maintain a certain high temperature during the process. Oxygen can be supplied mainly by natural ventilation or through perforated pipes installed underneath (Haug, 1993; Gajalakshmi and Abbasi, 2008). The disadvantages of windrows are: (a) requirement of large areas of land, (b) odor problems caused during the turning operations, (c) the release of fungal spores and other bioaerosols, as well as volatile compounds which cause environmental and public health impacts (Cookson, 1995; Gajalakshmi and Abbasi, 2008).

A static pile system, like windrows, consists of waste laid out in parallel rows. But the static pile is not turned and always built taller and more widely than windrows. Typically, 1.5-2.5 meters of height could be attained depending on the porosity of feedstock, weather conditions and operation equipments used (Gajalakshmi and Abbasi, 2008). Extra height retains heat inside piles, especially in wintertime. Because typically no further turnings will be applied, the initial selection and characteristics of raw materials is very important. Poultry and hog carcasses, as well as yard and garden trimmings, are common feedstock (Gouin, 1998). Bulk agents like wood straw or wood chips provide structural stability to the material, relatively constant porosity and maintain air voids without the need for periodic agitation. However, recyclable use of these bulk agents is required because they might be large enough and cannot be thoroughly digested during one cycle (Haug, 1993; Gajalakshmi and Abbasi, 2008). Aeration and exhaust pipes can be installed to provide active or passive air flow as shown in Figure 3.9d (Gajalakshmi and Abbasi, 2008). Agitated static piles (Figure 3.9e), have been designed to provide

operations such as tearing the pile, depluming solids and reforming for additional composting. However the agitation is considerably less than that normally conducted on the windrow system (Haug, 1993). In spite of large land and long time requirements, the static pile system tends to be significantly cheaper with respect to equipment, manpower and running cost (Haug, 1993). However, static pile composting generally does not generate uniform compost (the outer layer does not decay) and the potential of developing anaerobic environment is high, especially in high precipitation regions (Gouin, 1998).

3.7 Indices of Product Maturity and Stability

Since the bioremediation is conducted in a bioreactor that involves thermophilic phases, the quality of product can be assessed using composting indices for maturity and stability. The residual concentration of contaminants, combined with several operating indices would give a good evaluation of the product of the bioremediation. The ultimate goal of bioremediation composting is to eliminate contaminants and to produce a humus-like product that can be used for soil improvement and plant growth. The composting process is considered finished or the product is stable only when the compost does not generate appreciable heat, is low of carbon-nitrogen ratio and has low bio-available carbon (Epstein, 1997). Analytical procedures for determining inorganic elements and organic compounds that can affect plant growth have also been well defined. Chemical and biological parameters of the organic material can be considered as indicator of maturity. Physical characteristics such as color, odor and temperature give a general idea of the decomposition stage, but give little information about the degree of maturation (Bernal et al., 1998). Other chemical and biological parameters widely used include: drop in temperature, degree of self-heating capacity, oxygen consumption, microbial activities, biodegradable constituents, phytotoxicity assays, enzyme activity, cation-exchange capacity (CEC), organic matter nutrient content, C:N ratio, humus content and quality and water soluble organic-C:total organic-N ratio (Bernal et al., 1998; Gajalakshmi and Abbasi, 2008; Hue and Liu, 1995; Khan and Anjaneyulu, 2006; Raut et al., 2008).

3.7.1 Respiration Rate

Respiration rate is a widely accepted and standardized parameter for assessing compost stability, by measuring O_2 uptake or CO_2 evolution in the respirometer over a period of 4 days. Their relationship in evaluating compost maturity is shown in Table 3.6. Because this method requires a small amount of sample material (20 g), it is convenient to conduct at the lab-scale. However, moisture and temperature conditions must be controlled during measurement (Korner et al., 2003; Goméz et al., 2006; Gajalakshmi and Abbasi, 2008). Overall, respiration measurement can provide useful information for metabolic activity of composting material as well as the progress of degradation, but the full assessment of product quality must take other parameters into consideration (Korner et al., 2003; Goméz et al., 2006).

3.7.2 Carbon:Nitrogen Ratio

Carbon and nitrogen are the main two building blocks of microorganisms, so evaluate their relative amount in compost is an indicative way of maturity. Compost is assumed to be mature if the C:N ratio is below 12 (Bernal et al., 1998; Brewer and Sullivan, 2003). However, the final ratio may vary from 5 to 20 due to variations in feedstocks (Epstein, 1997). Typically, C:N ratios will decrease sharply during the first days of compost (Hsu and Lo, 1999; Gajalakshmi and Abbasi, 2008; Gómez-Brandón et al., 2008). Without any other indicators, it is difficult to determine when the maturity is really attained on the basis of C:N ratio alone.

3.7.3 pH

The change of pH is an important character in a composting process. After a short drop of pH at the beginning of composting, pH will go back to 6.5-7.5 (Epstain, 1997; Gajalakshmi and Abbasi, 2008). In this case, acid pH values can serve as an indicator of immaturity, because the existence of acid indicates the existence of anaerobic products in most of cases. Under aerobic conditions, there is no further change in pH in stable compost and odor is not produced (Epstein, 1997). However, due to less change after a period of time, pH is not a good indicator of stability without taking other parameters into consideration.

Table 3.6 Relationship between CO_2 evolution and O_2 consumption in compost maturity evaluation (Korner et al., 2003; Goméz et al., 2006)

CO_2 evolution (mg CO_2-C/g C)	**O_2 consumption (mg O_2/g DM)**	**Respiration rate**	**Material status**
0-2	≤ 20	Very slow	Stable
2-8	30-20	Moderately slow	Stable
8-15	50-30	Medium	Fresh
15-25	80-50	Medium – high	Fresh
>25	> 80	High	Raw

C: Carbon
CO_2-C: Carbon dioxide described as carbon
DM: Dry Matter

3.7.4 Phytotoxic Index

Inbar et al. (1993) and Ramirez-Perez et al. (2007) suggested that determining compost stability/maturity should emphasize plant response, or the phytotoxicity from organic compounds in compost on plants, and other indicators should be compared with it. The degree of maturity can, also, be revealed by biological methods concerning the seed germination rate and measuring the root length of the ensuing plants (Zucconi et al., 1981a). Immature composts may contain phytotoxic substances due to anaerobic conditions, thereby inhibiting the germination and growth of angiosperms. These substances include low-molecular-weight organic acids such as: acetic, propionic, and butyric acids. Cress seed, cucumber, ryegrass and radish can be used for the determining of germination index (GI) of compost (Epstein, 1997). A germination index (obtained by multiplying the percent germination by the percent root growth as related to control) of ≥80% indicates the disappearance of phytotoxins in composts (Tiquia et al., 1996). GI values greater than 50% may indicate phytotoxin-free compost (Gajalakshmi and Abbasi, 2008). However, dynamic toxicity effects and different sensitivities have been observed for various plants or stages of development. Some plants' sensitivity to toxins tends to be a transient condition and they have the capability to recover and even thrive in soils amended with organic matter (Zucconi et al., 1981b).

3.7.5 Water-soluble Organic Carbon : Organic Nitrogen Ratio

The water-soluble organic C : organic N ratio has been suggested as a maturity index (Bernal et al., 1998; Hue and Liu, 1995). Hue and Liu (1995) proposed a value of <0.70 as an index of compost stability. However, the analysis procedure should avoid nitrogen losses and accurately include all organic N species when using this ratio as a maturity index (Gajalakshmi and Abbasi, 2008).

3.7.6 Enzyme Activity

In a composting system, microbial activities decide the overall efficiency of catabolism of substrates. Microbial degradation of organic materials is based on different kinds of oxidation enzymes. The enzymes released by the microorganisms during composting breakdown several complex organic compounds to simple and water-soluble compounds (Benitez et al., 1999). Therefore, the activities of microbial enzymes may be good indicators of the status of the composting process. Various hydrolytic enzymes are

believed to control the rate at which various substrates are degraded. Important enzymes involved in the composting process include: (a) cellulases, which depolymerize cellulose, (b) β-glucosidases, which hydrolyse glucosides, (c) urease, which is involved in N-mineralisation, (d) phosphatases and arylsulphatase, that remove phosphate, (e) lipase, which depolymerize lipids and (f) sulphate groups from organic compounds (Benitez et al., 1999; Mondini et al., 2004). Dehydrogenase (as a group of mostly endocellular enzymes that catalyze the oxidation of organic matter) activity decreases with composting time of different organic feedstock and remains stable after 2 or 3 months of the process (Gajalakshmi and Abbasi, 2008). Characterizing and quantifying enzymatic activities during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations and provide information about the maturity and stability of composted products (Tiquia et al., 2002; Raut et al., 2008).

3.7.7 Nitrification

Compost maturity can, also, be defined in terms of nitrification. The presence of ammonia indicates immature compost, while the presence of nitrate indicates the maturity (Epstein, 1997; Bernal et al., 1998). However, nitrogen tests alone should not be sufficient and a sulfide test and a cress seed germination test are always required (Epstein, 1997).

3.7.8 Humification

Since stabilization or maturation implies the formation of some humic-like substances, the degree of organic matter humification is generally accepted as a criterion of maturity. Studies in this respect refer to the humification ratio, humification index, percent of humic acid, humic acid to fulvic acid ratio and the chemical, physico-chemical and spectroscopic characterization of humic-like substances (Epstein, 1997; Khan and Anjaneyulu, 2006). Humification ratio (HR) is the percentage of total extractable humic carbon (C-ext) of the total organic carbon (C-org) described as following (Epstein, 1997):

$$HR = C_{ext} \times 100/C_{org} \tag{3.1}$$

3.8 Regulations

Even though the complexity of the bioremediation technology makes it hard for the government to develop practical regulations, the development of a new bioremediation/composting technology is basically encouraged (Sikdar and Irvine, 1998). For example, USEPA promotes the laws that restrict the passing on of the liability for contaminated material to landfill operators, hence strongly encourages the remediation of contaminated sites (Sikdar and Irvine, 1998; Swannell, 2003). Locally, Nova Scotia is pursuing the goal of waste disposal rate under 300 kilograms per person per year by 2015 (ENS, 2009). This is a strong pull to technologies such as bioremediation, which divert wastes from going to landfills. However, there are several points one should pay attention to when developing or using a bioremediation technology (Strauss, 1997):

1. Are the partial products or metabolites of the bioremediation process toxic?

2. Is it causing a health problem if the growth of some kinds of microorganisms is enhanced?

3. Is there a risk caused by the interaction between microorganism and chemicals at the bioremediation site?

Concerning about all this questions, the maker of regulations and bioremediation guidelines would have a clear idea of ensuring a safe and successful bioremediation process. There were not many regulations concerning the feedstock of compost and the existing regulations are almost all focusing on the quality of compost products. The Canadian government has established guidelines for compost quality. In addition, provinces can issue regulations or guidelines (Epstein, 1997). The national guidelines are published under the auspices of the Environmental Choice Program which certifies products and services that are proven to have less impact on the environment because of how they are produced, consumed or disposed. Producers or importers are encouraged to apply for the Environmental Choice Program for verification and subsequent authority to label qualifying products with the environmental choice Ecologo label.

Several requirements are imposed on the producers to meet the requirement for compost product quality. Many feedstocks are eliminated and consequently requirements

tend to discourage the manufacture of compost products (Epstein, 1997). The requirements are (ECP, 1996):

1. Meet or exceed all applicable governmental and industrial safety and performance standards.
2. Meet the requirements of all governmental acts and regulations including the Canadian Environmental Protection Act (CEPA).
3. Adhere to the policies and targets of the national packaging protocol and adopt the code of preferred packaging.

The following are the product criteria that must be met (ECP, 1996):

1. Be uniformly exposed to temperatures exceeding 55˚C for three consecutive days
2. Have a pH between 5.0 and 8.0
3. Not to exceed the following amounts of heavy metals in mg/kg dry weight:
    - Arsenic (As): 13
    - Cadmium (Cd): 2.6
    - Cobalt (Co): 26
    - Copper (Cu): 128
    - Lead (Pb): 83
    - Mercury (Hg): 0.83
    - Molybdenum (Mo): 7
    - Nickel (Ni): 32
    - Selenium (Se): 2.6
    - Zinc (Zn): 315
4. Have a minimum of 30% organic matter content on a dry-weight basis
5. Have a maximum of 50% water content
6. Have a sodium adsorption ratio (SAR) less than 5
7. Have a maximum particle size of 13 mm
8. The products must not contain plastic in exceed of 0.4% dry weight and any combination of glass, rubber, and/or metal in excess of 1% by weight if such particles have a dimension in excess of 2 mm.
9. Have PCBs < 1.0 ppm
10. Have a maximum electrical conductivity (Ec) of 3 ms/cm
11. Be derived from source-separated municipal wastes

12. Have undergone the entire composting process (compost must be stable; i.e., no longer decaying) under aerobic conditions

If packaged in a plastic bag, it must be labeled in accordance with the Society of the Plastics Institute of Canada; plastic bottle and container material code system. It must contain over 15% recycled plastic by weight and a minimum 5% of the total weight must be post-consumer plastic. It must not be formulated with inks, dyes, pigments or other additives that contain lead, cadmium and mercury or hexavalent chromium and must not have a combined contaminant concentration exceeding 250 ppm.

4. EXPERIMENTAL APPARATUS

The experiments were carried out in a specially designed multiple reactor bioremediation system which consisted of a frame, three bioreactors, air supply units and data acquisition unit as shown in Figure 4.1.

4.1 The Frame

The frame was made of two parts as shown in Figure 4.2. The main part was made of three aluminium sheets (3.2 mm thick). The central sheet measured 330 mm × 1100 mm and the two side sheets measured 140 mm × 1100 mm each. The sheets were soldered together making a U-shaped vertical channel with a length of 1100 mm, a width of 330 mm and a depth of 140 mm. This U-shaped stand held the mixing motors, flow meters, air and exhaust gas manifolds and tubing, and the thermocouple wires. The second part of the frame was a horizontal supporter made of three 50 mm × 50 mm aluminium angles (3.2 mm thick), two of which measured 700 mm and were fixed to the main part by means of four 6 mm diameter stainless steel bolts and nuts. The third part measured 328 mm and kept the other two angles 330 mm apart. The three aluminium angles were permanently soldered together.

4.2 The Bioreactor

Each bioreactor provided space for 3.0-3.5 kg (wet-basis) of the compost mixture plus 25% of the volume as a head space. The bioreactor (Figure 4.3) was made of a polyvinyl chloride (PVC) cylinder with an inside diameter of 203 mm, a length of 520 mm and a thickness of 5 mm. Three bioreactors were horizontally fastened into the main frame. One end of each cylinder was fastened onto the frame horizontally. A removable circular Plexiglas plate of 203 mm diameter and 6 mm thickness was installed on the other end of the cylinder. It was sealed to the cylinder with the help of a rubber gasket lining ring (O-ring, 2.5 mm thick) in the inner side. A circular window of 64 mm in diameter was left on the removable circular plate for sampling purposes. The window was

