In-vessel composting as a sustainable bioremediation technology of contaminated soils and waste

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Chapter 1

IN-VESSEL COMPOSTING AS A SUSTAINABLE BIOREMEDIATION TECHNOLOGY OF CONTAMINATED SOILS AND WASTE

B. Antizar-Ladislao* and N. J. Russell
Imperial College London, Wye campus, Wye,
Ashford, Kent, TN25 5AH, UK

ABSTRACT

This chapter will provide a comprehensive review of research to date on the use of composting for bioremediation of contaminated soils and waste during the last twenty years.

Environmental pollution is of current environmental concern. In an effort to find solutions, bioremediation techniques have shown promising results in the treatment of contaminated soils and waste. Composting approaches as a bioremediation technology to treat contaminated waste were first reported in the 1980s. Currently, new sustainable technologies are demanded by the composting industry due to recent regulatory changes moving waste management away from landfill towards more integrated, practical, sustainable and economic schemes.

Thus, in-vessel composting is presented as a sustainable bioremediation technology to treat contaminated soils amended with biodegradable municipal solid waste. Special attention will be given to a case study of the disappearance of polycyclic aromatic hydrocarbons (PAHs) present in an aged coal-tar contaminated soil from a former manufactured gas plant site during in-vessel composting using green waste as an amendment. This chapter will discuss in detail a set of operational parameters to maximize contaminant disappearance and microbial activity towards the production of a mature and stable final compost using in-vessel composting as a sustainable bioremediation technology. The effect of temperature, soil to amendment ratio, moisture content and type of amendment on the fate of contaminants, organic matter and microbial communities during in-vessel composting using a multi-parameter factorial design and advanced analytical and microbiological techniques will be presented.

* Current address: University of Cantabria, Department of Water and Environment Science and Technology, Bulevar Ronda Rufino Peón, 254, Torrelavega, Cantabria, 39316 Spain. E-mail: blanca.antizar@unican.es

In summary, this chapter will determine optimal operation conditions during in-vessel composting of contaminated soils and waste, used as a sustainable bioremediation technology, will identify environmental indicators, and will include discussion of technological, regulatory and sustainable aspects.

I. COMPOSTING

Composting is an aerobic process where organic materials are biologically decomposed. Heat produced during this process leads to elevations in temperature characteristic of composting. Conventional composting processes typically comprize four major microbiological stages in relation to temperature: mesophilic, thermophilic, cooling and maturation, during which the structure of the microbial community also changes [1-3].

Composting systems are generally divided into three categories: windrow, static pile, and in-vessel. In the windrow approach, the solid waste mixtures are composted in long rows and aerated by convective air movement and diffusion. The mixtures are mechanically turned periodically to expose the organic matter to ambient oxygen. This approach to composting leads to a characteristic fluctuating temperature regime as core temperatures will fall on turning due to introduction of cooler masses of air and release of entrapped heated air, followed by a subsequent rise in temperature and a new temperature peak as maximum microbial activity resumes. Over time the peak temperatures fall as the level of substrates declines. Recording of temperature fluctuations results in the characteristic jagged tooth thermal pattern of windrowing. Windrowing is a simple mechanical system with no effective control of either oxygen or temperature levels within the organic mass undergoing decomposition. The technique, though popular commercially due to its ease of implementation and relative low cost of installation, has been criticized microbiologically since considerable periods of time elapse when either oxygen or temperature or both will become limiting for microbial diversity and decomposition.

In the static pile approach, piles of solid waste mixture, often with bulking agents (wood chips, straw) as a matrix improver, are aerated by using a forced-aeration system, which is installed under the piles to maintain a minimum oxygen level throughout the compost mass. The forced aeration system may be in either the positive or negative mode which leads to maximum core temperatures slightly above or below the central point respectively. Aeration may be on a timed basis [4] leading to high core temperatures and a severely restricted microbial diversity or by temperature feedback control [5]. This latter approach is based on a greater understanding of the microbial ecology involved and in which the control of air flow is dictated by reference to an upper temperature limit above which the fans blow continuously until heat loss through latent heat of vaporization of water (seen as steaming) reduces the core temperature to an acceptable operational level. In both the Epstein [4] and the Finstein *et al.* [5] static systems there are substantial variations in temperature throughout the composting mass and the cooler edges are routinely covered with a thick layer of finished compost acting as a thermal blanket to ensure that these edges reach regulatory temperature levels. Although the Finstein *et al.* [5] system allows for the control of maximum core temperature, and in theory supplies excess of oxygen for decomposition, both static approaches suffer from this gradation of temperature (and oxygen) throughout the organic waste mass. In static systems active composting will occur over a three to four week period depending on the nature of the

substrate being processed. This active phase is often followed by a two to three month period of maturation.

In-vessel composting takes place in a partially or completely enclosed container in which environmental conditions can be controlled. Enclosed vessels more closely approximate a laboratory incubator where the organic mass and its associated microflora should be exposed to a more even temperature profile. Control of temperature in in-vessel systems is usually achieved through recycling of exhaust gases with intermittent mixing of fresh air to maintain an agreed temperature [6-8]. In theory such systems should allow for excellent control of temperature within the vessel and considerably less variation of temperature through the composting mass. However in-vessel systems have serious limitations in general composting due to limited throughputs and the high installation costs [9]. Because of this, they are often used as a form of pre-treatment "bioreactor" for up to 5 days prior to conventional composting (usually windrowing), where further decomposition, stabilization, and degassing takes place [4,10,11].

The capability of microorganisms to biodegrade specific contaminants may not differ significantly from the ambient soil environment to that of composting but the transformation potential differs for several reasons [12]. Firstly, elevated temperatures of composting (> 50^0C) will increase the rate of enzyme action involved in biodegradation processes. Secondly, co-oxidation may be enhanced due to the range of alternative substrates present. Thirdly, modifications in the physical and chemical microenvironments within the composting mass can serve to increase the diversity of the microflora to which the contaminant is exposed. Lastly, high temperatures will typically increase the solubility and mass transfer rates of the contaminant, thereby making them more available to metabolism. However, some of these positive attributes listed above may be in conflict with the overall impact of temperature and microbial activity.

Over the past two decades there has been much discussion concerning the appropriate levels of temperature for maximizing decomposition rates in composting [5,13]. Reviews of the literature during this period established unequivocally that lower temperatures favour more efficient composting [14]. In practice however composting processes are often subject to regulatory levels of temperature (e.g. 70^0C during 1 hr; 55^0C during 72 hrs) in order to meet United Kingdom national levels of pathogen reduction [15,16]. Emphasis is therefore placed on pathogen reduction above that of composting process efficiency. The impact of this approach to composting is important when considering organic wastes that are likely to be non-pathogen or low pathogen containing materials and whose contaminant substrates are often complex requiring multiple enzyme systems for degradation. Research into bioremediation of PAH contaminated wastes has clearly shown that the most effective degraders of such substrates belong to the fungal class [10,17-22]. More specifically the majority of these belong to the *Basidiomycota* which contain the fungal genera responsible for wood decomposition and hence possess the necessary complex array of ligninolytic enzymes and non-enzymatic mechanisms for the degradation of lignin whose basic chemical structures are similar to the PAHs. Lignin degraders have been shown to be amongst the most potent degraders of PAHs, as epitomized by *Phanaerochaete chrysosporium* [22].

The relationship of these organisms to temperature in the context of composting as a bioremediation technology therefore becomes critical. Microorganisms exhibit a wide range of temperature adaptation and evolution. Microorganisms are found in the coldest and hottest regions of Earth [23-25]. At the extreme ends of this range are a very few highly specialized

genera whose wall and cell membrane modifications allow them to survive and multiply in such conditions. As has been pointed out above, current composting approaches and technologies tend to emphasize the higher end of the range (> 70^0C) in order to meet regulatory requirements for pathogen control. Such temperatures, which will be easily reached in uncontrolled operations, i.e. windrowing [4], severely inhibit the microbial diversity and hence enzymatic potential of the system. Those organisms of particular interest in PAH degradation will be eliminated above approximately 45^0C.

Some speculation has been given to the temperature range at which microbial diversity is maximized. What little research has been done suggests that the range between 40-45^0C allows maximum diversity to be expressed for both, bacteria, actinomycetes and fungi [13,26]. It is of interest to note that this range represents an overlap point at which both mesophilic and moderately thermophilic microorganisms will be viable and active. A strategy of temperature control at this range would seem therefore to be appropriate for the bioremediation of PAH contaminated waste.

