Stability and Maturity of Composted Creosote Treated Wood Waste with an Aerobic Mixed Culture Augmented with the Thermophilic Actinomycete *Thermomonospora curvata*

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Abstract— Creosote has been used as wood preservative and water proofing agent in railway sleepers and utility poles for centuries. However, creosote contains polycyclic aromatic hydrocarbons, phenolic compounds and other heterocyclic organic compounds which can cause potential contamination of soil and water, and threaten the health of human and animals. The bioremediation of phenolic compounds in composting bioreactor with a mixed aerobic culture augmented with the thermophilic actinomycete *Thermomonospora curvata* was investigated. The temperature profiles showed that the thermpophilic phase (>45°C) was achieved and successfully maintained in both the control and the inoculated experiments due to the addition of used cooking oil as a bioavailable carbon source and urea as a nitrogen source. The moisture content decreased in both control and inoculated experiments because the water produced by microbial respiration did not compensate for the water lost as vapour with the exhaust gases. The final moisture contents of 42.9% and 39.1% for the control and the inoculated experiments were within the optimum range for composting. An initial increase in the pH was caused by the breakdown of organic nitrogen to ammonium which was then followed by a decrease due to the formation of organic acids from the decomposition of fats and grease and the loss of ammonium with the exhaust gases. The inoculated experiments achieved higher reductions of volatile solids, total carbon and TKN indicating a higher level of activity of microorganisms during the composting process compared with the control. As a result, higher degradation of phenolic compounds, cellulose and lignin were observed in the inoculated experiments. Different degradation rates were observed in the mesophilic and thermophilic stages of composting. The results indicated that the reduced products from both experiments have improved stability and phytotoxicity. The inoculation of cellulolytic-thermophilic actinomycete *Thermomonospora curvata* accelerated the bioremediation process and as a result higher degradation levels of phenols and lignocellulose were achieved.

Keywords— *Wood Waste; Composting; Bioremediation; Mesophilic; Thermophilic; Phenols; Cellulose; Carbon; Nitrogen; Maturity; Stability*

I. INTRODUCTION

Creosote is distilled from coal tar as a byproduct of the coking process. It is a brown to dark liquid consisting of 80-85% polycyclic aromatic hydrocarbons (PAHs), 5-12% phenolic compounds and 10-20% heterocyclic organic compounds, depending on the sources and preparation procedures [1, 2]. PAH's are carcinogenic and genotoxic to human and animals, have low water solubility and are easily absorbed into organic matter. Phenols cause respiratory irritation and cancer and unlike most PAHs, they are all water-soluble, easily leach through soil to groundwater and can be quickly broken down in air, soil and water [3].

Creosote has been extensively used as a wood preservative and water proofing agent in North America since 1838 to prevent insect and fungal decay in railway sleepers, utility poles and other outdoor agricultural and recreational facilities [1-2, 4-6]. However, creosote application is restricted to commercial sectors and there is no registered use in the residential sector [1].

Direct and indirect human exposure to creosote is associated with the widespread use of creosote treated lumber. Acute exposure to large amounts of creosote may result in irritation of the skin, chemical burns of the eye, convulsions and confusion, kidney and liver problems, unconsciousness and even death. Longer exposures to lower levels of creosote may result in increased sensitivity to sunlight, damage to the cornea and skin cancer [2]. Creosote is also toxic to plants and algae; the toxicity of creosote to photosynthesis in soil and aquatic environments has been reported [7].

Current disposal options for creosote-treated wood waste include incineration and landfill. However, the release of components such as PAHs and furans into the atmosphere and the possible migration of phenolic compounds into soil and groundwater are of concern [5]. Compared with combustion and landfill, bioremediation is a less expensive, more environmentally friendly and highly effective in degrading pollutants in creosote-treated wood waste. Also wood substance can be converted into humus and plant nutrients during the volume-decreasing processes of mineralization and humification. The final value-added product is free of contaminants and could be used as a soil amendment [8, 9].

In the process of composting, temperature is important because it directly affects the rate of metabolism of microorganisms. In order to produce a stable compost product, a long hermophilic phase (temperature higher than 65°C) is required. However, in typical composting operations, the thermophilic phase lasts no long than 2-3 days [10-12]. In order to accelerate the degradation process, a controlled prolonged thermophilic phase is required. Given that mesophilic cellulolytic microorganisms would be severely deactivated at temperatures higher than 37°C, thermophilic fungi or bacteria would serve as better decomposers in an elevated temperature environment [13]. The thermophilic actinomycete (*Thermomonospora curvata*) is a dominant bacterial

population in a variety of aerated composts which secretes a variety of thermostable extracellular enzymes during growth including cellulase, making it a possible major cellulose decomposer in the composting process [14-16].

Scarcely explored phenolic compounds of creosote treated wood waste were our targeted contaminants during a composting process. The main objective of the present study was to explore the ability of the actinomycete *Thermomonaspora curvata* to enhance the degradation of the phenolic compounds in creosote treated wood waste during an in-vessel composting process.

II. MATERIALS AND METHODS

A. Experimental Apparatus

The experiments were carried out in a specially designed composting system which consisted of a frame, three bioreactors with air supply units and data acquisition unit (Fig. 1). Three bioreactors, all made of a polyvinyl chloride cylinders (PVC/711, IPS Corporation, Gardena, Canada), were horizontally fastened into the main frame. Each cylinder had an inside diameter of 203 mm, a length of 520 mm and a wall thickness of 5 mm. A fixed circular PVC plate of 203 mm diameter and 6 mm thickness was glued into the back end of the cylinder. A removable circular plexiglass plate of 203 mm diameter and 6 mm thickness was installed on the front end of the cylinder. A circular window of 64 mm diameter was left on the removable circular plate for sampling (Fig. 2). The window was closed with a rubber stopper (No. 13) when it was not in use. Each cylinder provided space for 3.5 kg (wet-basis) of the compost mixture plus 25% of the volume as a head space. The three cylinders were insulated with a 38.1 mm thick Fibreglass insulation while the removable circular plates were insulated with a 38.1 mm thick Styrofoam layer.

Each bioreactor had three holes at the bottom which were used for supplying air to the bioreactor. A top hole was used for the removal of exhaust gas. The pressure regulated air passed through a desiccator and then through a flow meter (No 32461-14, Cole-Parmer Instrument Company, Vernon Hills, Illinois, USA). The flow meter had a 10 cm (4-inch) scale and a range of 0.0566-0.566 m3/h (2-20 standard cubic feet per hour). When the gas left the bioreactor, it passed through a scrubber that contained water to remove aerosol and water soluble organic compounds, then through another scrubber to eliminate possible airborne PAH compounds in the exhaust gas.

Inside each bioreactor, a removable 10.5-mm diameter solid stainless steel shaft was mounted on two bearings. 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. A thermally protected electric motor (Model No. 127P1486/B, D.C., Sigma Instruments Inc., Braintree, Massachusetts, USA) provided power to rotate the mixing shaft at speed of 6 rpm.

The data acquisition unit consisted of a master unit (Multiscan 1200, Omega, Stamford, Connecticut, USA), thermocouple/volt scanning card (MTC/24, Omega, Stamford, CT), Tempview software (Omega, Stamford, Connecticut, USA), temperature sensors (type T thermocouples, Cole Parmer, Chicago, Illinois) and a personal computer. The thermocouples were inserted through specially constructed

fitting. 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 the inlet air temperature.