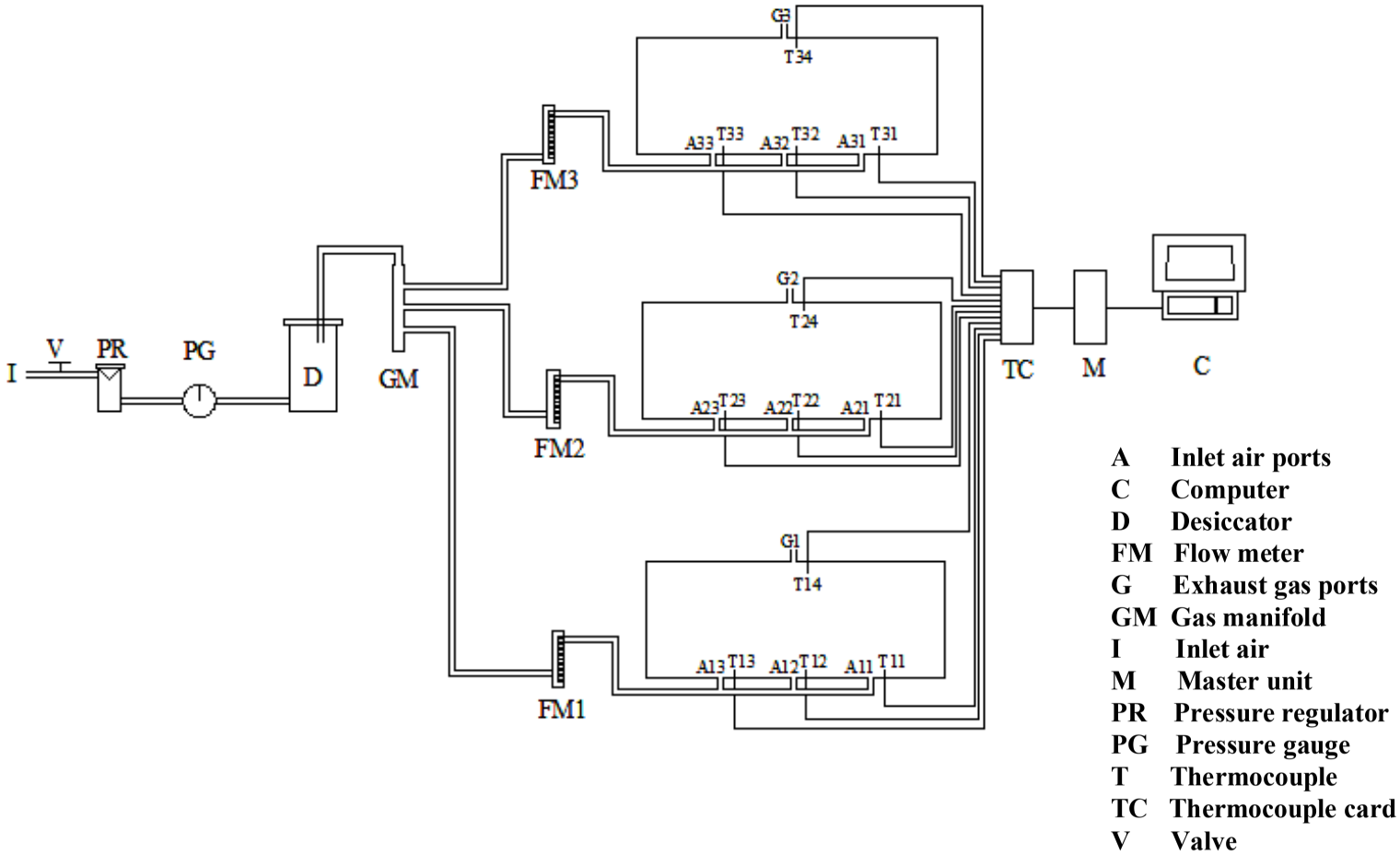


Figure 4.1 The experimental set up of the bioremediation system

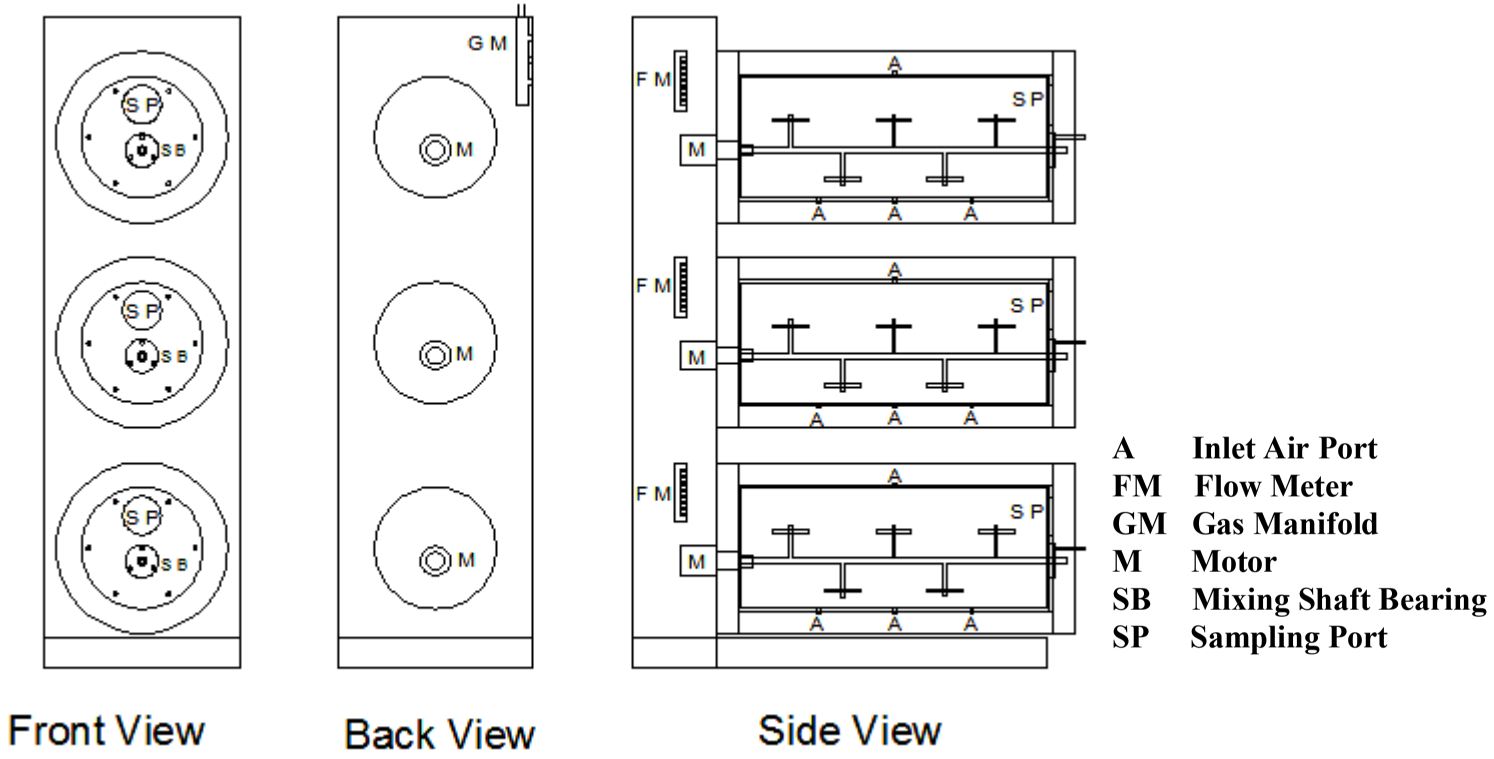


Figure 4.2 Schematic of the frame and the bioreactors arrangement

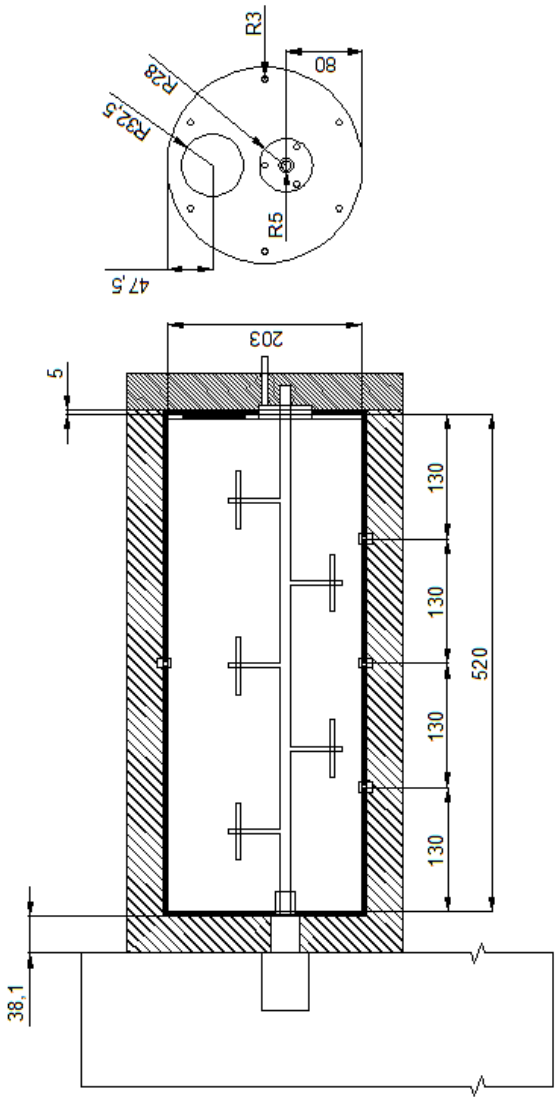


Figure 4.3 Bioreactor (dimensions in mm)

closed with a rubber stopper (No. 13) when it was not in use. The other side of the cylinder was covered with a fixed circular PVC plate of 203 mm diameter and 6 mm thickness.

This plate (PVC/711, IPS Corp., Gardena, CA) was glued into the cylinder and secured by six stainless steel screws (6 mm diameter). The fixed circular plate was fitted into an aluminium ring, which was fastened into the frame by four bolts (6 mm) and nuts. Each bioreactor had three holes at the bottom, connected to a manifold by 6.4-mm diameter tygon tubing, which were used for supplying air to the bioreactor. A top hole was used for the exhaust gas. The cylinder was insulated with a 38.1 mm thick Fibreglass while the removable fixed circular plates were insulated with a 38.1 mm thick Styrofoam layer.

Inside each bioreactor, a removable 10.5-mm diameter solid stainless steel shaft was mounted on two bearings (Figure 4.4). There were 5 stainless steel collars on the shaft on each of which, a bolt of 69 mm in length and 6 mm in diameter was mounted. Thermally protected electric motor (Model No. 127P1486/B, D. C., Sigma Instruments Inc., Braintree, Mass, USA) provided power to rotate the shaft (5.76 rpm).

4.3 Air Supply

Air was supplied continuously to the bottom of the bioreactor from the laboratory air supply (Figure 4.5) at the chosen airflow. The air passed through a regulator and a pressure gauge to maintain a pressure around 5 kpa in order to ensure equipment and connections safety. The regulated air then passed through a desiccator and then through a flow meter (No 32461-14, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) which was used to control and measure the volume of air entering the bioreactor. The flow meter had a 4-inch scale and a range of 2-20 standard cubic feet per hour (0.0566-0.566 m^3/h).

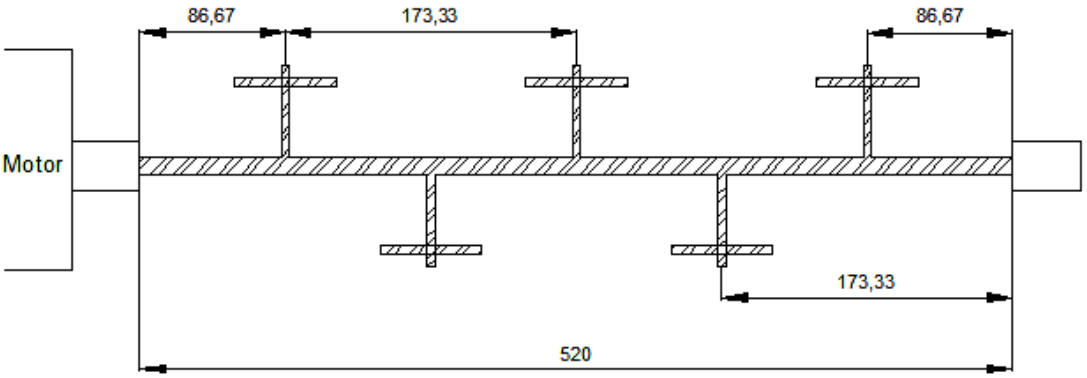


Figure 4.4 The mixing shaft (dimensions in mm)

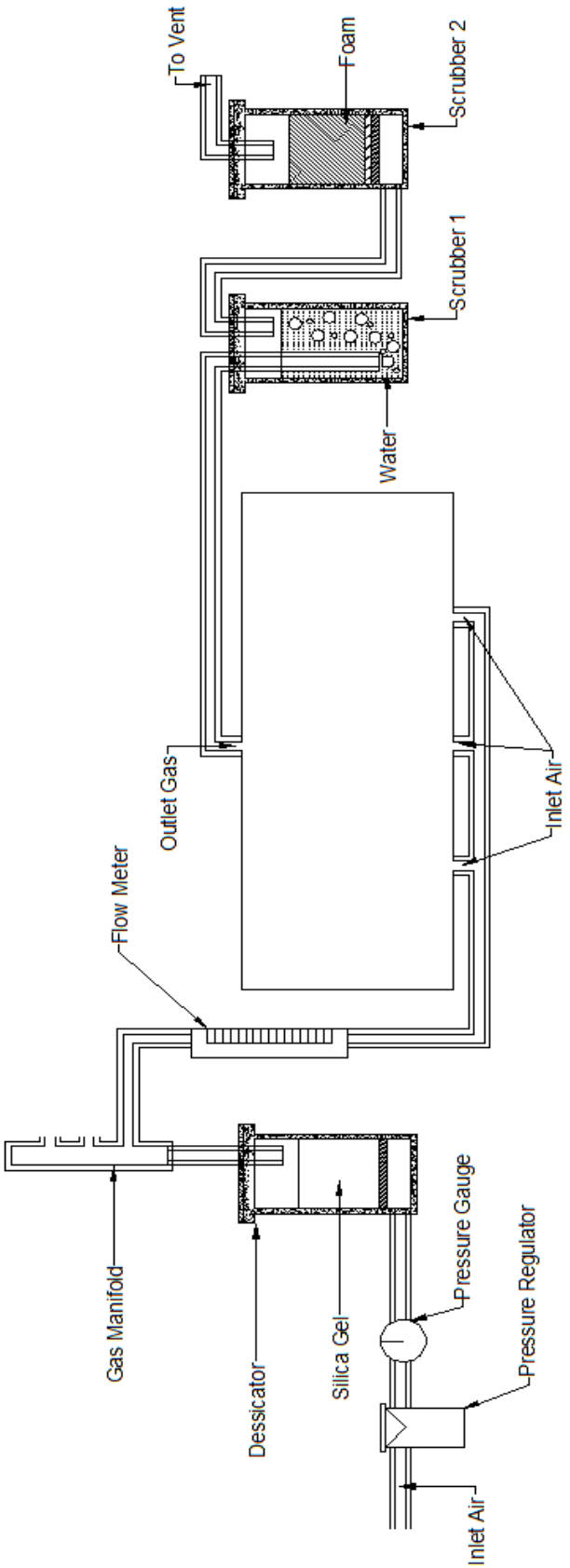


Figure 4.5 The aeration system and scrubbers used for each bioreactor

After the air entered the bioreactor, it passed vertically upward through the mixture and exited at the top of the bioreactor. When the gas left the bioreactor, it passed through a scrubber that contained water to get rid of aerosol and water soluble organic compounds, then through another scrubber to eliminate possible airborne PAH compounds in the exhaust gas. Both air entrance and exit tubes were covered, along with the bioreactor, with insulation material.

4.4 Data Acquisition Unit

The data acquisition unit consisted of a master unit, scanning card, software, temperature sensors, and a personal computer.

4.4.1 Scanning Card

The Thermocouple/Volt Scanning Card (MTC/24, Omega, Stamford, CT) contained 24 isolated differential input channels. Each channel could be programmed to receive inputs from thermocouple type J, K, T, E, R, S, B and N, or from any of the several voltage ranges: ± 100 millivolts, ± 1 volt, ± 5 volts and ± 10 volts with an accuracy of ± 0.02% of each range. Temperature values could be returned in any of the following units: $^{\circ}$C, $^{\circ}$F, $^{\circ}$K, $^{\circ}$R, and mV.

4.4.2 Software

The Tempview (Omega, Stamford, CT) was a Microsoft-Windows-Based setup and acquisition application software that featured a graphical spreadsheet-style user interface, which allowed easy configuration of hardware and acquisition and display parameters. The Tempview software provided a non-programmable approach that enabled data collection and display. Also, connected with Microsoft Excel worksheet, the software copied temperature data to Excel at any given intervals of measurement set by operator.

4.4.3 Temperature Sensors

Temperature measurement was conducted using four type T (copper-constantan) thermocouples (Cole Parmer, Chicargo, IL, USA) for each bioreactor. The thermocouples

were inserted through specially constructed fitting as shown in Figure 4.1. For bioreactor 1, three of the four thermocouples (T11, T12, and T13) were installed at the bottom of the bioreactor to measure the temperature of the compost mass and the fourth one (T14) was installed at the top near the outlet air hole (21 mm away) to measure the temperature of the exhaust gas. A similar arrangement of thermocouples was made in bioreactor 2 (T21-T24) and bioreactor 3 (T31-T34). The thermocouples on the bottom of all bioreactors were located far enough from the inlet air (65 mm away) to minimize the negative influence of inlet air temperature.

4.4.4 Computer

A personal computer (Pentium IV) with 256 MB of random access memory (RAM), 40 GB hard disk and 1.2 MB (3 1/2″) and a floppy drive was used to run the data acquisition system. The master unit (Multiscan 1200, Omega, Stamford, CT) used was a high-speed instrument that was connected to a computer via RS 232 interface.

5. EXPERIMENTAL DESIGN

An experiment was carried out to establish the optimum conditions of the bioremediation process. Chemical and physical analyses were first conducted on the raw materials. The results of these analyses are shown in Table 5.1, and they were used to adjust the carbon to nitrogen ratio and MC to optimum values (30:1 and 60%) using urea ($CO(NH_2)_2$) and distilled water. Used cooking oil was used as a bio-available carbon source to control the temperature.

Three kinds of microorganisms (Table 5.2) were used as inoculums in the main experiments. Their abilities to degrade different main contaminants during the composting process were tested separately and collectively and the results were compared to the control (Table 5.3). Three replicates of each trial were carried out, which resulted in a total of 15 experimental runs. Three bioreactors were used for each treatment at the same time and contained exactly the same raw materials for replicates. Each experiment was completed in 15 days.

Table 5.1 Carbon and nitrogen contents in the feedstock

Element	**Wood Waste (%)**	**Compost (%)**
Carbon	45.5	44.0
Nitrogen	0.671	1.8

Dry basis

Table 5.2 Three microorganisms used as inoculums (ATCC, 2009)

Microorganisms	**ATCC Number**	**Optimum Growth Temperature**	**Application**
Thermobifida fusca	BAA-630	50.0˚C	Degradation of cellulose and xylan
Thermomonospora curvata	19995	50.0˚C	Degradation of cellulosic substrates
Thermoascus aurantiacus	204492	45.0˚C	Degradation of lignocelluloses

Table 5.3 Experimental design

Experiment	Inoculums		
	T. curvata	*T. aurantiacus*	*T. fusca*
Control	×	×	×
1	√	×	×
2	×	√	×
3	×	×	√
4	√	√	√

6. EXPERIMENTAL PROCEDURE

6.1 Preparation of Raw Materials

The C&D wood waste was obtained from C&D site in Yartmouth, Nova Scotia. It was screened to remove visible non-biodegradable materials such as glass, metal, and plastic. In order to have a good mixture structure and large contact surface with microorganisms, wood waste was sieved using USA Standard Testing Sieve with 12.5 mm opening (USA Standard Testing Sieve, Opening in 12.5 mm, ATM, Milwaukee, Wisconsin). It was then placed in polyethylene bags and stored in a freezer at -10˚C in the Biotechnology Laboratory until required for the experiment.

6.2 Preparation of Culture Media

Broth culture, slant solid media and Petri dish solid media were made for the three microorganisms used in the study. For *T. aurantiacus*, potato dextrose agar (PDA) medium were used for slants and Petri dishes, and potato dextrose broth was used as liquid medium. For *T. fusca*, TYG medium was used for slants and Petri dises, and TYG broth medium was used for liquid media. For *T. curvata*, Hichey-Tresner agar was used for slants and Petri dishes, and Hichey-Tresner broth medium was used for liquid medium. All three kinds of solid or broth media were made in the laboratory using distilled water and reagents (Sigma Alderich, St. Louis, Missouri) as listed in Tables 6.1 and 6.2.

The agar media were autoclaved (Sterilmatic Autoclave, Market Forge Industries Inc., Everett, MA, USA) at 125˚C for 20 min. After cooling to about 70˚C, approximately 25 ml the medium were poured on to each Petri dish and approximately 20 ml were poured to each slant tube. The Petri dishes and slants were cooled to solidify before use.

The broth media were autoclaved (Sterilmatic Autoclave, Market Forge Industries Inc., Everett, MA, USA) at 125˚C for 20 min and cooled to room temperature before use.

Table 6.1 Culture media for slants and Petri dishes

Contents	**TYG Medium**	**Hichey-Tresner Agar**	**Potato Dextrose Agar (PDA)**
Distilled Water	1.0 L	1.0 L	1.0 L
Agar	15.0 g	15.0 g	15.0 g
Tryptone	3.0 g	2.0 g	/
Yeast Extract	3.0 g	1.0 g	/
Glucose	3.0 g	/	/
K_2HPO_4	1.0 g	/	/
Dextrin	/	10.0 g	/
Beef Extract	/	1.0 g	/
$CoCl_2$	/	2.0 mg	/
Potato Dextrose Powder	/	/	24.0 g

Table 6.2 Culture media for broth

Contents	**TYG Medium Broth**	**Hichey-Tresner Broth**	**Potato Dextrose Broth**
Distilled Water	1.0 L	1.0 L	1.0 L
Tryptone	3.0 g	2.0 g	/
Yeast Extract	3.0 g	1.0 g	/
Glucose	3.0 g	/	/
K_2HPO_4	1.0 g	/	/
Dextrin	/	10.0 g	/
Beef Extract	/	1.0 g	/
$CoCl_2$	/	2.0 mg	/
Potato Dextrose Powder	/	/	24.0 g

6.3 Preparation of Inoculums

The cultures of the three microorganisms were ordered from ATCC (Manassas, VA). The cultures were obtained in freeze-dried status in vials and were revived first. For revival of fungus, 1 ml sterile water was added to the freeze-dried pellet. Then, the entire liquid contents were transferred into a test tube with 5 ml sterile water. The liquid was kept overnight at 25˚C and then transferred to the Petri dish media and the agar slants. For bacteria, the cultures were revived by adding approximately 1 ml sterile broth medium to the freeze-dried material after opening the vials. The liquid contents were then transferred into a test tube containing 5 ml broth media. Then, the liquid containing microbial cultures was spread on the Petri dish media and the agar slants in test tubes. The agar slants were stored at 4˚C in a refrigerator for future use and the Petri dish cultures were used for further steps. Every two months, the Petri dish cultures were renewed from the slant culture. All Petri dishes and agar slants were incubated at 45˚C for fungus and 50˚C for bacteria in an incubator (Isotemp® oven, Model 106G, Fisher Scientific, Hampton, New Hampshire) for 48 h.