II. POLYCYCLIC AROMATIC HYDROCARBONS (PAH)-CONTAMINATED SOILS AND WASTE

PAHs are a class of organic chemicals consisting of two or more benzene rings fused in a linear, angular or cluster arrangement. Their occurrence in the environment is partly the result of natural processes including forest fires and volcanic eruptions, and partly due to anthropogenic activities including the incomplete combustion of fossil fuels, accidental discharge during transport, use and disposal of petroleum products, and incineration of refuse and wastes [27-29]. Thus, in areas of high population density and industrial activity PAH releases to soils and the wider environment have led to higher concentrations of these contaminants than would be expected from natural processes alone. The problem is exacerbated because PAHs have accumulated in soils, sediments and animals after their release to the environment because of their hydrophobicity (Table 1). This gives cause for concern because they have been implicated in many adverse effects on wildlife and human health – most notably carcinogenesis [30-32]. As a result there is a need to identify and clean up sites that have become heavily contaminated so that they do not pose unnecessary risks to health. A further incentive for clean up is the presence of many highly contaminated sites on the premises of former manufactured gas plants which were typically found on land close to the center of towns and cities which is often now a valuable capital asset if it can be cleaned up to a saleable condition [33]. Thus, the potential of using physical, chemical or biological technologies (or hybrid combinations of these) to remediate PAH-contaminated sites has received much attention in recent years [34-36]. Since the 1970s, research on the biological degradation of PAHs has demonstrated that bacteria, fungi and algae possess catabolic abilities that may be used for the bioremediation of PAH-contaminated waste and water [18,37,38]. Bioremediation technologies such as phytoremediation [39,40], land farming [41] and composting [10,42,43] have been used for biodegradation of PAH-contaminated wastes.

III. Chronological Evolution of Composting of PAH-Contaminated Soils and Waste as a Bioremediation Technology

Composting bioremediation approaches consist of the addition of compost ingredients to a contaminated soil or waste, where the compost matures in the presence of the contaminated soil or waste. Composting is a relatively new sustainable bioremediation technology, so few investigations have been conducted and most of them have been performed at the laboratory scale [6]. In this section, the application of composting as a bioremediation technology to treat PAHs contaminated soils and waste is presented, with emphasis on the progressive development of this technology on the treatment of PAHs either as single compound or a mixture of pollutants.

Table 1. Structure and physico-chemical properties of 16 USEPA regulated PAHs

PAH	Molecular weight	Formula	log K_{ow}	Solubility ($mmol \cdot l^{-1}$)	Henry constant ($atm.m^3 \cdot mol^{-1}$)
Naphthalene	128	$C_{10}H_8$	3.00-4.00	$2.4x10^{-1}$	$4.5x10^{-3}$
Acenaphthylene	152	$C_{12}H_8$	3.70	-	-
Acenaphthene	154	$C_{12}H_{10}$	3.92-5.07	$2.9x10^{-2}$	$2.4x10^{-4}$
Fluorene	166	$C_{13}H_{10}$	4.18	$1.2x10^{-2}$	$7.4x10^{-5}$
Anthracene	178	$C_{14}H_{10}$	4.46-4.76	0.07	-
Phenanthrene	178	$C_{14}H_{10}$	4.45	$7.2x10^{-3}$	$2.7x10^{-4}$
Fluoranthene	202	$C_{16}H_{10}$	4.90	$1.3x10^{-3}$	$1.9x10^{-3}$
Pyrene	202	$C_{16}H_{10}$	4.90	$7.2x10^{-4}$	$1.3x10^{-5}$
Benzo[a]anthracene	228	$C_{18}H_{20}$	5.61-5.70	-	$1.2x10^{-6}$
Chrysene	228	$C_{18}H_{20}$	5.61	$1.3x10^{-5}$	$6.7x10^{-7}$
Benzo[b]fluoranthene	252	$C_{20}H_{12}$	6.57	-	-
Benzo[k]fluoranthene	252	$C_{20}H_{12}$	6.84	-	-
Benzo[a]pyrene	252	$C_{20}H_{12}$	6.04	$1.5x10^{-5}$	$2.7x10^{-7}$
Dibenzo[a,h]Anthracene	278	$C_{22}H_{14}$	5.80-6.50	$1.8x10^{-6}$	$2x10^{-9}$
Indeno[1,2,3-cd]pyrene	276	$C_{22}H_{12}$	7.66	-	-
Benzo[g,h,i]perylene	276	$C_{22}H_{12}$	7.23	$2x10^{-5}$	$2x10^{-7}$

Source: International Society for Polycyclic Aromatic compounds [44].

A. Initial Studies

Crawford *et al.* [45] were one of the earliest research groups to report on a composting bioremediation approach to remove PAHs from a contaminated soil. They reported a controlled composting study in the USA at the beginning of the 1980s, although they do not give details on the technology, scale or conditions used. In their study, naphthalene, pyrene and benzo[a]anthracene, at initial concentration of 500 $mg \cdot kg^{-1}$, showed varying degrees of degradation. Naphthalene was completely removed during the first 7 days of composting; benzo[a]anthracene showed 25% removal during the first 7 days and 42% total removal over

30 days of composting; pyrene showed no decrease during the first 7 days of composting and a moderate removal (not percentage removal available) during the last 23 days of experiment.

Racke and Frink [46] studied the fate of phenanthrene during sewage sludge composting at laboratory scale, using radiolabel phenanthrene (1.3-1.6 $mg \cdot kg^{-1}$ dry weight), which allowed them to study the fate of phenanthrene. After 18-20 days of composting, between 10-11% of the phenanthrene was degraded and between 15-17% of unextractable phenanthrene metabolites remained in the compost, either bound to organic matter or incorporated into microorganisms.

Adenuga *et al.* [47] investigated the biodegradation of pyrene (13 $mg \cdot kg^{-1}$) using in-vessel composting technology at the laboratory scale. They amended a spiked soil with composted sewage sludge (40 g mixture), in quantities ranging from 0-50% dry weight basis and the moisture was adjusted to 40%. The mixture was placed in amber-flasks connected to an airflow system, in a water bath. Then the temperature was raised from 20^0C to 60^0C over a period of 21 days, probably trying to stimulate the mesophilic and thermophilic microbiological stages characteristic of composting. The results of their preliminary studies showed that pyrene could be degraded in the composting of soil/sludge mixture although the rate and extent were not fully described.

Crawford *et al.* [45] reported a pilot study to assess the feasibility of treating 2-, 3- and 4-ring PAH-contaminated soils by composting leaves. The study used small windrows (about 19 m^3 each) of varied ratios of soils from a former industrial site contaminated with low levels of 2-, 3- and 4-ring PAHs (about 100 $mg \cdot kg^{-1}$) and other semi-volatile compounds (less than 10 $mg \cdot kg^{-1}$). During their study, it was observed that temperature, moisture contents and ratio of carbon to nitrogen were deficient for optimal composting operation, which indicated the need for larger windrows and increased process control. Complete removal of these PAHs due to mesophilic degradation, abiotic breakdown, volatilization or a combination of them, occurred within 150 days with most losses during the first 63 days. They found that the amendment ratio did not affect the extent of degradation of the PAHs, and appeared to only slightly decrease the rate of degradation of semi-volatile compounds with increasing soil content.

After the completion of these preliminary studies, it was observed that disappearance of PAHs from contaminated waste was feasible using composting approaches, although its optimization required adequate oxygen supply, sufficient nutrients, suitable pH, temperature and moisture for the microbial activity. The ratio of PAH-contaminated waste in the composting mixture needed to be optimized as well to avoid toxicity effects. Attempts to study the fate of the target PAHs were already observed, with special emphasis on the entrapment of PAHs in the solid matrix or incorporation into the microbial biomass.