B. Collection and Preparation of Wood Waste

The Construction and Demolition (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, the wood waste was sieved using a sieve with 12.5 mm opening (USA Standard Testing Sieve, ATM, Milwaukee, Wisconsin). Fresh compost (Miller Compost Corporation, Dartmouth, Nova Scotia) was mixed with the wood waste at a ratio of 1:1. The carbon and nitrogen analyses were first performed on the raw materials and the results were used to adjust the carbon to nitrogen ratio (C:N) to 30:1 and the moisture content to 60% using urea $(CO(NH_2)_2)$ and distilled water. The mixture was placed in polyethylene bags and stored in a freezer at -10˚C in the Biotechnology Laboratory, Dalhousie University until required for the experiment.

C. Preparation of Inoculums

Thermomonospora curvata (ATCC 19995) was obtained from the American Type Culture Collection (Manassas, Virginia, USA) and used as inoculum in one of the experiments. Slants and Petri dishes were made using Hickey-Tresner agar (1 L distilled water, 15 g agar, 2 g tryptone, 1 g yeast extract, 10 g dextrin, 1 g beef extract and 2 mg $CoCl_2$) and Hickey-Tresner broth (1 L distilled water, 2 g tryptone, 1 g yeast extract, 10 g dextrin, 1 g beef extract and 2 mg $CoCl_2$) was used as liquid medium. The agar medium was autoclaved (Sterilmatic Autoclave, Market Forge Industries Inc., Everett, Massachusetts, USA) at 125C for 20 min. After cooling to about 70C, approximately 25 mL the medium was poured into each Petri dish and approximately 20 mL were poured into each slant tube. The Petri dishes and slants were cooled to solidify before use. The broth medium was autoclaved (Sterilmatic Autoclave, Market Forge Industries Inc., Everett, Massachusetts, USA) at 125˚C for 20 min and cooled to room temperature before use. The freeze dried culture was hydrated in Hickey-Tresner broth and then plated on Hickey-Tresner agar. After 48 h, colonies with light yellow color start forming on the Hickey- Tresner agar (Fig. 3a). *Thermomonospora curvata* produced long aerial hyphae with single spores on simple or dichotomously branching sporophores (Fig. 3b). Spore suspension of *Thermomonospora curvata* was prepared by cutting a 1 cm^2 Petri dish culture into 25 mL of sterile Hickey-Tresner broth medium. In order to activate the microbial culture, the inoculated broth was incubated (Isotemp® oven, Model 106G, Fisher Scientific, Hampton, New Hampshire, USA) at C50for 48 h. Then, colony forming units (CFU) were determined using the plate counts method. Aliquots of 5 mL of media containing a cell count of 1.0×10^6/mL were transferred into Fernbach flasks containing 250 mL of liquid culture media and agitated on a rotary shaker (Series G-25 Incubator Shaker, New Brunswick Company, New Jersey, USA) at 120 rpm and 50˚C for 48 hours. The final cultures were used as inoculum in the experiment in the amount of 10% (by weight) of composting material.
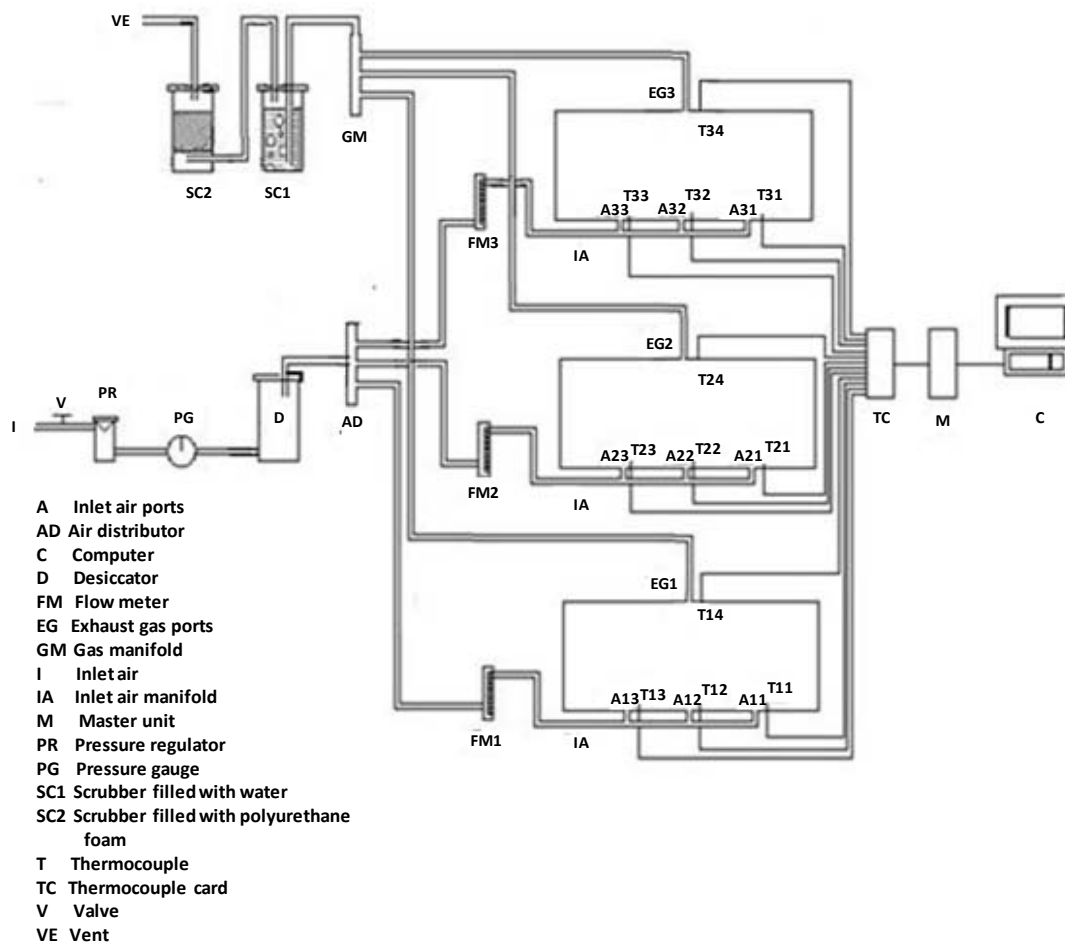
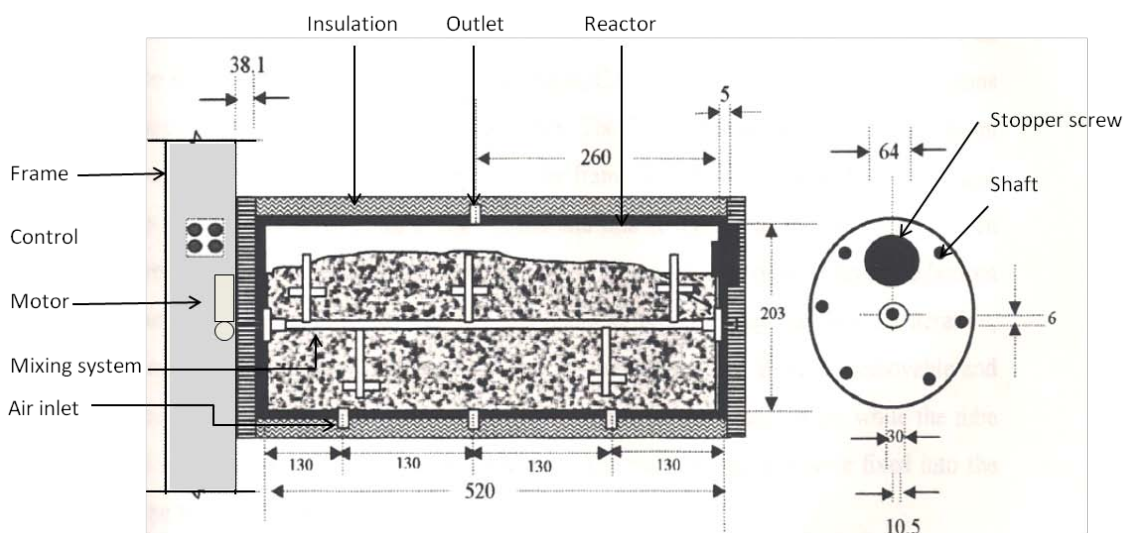Fig. 1 The experimental set up of the bioremediation system