Spore suspension of *T.* aurantiacus was prepared by cutting 1 cm^2 Petri dish culture into 25 ml sterile potato dextrose broth medium and incubating in an incubator (Isotemp® oven, Model 106G, Fisher Scientific, Hampton, New Hampshire) at 45˚C in order to activate the fungal culture. After 48 h of incubation, plate counts were conducted to detect colony forming units (CFU). An amount of 5 ml media containing the CFU of 2.0×10^4/ml was transferred into Fernbach flasks containing 250 ml of liquid cultural media and agitated on rotary shaker (Series G-25 Incubator Shaker, New Brunswick Company, New Jersey, USA) at 120 rpm and 45˚C for 48 hours. And the final culture was used as inoculums. The entire procedure is shown in Figure 6.1.

For bacteria *T. fusca* and *T. curvata*, the suspension was made by cutting 1 cm^2 Petri dish culture into 25 ml sterile TYG and Hichey-Tresner liquid media, respectively and incubated at 50˚C for 48 hours in order to activate bacterial cultures from low temperature in flasks containing 250 ml of culture media. For *T. curvata*, 5 ml of liquid medium containing CFU of 1.0×10^6/ml was transferred into Fernbach flasks containing 250 ml of culture media. All flasks were agitated on rotary shaker (Series G-25 Incubator Shaker,

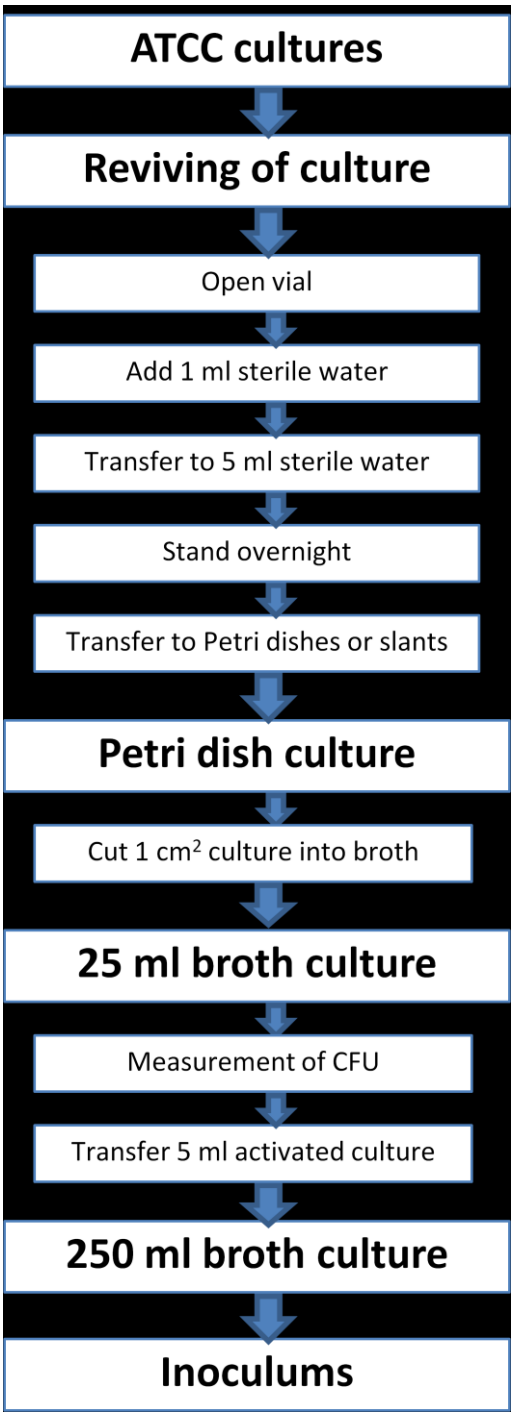


Figure 6.1 Preparation of fungal inoculums

New Brunswick Company, New Jersey, USA) at 150 rpm for 48 hours at 50˚C. The media containing bacteria spores were used as inoculums. The entire procedure is shown in Figure 6.2.

All equipments were autoclaved (Sterilmatic Autoclave, Market Forge Industries Inc., Everett, MA, USA) for 20 min at 121˚C before use. The final cultures were used as inoculums in the experiment in the amount of 10% (weight) of composting materials.

6.4 Measurement of CFU

In order to measure the number of CFU, a series of dilutions were carried out. A 0.5 ml aliquot of spore solution was added to an autoclaved test tube containing 4.5 ml of autoclaved distilled water. The liquid was mixed and homogenized to evenly distribute spores. From this tube, 0.5 ml suspension liquid was transferred into another autoclaved test tube containing 4.5 ml of autoclaved distilled water. The same process was carried out several times until the desired dilutions (10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}) were obtained. From each dilution, 0.1 ml was added to a Petri dish containing PDA (two duplicates were performed). The plates were sealed with parafilm, inverted and incubated at 45˚C for 24 h. After the incubation, colonies were counted on plates that had between 30-300 CFU present.

6.5 Experimental Protocol

The wood waste material was thawed first at room temperature. Then, the wood chips were well mixed with fresh compost (Miller Compost Corporation, Dartmouth, NS) at a ratio of 1:1. The total mixture was 2.5 kg. The C:N ratio was adjusted using urea ($CO(NH_2)_2$) and the MC was adjusted to 60% using distilled water. The microbial inoculums were added to the mixture according to the experimental design (Table 5.2).

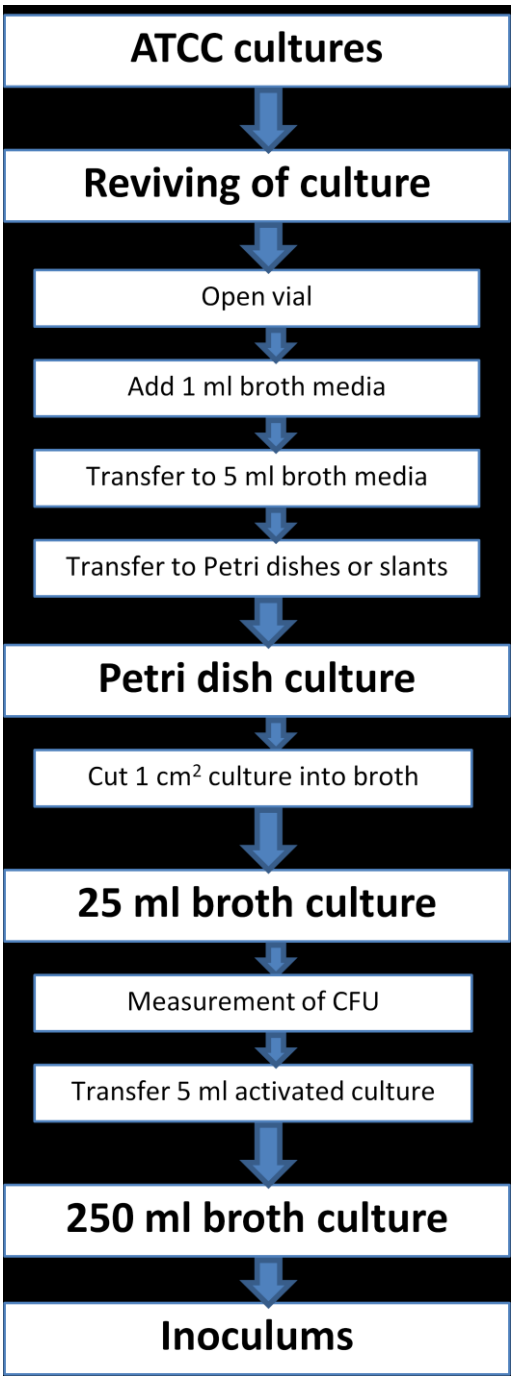


Figure 6.2 Preparation of bacterial inoculums

Approximately 3 kg of the final mixture were placed in each bioreactor. The pressure-regulated air was supplied continuously to the bottom of the bioreactor. The flow rate was measured by a flow meter (No 32461-14, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA) and adjusted to 0.05 m^3/h. Temperature was monitored throughout the process by temperature sensors (Cole Parmer, Chicago, IL, USA) and the data was stored in a Microsoft Excel® file in a computer at the interval of 30 min. The temperature of each sampling time was then generated using average temperature from 12 thermocouples in three bioreactors.

Used cooking oil was used in this study as a source of bio-available carbon. An amount of 36 ml used cooking oil were added into the bioreactor every 12 h for the duration of each experiment as recommended by Alkoaik (2005). The environmental parameters (pH, temperature and moisture content) were monitored and representative samples were taken from the bioreactors and analyzed according to the schedule shown in Table 6.3.

After 21 days of bioremediation, the quality, stability and maturity of the end product were evaluated. The quality of the end product was determined by quantifying the following parameters:

(a) C:N ratio
(b) pH
(c) CO_2 evolution
(d) Phytotoxicity (evaluated by calculating germination index of cress (*Lepidium sativum*) (Iannotti et al., 1994; Jiang et al., 2006)
(e) The degradation extent of cellulose and lignin materials
(f) The remaining contaminants of phenolic compounds

The following indices were used in evaluating the maturity/stability of the final product:

(a) C:N (< 12)
(b) CO_2 evolution (< 5 mg C-CO_2 per gram of volatile mass of sample per day)
(c) pH of compost (in the range of 5-7)
(d) The germination index (> 50%)

Table 6.3 Frequency of sample collection

Parameters	Sampling Schedule				
	1st day	**4th day**	**7th day**	**11th day**	**15th day**
pH value	√	√	√	√	√
Phenolic compounds	√	√	√	√	√
Moisture content	√	√	√	√	√
Total carbon	√		√		√
Total Kjeldahl Nitrogen	√		√		√
Solid analysis	√		√		√
Cellulose and lignin	√				√
Germination index	√				√
CO_2 evolution					√

The quality of the end product was compared to the guideline on compost published by the Canadian Minister of the Environment shown in Appendix A (ECP, 1996).

6.6 Statistical Analysis

The average values and standard errors were calculated for each treatment. The data collected was subjected to one-way analysis of variance (ANOVA) followed by pair-comparison using Bonferroni Analysis. The α-level was chosen as 0.05. All the statistical analysis of data was conducted using SigmaPlot (Version 11, Systat Software, Inc., Califonia).

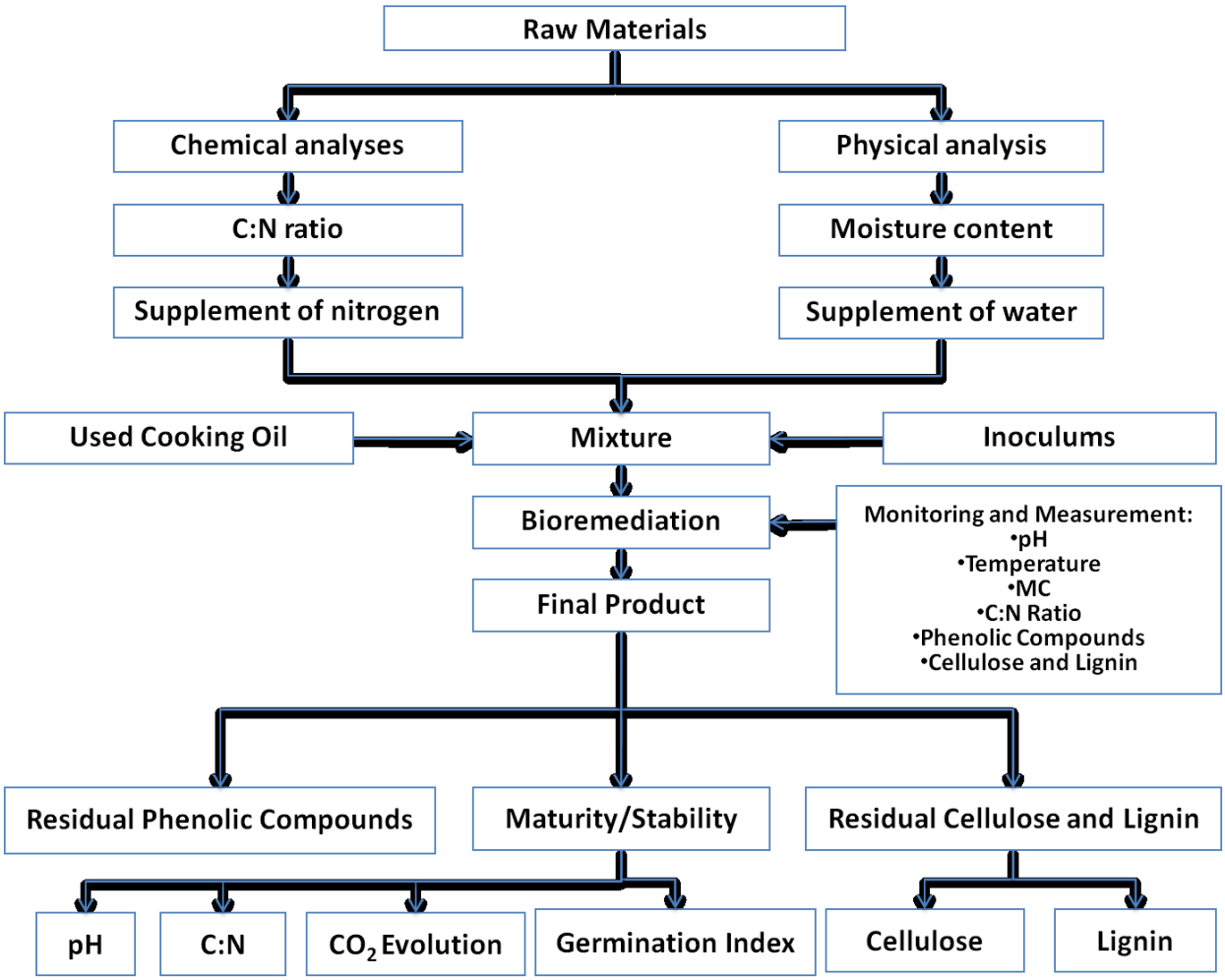


Figure 6.3 Experimental procedure

7. EXPERIMENTAL ANALYSES

7.1 Moisture Content

The moisture content (MC) was measured following the ASTM (D4442-07) oven-drying method (ASTM, 2007a). Samples (approximately 10 g each) were taken from the bioreactor and weighed using an electric balance with a minimum readability of 0.1 mg (Mettler, Model AE 200, Greifensee, Switzerland). The samples were dried in a forced-convection oven (Isotemp® Oven, Model 655F, Fisher Scientific, Hampton, New Hampshire) at 103°C for 20 hours. The end point was assumed to have been reached if the mass loss in a 3 hour interval was less than 0.2 mg. The dry weight was recorded and the MC of sample was calculated as follows:

$$\mathrm{MC} = \frac{\mathrm{A}-\mathrm{B}}{\mathrm{A}} \times 100 \qquad (7.1)$$

Where:

MC = Moisture content in percent (%)

A = Original weight (g)

B = Over dry weight (g)

7.2 pH

About 10 g of material were added to 50 ml distilled water. The mixture was agitated vigorously in a beaker with a magnetic stirrer for 30 min. After standing for 30 min, the pH value was determined with a pH meter (Fisher Accumet®, Model 805 MP, Fisher Scientific, Hampton, New Hampshire). Cautions had been taken to make sure that the electrode was in the supernatant and not in contact with the settled particles.

7.3 Total Carbon

Approximately 1.0 g of the material was sampled for total carbon analysis. Carbon dioxide was determined with a Leco carbon analyzer (Model 516-000, Leco Corporation,

St. Joseph, Michigan) along with an induction furnace (Leco HF_2O Furnace, Leco Corporation, St. Joseph, Michigan).

7.4 Total Kjeldahl Nitrogen

The total kjeldahl nitrogen (TKN) was determined at Maxxam Analytical Testing Laboratory in Mississauga, ON, following the procedure of USEPA Method 351.2 (USEPA, 1993b). Approximately 0.5 g of sample was weighed into a centrifuge tube. Samples were digested on a wet weight basis, and the moisture was determined on a separate sub-sample. The weighed sample was transferred into the digestion tube and rinsed with RO/DI (Reverse Osmosis / De-Ionization) water.

For digestion, 8.0 ml of digestion reagent (sulfuric acid-mercuric sulfate-potassium sulfate solution made by dissolving 133 g of K_2SO_4 in 700 ml of reagent water and 200 ml of concentrated sulfuric acid and adding 25 ml of mercuric sulfate solution and diluting to 1 L) was added into each digestion tube with about 15 bumping granules. The digestion sequence was as follows:

(a) Heat from room temperature (22˚C) to 180˚C at a rate 5˚C/minute;
(b) Keep at 180˚C for 90 minutes,
(c) Heat from 180˚C to 210˚C at a rate of 5˚C/minute,
(d) Keep at 210˚C for 90 minutes,
(e) Heat from 210˚C to 360˚C at a rate of 5˚C/minute,
(f) Keep at 360˚C for 40 min.

The digestion tubes were then cooled to room temperature, and 20 ml of RO/DI water were added into each tube. A solid cake was formed after centrifuge. The solid content was transferred into test tubes and topped up to 50 ml with RO/DI water. The content was then vialed and analyzed by Konelab 20/20XT Analyser (Konelab 20XT Clinical Chemistry Analyzer, Thermo Fisher Scientific, Waltham, Massachusetts).

7.5 Solid Analysis

The tests for various forms of solids were performed according to the procedures described in the USEPA Method 1684 (USEPA, 2001). An aluminum dish was weighed (W_1) with a balance with a minimum readability of 0.1 mg (Mettler, Model AE 200, Greifensee, Switzerland). Approximately 10 g of material was weighed and the weight of the dish and sample was recorded as W_2 using the balance (Mettler, Model AE 200, Greifensee, Switzerland). The samples were dried in a forced-convection oven (Isotemp® Oven, Model 655F, Fisher Scientific, Hampton, New Hampshire) at 103˚C for at least 3 h to evaporate all water contents. The weight of dry samples was measured as W_3. The dish was then put into a muffle furnace (Isotemp® Muffle Furnace, Model 186A, Fisher Scientific, Hampton, New Hampshire) at a temperature of 550˚C for 20 minutes. After cooling down in desiccator, the weight of dish and the sample was recorded as W_4. Then, the ash contents and volatile solids in percentage were calculated as follows:

$$\text{Ash Contents (\%)} = \frac{W_4 - W_1}{W_3 - W_1} \times 100 \tag{7.2}$$

$$\text{Volatile Solids (\%)} = \frac{W_3 - W_4}{W_3 - W_1} \times 100 \tag{7.3}$$

Where:

W_1 is the tare weight of the aluminium dish
W_2 is the wet weight of the sample and the dish
W_3 is the dry weight of the dry sample and the dish
W_4 is the weight of the ash and the dish

7.6 Phenolic Compounds

Approximate 3 g of material were extracted with 50 ml deionized water in centrifuge tubes. The material was mixed with a glass rod to make homogeneous slurry and then centrifuged for 20 min at 2400 rpm. The supernatant was filtered under vacuum through a

0.4 μm polycarbonate filter paper (47 mm diameter polycarbonate filter paper, Fisher Scientific, Hampton, New Hampshire) as described by Chantigny et al. (2007). The supernatant was transferred into a flask and analyzed for the presence of phenol using the 4-aminoantipyrine colorimetric test following the ASTM procedure (ASTM, 2007b). Samples were analyzed at 510 nm using spectrophotometer (Spectronic 601, Milton Roy, Ivyland, PA) and the results were correlated to the calibration curve. Three replicas for each sample were tested.