B. Towards Optimization of Physico-Chemical Parameters in Composting

Civilini [48] described a laboratory scale in-vessel-composting process (2 kg mixture) operated at a constant temperature of 45^0C during 15 days following the Finstein approach [5]. Civilini [48] used municipal solid wastes and fertilizer, to clean up PAHs present in creosote-contaminated soil, using an optimal ratio of starting material to creosote [80% compost, 5% fresh organic matter, 5% fresh organic mater mixed with fertilizer (N:P:K 20:50:5), 8% creosote-contaminated soil, 2% fertilizer (N:P:K 20:50:5), 0.2% creosote] to

avoid toxicity effects. Water moisture and airflow were continuously controlled, and samples were taken at 0, 5, 10 and 15 days. He investigated the fate of 2-, 3- and 4-ring PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene and chrysene). He reported a PAH removal between 81.63% (benzo[a]anthracene) to 98.63% (fluorene) after 15 days treatment, although the initial PAH concentration was not given. The author accounted for volatilization in this study, which was found to be less than 10% of total losses for all the PAHs with the exception of acenaphthene, which was found to be approximately 54%. The lack of thermophilic phase must be considered in this study, since it allows the development of important degraders of PAHs. Civilini [48] observed a selection of microorganisms during days 5 to 10, in which only sporogenic aerobic and/or facultative Gram-positive bacteria increased and all other groups decreased, maybe caused by the reduction of water-soluble PAHs. During days 10 to 15, a pathogenic problem was identified (*Escherichia coli*) which confirmed the unsatisfactory sanitization activity of the process at 45^0C [11].

So far, there was no report of the bioremediation of PAH-contaminated wastes using composting approaches where the mixture was inoculated. By the mid-1990s, research on biodegradation had shown that special groups of microorganisms (i.e. white-rot fungi) have a remarkable potential to degrade PAHs. These fungi naturally degrade lignin to obtain the cellulose that is inside wood fibre, but the non-specific mechanisms that enable them to degrade lignin also allow them to degrade a wide range of pollutants.

C. Towards Optimization of Microbiological Parameters in Composting

McFarland and co-workers [49-51] investigated the removal of PAH in a contaminated soil using fungi. They followed the fate of benzo[a]pyrene in a silt loam soil at laboratory scale under an in-vessel-composting regime (reactor volume, 125 ml) in the presence and absence of *P. chrysosporium* [51]. The soil spiked with benzo[a]pyrene (150 $mg \cdot kg^{-1}$) was amended with corn cobs (primary growth substrate) using a soil to amendment ratio of 2:1 (dry weight) and the reactor head space was periodically purged with humidified oxygen to keep aerobic conditions and water moisture. PAHs were also monitored in $HgCl_2$ (4%) treated systems to compare the impact of biotic and abiotic processes. Information on the temperature profile during composting was not given. Samples were taken after 1, 7, 14, 21, 28, 35, 84, 91 and 95 days. This study showed that although the benzo[a]pyrene appeared to be removed, there was no appreciable difference between the uninoculated and inoculated systems with 65.6 and 62.8% removal, respectively, after 95 days, although initial rates of removal were faster in the inoculated incubations. During poison test conditions, removal of benzo[a]pyrene was observed, which suggested the possibility of irreversible adsorption of benzo[a]pyrene to compost materials or a lack of complete microbial inhibition by the $HgCl_2$. Substantial concentration of *P. chrysosporium* (> 1×104 $CFU \cdot g^{-1}$) was found in both the inoculated and uninoculated compost systems at the end of the treatment period, which explained the similarity in removal in both systems. This suggests that amending soils with suitable fungal substrates may be sufficient to encourage the growth of *P. chrysosporium* populations already present in soil. Other studies have found that autochthonous microflora, with no introduction of foreign microorganisms, offers the greatest potential for PAH degradation in contaminated soils when an organic substrate is added [19-21].

McFarland and Qiu [51] reported the loss of benzo[a]pyrene by first order kinetic during composting with rate constants of 0.003 day^{-1}, 0.08 day^{-1} and 0.06 day^{-1} for poisoned control, fungal unamended and fungal amended treatments respectively. Analysis of gaseous traps indicated that there was no loss through volatilization or mineralization and that nearly 100% of the benzo[a]pyrene removed was attributable to bound residues as the parent compound (approx. 60%) or as chemical intermediates. Furthermore, this study highlighted that the presence of the fungi increased the rate of bound residue formation in the first 30 days of the composting study, where the rate increased from 0.73 $mg \cdot kg^{-1} \cdot day^{-1}$ in the absence of the fungi to 1.58 $mg \cdot kg^{-1} \cdot day^{-1}$ in the presence of the fungi. The authors concluded that the bioaugmentation of a soil-composting system with *P. chrysosporium* was ineffective in degrading benzo[a]pyrene during 95 days of incubation. However, in terms of 'locking up' the PAH within the compost matrix, this technique proved very successful, although the long-term implications for the fate of benzo[a]pyrene are unknown. In addition, when bioaugmentation is practised the rates of nutrient uptake may increase due to a higher metabolism of the target contaminant, which might result in nutrient-limiting conditions leading to a reduction in microbial activity during long-term soil treatment.

Similarly, Joyce *et al.* [52] investigated the fate of a mixture of 3- and 4-ring PAHs (fluorene, anthracene, phenanthrene, pyrene, benzo[a]anthracene) under laboratory scale in-vessel composting conditions of spiked simulated solid municipal waste, monitored over a 60 day period (30 days of active composting followed by 30 days of compost maturation). PAHs were also monitored in $HgCl_2$ (2%) treated systems to compare the impact of biotic and abiotic processes. The moisture of the compost was kept to 50-60% during the first 30 days and to 30% during the last 30 days. The reactors were aerated and/or stirred periodically to maintain aerobic conditions; temperature was kept at 50^0C during the first 30 days and nutrients (0.8% ammonia-nitrogen, 2.3% nitrate, 32.9% urea) were added only during the first 30 days. The results of this study showed that the loss processes occurred during the active phase of composting (first 30 days), when all investigated PAHs showed some abiotic losses in addition to their biological removal from the compost. Anthracene, phenanthrene and pyrene were removed during the composting process by a combination of biotic and abiotic mechanisms, but biotic processes were predominant. However, fluorene was readily lost with abiotic processes accounting for approximately 75% of the removal of this PAH. Benzo[a]anthracene was resistant to biodegradation throughout composting but 40-50% was lost abiotically. These results suggest that the PAHs present in the contaminated soil should be carefully screened, considering the potential for volatilization losses to prevent the process from becoming an air-stripping process.

D. Using Different Amendments

Kirchmann and Ewnetu [53] investigated the biodegradation of petroleum-based oil wastes in 280 litre-aerated-composting bins using horse manure as amendment. PAH concentrations in oil sludge and petroleum residues was 16,800 and 78,500 $mg \cdot kg^{-1}$ dry matter respectively. They studied 4 treatments, using 1.8% oil sludge (5,400 mg $PAH \cdot kg^{-1}$ dry matter), 2.1% petroleum residues (6,100 mg $PAH \cdot kg^{-1}$ dry matter), 7.1% petroleum residues (1,000 mg $PAH \cdot kg^{-1}$ dry matter) and 7.0% paraffin oil (5,300 mg $PAH \cdot kg^{-1}$ dry matter). During all the treatments, the temperature increased from 15^0C to 30^0C during the

first 12-17 days and then decreased to 22-25^{0}C. In the treatment using 7.1% petroleum residues, additions of horse manure were applied repeatedly, which resulted in repeated increases in the temperature following each addition. At the start of the composting period, the dominant PAH was naphthalene, which accounted for more than 50% of the measured PAHs. After 135 days of treatment, Kirchmann and Ewnetu [53] reported that the majority of the PAHs were removed with measurable quantities close to or below the detection limit of 0.1 mg·kg^{-1} dry matter, but pyrene, chrysene and dibenzo[a,h]anthracene were only partially removed (10%) and thus still present at concentrations of 0.2-0.8 mg·kg^{-1} dry matter. Thus, Kirchmann and Ewnetu [53] proved that composting PAH-oil wastes with horse manure was a suitable environmental approach. Horse manure is high in lignocellulosic residues and at the temperatures cited considerable impact would have been expected from the natural mycoflora including members of the *Basidiomycotina*.