A   Inlet air ports
AD Air distributor
C   Computer
D   Desiccator
FM Flow meter
EG Exhaust gas ports
GM Gas manifold
I   Inlet air
IA   Inlet air manifold
M   Master unit
PR   Pressure regulator
PG   Pressure gauge
SC1 Scrubber filled with water
SC2 Scrubber filled with polyurethane
      foam
T   Thermocouple
TC   Thermocouple card
V   Valve
VE   Vent

Fig. 2 Bioreactor (dimensions in mm)

D. Experimental Protocol

Two experiments were carried out: a control experiment which contained no inoculum of *Thermomonospora curvata* and an experiment which contained the bacterial inoculum added to the mixture. Three replicates were conducted simultaneously for each experiment using the three bioreactors. Each group of experiments lasted 15 days. Approximately 3 kg of the final mixture were placed in each bioreactor. Aliquots of 36 mL of used cooking oil were added into the bioreactor every 12 h for the duration of each experiment as recommended by Alkoaik [17]. The pressure-regulated air was supplied continuously to the bottom of the

bioreactor. The flow rate was adjusted to 0.05 m^3/h (3 V/V/h). The temperature was monitored throughout the process and the data were stored in a Microsoft Excel® file on the computer every 30 min. The average temperature of each experimental group was then generated using the temperature data of the 12 thermocouples in three bioreactors. Samples were collected every 3 days from the bioreactors and analyzed for pH, moisture content, TC, TKN, solids and phenolic compounds. After 15 days of bioremediation, the quality, stability and maturity of the end product were determined by quantifying C:N ratio, pH, CO_2 evolution, phytotxicity.
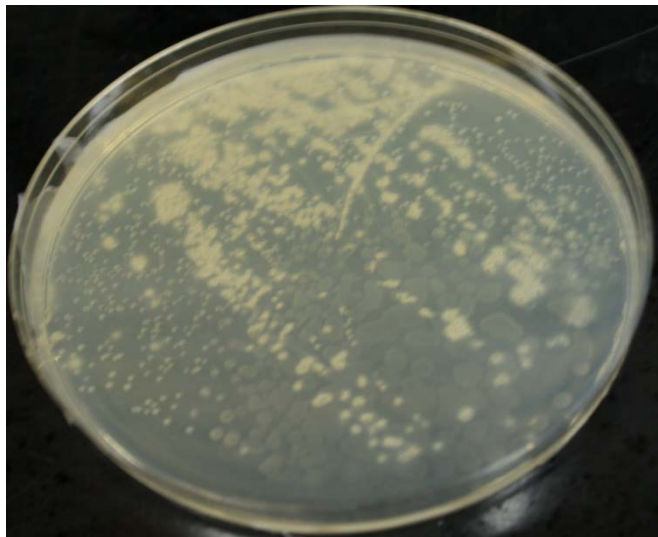
(a) Colonies with light yellow colour on Hichey-Tresner agar
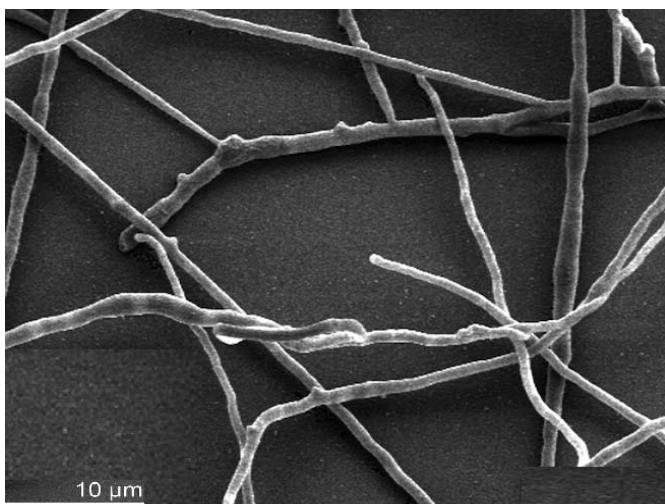
(b) Long aerial hyphae observed under the SEM microscope

Fig. 3 *Thermomonospora curvata*

E. Moisture Content and pH Measurements

The moisture content (MC) was measured following the ASTM (D4442-07) oven-drying method [18]. A slurry made of about 10 g of material and 50mL distilled water was used to measure the pH value using a pH meter (Fisher Accumet®, Model 805 MP, Fisher Scientific, Hampton, New Hampshire, USA).

F. Solids Analyses

The solids analyses were performed according to the procedures described in the USEPA Method 1684 [19]. The ash content was determined by burning the sample in a muffle furnace (Isotemp® Muffle Furnace, Model 186A, Fisher Scientific, Hampton, New Hampshire) at a temperature of 550°C for 20 minutes.

G. Determination of C:N Ratio

Approximately 2.0 g of the material were sampled for total carbon and total nitrogen analyses. Carbon dioxide was determined with a Leco carbon analyzer (Model 516-000, Leco Corporation, St. Joseph, Michigan, USA) along with an induction furnace (Leco HF$_2$O Furnace, Leco Corporation, St. Joseph, Michigan, USA) at the Mineral Engineering Centre of Dalhousie University, Halifax, Nova Scotia, Canada and the total carbon content was determined. The total kjeldahl nitrogen (TKN) was determined at Maxxam Analytical Testing Laboratory in Mississauga, Ontario, Canada following the procedure of USEPA Method 351.2 [20]. The C:N ratio was then determined from the total carbon and total nitrogen value.

H. Determination of Phenols

The phenols were extracted from 3 g of material with 50 mL of deionized water and centrifuged for 20 min at 2400 rpm. The supernatant was vacuum filtered through a 0.45 µm polycarbonate filter paper (Fisher Scientific, Montreal, Quebec, Canada) as described by Chantigny et al. [21]. The supernatant was transferred into a flask and analyzed for the presence of phenolic compounds using the 4-aminoantipyrine colorimetric test following the ASTM procedure [22]. A standard curve was prepared (Fig. 4) and the absorbance was measured at 510 nm using a spectrophotometer (Spectronic 601, Milton Roy, Ivyland, Pennsylvania, USA).