The standard curve for phenolic compounds analysis was generated following the ASTM procedure (ASTM, 2007b). A series of 100-mL phenol standard solutions were prepared which contained 0, 10, 20, 30, 40 and 50 mL of intermediate standard phenol solution (1 mL = 0.01 mg phenol) under room temperature. 5 ml of NH_4Cl solution (20 g/L) was added into each of the phenol standard solution. The pH of the solution was then adjusted to between 9.8 and 10.2 using concentrated ammonium hydroxide. The solution was mixed immediately after adding 2.0 ml of 4-aminoantipyrine solution (having a concentration of 20 g/L). 2.0 ml of $K_5Fe(CN)_6$ solution (having a concentration of 80 g/L) were added to the mixture. After 15 min, the solution was transferred to an absorption cell and measurements of absorbance of the sample were conducted against the zero absorbance of the reagent blank at 510 nm using spectrophotometer (Spectronic 601, Milton Roy, Ivyland, PA). The standard curve was developed by plotting the absorbencies against the corresponding weight in milligrams of phenol and shown in Figure 7.1. The linear relationship of phenolic compounds concentration to the absorbance reading at 510 nm was describe by Equiation 7.4 ($R^2 = 0.9972$):

$$Absorbance\ (510\ nm) = 0.0908 \times [Phenolic\ compounds] \tag{7.4}$$

The concentration of PC in the sample was expressed in terms of milligrams per litre of phenol (C_6H_5OH).

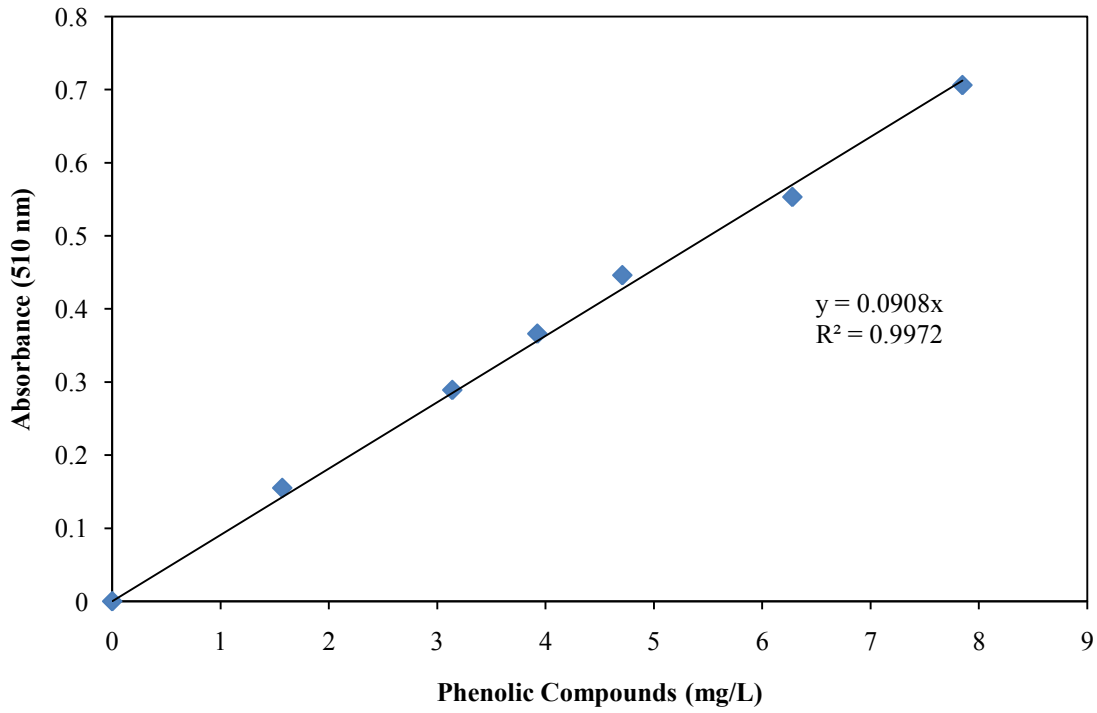


Figure 7.1 Standard curve relating concentration of phenolic compounds and absorbance at 510 nm.

7.7 Germination Index

The germination index (GI) was measured following the procedure described by Iannotti et al. (1994) and Jiang et al. (2006). About 10 g of compost sample were shaken mechanically with distilled water at a solid/liquid ratio of 1:10 (w/v, dry weight basis). Then, 5.0 ml of the extract was transferred into a sterilized plastic Petri dish lined with a filter paper (Whatman® 40, Whatman Inc., Clifton, New Jersey) using a volumetric pipette. Ten cress seeds (*Lepidium sativum* L.) were evenly placed on the filter paper and incubated at 25˚C in the dark for 48 hours. Three replicates were conducted for each compost sample. The results were evaluated by counting the number of germinated seeds and measuring the length of roots. The germination index (GI) was determined as follows:

$$\mathrm{GI}(\%) = \frac{\text{Seed germination (\%)} \times \text{Root length of treatment(cm)}}{\text{Root length of control(cm)}} \times 100 \qquad (7.5)$$

7.8 CO_2 Evolution

CO_2 evolution was determined as described by Benito et al. (2003). Approximately 25 g of the material were sampled and pre-incubated at room temperature for 3 days. The MC was adjusted to 60% and each sample was separately sealed in containers containing a beaker with 10 ml of 1 M NaOH solution. The samples were incubated at 25˚C and the CO_2 generated was determined by titrating NaOH solution with 1 M HCl solution every day for 5 consecutive days. The rate of CO_2 evolution was calculated as mg C-CO_2 per gram compost per day.

7.9 Lignin and Cellulose

The cellulose and lignin contents were measured following the standard methods published by AOAC International (AOAC, 2005) for acid detergent fiber (ADF) and acid detergent lignin (ADL). Cellulose was estimated as the difference between ADF and

ADL. Lignin was estimated as the difference between ADL and ash content as described by Yu et al. (2007).

7.9.1 Acid Detergent Fiber

Approximately 1 g of sample was weighed using an analytical balance (Mettler, Model AE 200, Greifensee, Switzerland) to an accuracy of ±2mg (W_1) and then placed in a beaker. 100 ml of acid detergent solution (prepared by adding 20 g technical grade cetyl trimethylammonium bromide to 1 L previously standardized 0.5 M H_2SO_4) was added at room temperature. The solution was heated to boil for 10 min and foaming was avoided by reducing heat slightly and providing moderate particle agitation. After 10 min of refluxing, a fine stream of acid detergent solution (5 mL) was used to rinse down the sides of the beaker. The refluxing continued for 60 min from the time of onset of boiling.

Empty crucibles were dried in an oven (Isotemp® Oven, Model 655F, Fisher Scientific, Hampton, New Hampshire) at 105˚C for 4 h and weighed (W_2) using an analytical balance (Mettler, Model AE 200, Greifensee, Switzerland). The solution was then stirred and transferred into crucible set up on a vacuum system using a pump (Edwards, Model A4C17DC428, Mississauga, Ontario). All particles left in the beaker were rinsed down into the crucible using a fine stream of boiling water. The acid detergent and rinse water were removed by vacuum filtration. The material was then soaked in about 40 ml of hot (90–100˚C) water in the crucible for 3-5 minutes and stirred to break up the residue filter mat. The soaking process was repeated twice. All traces of acid were removed by rinsing sides and bottom of crucible (any residual acid would be concentrated during drying and cause charring of residues and low fiber values). All particles were soaked in about 30–40 ml of acetone for 5 min and the process was repeated until no color was removed (typically, 2 acetone soakings were sufficient). The residual acetone was removed by vacuum filtration system. The crucibles were dried for 3 h at 105˚C in a forced-draft oven (Isotemp® Oven, Model 655F, Fisher Scientific, Hampton, New Hampshire). The crucibles were cooled for 15 min in desiccator and weighed (W_3) in the same order using an analytical balance (Mettler, Model AE 200, Greifensee, Switzerland). The % ADF (DM basis) was calculated as following:

$$\text{ADF (DM basis)} = 100 \times (W_3 - W_2)/(W_1 \times \frac{\%DM}{100}) \qquad (7.6)$$

Where:

W_1 is the test sample weight

W_2 is the crucible weight

W_3 is the crucible and residue weight (%DM is the dry matter content)

7.9.2 Acid Detergent Lignin

The crucibles containing fiber were arranged in 50 mL beakers for support. The contents of crucible were stirred to break all lumps and digested with cooled (15˚C) 72% H_2SO_4 with about halfway of crucible with acid. H_2SO_4 was refilled hourly and contents were stirred hourly as acid drained to a beaker. After 3 h, the contents in the crucibles were filtered as completely as possible with vacuum system, and washed with hot distilled water until was acid-free as indicated with pH paper. The sides of crucibles were rinsed thouroughly before they were dried for 3 h at 105˚C in a forced-draft oven (Model 655F, Fisher Scientific, Isotemp® Oven, Hampton, New Hampshire). The crucibles were cooled in desiccator for 15 min, and weighed (W_4). Contents in the crucibles were ignited in 525˚C furnace for 3 h or until C-free. The weighted crucibles were transferred into forced-draft oven (Isotemp® Oven, Model 655F, Fisher Scientific, Hampton, New Hampshire) at 105˚C while the crucibles were stilled hot. After 1 h, the crucibles were transferred to desiccator to cool for 15 min, and weighed (W_5). The % ADL (DM basis) was calculated as follows:

$$\text{ADL (DM basis)} = 100 \times (W_4 - W_5)/(W_1 \times \frac{\%DM}{100}) \qquad (7.7)$$

Where:

W_4 is the crucible and residue weight after drying

W_5 is the crucible weight after ashing

8. RESULTS

8.1 Environmental Parameters

Several environmental parameters were monitored during the bioremediation process including temperature, moisture content, pH, total carbon, total Kjeldahl nitrogen, C:N ratio, volatile organic contents and ash content.

8.1.1 Temperature Profiles

The temperature was monitored all through the bioremediation process. The temperature profile was generated by plotting time series of average temperature data of each experimental trial as shown in Figure 8.1. After loading the materials into the bioreactors at room temperature (around 22˚C), there was an obvious lag time after which the temperature increased. The temperature profile of all trials showed, also, a lag time for the thermophilic phase which started at 33, 35, 35, 38 and 35˚C for the Control, Trial 1 (*T. curvata*), 2 (*T. aurantiacus*), 3 (*T. fusca*) and Trial 4 (all organisms), respectively.

The fluctuation in the temperature during the thermophilic phase (between 40 and 50˚C) was due to the process of adding bio-available carbon source (used cooking oil) which required opening of the system every 12 h. Even though the addition of bio-available carbon source maintained the temperature within the thermophilic range for the first few days, there was a decreasing trend of temperature at the end of each trial including that the energy losses from the bioreactors exceeded the energy input from the utilization of bio-available carbon.

The peak temperature was above 50˚C for all experimental runs except Trial 2 (*T. aurantiacus*) which was only 48.8˚C. The peak temperature ranged from 48.8 to 54.5˚C as shown in Table 8.1. The temperature was maintained above 50˚C for 0, 11, 14, 23 and 49 h, above 45˚C for 59, 71, 99, 106 and 108 h, above 40˚C for 146, 170, 186, 192 and 191 h for Trial 2 (*T. aurantiacus*), Trial 3 (*T. fusca*), Control, Trial 1 (*T. curvata*) and Trial 4 (all organisms), respectively. The room temperature was stable around 22-24˚C.

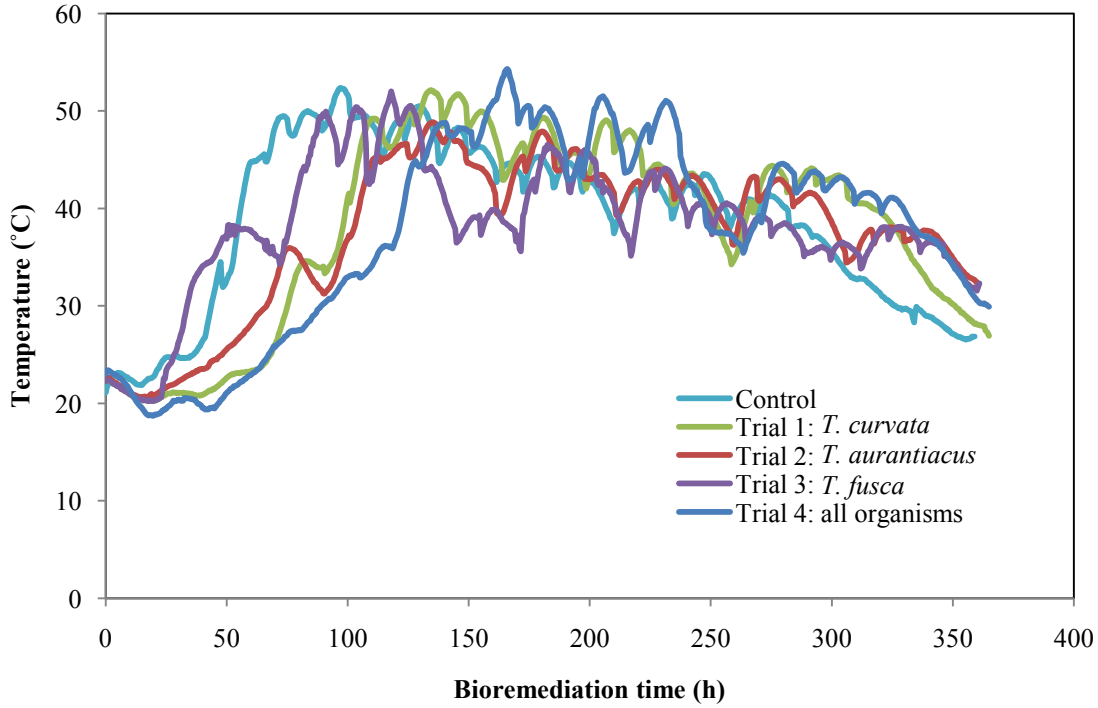


Figure 8.1 Temperature profiles

Table 8.1 Temperature ranges and durations of experimental trials

Trial	Average room temperature	Peak		Duration of temperature above		
		Temperature	Time to reach	40˚C	45˚C	50˚C
Control	24.1˚C	51.7˚C	97 h	186 h	99 h	14 h
1 (*T. curvata*)	22.2˚C	52.1˚C	134 h	192 h	106 h	23 h
2 (*T. aurantiacus*)	22.7˚C	48.8˚C	135 h	146 h	59 h	0 h
3 (*T. fusca*)	22.0˚C	52.0˚C	69 h	170 h	71 h	11 h
4 (All organisms)	22.9˚C	54.5˚C	165 h	191 h	108 h	49 h

8.1.2 Moisture Content

The initial moisture content (MC) of each experiment trial was adjusted to 60% using distilled water. During the bioremediation process, the MC was monitored five times (days 1, 4, 7, 11 and 15). The MC in each trial decreased gradually during the 15 days of bioremediation. This could be due to the loss of water (in a form of vapor) with the exhaust air. The changes of MC are shown in Figure 8.2 and Table 8.2.

After 15 days of bioremediation, the moisture content decreased to 48.0% in Trial 2 (*T. aurantiacus*), to 43.8% in Trial 3 (*T. fusca*), to 42.9% in Control, to 39.1% Trial 1 (*T. curvata*) and to 35.3% in and Trial 4 (all organisms).

8.1.3 pH

The change of pH value of the material was monitored five times for each run (days 1, 4, 7, 11 and 15). The change in pH is shown in Table 8.3 and Figure 8.3. All the five trials showed similar plot patterns. The initial material was acidic with a pH of 6.0. In the first week of bioremediation, the pH value tended to increase to the basic range (7.4 - 8.5) and then decreased back to neutral/weak acid range (5.5 - 6.8). However, the increase in the pH of Trial 2 (*T. aurantiacus*) during the first week was slower than other trials.

8.1.4 Solid Contents

The volatile solid and ash contents were monitored three times for each run (days 1, 7 and 15). The changes of volatile solid and ash content are listed in Tables 8.4 and 8.5 and presented in Figures 8.4 and 8.5. The volatile solid contents of the materials on the first day were 805 ± 10 g/kg material (DM based). The volatile solid contents showed a decreasing trend in all trials. As shown in Table 8.4, the reduction rates were 4.6, 8.2, 0.1, 3.6 and 12.2%, Control, Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) respectively.

The initial ash content was 195 ± 12 g/kg material (DM based). The ash content basically stayed the same at the end of the bioremediation experiments. The variations in the final ash contents were within the experimental errors as shown in Table 8.5.