Loser *et al.* [54] investigated the removal of PAHs during composting of wood containing PAHs with liquid pig manure. They compared the removal of PAHs in artificially contaminated pine-wood (1,000 mg phenanthrene·kg^{-1} and 1,000 mg pyrene·kg^{-1} aged by autoclaving) and in real PAH-polluted waste wood (5,485 mg PAH·kg^{-1}) in a pilot-scale percolator system. They inoculated both kinds of wood with 50 g decomposed wood·kg^{-1}, and mixed with 26 litres of liquid pig manure each. After 31 days of composting treatment, the PAH concentration of the real polluted waste wood was higher than the concentration of the artificially contaminated pine-wood (1,470 mg·kg^{-1} to 170 mg·kg^{-1}) which was probably due to the lower bioavailability of PAHs in the 'naturally' polluted waste and thus slower biodegradation as compared to the artificially contaminated wood. In addition, in pine-wood 93% of phenanthrene and 90% of pyrene were removed, but in 'naturally' polluted waste wood 86% and 32% of phenanthrene and pyrene respectively were degraded. Another possible reason for the higher residual hydrocarbon ratio in the 'naturally' polluted soil could be the presence of higher molecular weight PAHs. In the 'naturally' polluted soil 2- and 3-ring PAHs were decreased by 75 to 98% and 4-ring PAHs by 40 to 45%, but 5- and 6-ring PAHs were reduced by 15% only. Loser *et al.* [54] concluded that remediation of PAH-polluted waste wood by means of microorganisms is possible. Despite using a different PAH-contaminated waste to enhance composting, Loser *et al.* [54] corroborated the suitability of composting approaches to bioremediate highly PAH-contaminated wastes.

Potter *et al.* [42] investigated the degradation of 19 PAHs from a Reilly soil (creosote manufacturing and wood preserving) during in-vessel composting at the laboratory scale. Each of the test conditions in their experiments utilized a 70% soil and 30% corn cob mixture on a dry weight basis, amended with cow manure, a modified OECD fertilizer or activated sludge to adjust the nutrient content (C:N:P 100:5:1). Moisture content in each 208 litre compost-reactor was adjusted to 30-35%, and aerobic conditions facilitated by a continuous vertical air flow. Samples for analyses were taken after 7, 14, 28, 56 and 84 days of treatment. Temperatures in all reactors increased to the upper mesophilic and lower thermophilic ranges (41-53^{0}C) during the first 15 days of treatment and subsequently decreased to ambient temperature, which confirmed aerobic conditions. Compost reactors amended with sludge sustained higher biomass concentrations than those amended with cow manure during the first 28 days. In addition, the greatest amount of biomass appeared between the 15 days of composting corresponding to the highest temperatures, and thus greatest aerobic activity. Following 56 days of composting, all compost-reactors contained similar amounts of biomass. Starting concentrations of total PAHs ranged from 1,606 to 4,445 mg·kg^{-1}, and final

concentrations ranged from 888 to 1,556 mg·kg^{-1} in the reactors. They reported that 2- and 3-ring PAHs were removed by an average of 87% in all composters after 84 days of treatment, 4-ring PAHs were reduced by an average of 61%, however, none of the amendment conditions appeared to be effective in degrading 5- to 6-ring PAHs. Most of the concentration reduction occurred within the first 28 days of treatment with a plateau forming by 56 days, suggesting first order kinetics. Potter *et al.* [42] reported removal rates of small PAHs ranging from 0.012 day^{-1} (5% activated sludge) to 0.081 day^{-1} (OECD corn cobs + 1% cow manure), and removal rates of medium PAHs ranging from 0.004 day^{-1} (5% autoclaved sludge) to 0.033 day^{-1} (1% cow manure) during the first 28 days. The results of Potter *et al.* [42] showed a general similarity of final PAH concentrations across all treatments, which might reflect the recalcitrance of PAHs during the composting-bioremediation process whereby different types of amendments did not significantly alter the final results.

Ahtiainen *et al.* [55] constructed 2 pilot compost piles (5 m^3) with soil from a sawmill area heavily contaminated with creosote oil (23,600 mg PAHs·kg^{-1} fresh weight soil) and metals (As, Cr, Cu) and they added spruce bark chips (mixture rate not available) as a bulking agent. One pile was inoculated with *Mycobacterium*, and the other pile was left uninoculated. At the start of composting most of the PAHs (about 78%) constituted small PAHs and hence rather quickly biodegradable. However, the total amount of PAHs was high enough to cause potential inhibition of biological activity. Their data shows that soil native microbes were able to degrade PAHs under composting conditions. Furthermore, inoculation of the compost with known PAH degraders did not speed up the process, in contrast to the findings reported by McFarland and Qiu [51], who used *P. chrysosporium* as the inoculum. The increase in certain PAHs was probably due to the heterogeneous nature of the compost. These results support the recommendations given by Guerin [41] regarding the importance of soil homogenization previous to composting treatment.

Ahtiainen *et al.* [55] also investigated the removal of PAHs during composting at a larger scale, constructing one compost pile (100 m^3) with soil from an old wood-preserving facility heavily contaminated with creosote oil (10,960 mg PAHs·kg^{-1} fresh weight soil) and metals (As, Cr, Cu). The soil was pre-treated with 50% hydrogen peroxide to speed up breakdown of the recalcitrant medium and large PAHs. After the hydrogen peroxide was completely degraded, a microbial inoculum of PAH degraders, nutrients and spruce bark chips were added. Ahtiainen *et al.* [55] found that by pre-treating the soil with hydrogen peroxide, they could achieve similar removal rates of about 96% of small-PAHs, and 57% medium and large PAHs, in shorter periods of time.

Amir *et al.* [56] investigated the removal of PAHs present in lagoon-derived sewage sludge (165 kg) during composting with straw (20 kg) in a heap turned every 15 days to provide aeration, during 180 days of treatment. The initial total extractable PAH concentration after mixing the sludge with straw was 0.21 mg·kg^{-1}. They reported an 88% decrease in the concentration of total extractable PAH, and a decrease in the content of organic matter from 30.6% to 24.6% following the composting treatment. During the same period of time, the amount of humic substances increased from 17.5 to 20.8 mg·g^{-1}. They suggested two mechanisms of removal, namely biodegradation and adsorption of PAH. These mechanisms depended on the hydrophobicity, number of aromatic rings and molecular structure of the various PAHs present, and possible PAH bioavailability, although the latter finding was not conclusive.

Oleszczuk [57] investigated the effect of different amendments (fly ash and sawdust) on the disappearance of PAHs during sewage sludge composting in 100 dm^3 plastic containers keeping humidity at a constant level (55 – 60%). The initial concentration of PAHs was 10,385 μg kg^{-1} and 5.4 μg kg^{-1} in sewage sludge and fly ash respectively. Sawdust did not contain PAHs. Three-ring PAHs were predominant (48.7%) in the sewage sludge. Following composting treatment for 353 days, Oleszczuk reported a decrease in the total PAH load of 87.5, 83.4, 82.9 and 88.1% after composting with sludge alone, and with additions of fly ash (20% and 30% w/w dry weight) and sawdust (30% w/w dry weight), respectively. These results indicated that the composting of sewage sludge alone and the treatment with addition of sawdust were not statistically different.

E. Treatment of PAH Contaminated Soils and Waste with Compost

Mature compost can also be added to contaminated waste for bioremediation purposes. The composts sustain populations of microorganisms with the potential to degrade a variety of organic contaminants [58] and they can improve the contaminated soil environment for indigenous or introduced microorganisms by changing the soil pH, nutrient status, aeration and moisture retention characteristics. However, one major concern of using compost as a bioremediation approach is the problem of mixing non-contaminated material with contaminated soil resulting in a greater quantity of contaminated material if the treatment does not succeed. In fact, dilution has frequently been practised as a simple means of getting contaminated sites within regulatory limits. Research in using compost as a bioremediation approach is limited and most of it has been done in the laboratory scale.

Mahro and Kästner [59] investigated the fate of pyrene in soil and soil-compost mixtures over a period of 100 days, finding that the degradation of pyrene was enhanced significantly with the addition of mature compost with > 80% pyrene removal after 20 days in the presence and < 5% pyrene removed in the absence of the compost. Further, Kästner *et al.* [60] investigated the impact of mature compost addition on the fate of ^{14}C-labeled anthracene in soil at laboratory scale. They used 3-litre volume closed compost reactors incubated at 21 ± 2^0C, continuously aerated with humidified air to keep 60% moisture. In soil-compost incubations, 23% of the ^{14}C-labelled anthracene was mineralized to $^{14}CO_2$ and 42% was irreversibly sequestered/bound to the soil-compost matrix after 103 days (the authors suggested biogenic binding). However, in soil-only incubations, approximately 88% of the PAH was recoverable by solvent extraction with the formation of bound residues being less significant.