I. Determination of Germination Index

The germination index (GI) was measured following the procedure described by Iannotti et al. [23] and Jiang et al. [24]. About 10 g of compost sample were mixed with 100 mL of distilled water. Ten cress seeds (*Lepidium sativum* L.) were evenly placed on the filter paper (Whatman® 40, Whatman Inc., Clifton, New Jersey, USA) in a sterilized petri dish. Then, 5.0 mL of the extract was transferred onto the filter paper. Three replicates were carried out for each sample. The Petri dishes were incubated at 25C in the dark for 48 hours. The number of germinated seeds was counted and the length of the roots was measured. The germination index (GI) was determined as follows:
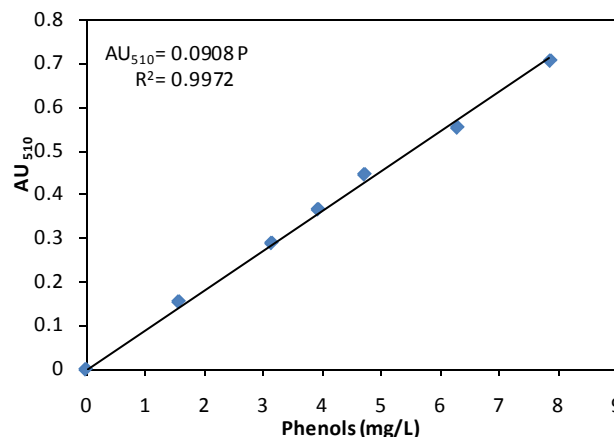

Fig 4. Standard curve for the phenolic compounds

$$GI = \frac{\text{Seed germination } (\%) \times \text{Root length of treatment (cm)}}{\text{Root length of control (cm)}} X\ 100 \quad (1)$$

J. Determination of CO_2 Evolution

CO_2 evolution was determined as described by Benito et al. [25]. Approximately 25 g of the final compost were sampled and incubated at room temperature for 3 days. The moisture content was adjusted to 60% and each sample was separately sealed in containers with a beaker containing 10 mL of 1.0 M NaOH solution. The samples were incubated at 25°C and the CO_2 generated was determined by titrating with NaOH solution with 1.0 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.

K. Determination of Cellulose and Lignin Contents

The cellulose and lignin contents were measured following the Standard Methods published by AOAC International [26] 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. [27].

III. RESULTS

A. Environmental Parameters

1) Temperature:

The average temperature was calculated using the data collected from 12 thermocouples during each experimental run. The average temperatures of the control experiment and the experiment inoculated with *Thermomonospora curvata* and the ambient temperature are plotted against time as shown in Fig. 5(a). The room temperature remained around 22±1°C. The temperatures of the control and inoculated bioreactors increased with time due to microbial activity. The peak temperatures for the control and inoculated experiments were 51.7°C and 52.1°C, respectively. The temperature was maintained above 45°C for 106 and 99 h, and above 40°C for 192 and 186 h for the inoculated and control experiments, respectively.

2) Moisture Content:

The moisture content decreased gradually during the bioremediation process as shown in Fig. 5(b). The final moisture contents for the control and inoculated experiments were 42.9% and 39.1%, respectively.

3) pH:

The changes in the pH of the compost mixture are shown in Fig. 5(c). In the first week, the pH increased from the initial weak acidic value of 6.0 to the basic values of 8.2 and 8.5 for the inoculated and control experiments, respectively.

B. Chemical Parameters

1) Solids:

The changes in volatile solids and ash contents are presented in Fig. 6(a). At the beginning of the experiments, the volatile solids content of the material was 805 ± 10 g/kg material. The volatile solids content decreased in both the control and the inoculated experiments to 775 ± 12 and 739 ± 8 g/kg material (db) resulting in reductions of 4.6 and 8.2% for the control and the inoculated experiments, respectively.
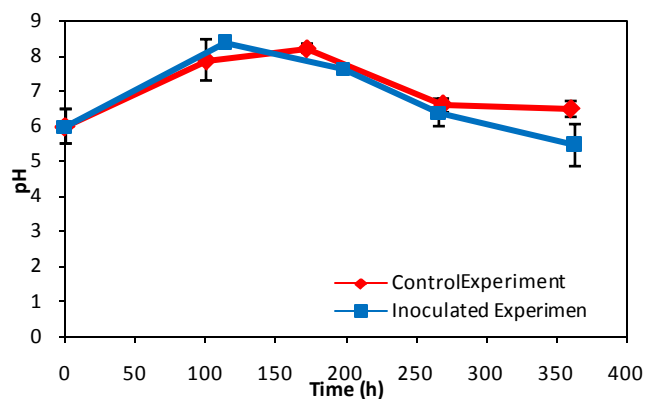
The ash content basically stayed constant at 195 ± 12 g/kg material (db) until the end of the composting process.
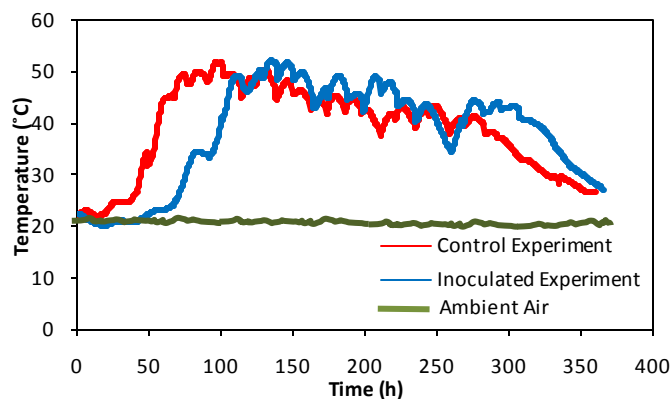
(a) Temperature
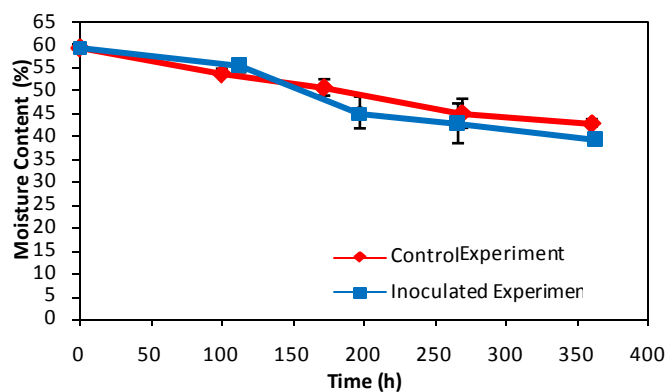
(b) Moisture Content

(c) pH

Fig. 5 Changes in environmental parameters
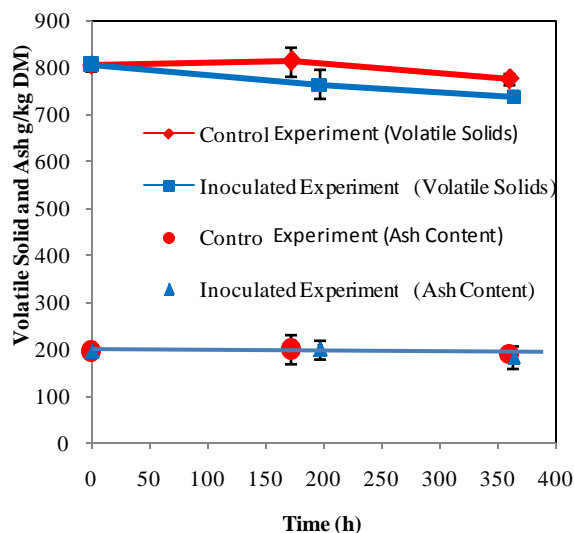
2) Total Carbon:

The changes in total carbon content are shown in Fig. 6(b). The initial total carbon was 392 g/kg material (db). The final total carbon for the control and the inoculated experiments was 358 and 330 g/kg material (db), respectively. This resulted in total carbon reductions of 8.7 and 15.8% for the control and inoculated experiment, respectively.
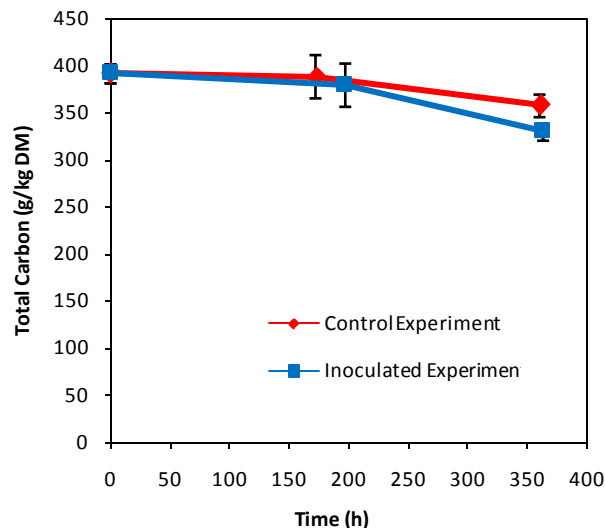
3) Total Kjeldahl Nitrogen:

The changes in total Kjeldahl nitrogen (TKN) are shown in Fig. 6(c). The initial TKN was 24.94 g/kg material (db) which decreased to 12.5 and 7.1 g/kg material (db) for the

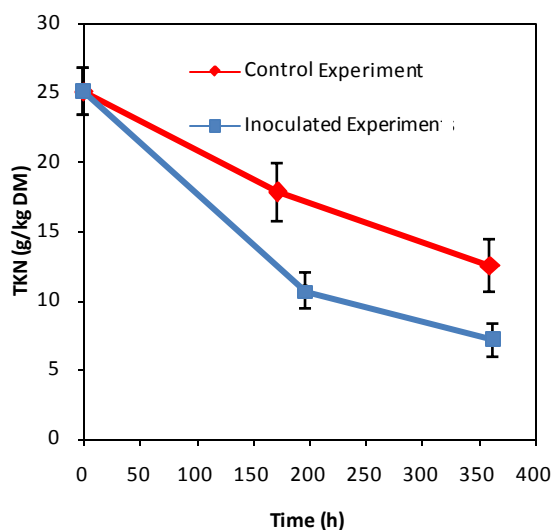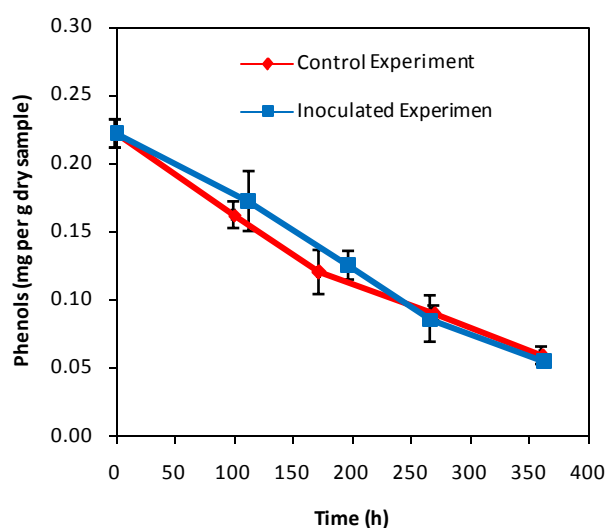control and the inoculated experiments resulting in reductions in TKN of 50.2% and 71.7%, respectively.

(a) Volatile Solids and Ash

(b) Total Carbon

(c) TKN

(d) Phenols

Fig. 6 Changes in chemical parameters

4) C:N Ratio:

Due to the rapid consumption of nitrogen by microorganisms (to metabolize bio-available carbon for cell growth and energy production), the C:N ratio increased from the initial value of 15.6:1 to final values of 28.6:1 and 46.5:1 for the control and the inoculated experiments, respectively.

5) Phenols:

The initial concentration of PC was 0.222 ± 0.010 mg /g material (db) which decreased gradually in both experimental trials, reaching the final concentrations of 0.058 and 0.054 mg/g material (db) for the control and the inoculated experiments as shown in Fig. 6(d). The total reductions were 73.9% and 75.7% for the control and the inoculated experiments, respectively.

6) Lignocellulose:

The results of the degradation of cellulose and lignin are shown in Table I. The initial content of cellulose was $24.8 \pm 1.5\%$ which decreased after 15 days to 19.2 and 19.0% resulting in reductions of 22.6 and 23.4% for the control and inoculated experiments, respectively. The initial content of lignin was $19.8 \pm 0.9\%$ which decreased after 15 days to 16.3% for the control and 15.5% for the inoculated experiments resulting in reductions of 17.7% and 21.7%, respectively.

7) Maturity and Stability:

In the present study, the maturity and stability of the final products were evaluated by the CO_2 evolution (as mg of CO_2-C/g VS-day and mg of CO_2-C/g C-day), the germination rate (GR) and the germination index (GI) and the results are shown in Table II. The CO_2 evolution rates from the final product were 3.18 and 3.10 mg CO_2-C/g VS-day for the control and inoculated experiments, respectively. The initial GI was 0% for both the control and the inoculated experiments which improved significantly after composting

reaching final values of 20% and 22% for the control and inoculated experiments, respectively. As indicated by the GI,

the products in both trials were not free of phytotoxicity.

TABLE I DEGRADATION OF CELLULOSE AND LIGNIN

Trial	Cellulose Content (% DM)			Lignin Content (% DM)		
	Initial	*Final*	*Degradation Percent*	*Initial*	*Final*	*Degradation Percent*
Control Experiment	24.8 ± 1.5	19.2 ± 0.7	22.6	19.8 ± 0.9	16.3 ± 0.3	17.7
Inoculated Experiment	24.8 ± 1.5	19.0 ± 0.8	23.4	19.8 ± 0.9	15.5 ± 0.9	21.7

TABLE II CO_2 EVOLUTION, GERMINATION RATE AND GERMINATION INDEX

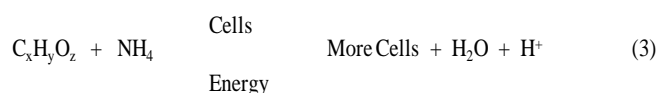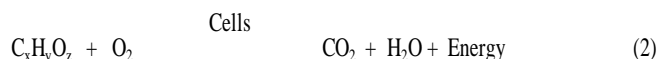| Trial	CO_2 Evolution		GR (%)		GI (%)	
	(mg CO_2-C/g VS-d volatile solid-day)	*(mg CO_2-C/g C-d carbon-day)*	*Initial*	*Final*	*Initial*	*Final*
Control Experiment	$3.18 \pm 0.19^{*}$	6.89 ± 0.41	0	90	0	20
Inoculated Experiment	3.10 ± 0.20	6.94 ± 0.45	0	90	0	22

$\pm$ Standard deviation

However, CO_2 evolution rate of both trials indicated improved stability of the products. The pH of both products was 5.5-6.8 which was within the range of 5-7 for stable compost.

IV. DISCUSSION

A. Operating Parameters

1) Temperature:

The aerobic decomposition of organic matter is a process that provides the energy required for microbial growth and supply carbon and nitrogen and other nutrients for the synthesis of new microbial cells as follows:

$$C_xH_yO_z + O_2 \xrightarrow{Cells} CO_2 + H_2O + Energy \qquad (2)$$

$$C_xH_yO_z + NH_4 \xrightarrow[Energy]{Cells} More Cells + H_2O + H^+ \qquad (3)$$

The release of energy caused the rise in the temperature of both bioreactors. The shape of the temperature curve is similar to that of the microbial growth curve. They have an initial lag phase, an exponential phase, a stationary phase and a declining phase. The mesophilic and thermophilic stages are clearly identified in the exponential phase. The fluctuation in the temperature during the stationary phase (40-50°C) resulted from the opening of the sampling port every 12 hours to add bio-available carbon (used cooking oil) into the system. As the biodegradable carbon in the wood waste mixture declined at the end of each trial, the thermal energy lost from the bioreactors exceed the energy input from the degradation of bio-available carbon and the temperature declined.