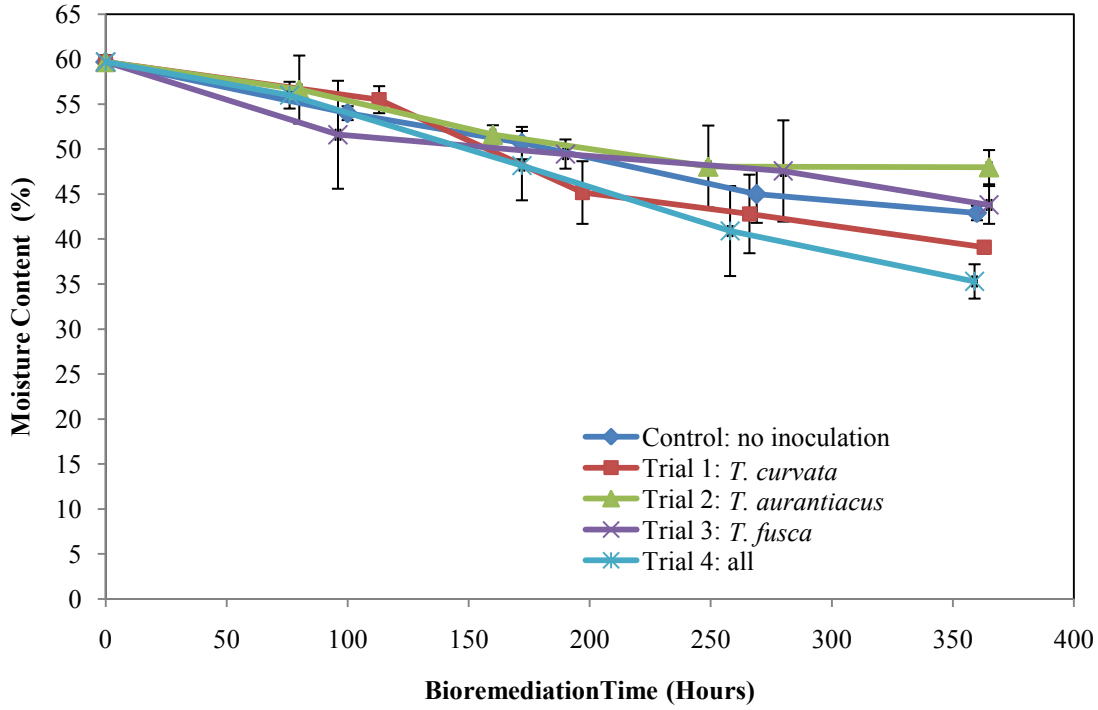


Figure 8.2 Changes of moisture content during the bioremediation process

Table 8.2 Moisture content changes during the bioremediation process

Trial	Initial MC (%)	Final MC (%)	Change (%)	Reduction (%)
Control	59.7 ± 0.8	42.9 ± 1.1	16.8	28.1
1 (*T. curvata*)	59.7 ± 0.8	39.1 ± 0.7	20.6	34.5
2 (*T. aurantiacus*)	59.7 ± 0.8	48.0 ± 1.9	11.7	19.6
3 (*T. fusca*)	59.7 ± 0.8	43.8 ± 2.1	15.9	26.6
4 (All organisms)	59.7 ± 0.8	35.3 ± 1.9	24.4	40.9

± SD

Table 8.3 Changes of pH value during the bioremediation process

Trial	1st Day	4th Day	7th Day	11th Day	15th Day
Control	6.0 ± 0.5	7.9 ± 0.6	8.2 ± 0.1	6.6 ± 0.2	6.5 ± 0.2
1 (*T. curvata*)	6.0 ± 0.5	8.4 ± 0.1	7.7 ± 0.1	6.4 ± 0.4	5.5 ± 0.6
2 (*T. aurantiacus*)	6.0 ± 0.5	6.4 ± 0.8	8.5 ± 0.1	7.5 ± 0.5	6.0 ± 0.1
3 (*T. fusca*)	6.0 ± 0.5	8.7 ± 0.1	7.4 ± 0.4	6.5 ± 0.3	6.2 ± 0.2
4 (All organisms)	6.0 ± 0.5	8.5 ± 0.0	8.0 ± 0.2	6.9 ± 0.3	6.8 ± 0.4

± SD

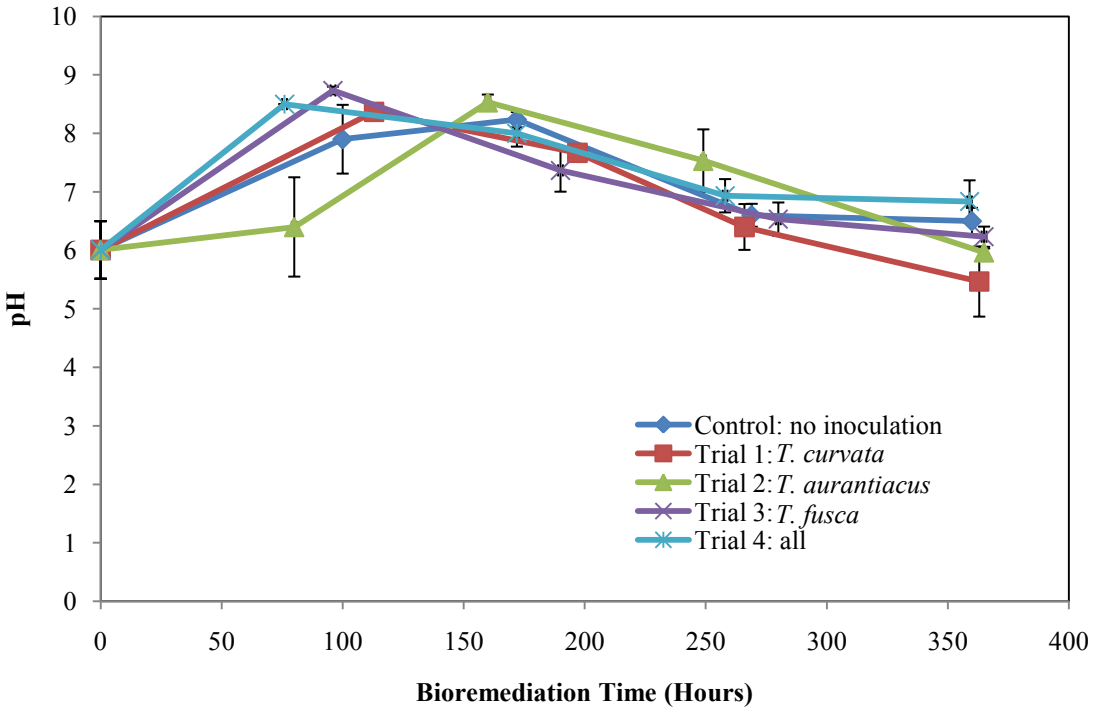


Figure 8.3 Changes in the pH during the bioremediation process

Table 8.4 Changes in the volatile solids during the bioremediation process

Trial	1st day (g/kg DM)	7th day (g/kg DM)	15th day (g/kg DM)	Reduction (%)
Control	805 ± 10	812 ± 12	775 ± 12	4.6%
1 (*T. curvata*)	805 ± 10	765 ± 24	739 ± 8	8.2%
2 (*T. aurantiacus*)	805 ± 10	806 ± 24	804 ± 9	0.1%
3 (*T. fusca*)	805 ± 10	797 ± 13	776 ± 14	3.6%
4 (All organisms)	805 ± 10	735 ± 32	707 ± 12	12.2%

± SD

Table 8.5 Changes in ash content during bioremediation process

Trial	1st day (g/kg DM)	7th day (g/kg DM)	15th day (g/kg DM)	Change (%)
Control	195 ± 12	200 ± 21	188 ± 12	-3.6%
1 (*T. curvata*)	195 ± 12	199 ± 20	183 ± 24	-6.2%
2 (*T. aurantiacus*)	195 ± 12	205 ± 22	200 ± 24	+2.5%
3 (*T. fusca*)	195 ± 12	186 ± 14	185 ± 29	-5.1%
4 (All organisms)	195 ± 12	198 ± 14	183 ± 21	-6.2%

± SD

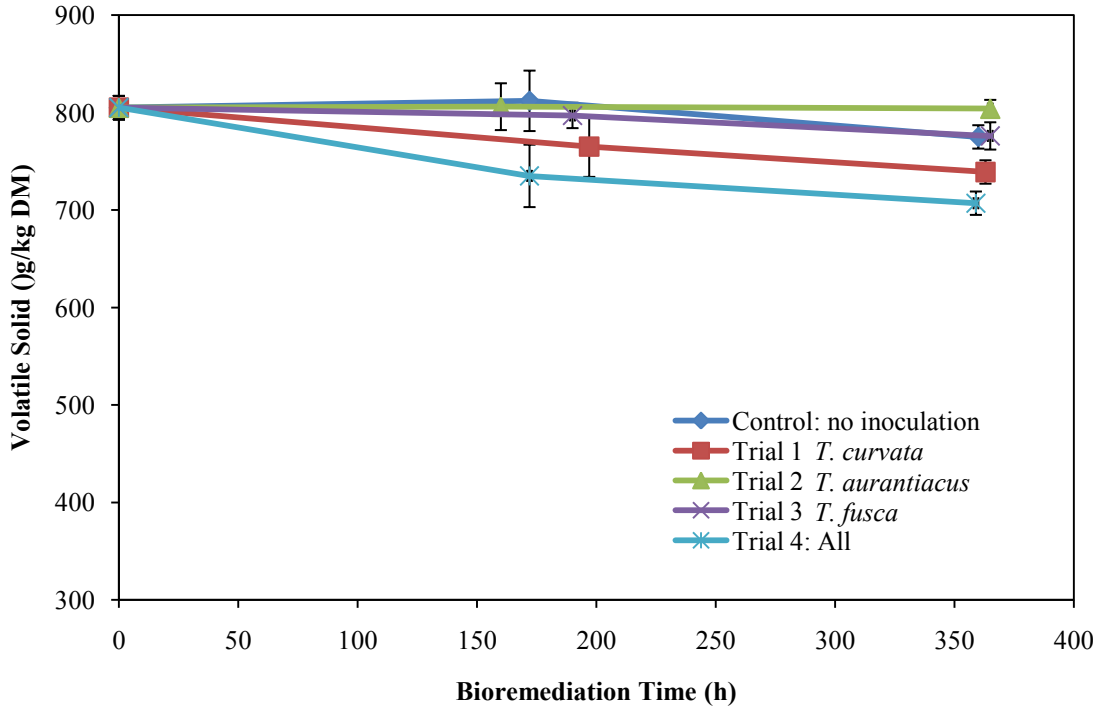


Figure 8.4 Changes of volatile solid during bioremediation process

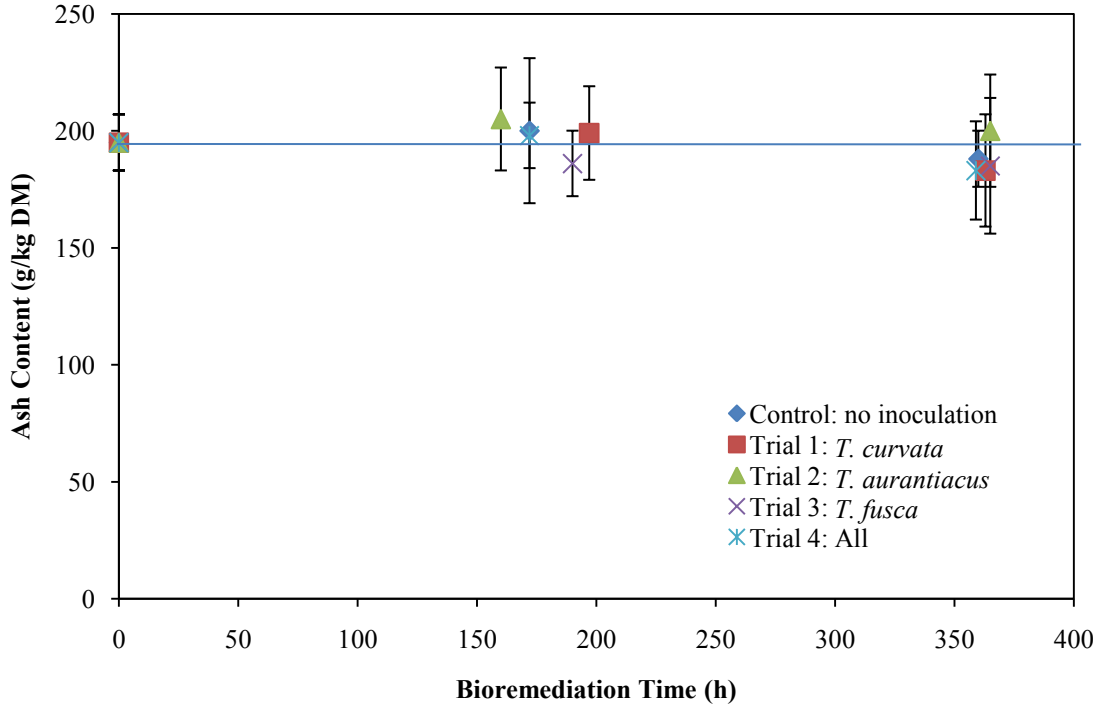


Figure 8.5 Ash content measured during bioremediation process

8.1.5 Total Carbon

The total carbon was monitored three times in each experimental run (days 1, 7 and 15) as shown in Table 8.6 and Figures 8.6. The initial total carbon was 392 g/kg DM. The total carbon decreased in all trials. For Control, Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*), the final total carbon was 358, 330, 386 and 358 g/kg DM with reductions of 8.7, 15.8, 1.5 and 8.7%, respectively. However, the total carbon decreased from 392 g/kg DM to 302 g/kg DM in Trial 4 (all organisms) which resulted in the highest reduction (22.9%).

8.1.6 Total Kjeldahl Nitrogen

The total Kjeldahl nitrogen (TKN) was monitored three times in each experimental run (days 1, 7 and 15) as shown in Table 8.7 and Figures 8.7. The initial TKN was 24.94 g/kg DM. For Control and Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*), the TKN decreased to 12.5, 17.1 and 11.9 with reductions of 50.2%, 31.9% and 52.6%, respectively. However, the TKN in Trial 1 (*T. curvata*) and Trial 4 (all organisms) decreased more rapidly than other trials to 7.1 and 2.9 g/kg DM with reductions of 71.7% and 88.4%, respectively.

8.1.7 C:N Ratio

The changes of C:N ratio were calculated from total carbon and total Kjeldahl nitrogen data. The value of C:N ratio are shown in Table 8.8 and Figure 8.8. The initial C:N ratio was found to be 15.6:1. Due to continuous addition of used cooking oil and the rapid consumption of nitrogen by microorganisms to metabolize bio-available carbon for cell growth and energy production, the C:N ratio increased significantly.

Trial 1 (*T. curvata*) and Trial 4 (all organisms) resulted in a more rapid increase of C:N ratio compared with other trials because of more rapid decreases of TKN. The final C:N ratios were 46.5:1 and 104:1, respectively. For the other trials, the final C:N was in the range of 22.6:1 to 30.1:1.

Table 8.6 Changes in total carbon during the bioremediation process

Trial	Total Carbon (g/kg DM)			Reduction (%)
	1st day	7th day	15th day	
Control	392 ± 10	390 ± 23	358 ± 12	8.7%
1 (*T. curvata*)	392 ± 10	380 ± 21	330 ± 9	15.8%
2 (*T. aurantiacus*)	392 ± 10	389 ± 30	386 ± 9	1.5%
3 (*T. fusca*)	392 ± 10	387 ± 23	358 ± 11	8.7%
4 (All organisms)	392 ± 10	343 ± 29	302 ± 11	22.9%

± SD

Table 8.7 Changes in TKN during the bioremediation process

Trial	TKN (g/kg DM)			Reduction (%)
	1st day	7th day	15th day	
Control	25.1 ± 1.7	17.8 ± 2.1	12.5 ± 1.9	50.2%
1 (*T. curvata*)	25.1 ± 1.7	10.7 ± 1.3	7.1 ± 1.2	71.7%
2 (*T. aurantiacus*)	25.1 ± 1.7	20.5 ± 2.2	17.1 ± 2.1	31.9%
3 (*T. fusca*)	25.1 ± 1.7	16.1 ± 1.7	11.9 ± 1.5	52.6%
4 (All organisms)	25.1 ± 1.7	9.5 ± 2.2	2.9 ± 1.0	88.4%

± SD

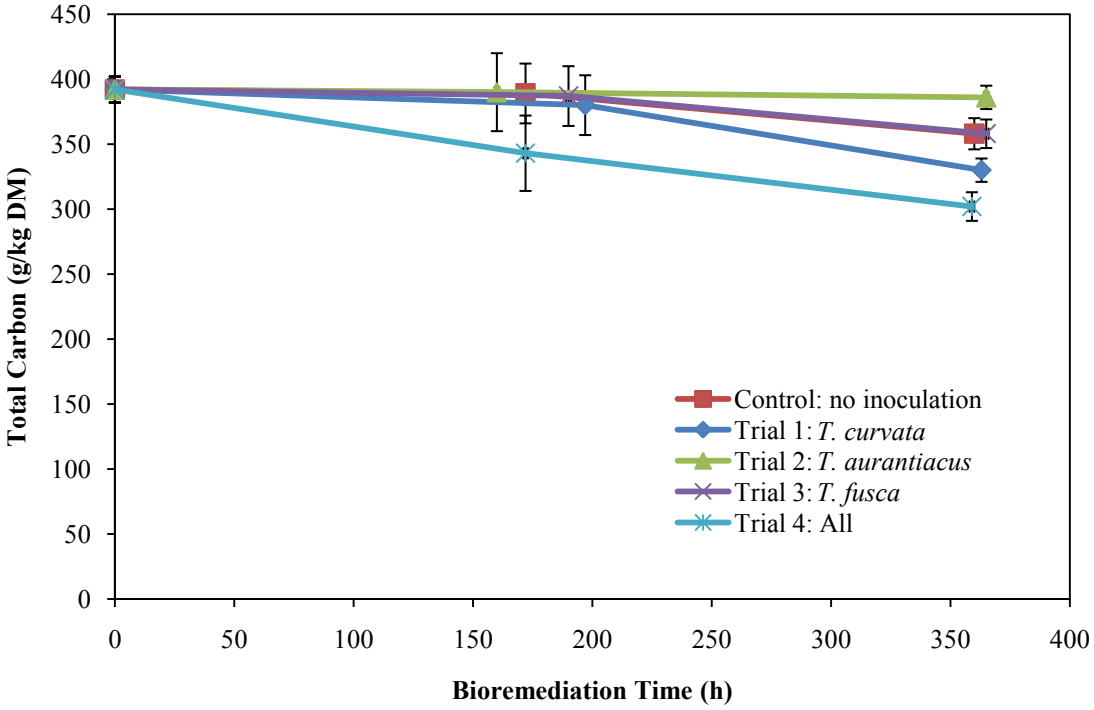


Figure 8.6 Changes in total carbon during the bioremediation process

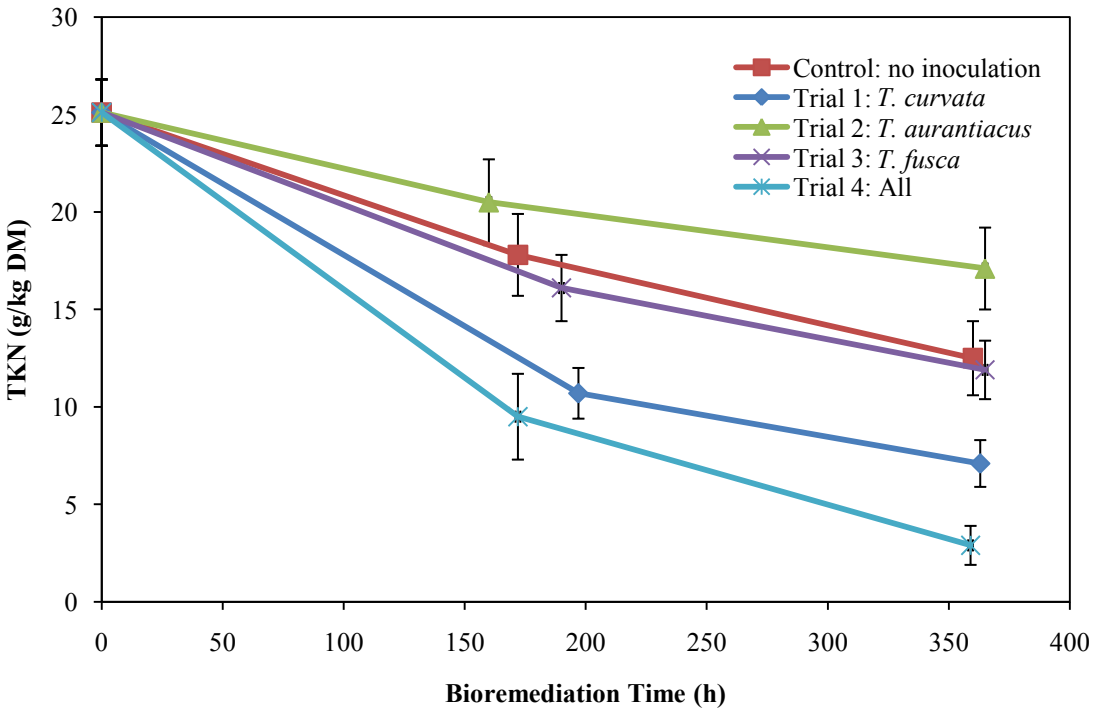


Figure 8.7 Changes in TKN during the bioremediation process

Table 8.8 Changes of C:N during the bioremediation process

Trial	**1st day**	**7th day**	**15th day**
Control	15.6:1	21.9:1	28.6:1
1 (*T. curvata*)	15.6:1	35.5:1	46.5:1
2 (*T. aurantiacus*)	15.6:1	19.0:1	22.6:1
3 (*T. fusca*)	15.6:1	24.0:1	30.1:1
4 (All organisms)	15.6:1	36.1:1	104:1