Following this study, an interest in the understanding of the interaction between the PAHs and the soil-compost matrix on biodegradation and binding mechanisms increased. Thus, Kästner and Mahro [61] continued this work by investigating the degradation of naphthalene (500 $mg \cdot kg^{-1}$), phenanthrene (100 $mg \cdot kg^{-1}$), anthracene (100 $mg \cdot kg^{-1}$), fluoranthene (100 $mg \cdot kg^{-1}$) and pyrene (100 $mg \cdot kg^{-1}$) in soil (Ah horizon of a para brown soil at a non-contaminated, rural area) and soil-compost (3:1, w/w) incubations (25^0C, 60% of the water-holding capacity) at laboratory scale during 100 days. The authors found that the presence of the compost enhanced the removal of the PAHs and that the presence of the organic matrix of the compost was essential for enhanced degradation. In contrast to the pure soil, naphthalene, phenanthrene and anthracene were degraded after 20 days in the soil-

compost mixture. Fluoranthene and pyrene showed lag phases of 10 days, and complete degradation occurred after 35 days. The authors suggested that the stimulating effect on the PAH degradation was a function of the organic matrix of the compost (humic substances) and the ecological niches of the compost, which have to be colonized by the respective microorganisms. Kästner and Mahro [61] suggested that the addition of compost to contaminated soils may enhance the biodegradation and bioavailability of PAHs, and retain certain volatile PAHs as well as reduce the sorptive effects in soils that prevent the compounds from analytical detection.

Kästner *et al.* [62] investigated the fate of [^{14}C]anthracene (100 mg·kg^{-1}) in soil (particle size < 2 mm) and in soil-compost mixtures (particle size < 4 mm) in a continuously aerated bioreactor at laboratory scale during 176 days. Soil and compost were mixed at a ratio of 4:1 (dry wt), with a moisture content adjusted to 60%. They reported complete transformation of the parent compound (anthracene). Although the amount of organic carbon, which might act as an additional binding substrate, was larger in the soil-compost mixture (12.7%) than in the native soil (1%), Kästner *et al.* [62] reported less formation of bound residues from [^{14}C]anthracene and a higher mineralization in the soil-compost mixture (67.2% mineralized, 20.7% transformed into bound residues) than in the native soil (43.8% mineralized, 45.4% transformed into bound residues).

Haderlein *et al.* [63] also investigated the impact of humic matter present in the compost on the mineralization of PAH-contaminated wastes. They prepared mixtures of 640 g of a silty soil contaminated with aliphatic hydrocarbons (TPH = 40,000 mg·kg^{-1}) and PAH (630 mg·kg^{-1}), 250 g maple leaves, 750 g alfalfa and 80 g (w/w) $CaCO_3$ with a moisture content about 50% (w/w), incubated at 55^{0}C, 50% moisture and aerated either continuously or intermittently during 35 days and left to mature for 90 days at ambient temperature. This mature composted PAH-contaminated soil had a pyrene concentration of about 16 mg·kg^{-1}. Then Haderlein *et al.* [64] mixed this resulting mature compost with PAH-contaminated soil (80% soil, 20% compost) and left the mixtures for further composting during 100 days. Abiotic controls contained 0.4% NaN_3. Pyrene was rapidly mineralized (> 50% mineralization after 15 days) whereas mineralization in unamended soil was limited to < 3% during the same period. Abiotic controls had a maximum total mineralization of 0.7% of the initially added pyrene. Handerlein *et al.* [64] focused their efforts to elucidate the link between PAH mineralization and humic matter based on their preliminary studies where they found that pyrene mineralization potential during the composting of contaminated soil increases with time, as does humification. The addition of humic acid (previously extracted from the mature compost) to the soil-compost mixture enhanced pyrene mineralization, reaching an increase of 18±14% mineralization values at the end of the experiment. However the addition of fulvic acid (previously extracted from the mature compost) inhibited pyrene mineralization, probably due to the high content on mineral salts remaining in the fulvic acid after extraction and high pH (8-10). This study corroborated the conclusion that humic acid is a major but not sole reason why the addition of compost stimulates PAH mineralization. This is probably due to the increased bioavailability of contaminants sorbed to mineral-humic acid complexes as suggested by Laor *et al.* [65] and the fact that sorption of the microorganisms and PAHs to the colloidal surfaces of humic matter stimulates PAH biodegradation.

Wischmann and Steinhart [66] investigated the removal of PAH/N-PAH in soil-compost mixtures (415 g mixture) as compared to unamended soil (Ah/Al horizon soil material; 400 g) in a 1 litre bioreactor at laboratory scale for 180 days. Soil and soil-compost mixtures were

spiked with a PAH/N-PAH standard solution in dichloromethane, resulting in concentrations from 28 to 181 mg·kg^{-1} dry weight. The moisture of the mixture was kept to 50% during the length of the treatment. The compost used in this investigation had a degree of maturity V. Abiotic removal of PAH / N-PAH was investigated in poisoned controls (soil autoclaved 35 min at 130^{0}C, 1.7 bar, 1 g·kg^{-1} $HgCl_2$). Samples were taken after 1, 3, 8, 14, 21, 28, 42, 54, 77, 105 and 180 days. In unamended soils, only PAHs with up to 3-rings were degraded over 105 days. In the soil-compost mixture there was a lag phase of 8 days, and the 2-ring PAHs depleted within the following 49 days. The 3-ring PAHs were eliminated to < 3% during 105 days. Of the PAHs with more than 3-ring PAHs only fluoranthene and pyrene were almost completely transformed within 105 days. The residual concentration of benzo[a]anthracene, chrysene and benzo[a]pyrene decreased to 2, 3 and 27% compared to the initial estimated amounts within 180 days of treatment. PAH removal in the poisoned soil showed similar elimination rates to those in the unamended soil, which suggested abiotic losses predominated in the unamended soil. Microbiological analyses indicated non-sterile conditions in the poisoned soil-compost mixture that suggested potential biotic losses, although they occurred to a lesser extent as compared to the soil-compost mixtures. Longer treatment times were required in the investigation by Wischmann and Steinhart [66] than in the investigation by Kästner and Mahro [61] for complete removal of 2- and 3-ring PAHs, although both investigations used mature compost and not aged-soils. These different results might be explained by the use of different operational parameters and different origins of soil and compost.

Carlstrom and Tuovinen [67] investigated the mineralization of phenanthrene in domestic-waste compost in biometers to assess the impact of the origin of the compost on the mineralization of PAHs. The compost was collected in four different sampling events from the interior thermophilic zone and from the exterior mesophilic zone of compost piles between 3 to 6 months old. Replicates of 5g compost were spiked with phenanthrene (100mg·kg-1) and incubated in the dark at 22±2 or 60±2^{0}C during 90 days. Carlstrom and Tuovinen [67] reported a dominant effect due to the heterogeneity of the yard-waste samples, which obscured the possible effect of surfactant addition, particle size, and moisture content. Nevertheless they reported an effect of the origin of the compost on the mineralization of phenanthrene. Yard- waste compost samples collected from the 50-60^{0}C thermophilic interior zone and incubated at 60±2^{0}C yielded 1-2% mineralization, whereas their incubation at 22 ± 2^{0}C resulted in 17% average mineralization of phenanthrene. These results suggested that phenanthrene-mineralizing microorganisms present in the biometer were not thermophiles, but were able to survive in the thermophilic zone of the compost, probably spore-formers. Carlstrom and Tuovinen [67] were one of the first laboratories to report thermophilic biomineralization of PAHs. Samples collected from an outer 30-40^{0}C mesophilic zone and incubated at 60±2^{0}C showed negligible mineralization, while parallel sub-samples incubated at 22±2^{0}C yielded about 8% mineralization. The removal of phenanthrene was attributed to microbial activity because sterile samples showed negligible $^{14}CO_2$ evolution (< 1%). The rate constants were in the range of 0.083-0.033 day^{-1} for the mineralizable fraction of phenanthrene.