A temperature range of 50-60°C in the composting mixture is optimum for high level thermophilic activity [28]. Guardia et al. [29] compared the composting of household waste, food waste and pig slaughterhouse sludge with wood chips and found that only composting pig slaughterhouse sludge with wood chips achieved a peak temperature above 60°C while the other two groups (household waste with wood

chips and food waste with wood chips) reached peak temperatures around 55°C. Tang et al. [28] evaluated the composting of cattle manure with rice straw in a laboratory-scale composting system and observed a peak temperature of 65°C and a temperature above 50°C for 21 days. The lower peak temperature achieved in the present study (51.7-52.1°C) was due to the low bio-available carbon in the wood waste.

Stutzenberger and Jenkins [30] reported optimum growth of *Thermomonospora curvata* within the temperature range of 38-65°C. The temperatures recorded in the present study were within the range of temperature required for the growth of *Thermomonospora curvata*. The temperature curve can be used to determine the specific growth rates (μ) for the heterogeneous composting population during the mesophilic and thermophilic stages. The *ln* of T/T_0 (the measured temperature divided by the initial temperature) was plotted versus time to linearize the exponential portion of the temperature curve as shown in Fig. 7. The slopes of the curves can be used to calculate the specific growth rates (μ) according to the following equation [31]:

$$\mu = 2.303 \, (Slope) \qquad (4)$$

The lag period and specific growth rate for control experiment were 15 h and 0.9 h^{-1} for the mesophilic stage and 4 h and 1.10 h^{-} for thermophilic stage while the lag period and specific growth rate for inoculated experiment were 43 h and 0.7 h^{-1} for the mesophilic stage and 12 h and 0.9 h^{-1} for thermophilic stage, respectively.

Thies et al. [32] evaluated the growth of *Thermomonospora curvata* on cellobiose/minimal medium and reported specific growth rates of 0.24 h^{-1} and 0.32 h^{-1} and lag periods of 25 h and 6 h for the mesophilic and thermophilic stages, respectively. Busch and Stutzenberger [33] studied the growth of *Thermomonospora curvata* on cellobiose, glucose and xylose as sole carbon sources in mineral salts media and reported specific growth rates in the range of 0.31-0.33 h^{-1}. Fennington et al. [34] observed doubling of the specific growth rate (from 0.35 to 0.7 h^{-1}) after the addition of glucose to cotton fibres. In the present study, the specific growth rates for the mesophilic and thermophilic stages were higher than

those reported in the literature due to the addition of used cooking oil as a bioavailable carbon source and urea as a source of nitrogen. However, the lag periods for both the mesophilic and thermophilic stages were longer in the present study. This could be due to the acclimatization to a new mixture containing phenolic compounds.

Both the control and inoculated experiment had identical composting mixtures (wood waste, municipal solid waste compost, urea and phenols) with the same moisture content and C:N ratio. The substantially longer initial lag period observed with the inoculated experiment could be due to the production of an extracellular metabolite that inhibited the growth of the composting microbial population in the inoculated experiment. However, no information was found in the literature to support this theory. Since the experiment was repeated three times using the same mixture and same results were obtained every time, further work should be carried out to investigate this inhibition phenomenon. Competitive conditions may also have affected the growth of the microbial populations. Liang et al. [34] observed a specific growth rate in the range of 0.025–0.70 h^{-1} for a single culture of *Thermomonospora curvata* growing in different substrates but when *Thermomonospora curvata* was grown under competitive conditions with different microbial populations on the same substrates, the specific growth rate was in the range of 0.0222-0.20 h^{-1}, 0.0214–0.10 h^{-1} and 0.05–0.25 h^{-1} when grown with bacteria, actinomycetes and fungi, respectively [35-36].

2) Moisture Content:

In the composting process, moisture content has a vital effect on the biodegradation of organic materials as it influences microbial activity, the physical structure, biological reactions and nutrient transport. The reported optimum moisture content for metabolic activity in composting is in the range of 25-80% on a wet basis (w.b.) [12, 38-40]. However, the ideal moisture content is in the range of 50-70% with 60% being the optimal for in-vessel composting [41]. Petric et al. [42] found the moisture content of 69% to be optimal for the composting of poultry manure with wheat straw. Rao et al. [43] recommended 70% for the composting of poplar wood.

In the present study, the initial moisture content was adjusted the optimal value (60%) for the composting process. During the composting process, the initial moisture content of the compost mixture decreased from 59.69 ± 0.77% to 42.9% and 39.1% at the end of the process for the control and the inoculated experiments, respectively. This was due to the water loss as vapor in the exhaust gases. Guardia et al. [29] composted food waste with wood chips and observed a decrease in the moisture content of the mixture from 63.4% to 50.5% after 37 days of composting. Tomati et al. [44] composted olive mill waste with wheat straw in a forced aeration static pile and observed a decreasing trend in moisture content from 80% to 35% after 35 days of composting. In the present study, the final moisture contents of the compost mixture in both trials were not within the optimum range but were still high enough for the composting process to proceed [10].

3) pH:

The optimum pH range for the growth of *Thermomonospora curvata* is 7.2-7.5 [14, 15]. Janda et al. [45] studied the effect of pH (in the range of 4-9) on the growth of *Thermomonospora curvata* and found the optimum growth of

Thermomonospora curvata occured at pH of 7.5 which then declined with increase or decrease in pH. The pH recorded in the present study (5.5-8.5) provided favorable conditions for the growth of *Thermomonospora curvata*.
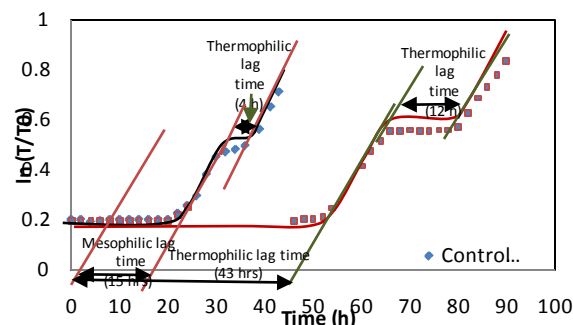

Fig. 7 Graphical determination of the lag period and specific growth rate

It has been reported that the initial pH of the composting process affects the temperature, volume reduction and compost product [28, 46]. A higher pH of compost material may increase the length of thermophilic phase during the composting process [47]. The pH of the control experiment increased to 7.9 on the 4th day and then gradually decreased back to a weakly acidic pH (6.5) by the end of the experiment. However, in the inoculated experiment, the pH increased to 8.4 on the 4th day of the experiment and then gradually decreased back to a weakly acidic pH (5.5). The decomposition of organic matter and the production of NH_4 caused the initial pH increase. Tang et al. [28] while composting cattle manure with rice straw observed an increase in the pH from 8.0 to 9.2 in the first week which then decreased back to 8.6 after 21 days of the process. In present study, the initial nitrogen content was relatively high (C: N ratio of 15.7:1) and the breakdown of organic nitrogen to ammonium resulted in the initial increase in pH. The final drop in pH was due to the formation of organic acids from decomposition of fats [40].