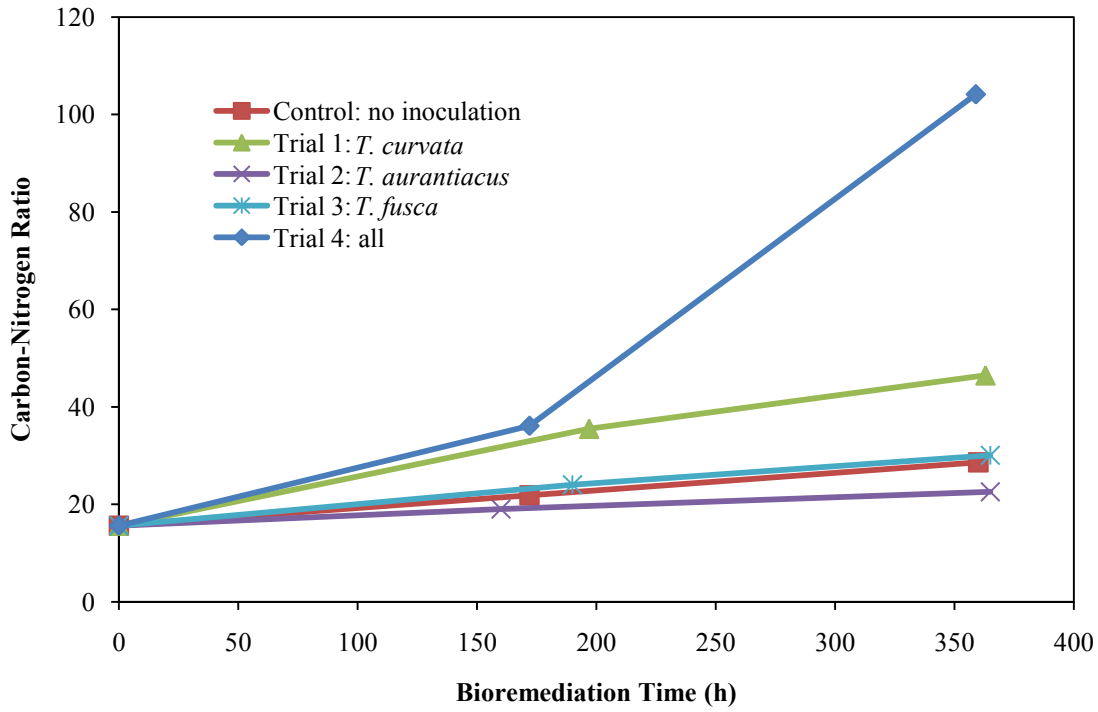


Figure 8.8 Changes of C:N during the bioremediation process

8.2 Degradation of Phenolic Compounds

In order to monitor the concentration of contaminants in the material, the concentrations of phenolic compound were measured during the five trials of bioremediation 5 times (1^{st}, 4^{th}, 7^{th}, 11^{th}, 15^{th} days) as shown in Table 6.1. The initial concentration of PC was 0.222 ± 0.010 mg per gram material (DM based). During the bioremediation process, the concentration of PC decreased gradually in all five trials as shown in Table 8.9 and Figure 8.9. Trial 4 (all organisms) achieved the highest degradation (77.0%) of phenolic compounds, while Trial 2 (*T. aurantiacus*) had the lowest degradation (68.0%). The Control and Trial 3 (*T. aurantiacus*) achieved similar degradation (73.9%).

Also, the degradation patterns of PC in each trial were different. For Trial 1 (*T. curvata*), the degradation of PC was slower in the first week compared with other trials. However, for Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*), the degradation of phenolic compounds started very early in the bioremediation process and resulted in a smooth and gradual decrease in concentrations. Even though the degradation of phenolic compounds in Trial 1 (*T. curvata*) were delayed at the first week, it accelerated during the second week of bioremediation and resulted in a similar concentration (compared with other trials) at the end of the bioremediation process.

8.3 Degradation of Lignocellulose

The results of the degradation of cellulose and lignin are shown in Table 8.10. The initial content of cellulose was 24.8 ± 1.5%. After 15 days of bioremediation, the content of cellulose decreased to the final contents of 16.8-19.8%. The highest degradation of cellulose (32.3%) was achieved in Trial 4 (all organisms) and the lowest (20.2%) was observed in Trial 2 (*T. aurantiacus*).

Table 8.9 Degradation of phenolic compounds

Trials	Initial Concentration (mg/g DM)	Final Concentration (mg/g DM)	Degradation (%)
Control	0.222 ± 0.010*	0.058 ± 0.006	73.9
1 (*T. curvata*)	0.222 ± 0.010	0.054 ± 0.004	75.7
2 (*T. aurantiacus*)	0.222 ± 0.010	0.071 ± 0.005	68.0
3 (*T. fusca*)	0.222 ± 0.010	0.058 ± 0.004	73.9
4 (All organisms)	0.222 ± 0.010	0.051 ± 0.006	77.0

* 95% confidence interval

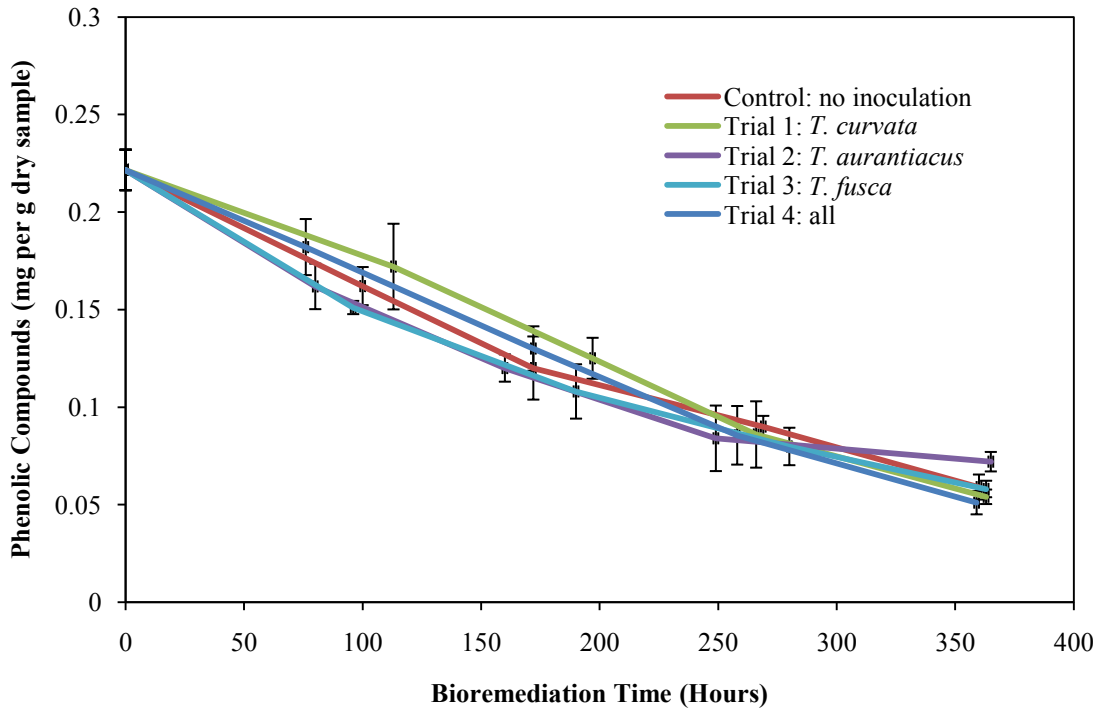


Figure 8.9 Degradation of phenolic compounds

Table 8.10 Degradation of cellulose and lignin

Trial	Cellulose			Lignin		
	Initial content (% DM)	Final content (% DM)	Degradation (%)	Initial content (% DM)	Final content (% DM)	Degradation (%)
Control	24.8 ± 1.5	19.2 ± 0.7	22.6	19.8 ± 0.9	16.3 ± 0.3	17.7
1 (*T. curvata*)	24.8 ± 1.5	19.0 ± 0.8	23.4	19.8 ± 0.9	15.5 ± 0.9	21.7
2 (*T. aurantiacus*)	24.8 ± 1.5	19.8 ± 1.2	20.2	19.8 ± 0.9	17.2 ± 0.7	13.1
3 (*T. fusca*)	24.8 ± 1.5	19.1 ± 0.6	23.0	19.8 ± 0.9	16.3 ± 0.4	17.7
4 (All organisms)	24.8 ± 1.5	16.8 ± 0.8	32.3	19.8 ± 0.9	13.7 ± 0.3	30.8

± SD

The initial content of lignin was 19.8 ± 0.9%. After 15 days of bioremediation, the final contents in five trials were in the range of 13.7-17.2%. The reductions in Control, Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*) were 17.7, 21.7, 13.1, and 17.7%. The highest degradation (30.8%) of lignin was achieved in Trial 4 (all organisms).

8.4 Maturity and Stability Tests on Bioremediation Product

The maturity and stability of the final bioremediation product was evaluated by analyzing the CO_2 evolution and the germination index (GI) of the bioremediation product.

8.4.1 CO_2 Evolution

The CO_2 evolution was measured for the final product of each run. The CO_2 evolution is described as mg of CO_2-C per g of VS per day as well as g of CO_2-C per g carbon per day. The results are shown in Table 8.11. The CO_2 evolution rates were in the range of 2.62-4.22 mg CO_2-C per g VS per day for the five experimental runs. The CO_2 evolution rate was highest (4.22 mg CO_2-C per g VS per day) in Trial 2 (*T. aurantiacus*) and lowest (2.62 mg CO_2-C per g VS per day) in Trial 4 (all organisms).

8.4.2 Germination Index

The germination rates were between 62% and 100%. Germination indices (GI) of the initial material and the final product were compared. The results are listed in Table 8.12. The initial GI of each experimental run was 0%. After the bioremediation process, the GI improved significantly after bioremediation for Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) (19-65%). However, for Trial 2 (*T. aurantiacus*), the final GI was 5%. The highest GI (65%) was achieved in Trial 4 (all organisms).

Table 8.11 CO_2 evolution of final products

Trial	CO_2 evolution	
	(mg CO_2-C per g volatile solid per day)	(mg CO_2-C per g carbon per day)
Control	3.18 ± 0.19*	6.89 ± 0.41
1 (*T. curvata*)	3.10 ± 0.20	6.94 ± 0.45
2 (*T. aurantiacus*)	4.22 ± 0.24	8.79 ± 0.50
3 (*T. fusca*)	3.21 ± 0.28	6.96 ± 0.61
4 (All organisms)	2.62 ± 0.18	6.11 ± 0.42

CO_2-C: Carbon in the form of CO_2
± SD

Table 8.12 Comparison of GI before and after bioremediation

Trial	Feedstock		Final product	
	Germination rate (%)	GI (%)	Germination rate (%)	GI (%)
Control	0	0	90	20
1 (*T. curvata*)	0	0	90	22
2 (*T. aurantiacus*)	3	0	62	5
3 (*T. fusca*)	3	1	85	19
4 (All organisms)	0	0	100	65

9. DISCUSSION

9.1 Environmental Parameters

9.1.1 Temperature

The organic materials were broken down and utilized by microorganisms for energy and cell growth. The utilization of bio-available carbon (used cooking oil and other biodegradable organic matters in the wood waste) generated heat inside the bioreactors which increased the temperature. For a period of time (from 50 to 225 h), the heat generated from microbial activities was equal to the heat lost from the material with the exhaust gas and through the body (sidewalls, top and bottom) of the bioreactor and as a result, the temperature in the bioreactor remained constant. The peak temperature achieved was above 50˚C in the bioreactors for all experimental trials except Trial 2 (*T. aurantiacus*) where it was 48.8˚C.

According to Black (1999), there are three groups of microorganisms characterized by their optimum growth temperature: psychrophilic (-10˚C to 20˚C), mesophilic (~15˚C to 45˚C) and thermophilic (35˚C to 68˚C). The temperature ranges of these three groups of microorganisms are shown in Figure 9.1. In this study, the lag and exponential phases usually encountered in a biological process were clearly observed for the psychrophilic, mesophilic and thermophilic phases. There was an initial psychrophilic lag phase observed for all trials except Trial 3 (*T. fusca*). The temperature increased and the mesophilic phase was observed for all the trials. The lag phase of the thermophilic stage (the third lag phase) was, also, identified in all trials. The lag phases (psychrophilic, mesophilic and thermophilic) were determined graphically according to the procedure described by Ghaly et al. (1989) as shown in Figure 9.2. The results are shown in Table 9.1. The psychrophilic lag phase was not observed in Trial 3 (*T. fusca*). The psychrophilic lag phase ranged from 15 h for the Control and 45 h for Trial 4 (all organisms). The temperature increase rates in psychrophilic phase were relatively low. The mesophilic lag phase ranged from 2 h for Trial 2 (*T. aurantiacus*) to 26 h for Trial 3 (*T. fusca*). The temperature increasing rates were higher in the Control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*) than in Trial 2 (*T. aurantiacus*) and Trial 4 (all organisms). The thermophilic lag

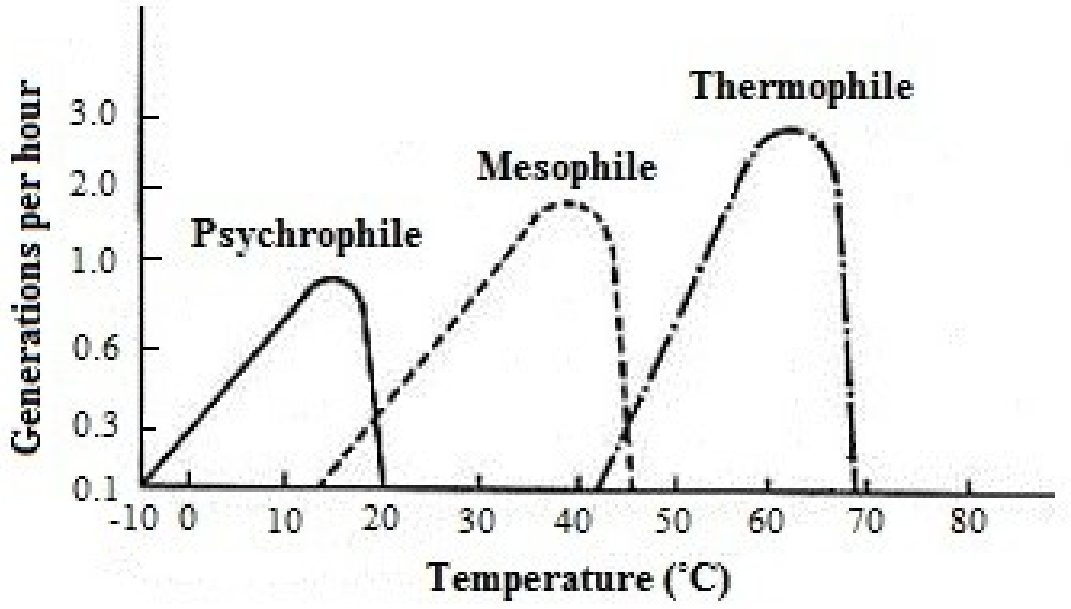


Figure 9.1 Growth rates and temperature ranges of psychrophilic, mesophilic and thermophilic microorganisms (Black, 1999)

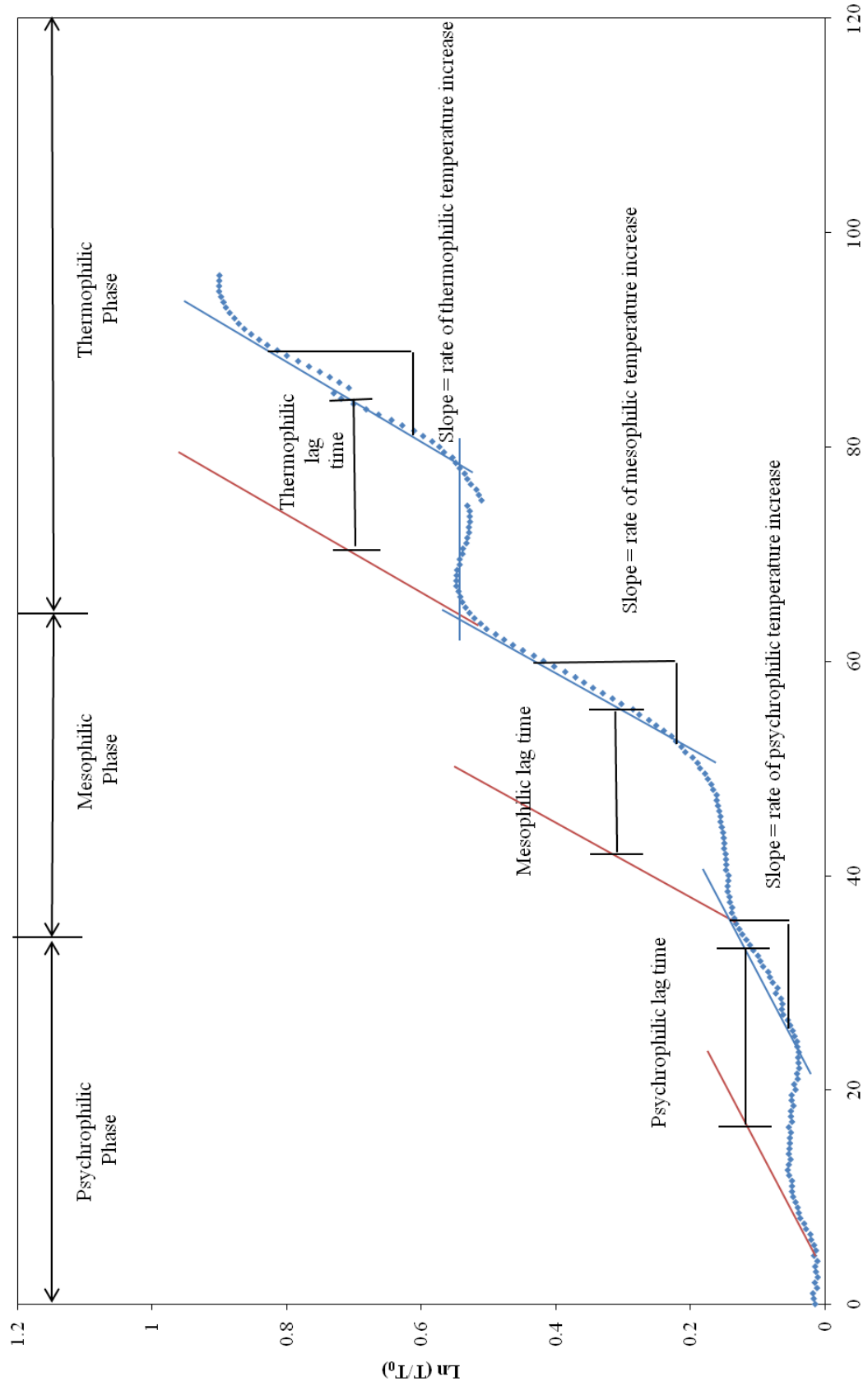


Figure 9.2 Determination of the psychrophilic, mesophilic and thermophilic lag periods and the rates of temperature increase during the bioremediation process

Table 9.1 Kinetic parameters of temperature increase

Trial	Psychrophilic Range		Mesophilic Range		Thermophilic Range	
	Lag Time (h)	**Rate (˚C/h)**	**Lag Time (h)**	**Rate (˚C/h)**	**Lag Time (h)**	**Rate (˚C/h)**
Control	15	0.31	12	0.84	2	1.10
Trial 1 (*T. curvata*)	40	0.17	13	0.70	15	0.90
Trial 2 (*T. aurantiacus*)	9	0.16	2	0.37	14	0.46
Trial 3 (*T. fusca*)	0	0	26	0.58	23	0.79
Trial 4 (all organisms)	45	0.25	5	0.25	4	0.64

phase ranged from 2 h for the Control to 23 h for Trial 3 (*T. fusca*). The temperature increasing rates were higher in the Control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*) than in Trial 2 (*T. aurantiacus*) and Trial 4 (all organisms).