Šašek *et al.* [10] investigated the bioremediation of an MGP site soil contaminated with PAHs by amending with mushroom compost in mid-phase I (consisting of wheat straw, chicken manure), and gypsum during 54 days in a thermally insulated composting chamber (approx. 1,000 kg mixture) followed by a further 100 days of maturation in windrows. The

total concentration of twelve US EPA PAHs in the soil was 610 mg PAH·kg^{-1} dry mass of soil. The mixture comprised 64% soil and 36% compost on a dry weight basis, and the moisture content was maintained at 64%. Changes in the temperature of compost were monitored, showing an initial increase of temperature up to 70^{0}C, and after 12 days of composting, the temperature progressively decreased, indicating the different stages of composting. The degradation of individual PAHs was in the range of 20-60% at the end of 54 days of composting followed by further PAH removal (37-80% maximum) after another 100 days of maturation. During composting, the outgoing air was passed through a filter and the filter was analyzed for possible volatilization losses of PAHs. The amount of PAHs retained in the filter was below detection limits, indicating that the removal of PAHs during composting was either due to compost microflora metabolism or irreversible sorption to the compost matrix.

Šašek *et al*. [68] investigated the same composting/compost approach in the treatment of a soil collected from an area of a former-tar-producing plant with a total concentration of 16 PAH listed by US EPA at a higher concentration of 2,832 mg·kg^{-1}. The mixture comprized approximately 47% soil and 53% compost on a dry weight basis, and the mixture moisture was maintained at 64%. Their results showed 42-68% removal of 3- and 4-ring PAHs, and 35-57% removal of 5- and 6-ring PAHs after 42 days of composting. However, an additional 100 days of compost maturation in open-air did not result in a further decrease of PAH concentrations.

Lau *et al.* [69] investigated the effect of temperature on the biodegradation of naphthalene, phenanthrene, benzo[a]pyrene and benzo[g,h,i]perylene using a compost approach in the laboratory. Sterilized garden soil (1g) was spiked with 1 ml of acetone containing the PAHs and mixed with a straw spent-mushroom compost (*Pleurotus pulmonarius*), which is a combination of wheat straw, dried blood, horse manure and ground chalk composted together. The moisture content of the mixture was adjusted to 60%, and the sample was incubated at 4 to 80^{0}C at 200 rpm. Removal of the PAH under investigation occurred due to biodegradation and sorption mechanisms. Under the experimental conditions of 1% spent mushroom compost treating 100 mg PAH·l^{-1} at room temperature, the removal of PAHs varied between 82% naphthalene and 59% phenanthrene. The highest sorption removal (46%) was with phenanthrene. Lau *et al.* [69] reported an increase in PAH removal as temperature was increased. At 50^{0}C, 3 PAHs except phenanthrene were completely removed. At 80^{0}C, 5% of the spent mushroom compost completely degraded the 4 PAHs at 200 mg·kg^{-1}soil. This mechanistic study indicated that increasing the temperature during bioremediation of PAH-contaminated waste using compost enhanced the removal of PAHs, with an optimal removal at 80^{0}C.

Atagana [70] investigated the removal of PAHs during composting of soil contaminated with > 30,000 mg·kg^{-1} creosote with poultry manure for 19 months. He treated a 350 kg mixture (PAH contaminated soil first homogenised and mixed with wood chips in a ratio 1:1 (v/v) and then mixed with solid poultry manure in a ratio 4:1 v/v) set up as static pile compost heap on a wood pallet overlaid with nylon fibre sheet in the open yard, and covered with hay for insulation, and compared PAH removal with the control (mixture of contaminated soil and wood chips without poultry manure). The pH of the compost for most of the experiment remained between 6.2 and 7.6. Temperature in the compost rose to 60^{0}C in the second month of treatment before decreasing to 45^{0}C in the fourth month, and thereafter remained between 35 and 45^{0}C. In general, 2- and 3-ring PAHs were removed below 1 mg·kg^{-1} by the third and

fourth months, whereas 4- and 5-ring PAHs were removed below the same limit by the tenth and eleventh months. Chrysene was removed after 16 months of treatment. Atagana reported that microbial activity correlated with temperature fluctuations and hydrocarbon degradation. At the beginning of composting, a mixed population of bacteria (*Pseudomonas* sp., *Bacillus* sp., *Rhodococcus* sp., *Mycobacterium* sp.) and fungi (*Rhizopus*, *Fusarium*, *Aspergillus*, *Penicillium*, *Pleurotus*) dominated the compost. After the fourth month the most dominant species were mainly fungi (*Pleurotus*). Additionally, the removal of PAHs from the control experiment was similar to that of the composting system in the first month, but then, it continued very slowly for the length of the treatment to a final value of 56% removal. Atagana [70] concluded that high nitrogen content (C:N 306:1) and high temperatures (60^0C) had limited effects on the microbial degradation capacity of the compost.

Moretto *et al.* [71] investigated the removal of PAHs during in-vessel composting of a mixture of 146 kg of soot and 1,029 kg of active mass (mixture of sewage biomass and yard waste, 1: 2.5, v/v) characterized by high initial pH (pH of soot = 12.53; pH of active biomass = 8.06). Composting occurred in a 1.86 m^3 closed tank under forced aeration regime during the first 60 days and then natural aeration during 70 days. The initial concentration of US PAHs and not-US PAHs in the soot component of the total mass was 157.62 and 60.52 $mg \cdot kg^{-1}$ (dry basis) respectively. Additionally, the mass was turned by shovel every 30 days. The initial moisture content in the mixture was 55-60%, and the initial C/N ratio was 15-30, within the range values for optimal composting. Temperature in the compost rose to 40-55^0C and remained in the thermophilic range during the 55 first days, and then dropped and remained within the range 35-44^0C until the end of the treatment. Moretto *et al.* [71] reported a 50% removal of 16 US PAHs after 60 days of composting treatment, increasing up to 68% after 130 days. They suggested a more effective degradation of lower molecular compounds, and volatilization of some 2-4 ring PAHs due to an increase in temperature during composting. Thus, this study indicated that composting technology can efficiently be applied to treat alkaline soils heavily contaminated by PAHs.

F. Comparing Composting with other Bioremediation Technologies

Guerin [41] investigated the removal of PAHs during bioremediation, comparing the use of mesophilic composting of soil with conventional land treatment or landfarming, both of them at field scale. This study was of special importance because it was amongst the first to quantitatively compare the treatment of a highly PAH-polluted soil using a composting approach with a different bioremediation technology. The treated contaminated soil (4.3 - 6,915 $mg \cdot kg^{-1}$ total PAH) had a silty clay texture, was visually contaminated with tar residues, and was initially blended with commercially based slow-release nutrients. A ratio of green tree waste to manure to soil of 15:5:80 was used, where green tree waste was fresh (< 5 days old) *Eucalyptus* spp. leaf and stem waste. The initial concentration of naphthalene was 180-300 $mg \cdot kg^{-1}$, phenanthrene was present at 70-230 $mg \cdot kg^{-1}$ and benzo[a]pyrene was present at 58-71 $mg \cdot kg^{-1}$. The soil compost mixture (130 m^3) was placed in a windrow 4 m (wide) × 24 m (long) × 1.5 m (high) and regularly mixed during 224 days. The moisture was maintained at 60-80% of the water holding capacity of the treatment soils during the course of the field program. Soil composting temperature reached maximum temperature (42^0C) after 35 days, while there was no self-heating of the soil observed in the land treatment. The soil

composting process conditions reduced the total PAH concentrations to below the target level given by the regulatory body (500 $mg \cdot kg^{-1}$) after 224 days and resulted in a final concentration of 120 $mg \cdot kg^{-1}$, which was lower than that obtained by land treatment. Losses of the low molecular weight PAH from volatilization throughout the treatment period, as determined by a portable Flame Ionization Detector (FID), were not detected. After 224 days of composting treatment, there was complete removal of the lower molecular weight PAH, 90% degradation of medium PAHs and 70% degradation of large PAHs. Indeno[1,2,3-cd]pyrene and benzo[g,h,i]perylene were most resistant to degradation but approximately 50% of each was lost. Guerin [41] also investigated changes in microbial populations during 224 days of composting. Total heterotrophic populations remained in the range 107-109 per gram soil, showing slightly higher values in the composting treatment than in the land treatment. Microorganisms capable of utilizing naphthalene (*Pseudomonas* spp.) remained in the range 107-108 per gram soil during the first 35 days. However after 224 days they considerably decreased as expected due to the reduction in PAH concentration, and in particular naphthalene concentration. In this study, composting proved to be a suitable field-scale environmental technology for PAH-contaminated soil treatment. Operational parameters such as appropriate amendment and ratio of amendment to soil, oxygen supply and moisture were critical factors in achieving effective soil composting. Contaminated soils can vary greatly in distribution of contaminants, thus special attention should be given to the appropriate soil preparation previous to composting treatment.