B. Chemical Parameters

1) Solids:

The biodegradable portion of volatile solids (bioavailable carbon in the form of carbohydrates, proteins and fats) are easily broken down by microorganisms and converted into energy, CO_2 and water which results in decreased solid content and increased temperature of the compost material [48,49]. Wang et al. [50] reported 25% reduction in the volatile solids after 12 days of thermophilic composting of a mixture of sewage sludge and solid food wastes. Ivanov et al. [51] reported a 21% reduction of volatile solids after 10 days during composting of sewage sludge and food waste. Ramaswamy et al. [52] reported a 3.5 % (from 86% to 82.5%) reduction in volatile solids after 38 days of composting pharmaceuticals (salinomycin -antibiotics) in poultry manure. In the present study, 36 mL of used cooling oil was added into the system every 12 hrs. As a result, the available organic carbon in the feed stock was not fully consumed because the used cooking oil was probably the preferred bio-available carbon by microorganisms. This resulted in limited reductions of the volatile solid in the feedstock (4.6 and 8.2% for control and inoculated experiment, respectively). The presence of low concentrations of immediately bioavailable carbon in the wood waste could be another reason for the limited reduction of the volatile solid in the feedstock.

2) Total Carbon:

The reduction of total carbon was higher (2 fold increase) in the inoculated experiment compared to the control which indicates a higher level of decomposition in inoculated experiment. Beck–Friis et al. [53] achieved total carbon reductions of 65% after 31 days and 68% after 22 days of composting chopped wheat straw and food waste, respectively. Bautista et al. [54] reported a reduction of 67% of total carbon during composting of a mixture of swine manure, sawdust and wood bark after 18 days. Michel et al. [55] reported a total carbon reduction of 24% while composting yard trimming waste for 45 days. Longer composting time and higher biodegradability of the materials used in these studies were the possible reason for the higher reductions of total carbon. In present study, the continuous addition of used cooking oil provided a preferred carbon source for microorganisms and as a result, the total carbon reduction was low.

3) Total Kjeldahl Nitrogen:

The reductions in TKN were more rapid and much higher than the reductions in the total carbon due to the high initial concentration of total nitrogen and the low concentration of bioavailable carbon [12, 43]. The rate of TKN reduction was 20% higher in the inoculated experiment than the control. Tiquia and Tam [56] reported a TKN reduction of 50% during 168 days of composting of chicken litter in forced-aeration piles. While composting greenhouse tomato plant residues, Alkoaik and Ghaly [57] observed TKN reductions of 3.50, 17.70 and 11.04% for the control and the mixtures of tomato plant residues with the cheese whey and used cooking oil, respectively.

4) C: N Ratio:

A critical parameter in the composting process is a balance between carbon and nitrogen (C: N ratio). If nitrogen is limited, the 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 [12, 40]. In a biological decomposition system, the C: N ratio would typically decrease because: (a) the organic carbon is 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 [50].

However, the initial concentration of nitrogen and the temperature also affect the change of C: N ratio. Higher temperature and/or longer thermophilic phase will result in higher rate of organic nitrogen decomposition and increased nitrogen loss [50, 56]. Tiquia and Tam [56] 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. A composting process that starts with low C: N ratio results in significant losses of nitrogen as to inhibit the composting process [12, 43]. Rao et al. [43] suggested an initial C: N ratio of 30:1 to 50:1 for composting poplar wood. Zenjari et al. [58] reported that the initial C: N ratio fell from 28.6 to 15.4 at the end of the 15 days of the reactor incubation period during composting of olive mill wastewater with barley straw. N'dayegamiye and Isfan [59] reported decreasing C: N ratio from 43 to 17 in wood shavings pile and from 48 to 35 in peat moss pile mixed with cattle manure at 2:1 ratio by volume under field conditions. Cofie et al. [60] reported a 1.8 fold reduction of in C: N ratio (from 24 to 13) during the co-composting process of fecal sludge and

municipal solid waste. In the present study, the C:N ratio increased by 83% and 198% for the control and the inoculated experimental trial, respectively. This was due to the rapid decomposition of nitrogen by microorganisms.

5) Phenols:

Atagana et al. [61] co-composted heavily creosote-contaminated soil with cattle manure and vegetable waste and reported final concentration of phenolic compounds (after 19 months) below the detectable limit. Atagana [62] used seven non-basidiomycetic fungi, *Aspergillus, Candida, Cladosporium, Fusarium, Monicillium, Trichoderma* and *Penicillium*, for the bioremediation of creosote-contaminated soil and reported 84-100% degradation of phenolic compounds after 70 days of treatment. In the present study, reductions of 73.9-75.7% of phenols were achieved in much shorter time (15days).

The degradation of organic substrate can be described with the following first order model [10]:

$$C_t = C_0\, e^{-kt} \qquad (5)$$

Where:

C_t is the concentration of the organic substrate at time t (mg/kg)

C_0 is the initial concentration of the organic substrate (mg/kg)

k   is the rate constant

The value Ln (C_t/C_0) has a linear relationship with the time (t) within a given temperature range. The linear relationships between Ln (C_t/C_0) and t for phenols in the mesophilic and thermophilic temperature zones are graphically presented in Fig. 8. The rate constant for the mesophilic and thermophilic phases were developed from the slope of the lines. The k values for the mesophilic phase were 0.0028 and 0.0022 h^{-1} and for the thermophilic phase were and 0.0040 h^{-1} and 0.0051 h^{-1} for the control and the inoculated experiments, respectively. To bring the concentration of phenols in the final compost to the accepted level of 1.2 mg/kg [63], 1223 h (51 days) and 933 h (39 days) of composting would be required for the control and inoculated experiments, respectively. These will result in a removal efficiency of 99.5%.

6) Lignocellulose:

Tomati et al. (1995) composted wheat straw with olive mill waste water and reported 70% of the lignin content degraded after 140 days of composting. Horwath and Elliott [64] composted ryegrass straw with an initial cellulose concentration of 562.1 g/kg and an initial lignin concentration of 12.15 g/kg in a laboratory-scale container at 25C for 45 days and reported reduction of 47% and 7% in cellulose and lignin, respectively. Reductions of 22.6 and 23.4% for cellulose were achieved in 15 days of composting for the control and inoculated experiments, respectively. Reductions of 17.7 and 21.7% for the lignin were achieved in 15 days of composting for the control and inoculated experiments, respectively. The thermophilic actinomycete *Thermomonospora curvata* has been reported as a predominant organism in compost material because of its ability to produce a variety of thermostable extracellular enzymes during growth on either complex plant materials or purified polymeric substrates [14-16]. Stutzenberger [13] achieved 75% breakdown of the cellulose in cotton fiber during a 10-day period using *Thermomonospora*

curvata at a temperature of 55˚C and a pH of 8.0. Stutzenberger [15] used bagasse as sole carbon source for extracellular enzyme from *Thermomonospora curvata* at 55°C. The combined activity of the extracellular enzymes cellulose and xylanase from *Thermomonospora curvata* caused a 27% solubilization of the bagasse fiber and yielded a mixture of cellooligosaccharides, cellobiose, xylobiose, glucose, xylos, fructose, arabinose and mannitol.
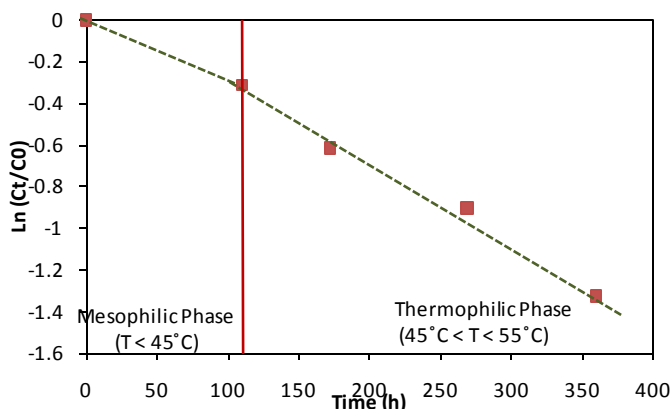
(a) Control Experiment
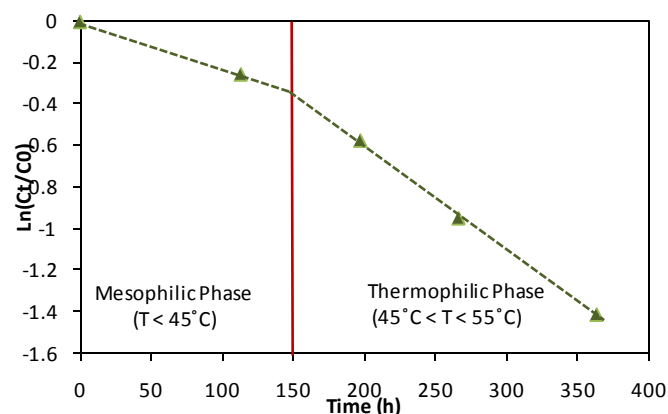
(b) Inoculated Experiment