The analysis of variance (ANOVA) are shown in Tables 9.2 and 9.3. The results showed that there was significant difference among the peak temperatures ($P<0.001$). However, the peak temperatures of Control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*) were not significantly different from each other, but both were significantly different from those of Trial 2 (*T. aurantiacus*) and Trial 4 (all organisms) at 95% confidence level. The lowest peak temperature was observed with Trial 2 (*T. aurantiacus*) and the highest peak temperature was observed with Trial 4 (all organisms).

Löser et al. (1999) used a pilot scale percolator to compost artificially PAH-contaminated pine wood waste with liquid hog manure and mineral medium. During the 61 days experiment, the highest temperature reached was 42˚C only in the group with liquid manure as nitrogen source while other groups treated with mineral medium and without nitrogen source did not result in a temperature above 30˚C. The higher temperature achieved in this study may due to the addition of bio-available carbon (used cooking oil) into the system and the use of better thermally insulated bioreactors used.

McMahon et al. (2009) composted mixture of board waste (C&D wood waste) to which poultry manure and green waste were added as nutrients and top soil and compost were added to provide active microorganisms. The peak temperature in their study was above 70˚C which is significantly higher than the peak temperature achieved in this study. This could be due to the fact that more nutrients were added in the system (poultry manure), thereby increased the bio-available carbon and nitrogen.

VanderGheynst et al. (1997) conducted composting process in a pilot-scale bioreactor of a synthetic food waste and digested biosolids. They observed a mesophilic lag time of 5 h and a thermophilic lag time of 12.5 h. The mesophilic temperature increasing rate was 0.05˚C/h and thermophilic rate was 0.02˚C/h. The temperature increasing rate in present study (0.16-0.31, 0.25-0.84 and 0.46-1.10˚C/h for psychrophilic, mesophilic and thermophilic phases respectively) was much larger than 0.05 or 0.02˚C/h

Table 9.2 ANOVA of the peak temperature

Source of Variation	DF	SS	MS	F	P
Between Groups	5	50.207	10.041	44.084	<0.001
Residual	12	2.733	0.228		
Total	17	52.940			

Table 9.3 Differences of the peak temperature

Trial	Number of replicates	Mean peak temperature	Duncan grouping*
Control	3	51.7	A
Trial 1 (*T. curvata*)	3	52.3	A
Trial 2 (*T. aurantiacus*)	3	48.8	B
Trial 3 (*T. fusca*)	3	52.0	A
Trial 4 (all organisms)	3	54.5	C

* Means with different letters are significantly different from one another at 95% confidence level.

reported in their study. This could be due to the continuous addition of used cooking oil as a bio-available carbon source.

Alkoaik and Ghaly (2006) conducted in-vessel composting of tomato plant residues using the same equipment used in this study. They observed a mesophilic lag time of 12 h and no thermophilic lag time. The rate of temperature increase was 2.32˚C/h for both mesophilic and thermophilic phases. The rates in this study were much smaller than the 2.32 ˚C/h reported by Alkoaik and Ghaly (2006). This is due to the fact that the tomato plant residues used in their study had a much higher bio-avalable carbon than the wood waste used in the present study.

The differences observed in the lag periods and the rates of temperature increase for both the mesophilic and thermophilic phases may be due to: (a) the use of different substrates and (b) the effect of the contaminants contained in the feedstock on the microbial activity in the early psychrophilic and mesophilic phases (when these concentrations were still high).

9.1.2 Moisture Content

The availability of nutrients and contaminants to microorganisms is affected by the water content in their micro-environment (especially in the thin liquid layers on the surfaces of particles) because water is the media for nutrient transportation and metabolic reactions (Golueke, 1977; Gajalakshmi and Abbasi, 2008). However, if too much water fills the voids, the pore space that allows air diffusion would be limited. Therefore, the optimum moisture content (MC) is in the range of 50-70% (Tiquia et al., 1996; Epstein, 1997; Gajalakshmi and Abbasi, 2008). In this study, the initial moisture content of the compost mixture was 59.69 ± 0.77% which was within the optimal range. However, the moisture content decreased significantly for all trials during the 15 days of bioremediation due to the water loss through vapor with the exhaust gases. The patterns of moisture content reduction for each trial were different.

The ANOVA results presented in Table 9.4 showed that there were significant differences among final values of moisture content ($P<0.001$). However, the final MC of the Control and Trial 3 (*T. fusca*) were not significantly different from each other but they

Table 9.4 ANOVA of the final moisture content

Source of Variation	DF	SS	MS	F	P
Between Groups	4	5.59	1.40	35.100	<0.001
Residual	25	0.994	0.0398		
Total	29	6.58			

Table 9.5 Differences of the final moisture content

Trial	Number of replicates	Mean of final MC	Duncan grouping*
Control	6	42.9%	A
Trial 1 (*T. curvata*)	6	39.1%	B
Trial 2 (*T. aurantiacus*)	6	48.0%	C
Trial 3 (*T. fusca*)	6	43.8%	A
Trial 4 (all organisms)	6	35.3%	D

* Means with different letters are significantly different from one another at 95% confidence level.

were significantly different from Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*) and Trial 4 (all organisms) at 95% confidence level. The differences in the final MC are due to the differences in the peak temperature and the duration of thermophilic phase. Trial 2 (*T. aurantiacus*) had the shortest thermophilic phase (59 h above 45˚C) and Trial 4 (all organisms) had the longest thermophilic phase (108h above 45˚C). The control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*) had similar thermophilic phases. Similar results were reported by Walker et al. (1999) for a laboratory composting system which lasted for 13-20 days with a peak temperature above 60˚C and a final MC of 45%.

According to Haug (1993) and Walker et al (1999), an intense decrease of MC will reduce the metabolic rate and, therefore, affects the effectiveness of the bioremediation process. If the MC is lower than 30%, the microbial activity will be significantly limited (Haug, 1993). For the Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms), the MC was still in proper range of 50-70%. The lower final MC for Trial 4 (all organisms) was due to higher rate of decomposition of volatile solids and the production of water in the system through mineralization process that compensated for the lost water with the exhaust gases.

9.1.3 pH

The pH changed during the bioremediation processes for all trials in similar patterns as shown in Figure 8.5. The pH of all trials was slightly acidic at the beginning of the experiments. The pH increased to 7.9-8.7 on the 4^{th} day and then gradually decreased back to neutral or weakly acidic (5.5-6.8). The optimum pH of *T. curvate*, *T. aurantiacus* and *T. fusca* are 7.2, 5.6 and 7.2, respectively (ATCC, 2009). The initial pH of each trial was around 6.0. As a result, the pH environment was only favorable for *T. aurantiacus* which showed shorter psychrophilic and mesophilic lag times.

The changes in the pH were due to the decomposition process and the production of byproducts. The initial nitrogen content in the material was relatively high (C:N ratio of 15.7:1). The initial increase in the pH observed in this study was due to the breakdown of organic nitrogen to ammonium (Epstein, 1997). The final drop in the pH could be due to

Table 9.6 ANOVA of the final volatile solids

Source of Variation	DF	SS	MS	F	P
Between Groups	4	16974.973	4243.743	45.648	<0.001
Residual	10	929.675	92.967		
Total	14	17904.648			

Table 9.7 Differences of the final volatile solids

Trial	Number of replicates	Mean of final VS (g/kg DM)	Duncan grouping*
Control	3	775	A
Trial 1 (*T. curvata*)	3	739	B
Trial 2 (*T. aurantiacus*)	3	804	C
Trial 3 (*T. fusca*)	3	775	A
Trial 4 (all organisms)	3	707	D

* Means with different letters are significantly different from one another at 95% confidence level.

the formation of organic acids from decomposition of fats and grease. A similar pH trend was observed by Khan and Anjaneyulu (2006) who explained the rise of pH as due to the breakdown of protein into ammonia.

9.1.4 Solid Contents

The ANOVA performed on the volatile solids data (Table 9.6) showed that there were significant differences among final volatile solid contents ($P<0.001$). The Bonferroni Analysis (Table 9.7) showed that there were no significant differences of final volatile solids between the Control and Trial 3 (*T. fusca*), but they were significantly different from the other trials at 95% confidence level.

Khan and Anjaneyulu (2006) conducted a study on the bioremediation of phenolic compounds and benzene contaminated soil and sediment using a composting technology. The volatile contents decreased by 42-48% after 40 days of composting. Saludes et al., (2007) achieved 43.89% reduction of volatile solids of dairy cattle manure after 35 days in a controlled thermophilic-mesophilic (55˚C) composting system. Lu et al. (2008) reported reductions of volatile solids of 16.71-22.97% after 7 days of composting barley dregs and sewage sludge.

The degradation of oil and fats by microorganisms is the major energy source during bioremediation process (Viel et al., 1987; Lemus and Lau, 2002). In this study, 36 ml of the used cooling oil was added into the system every 12. Therefore, the addition of used cooking oil as bio-available carbon was preferred by microorganisms over organic carbon in the feedstock, and as a result the reduction of volatile solid in the feedstock was limited (0.1-12.2%).

The ash contents stayed relatively constant since there was no input or output of ash into or from the system. The changes observed in the ash were within the experimental errors.

9.1.5 Total Carbon

The total carbon decreased in all trials in this study after 15 days of bioremediation. The ANOVA analysis performed on the total carbon data (Table 9.8) showed that there

were significant differences among the value of final carbon contents (P<0.001). However, the Bonferroni Analysis performed on the means (Table 9.9) indicated that the differences between Control and Trial 3 (*T. fusca*) were not significant, but these were significantly different from all other trials at 95% confidence level. The lowest final total carbon was in Trial 4 (all organisms) and the highest was in Trial 2 (*T. aurantiacus*). The results again indicate a higher rate of decomposition in Trial 4 (all organisms) which was, also, supported by the reduction of volatile solid.

Tiquia et al. (2002) studied windrow composting of manure for 42 days and reported total carbon reductions of 50-63% in turned windrows and 30-54% in unturned windrows. A carbon reduction of 24% was reported by Michel et al. (1995) while composting yard trimming waste in a bioreactor for 45 days. The higher reductions of total carbon in these two studies may be due to the longer bioremediation time and higher biodegradability of the materials used in their experiments.

Wang et al. (2003) achieved a total carbon reduction of 14% while the composting sewage sludge with solid food waste in a bioreactor for 5 days. Gómez-Brandón et al. (2008) reported a total carbon reduction of 3.5% while composting cattle manure for 15 days. These reductions are similar to those obtained in the present study. In this study, the continuous addition of used cooking oil provided a preferred carbon source for microorganisms and as a result, the total carbon reductions were low (1.5-22.9%).

9.1.6 Total Kjeldahl Nitrogen

The reductions in TKN (31.9-88.4%) were more rapid and much higher than the reductions in the total carbon (1.5-22.9%). The ANOVA analysis (Table 9.10) indicated that there were significant differences among the final TKN values (P<0.001). However, the Bonferroni analyses (Table 9.11) indicated that there was no significant difference between the Control and Trial 3 (*T. fusca*), but these were significantly different from all other trials at 95% confidence level. The highest reduction of TKN was achieved in Trial 4 (all organisms) and the lowest was achieved in Trial 2 (*T. aurantiacus*) which was the case as with the volatile solids and total carbon.

Table 9.8 ANOVA of the final total carbon

Source of Variation	DF	SS	MS	F	P
Between Groups	4	12287.067	3071.767	35.254	<0.001
Residual	10	871.333	87.133		
Total	14	13158.400			

Table 9.9 Differences of the final total carbon

Trial	Number of replicates	Mean of final total carbon (g/kg DM)	Duncan grouping*
Control	3	358	A
Trial 1 (*T. curvata*)	3	330	B
Trial 2 (*T. aurantiacus*)	3	386	C
Trial 3 (*T. fusca*)	3	357	A
Trial 4 (all organisms)	3	302	D

* Means with different letters are significantly different from one another at 95% confidence level.

Table 9.10 ANOVA of the final TKN

Source of Variation	DF	SS	MS	F	P
Between Groups	4	354.041	88.510	44.216	<0.001
Residual	10	20.018	2.002		
Total	14	374.059			

Table 9.11 Differences of the final TKN

Trial	Number of replicates	Mean of final TKN (g/kg DM)	Duncan grouping*
Control	3	12.5	A
Trial 1 (*T. curvata*)	3	7.1	B
Trial 2 (*T. aurantiacus*)	3	17.1	C
Trial 3 (*T. fusca*)	3	11.9	A
Trial 4 (all organisms)	3	2.9	D

* Means with different letters are significantly different from one another at 95% confidence level.

It was reported that the higher temperature and/or longer thermophilic phase will result in higher rate of organic nitrogen decomposition and increased nitrogen loss (Bishop and Godfrey, 1983; Tiquia and Tam, 2000; Wang et al., 2003). Tiquia and Tam (2000) stated that when the initial C:N ratio is low (<20), the nitrogen is lost via NH_3 volatilization, and the high temperature will accelerate the volatilization process. In this study, the C:N ratio increased due to the higher initial nitrogen content. This, also, explains the rise of pH in the first week of bioremediation

Studies of bioremediation/composting that started with low C:N ratio were reported by Tiquia et al. (2002), Tiquia and Tam (2000) and Beck-Friis et al. (2001) and all of which resulted in significant losses of nitrogen. Tiquia et al. (2002) composted manure with an initial C:N ratio in the range of 9:1-12:1 and reported nitrogen losses of 35-45% after 42 days. Tiquia and Tam (2000) reported a nitrogen reduction of 59% while composting chicken litter with an initial C:N ratio of 14.5:1. Beck-Friis et al. (2001) reported nitrogen reduction of 24-33% while composting household wastes with an initial C:N ratios of 21-23:1 under controlled conditions for 22-31 days.

9.1.7 C:N Ratio

The C:N ratio usually decreases in a biological decomposition system because: (a) the organic carbon is degraded and oxidized to CO_2 faster than ammonium is oxidized to NO_3^- and (b) nitrogen can remain relatively stable if the balance between mineralization of organic nitrogen to NH_4 and the immobilization of NH_4 to organic nitrogen (microbial growth) is maintained during the process (Wang et al., 2003; Alkoaik, 2005). However, if the initial concentration of nitrogen was high, the decrease of nitrogen will surpass the decrease of total carbon and result in a higher C:N ratio. In this study, the C:N ratio increased for all trials. Similar trends were reported by Morisaki et al. (1989) and Tiquia and Tam (2000). The results obtained from this study suggest that when calculating the C:N ratio for cellulosic material, the bio-available carbon and bio-available nitrogen should be used.

9.2 Degradation of Phenolic Compounds

About 68.0-77.0% of phenolic compounds were degraded after 15 days of bioremediation. The ANOVA analyses performed on the PC data (Table 9.12) indicated that there were significant differences among the final concentration of PC (P=0.001). However, the Bonferroni analysis (Table 9.13) indicated that the final concentrations of PC in the Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) were not significantly different from each other, but they all were significantly different from that of Trail 2 (*T. aurantiacus*). The higher final PC value observed with Trial 2 (*T. aurantiacus*) is the result of lower temperature and shorter thermophilic phase observed with this trial. This is in agreement with reduction results for the total carbon, nitrogen and volatile solids.

The degradation of phenolic compounds in wood substrate was reported by Galli et al. (2006) who used white-rot fungus *Pleurotus ostreatus* to degrade phenolic compounds in creosote-treated wood on Petri dish cultures. A decrease of phenol compounds greater than 75% was achieved after 30 days. The similar degradation of PC was achieved in the present study but with shorter period of time.

McMahon et al. (2008) studied the degradation of creosote components in the C&D wood waste using composting technique. The highest degradation of creosote (66%) was achieved after 10 days with a peak temperature of 45˚C. In the present study, the higher degradation of PC was due to the addition of bio-available carbon (used cooking oil) which helped to achieve a higher temperature.

Complete degradation of phenolic compounds in wastewater was reported. Ehlers and Rose (2005) used immobilized white-rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor* and *Lentinula* edodes in trickling packed-bed reactors and achieved 90% removal of phenol in 24-30 h. Godjevargova et al. (2006) used fungus *Trichosporon cutaneum* R57 immobilized on modified polymer membrane for the bioremediation of phenol and achieved a complete removal of phenol from the wastewater in 45-51 h. The PC degradation in this study was less than the PC degradation in the wastewater, because

Table 9.12 ANOVA of the final phenolic compounds

Source of Variation	DF	SS	MS	F	P
Between Groups	4	0.000753	0000188	9.244	0.001
Residual	10	0.000204	0.0000204		
Total	14	0.000957			

Table 9.13 Differences of the final phenolic compounds

Trial	Number of replicates	Mean of final PC (mg/g DM)	Duncan grouping*
Control	3	0.058	A
Trial 1 (*T. curvata*)	3	0.054	A
Trial 2 (*T. aurantiacus*)	3	0.072	B
Trial 3 (*T. fusca*)	3	0.058	A
Trial 4 (all organisms)	3	0.051	A

* Means with different letters are significantly different from one another at 95% confidence level.

the liquid media significantly enhances the bio-availability of water-soluble compounds such as PC and makes the degradation process much faster (Haug, 1993; Epstein, 1997).