Atagana [70] investigated the removal of PAHs using co-composting of PAH-contaminated soil with poultry manure, as described above mentioned, and compared it to landfarming. He reported that more that 20% of the 4- and 5-ring PAHs persisted after 11 months landfarming treatment as compared with all PAHs being removed (except chrysene) to below 1 $mg \cdot kg^{-1}$ after 11 months co-composting with poultry manure.

G. Current State of the Art

Most investigations of the bioremediation of PAH-contaminated soils and waste using composting approaches conducted to date have been at laboratory or pilot-plant scale. Furthermore, efforts have focused on operational considerations rather the fundamental physical, chemical and biological mechanisms that underpin bioremediation-composting technologies. Results from these investigations are difficult to compare due to the use of different experimental conditions: (i) temperature, (ii) moisture, (iii) soil/waste to amendment/compost ratio, (iv) aeration, (v) inoculation vs. non-inoculation; different composting technologies: (i) windrows vs. in-vessel approach; and different scales of operation: (i) laboratory vs. pilot vs. field. Further complications are treatment of wastes/soils of different origin with different total PAHs concentration, distribution of PAHs, bioavailability of PAHs, homogenization, and variations in the sources and types of organic wastes.

Most of the reviewed investigations, regardless the composting/compost approach, technology or scale used, agreed on the importance of optimizing the operations conditions during the application of the bioremediation technology. Emphasis was given to temperature, moisture and maintenance of aerobic conditions but particularly to the ratio of waste to amendment used. The majority of the laboratory scale investigations on the use of

composting/compost of PAH-contaminated wastes were kept in the mesophilic or lower thermophilic ranges [42,52,53] with a few investigations in the thermophilic phase [67,69]. Moisture differed from one investigation to another, ranging from 40% to 80%, although the majority of the studies emphasized the importance of keeping the moisture of the mixture constant throughout the treatment. Different technologies require different approaches to aeration, but was commonly achieved by aerating the mixture with humidified air. By doing so, both moisture and aerobic conditions were maintained. Although some of the earliest work on composting/compost bioremediation approaches suggested the importance of finding a suitable mixture ratio between the contaminated waste and the amendment/compost [45], there are still a few reports where the optimal mixture ratio has been investigated [48] and in general it does not exceed 80% soil content on a dry weight basis [62,63].

Composting of PAH-contaminated wastes has received more attention than the treatment of PAH-contaminated wastes with compost. One major concern of using compost as a bioremediation technology is the problem of mixing non-contaminated compost with contaminated waste, resulting in a greater quantity of contaminated material if the treatment does not succeed. Nevertheless, if using compost as a bioremediation technology proves to achieve similar removal levels of PAHs as using composting as a bioremediation technology, the application of compost-bioremediation technology may offer important operational advantages (i.e., homogenization) during the application of the technology at a field scale. Consequently, more mechanistic studies comparing the bioremediation of PAH-contaminated waste using composting and compost approaches applying the same technology and scale at optimal operational conditions are necessary. If fresh composts are mixed with the soil, a composting process may occur [10,68], and then a more appropriate definition would be composting/compost approach rather than composting or compost approach.

Composting and the use of compost have both been successfully applied to the bioremediation of PAH-contaminated wastes. The main mechanisms of PAH removal under both bioremediation regimes were mineralization, binding and volatilization. Mineralization was the more significant mechanism of removal reported in most studies to date. Mineralization of PAHs may be enhanced by increasing their availability to microbial attack during bioremediation processes. In order for the pollutants to become available to microbial attack, desorption from the waste-compost matrix may have to take place. In terms of composting, increases in temperature may enhance the rate of PAH desorption from the matrix and thus transfer to the aqueous phase (Pignatello and Xing, 1996). In terms of compost, enhanced PAH mineralization of PAHs present in soil-compost mixtures may occur due to the presence of humic matter in the compost matrix. This is supported by studies where either humic acid and/or fulvic acid have been added to a soil with very low endogenous humic acids/fulvic acids [64,65,72,73] and an increase in the bioavailable fraction has been observed. Another favourable factor inherent to composting approaches is that normally large amounts of organic matter, considered a major factor in the ‘locking-up’ of organic pollutants are added to the system[50,74]. Thus, by utilizing composting approaches, the bioavailable fraction will ideally be mineralized and the unavailable fraction will be ‘locked up’ in the soil-compost matrix, reducing the overall risk. However, the long-term fate or the ‘locked up’ organic contaminants remains uncertain.

IV. IN-VESSEL COMPOSTING OF AN AGED COAL-TAR CONTAMINATED SOIL: A CASE STUDY

This section focuses on the optimization of operational parameters at laboratory scale using an aged naturally PAH-contaminated coal-tar soil, which will facilitate the highest possible removal of the target PAHs. Complementary studies on the fate of organic matter and microbial community dynamics during in-vessel composting are also presented.

A. Materials and Methods

Contaminated Soil

The coal tar contaminated soil was obtained from a manufactured gas plant site commissioned in 1838 at Clitheroe, Lancashire, United Kingdom. An extensive description of the site and the procedures for soil sampling and preparation were reported by Birnstingl [75]. Prior to use in the present study the coal tar contaminated soil was air-dried and homogenized by passing through a 5 mm followed by a 2 mm sieve and stored in the laboratory at room temperature. Before experimentation the coal tar contaminated soil was diluted by homogenizing with silver sand (1:1) to provide a more homogeneous distribution of the coal tar residue. The results presented here refer to post dilution coal tar contaminated soil, and all results are expressed as post dilution. Thus, the soil contained 100.3 mg PAHs·kg^{-1} soil, organic matter content was 4.79±0.16% (wt/dry wt) and soil pH_w was 7.3±0.1.

Green Waste

The soil was conditioned with an artificial green waste for the composting studies, which represents green waste to be used at field-scale based studies. Artificial green waste was prepared by mixing foodstuff (mixture of carrots, cucumber, lettuce, onions, potatoes and tomatoes in equal amounts) (3% dw), sawdust (38% dw), leaves (17% dw), grass (27% dw) and wheat straw (14% dw). Foodstuff, sawdust, wheat straw and leaves where separately blended and grass was cut. The composition of the green waste satisfied the nutrient requirement (C:N 40-50) according to the calculations using Cornell's system [76].

Reactor Design

A set of 360 glass composting reactors (200 ml) was constructed, which allowed conditions to be easily monitored and controlled (Figure 1). These fully enclosed bench-scale reactors each held about 65 g total composting mixture. Composting reactors were placed in triplicates in temperature controlled incubators at 38^{0}C, 55^{0}C and 70^{0}C to simulate representative mesophilic and thermophilic microbiological stages during composting processes [13,26]. For each glass composting reactor, the ingredients of the artificial green waste and soil in the particular S:GW ratio under investigation were thoroughly mixed in a glass beaker (500 ml), and then the mixture introduced into the reactor. Initial moisture content of the composting mixture was measured and double distilled water (DDW) was added when needed to reach the desirable moisture content for the different experimental conditions under investigation. Compost moisture content was measured at intervals to ensure that it was maintained at the required level, and amended with DDW when needed.