Fig. 8 Graphical determination of rate constant (k) for PC degradation

Thermomonospora curvata produces a pool of extracellular enzymes including cellulases (endoglucanases and β-glucosidase), amylases, pectinases, and xylanases [15]. Stutzenberger [14] studied the effect of pH in the range of 6-9 on cellulase production by *Thermomonospora curvata* and reported zero and optimal cellulose production at pH 6 and 8, respectively. In cell free liquid, the optimal temperature and pH for the activity of cellulase produced by *Thermomonospora curvata* were 65˚C and 6.0 respectively. However, the results of present study indicated that the inoculation of cellulolytic-thermophilic *Thermomonospora curvata* had minima effect on the degradation of cellulose and lignin during the bioremediation process. This could be due to a slower rate of production of celluloytic and hemocelluloytic enzymes in the complex microbial ecosystem of the bioreactor caused by variation of temperature (40-52°C) and pH (5.5-8.5) throughout the composting experiment. Optimum production of cellulose by *Thermomonospora curvata* requires a pH and a temperature of 6 and 65˚C, respectively. Another possible reason for the low input of *Thermomonospora curvata* innoculum is the presence of heavy metals in the MSW compost (50% of the mixture which may have inhibited the production of cellulose. Lin and

Stutzenberger [16] reported that the activity of enzymes produced by *Thermomonospora curvata* may be inhibited by heavy metals including Fe^{2+}, Hg^{2+}, Ag^+ and Pb^{2+} however, Ca^{2+} and Mg^{2+} have a positive effect on the enzyme activity as reported by Janda et al. [45].

7) Maturity and Stability:

Maturity and stability of the final product was assessed by CO_2 evolution and germination index (GI). CO_2 evolution is a good indicator of the level of microbial activity and the stability of compost while GI provides information about the decomposition of phytotoxic organic substances. A more stable and mature product will have a lower value of CO_2 evolution and a higher value of GI [23, 65].

Benito et al. [25] monitored the germination index during the composting of pruning waste and reported higher initial GI value of 100 due to the absence of contaminants and increased GI values from 111.5 to 142.8 after 190 days of composting. Gómez-Brandón et al. [66] evaluated the maturity of cattle manure compost and reported a GI value of only 30% GI on the 15th day of composting (active phase) which then reached 60% after 180 days. Gao et al. [67] reported the high (94%) GI in composting mixture of initial C:N ratio of 28:1 during the composting of chicken manure and saw dust after 115 days and found the GIs in composting mixtures of initial C: N ratio of 12:1 or 18:1 never exceeded 80%. Brewer and Sullivan [68] evaluated a variety of stability and maturity indices for yard trimmings compost produced in the Puget Sound Region of Western Washington State and reported 1 mg CO_2-C per g VS per day after 113 days of composting. Changa et al. [69] used three types of manure (a) dairy manure amended with wheat straw, (b) dairy manure amended with sawdust and (c) swine manure amended with sawdust and ground wood pallets and composted in windrows for 120 days. The CO_2 evolution rates of the three composts decreased from initial values of 3.41, 3.42 and 9.35 to final values of 0.63, 0.76 and 0.31 mg CO_2-C g^{-1} VS day^{-1}, for the dairy manure-straw, dairy manure-sawdust, swine manure composts, respectively.

C. Comparative Analysis

The values of the performance evaluation parameters of the two bioreactors are presented in Table III. There appear to be slight improvements in the reductions of volatile solids, total carbon, total nitrogen, cellulose, lignin and phenols in the inoculated bioreactor. The peak temperature was sustained longer and the duration of thermophilic phase was longer in the inoculated bioreactor. However, there were no apparently significant differences in the maturity and stability (CO_2 and germination rate) of the compost obtained from both reactors.

V. CONCLUSION

The temperature profiles showed that the thermophilic phase (>45C) was achieved and maintained in both the control and inoculated experiments. The temperature curve was similar to the microbial growth curve. They had a lag period followed by an exponential growth period, a stationary growth period and a declining growth period. The mesophilic and thermophilic stages were clearly identified in the exponential growth phase. Higher specific growth rate were achieved due to the addition of used cooking oil and urea as sources of bioavailable carbon and nitrogen, respectively. The moisture content decreased in both control and inoculated experiments as the water produced by microbial respiration

did not compensate for the water lost as vapour in the exhaust gases. The final moisture contents of 42.9% and 39.1% for the control and the inoculated experiments were within the optimum range for composting. The initial increase in the pH was due to the breakdown of organic nitrogen to ammonium which then decreased due to the formation of organic acids from the decomposition of fats and grease and the loss of ammonium in the exhaust gases. The inoculated experiments achieved higher reductions of volatile solids, total carbon and TKN indicating a higher level of activity of microorganisms during the composting process compared with the control. As a result, higher degradation of phenols, cellulose and lignin were observed in the inoculated experiments. Different degradation rates were observed in the mesophilic and thermophilic stages of composting. The results indicated that the products from both experiments have improved stability and phytotoxicity. The inoculation of cellulolytic-thermophilic actinomycete *Thermomonospora curvata* accelerated the bioremediation process and as a result higher degradation levels of phenols compounds and lignocellulose were achieved.

TABLE III PERFORMANCE EVALUATION OF THE EXPERIMENTAL TRIALS

Activities/Parameters		Control Experiment	Inoculated Experiment
Microbial Growth			
Mesophilic specific growth rate (h^{-1})		0.9	0.7
Thermophilic specific growth rate (h^{-1})		1.1	0.9
Mesophilic lag period (h)		15	43
Thermophilic lag period (h)		4	12
Environmental Parameters			
Peak temperature (˚C)		51.7	52.7
Duration of temperature above 40	˚C	186	192
Duration of temperature above 45	˚C	99	106
Duration of temperature above 50	˚C	14	23
Final moisture content (%)		42.9	39.1
Final pH		6.5	5.5
Chemical Parameters			
Volatile solids reductions (%)		4.6	8.2
Total carbon reductions (%)		8.7	15.8
Total nitrogen reduction (%)		50.2	71.7
Final C:N ratio		28.6:1	46.5:1
Cellulose reduction (%)		22.6	23.4
Lignin reduction (%)		17.7	21.7
Maturity and Stability			
CO_2 evolution (mg CO_2-C/g C-d carbon-d)		6.89	6.94
Germination rate (%)		90	90
Germination index (%)		20	22
Phenol Degradation			
Mesophilic degradation rate constant (h^{-1})		0.0028	0.0022
Thermophilic degradation rate constant (h^{-1})		0.0040	0.0051
Phenols reduction in 15 d (%)		73.9	75.7
Time required for 99.5% removal (d)		51.3	40.2

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