The biodegradation of phenolic compounds in contaminated soil was studied by Prpich et al. (2006). The phenol in the contaminated soil was absorbed using polymer beads, the phenol absorbed on the beads was desorbed in distilled water to a concentration of 365 mg/L and was then degraded completely by phenol-degrading microbial culture in 10 h. Khan and Anjaneyulu (2006) achieved complete phenolic compounds and benzene degradation after 40 day composting contaminated soil and sediment.Galli et al. (2006) stated that the degradation rates of PC are much slower in solid medium than in the water. Generally, the degradation of PC in the current study was similar to those reported for solid media. The degradation of organic substrate can be described with the following first order model (Haug, 1993):

$$C_t = C_0 e^{-kt} \tag{9.2}$$

Where:

C_t is the concentration of the organic substrate at time t
C_0 is the initial concentration of the organic substrate
k is the rate constant

As shown in Equation 9.2, the value $Ln(C_t/C_0)$ has a linear relationship with the time t within given temperature range. The linear relationship between $Ln(C_t/C_0)$ and time for phenolic compounds was determined graphically for the mesophilic and thermophilic temperature zones as shown in Figure 9.3. The rate constant (k) was determined from the slope of the lines and the results are shown in Table 9.14. The mesophilic rate constants ranged from 0.0023 h^{-1} to 0.0033 h^{-1} while the thermophilic rate constant ranged from 0.0029 h^{-1} to 0.0051h^{-1}. During the thermophilic phase, the rate constant of Trial 2 (*T. aurantiacus*) was the lowest (0.0029). The rate constants of the Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) were not significantly different from one another. The Trial 4 (all organisms) also had the highest peak temperature and highest reductions of volatile solids, total carbon and TKN, indicating the presence of a more active microorganism consortium during the bioremediation process. The rate

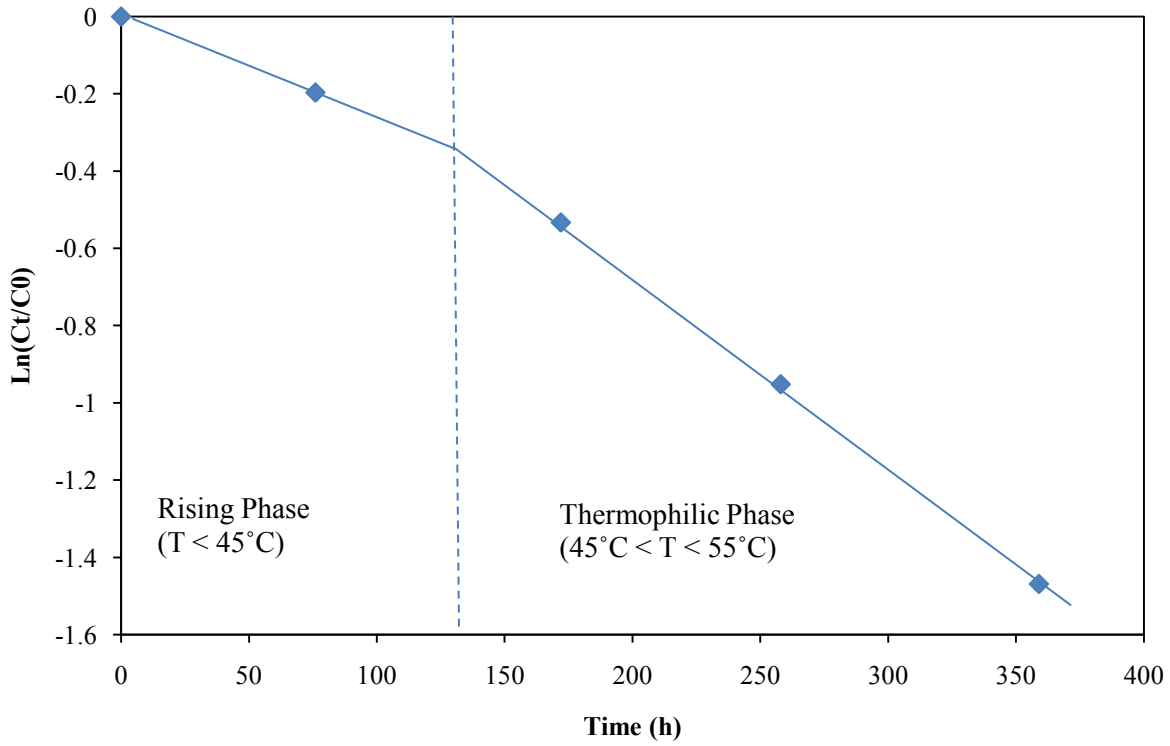


Figure 9.3 Determination of rate constant (k) for PC degradation for different phases

Table 9.14 Rate constants for PC degradation

Trial	Rate constant (h^{-1})	
	Rising Phase (T < 45˚C)	Thermophilic Phase (45˚C < T < 55˚C)
Control	0.0027	0.0047
1 (*T. curvata*)	0.0023	0.0049
2 (*T. aurantiacus*)	0.0033	0.0029
3 (*T. fusca*)	0.0032	0.0045
4 (all organisms)	0.0026	0.0051

constant of PC degradation was the lowest observed in Trial 2 (*T. aurantiacus*) which was correlated with reductions of volatile solids, total carbon and TKN.

9.3 Degradation of Lignocellulose

All experimental trials resulted in significant degradation of cellulose (20.2-32.3%) and lignin (13.1-30.8%). The ANOVA analyses performed on cellulose and lignin (Table 9.15 for cellulose and Table 9.17) for lignin indicated that there was significant difference among the final values of cellulose and lignin (P=0.005) (Table 9.16 for cellulose and Table 9.18 for lignin). However, the Bonferroni analyses indicated that the degradation rates of cellulose and lignin in the Control, Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*) were not significantly different from each other but these were all significantly different from that of Trial 4 (all organisms) at 95% confidence level.

The results indicated that the inoculation of individual cellulolytic and thermophilic microorganisms in Control, Trial 1 (*T. curvata*), Trial 2 (*T. aurantiacus*) and Trial 3 (*T. fusca*) had little effect on the degradation of cellulose and lignin during the bioremediation process. However, when the three microorganisms (*T. curvata*, *T. aurantiacus*, and *T. fusca*) were inoculated together into the system, the degradation of cellulose was significantly accelerated after 15 days of bioremediation.

Petre et al. (2000) used immobilized bacteria (*Bacillus subtilis*, *Bacillus licheniformis*), and fungus (*Trichoderma viride*) cells in the bioremediation of cellulosic wastes in a specially designed bioreactor for 240 h and reported cellulose degradations of 25%, 23% and 15%, respectively. Yu et al. (2007) studied the degradation of lignocellulose in a laboratory composting system during which the temperature reached a peak of 65˚C and reported degradation of cellulose and lignin of 11% and 18% on day 15 and 30% and 25% on day 45, respectively. Vikman et al. (2002) used controlled composting system to test the biodegradation of lignin-containing pulp and paper products. The degradation of stone-ground wood (lignin content of 23-27% DM) were

Table 9.15 ANOVA of the final cellulose content

Source of Variation	DF	SS	MS	F	P
Between Groups	4	15.368	3.842	7.220	0.005
Residual	10	5.321	0.532		
Total	14	20.689			

Table 9.16 Differences of the final cellulose content

Trial	Number of replicates	Mean of final cellulose content (%)	Duncan grouping*
Control	3	19.203	A
Trial 1 (*T. curvata*)	3	19.040	A
Trial 2 (*T. aurantiacus*)	3	19.780	A
Trial 3 (*T. fusca*)	3	19.072	A
Trial 4 (all organisms)	3	16.833	B

* Means with different letters are significantly different from one another at 95% confidence level.

Table 9.17 ANOVA of the final lignin content

Source of Variation	DF	SS	MS	F	P
Between Groups	4	20.789	5.197	20.955	<0.001
Residual	10	2.480	0.248		
Total	14	23.270			

Table 9.18 Differences of the final lignin content

Trial	Number of replicates	Mean of final lignin content (%)	Duncan grouping*
Control	3	16.265	A
Trial 1 (*T. curvata*)	3	15.528	A
Trial 2 (*T. aurantiacus*)	3	17.234	A
Trial 3 (*T. fusca*)	3	16.268	A
Trial 4 (all organisms)	3	13.710	B

* Means with different letters are significantly different from one another at 95% confidence level.

approximately 17% at 35˚C, 25% at 50˚C and 15% at 58˚C on the 15th day, and were 41.8% at 35˚C, 39.6% at 50˚C and 24.8% at 58˚C on day 45.

The current study resulted in similar degradation of cellulose and lignin but in a shorter period of time. This may be due to the addition of bio-available carbon (used cooking oil). Inoculation of cellulolytic microorganisms may have helped the degradation process as well. The degradation of lignin was similar to those reported in other studies.

9.4 Maturity and Stability Tests

The maturity and stability of the bioremediation product was evaluated by analyzing the CO_2 evolution rate of the final product and comparing the germination index (GI) of the final product with that of the feedstock. CO_2 evolution is a good indicator to determine the level of microbial activity and the stability of compost. Germination index provides information about the decomposition of phytotoxic organic substances which indicates the maturity of compost. The lower the CO_2 evolution, the more stable the product is, and the higher the GI, the more mature the product is (Iannotti et al. 1994; Wu et al. 2000; Boulter-Bitzer et al., 2006).

The ANOVA analysis performed on the CO_2 evolution data (Table 9.19) indicated that there were significant differences among the CO_2 evolution of final bioremediation products ($P<0.001$). However, the Bonferroni Analysis (Table 9.20) indicated that there was no significant difference between Control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*), but these were significantly different from Trial 2 (*T. aurantiacus*) and Trial 4 (all organisms) at 95% confidence level. Trial 2 (*T. aurantiacus*) had the highest CO_2 evolution rate and Trial 4 (all organisms) had the lowest CO_2 evolution rate. This indicates that the product of Trial 4 (all organisms) was more stable than other trials. This result correlates as shown by reductions of volatile solids, total carbon, total Kjeldahl nitrogen due to higher decomposition rate. Also, the GI of Trial 4 (all organisms) was highest.

Table 9.19 ANOVA of the final CO_2 evolution

Source of Variation	DF	SS	MS	F	P
Between Groups	4	20.432	5.108	26.844	<0.001
Residual	70	13.320	0.190		
Total	74	33.752			

Table 9.20 Differences of the final CO_2 evolution

Trial	Number of replicates	Mean of final CO_2 evolution (mg CO_2-C per g volatile solid per day)	Duncan grouping*
Control	15	3.184	A
Trial 1 (*T. curvata*)	15	3.096	A
Trial 2 (*T. aurantiacus*)	15	4.218	B
Trial 3 (*T. fusca*)	15	3.213	A
Trial 4 (all organisms)	15	2.619	C

* Means with different letters are significantly different from one another at 95% confidence level.

The final products for Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) had a threshold of under 8 mg CO_2-C per g carbon per day and are considered stable according to Korner et al. (2003) and Goméz and Lima (2006).

Boulter-Bitzer et al. (2006) assessed the maturity and stability of different compost product from horse manure, chicken manure, paunch manure, bone meal ash and bark mix. After 217-231 days of composting, the final products had the CO_2 evolution rates of 2.3-3.5 mg CO_2-C per g VS per day. In this study, the Control, Trial 1 (*T. curvata*), Trial 3 (*T. fusca*) and Trial 4 (all organisms) had similar CO_2 evolution rates but with much shorter periods of bioremediation.

Wu et al. (2000) used CO_2 evolution to evaluate the stability of compost products from different composting facilities in Florida. In two groups of samples of stable compost, the CO_2 evolution rates were around 0.5-0.8 mg CO_2-C per g VS per day in one group and 5.7 mg CO_2-C per g VS per day in another group.

Cooperband et al. (2003) conducted windrow composting of sawdust for 1 year. The final stable compost had a CO_2 evolution of approximately 2 mg CO_2-C per g compost carbon per day. The CO_2 evolution in the current study was higher but the period of bioremediation only lasted 15 days which is much shorter than the processes reported by other researchers.

Gómez-Brandón et al. (2008) evaluated the maturity of cattle manure compost. On the 15^{th} day of composting (active phase), the GI was only 30% and reached 60% after 180 days. Wu et al. (2000) used germination rate to evaluate the maturity of compost products from different composting facilities in Florida. The germination rate of tomato seeds (*Lycopersicon esculentum* L.) was 7-97%.

Rekha et al. (2006) used composting technology in the bioremediation of contaminated lake sediments. After 14 weeks of bioremediation, the GIs of two piles were 49% and 95%. The GI of 20-30% obtained in this study after only a short period of time (15 days) is reasonable. Longer bioremediation may be required to achieve more mature bioremediation products.

In general, the maturity and stability of bioremediation products were different. The phytotoxicity still existed in the final product in all trials except Trial 4 (all organisms). Trial 4 (all organisms) achieved maturity as well as stability. The products of Control, Trial 1 (*T. curvata*) and Trial 3 (*T. fusca*) were stabile but not mature. Wu et al. (2000) and Gómez-Brandón et al. (2008) stated that the evaluation of compost stability based on CO_2 evolution and the maturity based on seed germination are two different parameters of compost quality. The pH of all products was 5.5-6.8 which was within the range of 5-7 for stable compost.

10. CONCLUSIONS AND RECOMMENDATIONS

10.1 Conclusions

1. The temperatures of bioremediation process reached thermophilic phase (>45˚C) in all experimental trials. The mesophilic and thermophilic lag phases were clearly identified in each trial.

   (a) The psychrophilic, mesophilic and thermophilic lag phases were clearly identified and the kinetic parameters were graphically determined in all trials.

   (b) The temperature increasing rates were moderate in this study (0.28-0.84˚C/h for mesophilic phase and 0.46-1.10 ˚C/h for thermophilic phase) because even though the bio-available carbon was provided, the bio-available carbon in the wood waste was low.

   (c) The Trial 4 (all organisms) achieved the highest peak temperature and the longest thermophilic phase and Trial 2 (*T. aurantiacus*) had the lowest peak temperature and shortest thermophilic phase.

2. The moisture content decreased significantly in all experimental trials indicating that the water produced by microbial respiration did not compensate for the water loss as vapor with the exhaust gases. The final moisture content remained in the proper range of 40-60% except for Trial 4 (all organisms) which had the highest temperature and longest thermophilic phase.

3. Similar changing patterns for the pH were observed in all trials.

   (a) The initial increase in the pH observed was due to the breakdown of organic nitrogen to ammonium.

   (b) The final drop in the pH could be due to the formation of organic acids from decomposition of fats and grease in the bio-available carbon and the loss of ammonium with the exhaust gases.

4. The volatile solids decreased slightly while the ash content stayed relatively constant in all trials during the bioremediation process.

   (a) Except for the Control and Trial 3 (*T. fusca*), each trial showed significant reduction of volatile solids.

   (b) The low reductions in volatile solids (0.1-12.2%) were due to the addition of bio-available carbon which was easily available to microorganisms than the organic carbon in the feedstock.

   (c) The Trial 4 (all organisms) resulted in the highest volatile solids reduction because it had the highest temperature and the longest thermophilic phase while Trial 2 (*T. aurantiacus*) had the lowest volatile solids reduction because it had the lowest temperature and the shortest thermophilic phase.

5. The total carbon decreased in all trials after 15 days of bioremediation.

   (a) The reductions of total carbon were relatively low because there was limited bio-available carbon in the wood waste.

   (b) The Trial 4 (all organisms) resulted in the highest total carbon reduction because it had the highest temperature and the longest thermophilic phase and Trial 2 (*T. aurantiacus*) had the lowest total carbon reduction because it had the lowest temperature and the shortest thermophilic phase.

6. The TKN decreased significantly in all trials after 15 days of bioremediation.

   (a) The reduction of TKN was significant (31.9-88.4%) because the high initial content of organic nitrogen resulted in the nitrogen being lost through ammonium volatilization.

   (b) The Trial 4 (all organisms) resulted in the highest TKN reduction because it had the highest temperature and the longest thermophilic phase and Trial 2 (*T. aurantiacus*) had the lowest TKN reduction because it had the lowest temperature and the shortest thermophilic phase.

7. The increase of the C:N ratio indicated that the bio-available nitrogen was abundant in the system compared to the bio-available carbon. The C:N ratio should be calculated on the basis of bio-available carbon and bio-available nitrogen.

8. The bioremediation was successful in degradation the phenolic compounds as a target compound of creosote in all trials (68.0-77.0%). The results are comparable to those reported in other studies but were achieved at much shorter time in this study.

9. The cellulose and lignin contents were degraded significantly (20.2-32.3% of cellulose and 13.1-30.8% of lignin) in all trials.

   (a) The inoculation of individual cellulolytic microorganisms did not result in a significant enhancement in the degradation of cellulose compared with the Control.

   (b) The combination of three different cellulolytic microorganisms improved the degradation of cellulose and lignin significantly.

   (c) The cellulose and lignin results are comparable to those reported in other studies but were achieved in much shorter time in this study because the addition of bio-available carbon.

10. The maturity and stability results indicated that the bioremediation resulted in mature and stable products in some trials in a relatively short period of time (15 days).

    (a) The products of all trials except Trial 2 (*T. aurantiacus*) were stable as indicated by CO_2 evolution test.

    (b) The pH of final products were between 5.5-6.8 in all trials which was within the range of 5-7 for stable compost.

    (c) The phytotoxicity still existed in the final products in all trials except Trial 4 (all organisms) because the bioremediation time was relatively short (15 days).

10.2 Recommendations

1. A bio-available carbon source should be added if high temperature with long duration of thermophilic phase are desirable in the bioremediation of substrates that have low bio-available carbon content such as wood waste.

2. Inoculation of multiple microorganisms is recommended over the inoculation of individual microorganisms. However, a cost analysis should be performed to determine if their use is justified.

3. A better technique to measure the C:N ratio of the mixture should be developed based on bio-available carbon and nitrogen because the C:N ratio based on total carbon and total Kjeldahl nitrogen is not suitable for cellulosic material such as wood waste.

4. The PAHs in the creosote should be monitored in order to evaluate the bioremediation process of all compounds in creosote.

5. A larger scale operation should be conducted to verify the results obtained in insulated laboratory bioreactors for future application in the field.

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APPENDIX A

Compost Product Specific Requirements in Environmental Choice Program CCD-089

(a) have been uniformly exposed to temperatures in excess of 55°C for 3 consecutive days;
(b) have a pH range between 5.0 and 8.0;
(c) not exceed the following amounts of heavy metals (in mg/kg dry weight):

Arsenic (As)	13
Cadmium (Cd)	2.6
Chromium (Cr)	210
Cobalt (Co)	26
Copper (Cu)	128
Lead (Pb)	83
Mercury (Hg)	0.83
Molybdenum (Mo)	7
Nickel (Ni)	32
Selenium (Se)	2.6
Zinc (Zn)	315

(d) have a minimum of 30% organic matter content (of dry weight);
(e) have a maximum of 50% water;
(f) have a sodium adsorption ratio less than 5;
(g) have a maximum particle size of 13mm; not contain plastic in excess of 0.4% by dry weight and any combination of glass, rubber and/or metal in excess of 1% by dry weight if such particles have a dimension in excess of 2 mm;
(h) not contain PCBs in excess of 1 ppm;
(i) have a maximum electrical conductivity of 3 ms/cm; and
(j) be derived from source-separated municipal waste.