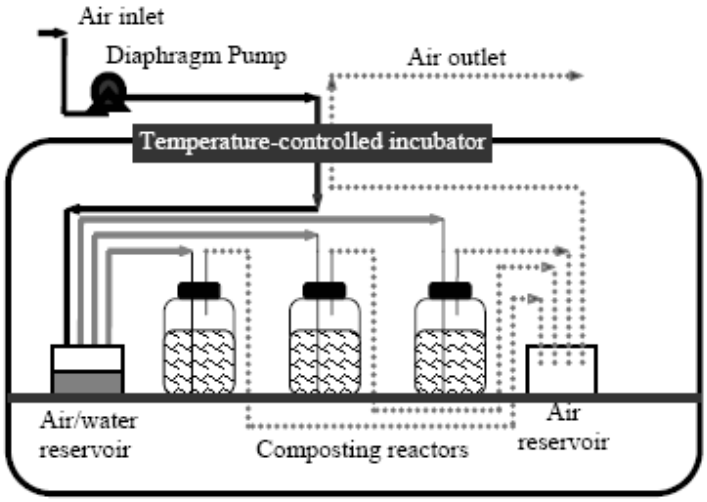

Figure 1. Laboratory set-up of composting reactors.

The reactor units stood vertically with air flowing continuously to avoid oxygen content limitation and vented outdoors to avoid volatiles accumulating in the composting reactors. Airflow up through the composting mixture via a stainless steel air-delivery tube inserted into the bottom of the composting reactors was provided by 100% oil free diaphragm pumps (Model PXW-600-DIOV, VP1, Fisher Scientific). The air inlet was bubbled through a DDW reservoir to avoid excessive water evaporation during aeration.

Sample Analysis

Destructive sampling, in triplicate, for each experimental treatment was executed periodically. The contents of each composting reactor were thoroughly mixed with a stainless steel spatula in a glass beaker (500 ml), and sub-samples were collected for analysis.

Total Organic Matter (TOM) in Composting Mixtures

Ash content was determined using a loss-on-ignition procedure [77]. Triplicate 5 g samples were dried at 110^0C for 24 h (moisture content) and then transferred to a muffle furnace at 550^0C for 12 h. Ash content was calculated from the ratio of pre- and post-ignition sample weights. The residual moisture of the samples was determined to produce the results on a dry matter basis (110^0C).

PAHs in Composting Mixtures

The extraction of 16-E.P.A. Priority PAHs from composting mixtures and soil was done by Accelerated Solvent Extraction (ASE^{TM} 200) and defined in this investigation as total PAHs for simplicity. Briefly, glass fibre disks were placed at the outlet end of the extraction cells and a 7g sample of compost was mixed with 3 g of sodium sulphate and 7 g of Hydromatrix^{TM} and introduced into each extraction cell. Surrogate standards (1-fluoronaphthalene, 2-fluorobiphenyl, purity > 97%, Greyhound Chromatography and Allied Chemicals, UK) were added to the cells prior to extraction to monitor PAH losses. Extraction cells were placed into the auto-sampler tray with copper turnings to remove sulphur. ASE^{TM}

200 conditions for PAH extraction were: 14 MPa (2000 psi), 100^0C, oven heat-up time = 5 min, static time = 5 min, solvent dichloromethane/acetone (1:1), (v/v), flush volume = 60% of extraction cell volume, nitrogen purge = 1 MPA (150 psi) for 60 s. The extracts were cleaned up on a column containing 1 g of activated-florisil (SiO_2, 84.0%; MgO, 15.5%; Na_2SO_4, 0.5%; 60/100 mesh; 130^0C; 12 h) and 2 g of anhydrous sodium sulphate. To remove hydrophobic impurities the column was initially washed with 10 ml dichloromethane before eluting 5 ml of extract and then leaving to dry for 1 min. The PAHs were then eluted with 10 ml dichloromethane.

Internal standards (napahthalene-d8, acenaphthene-d10 in a mixture with chrysene-d12, 1,4-dichlorobenzene-d4, perylene-d12, phenanthrene-d10, purity > 97%, Greyhound Chromatography and Allied Chemicals, UK) were added to the clean extracts prior to analysis. A Hewlett Packard 6890 series gas chromatograph with a 7673 series auto-sampler and a 5973 series mass selective detector was used for the analysis. The GC inlet was operated in pulsed (0.90 min, 30.0 psi) splitless mode at 270^0C with helium as carrier gas. The injection volume was 1 μl and the inlet purged at 50 ml·min^{-1} one minute after injection; the column flow was maintained at 1 ml·min^{-1}. Separation was achieved using an HP-5MS column (19091S-433 30 m × 0.25 mm × 0.25 μm). The temperature program comprized 70^0C for 2 min, 10 centigrade degrees per minute to 300^0C where it was held until the end of the analysis (10 min). The MS transfer line was 280^0C providing conductive heating of the MS source to about 230^0C. The instrument was tuned using perfluorotributylamine. The MS was operated in selective ion monitoring (SIM) mode. The 16 US EPA PAHs (quantification ion/confirmation ions), internal standards (quantification ion/confirmation ion) and surrogates (quantification ion/confirmation ions) for SIM GC-MS mode were: naphthalene (128/127, 129, 102), naphthalene-d8 (136/137, 134, 108), 1-fluoronaphthalene (146/120, 125), 2-fluorobiphenyl (172/171, 170), acenaphthylene (152/151, 153, 76), acenaphthene (154/153, 152), acenaphthene-d10 (164/162, 160, 163), fluorene (166/139, 165), phenanthrene (178/165, 163, 82, 176), anthracene (178/179, 176, 89), fluoranthene (202/200, 101, 203), pyrene (202/200, 201, 101, 203), benzo[a]anthracene (228/226, 229), chrysene (228/226, 230, 113), benzo[b]fluoranthene (252/250, 253, 126), benzo[k]fluoranthene (252/253, 250, 126), benzo[a]pyrene (252/207, 253, 250, 126), indeno[1,2,3-c,d]pyrene (276/276, 279, 138), dibenzo[a,h]anthracene (278/279, 139, 276), benzo[g,h,i]perylene (276/138, 137, 277). The GC-MS system was calibrated prior to the analysis of samples using seven calibration standards.

Organic Matter of Composting Mixtures

Contour maps of excitation-emission matrix (EEM) spectra were obtained on water extracts of whole compost (1 g composting mixture; 50 ml DDW) after shaking in an orbital shaker SO1 (Stuart Scientific, UK) for 5 min at 75 rev·min^{-1}, and filtration through Whatman No.2 filter paper. The pH of the extract was not adjusted but monitored. A Perkin Elmer LS45 fluorescence spectrophotometer was used, equipped with FL WINLAB Software for data processing. The excitation and emission slits were fixed to 10 nm. The emission (Em) wavelength range was fixed from 280 to 600 nm, whereas the excitation (Ex) wavelength was increased from 200 to 500 nm keeping a constant $\Delta\lambda$ of 20 nm steps at 500 nm·min^{-1}.

Biomass

Analysis of bacteria, fungi and actinomycetes were by the dilution and spread-plate method following the "Standard Methods for the Examination of Water and Wastewater" [78] with minor modifications. Briefly, 10 g of the soil green-waste mixture sample were mixed with 90 ml of Ringers' solution and shaken for 10 min. Consecutive 1:10 dilutions were prepared, starting with 1 ml of sample to produce 8 dilutions of each sample. Then 0.1 ml of each dilution was spread onto 5 plates of nutrient agar (with cycloheximide) for bacteria, 5 plates of starch casein (with cycloheximide) for actinomycetes and 5 plates of potato dextrose agar (with rose bengal) for fungi. Cycloheximide was used to inhibit the growth of fungi from the soil, and rose bengal was used to suppress the growth of bacteria. Samples from the soil green-waste mixtures treated at 38^0C, 55^0C and 70^0C were incubated at 38^0C, 55^0C and 70^0C respectively for 72 h. Following incubation, plate colonies were counted.

Additionally, sub-samples of compost (2 g) were spiked with 500 $\mu g \cdot l^{-1}$ ^{13}C-palmitic acid (hexadecanoic acid, internal standard), the total lipid was extracted using the Bligh and Dyer procedure, and the lipid acyl chains and internal standard fatty acid converted to fatty acid methyl esters (FAME) using $MeOH/H_2SO_4$ [79]. The FAME were analysed using the same Hewlett Packard GC/MS system described above. The GC inlet was operated in pulsed (1.40 min, 40.0 psi) splitless mode at 260^0C with helium as carrier gas. The injection volume was 1 µl and the inlet was purged at 50 $ml \cdot min^{-1}$ 20 min after injection; inlet pressure was controlled by electronic pneumatics to maintain a constant column flow of 1 $ml \cdot min^{-1}$. Separation was achieved using an HP-5MS column (19091S-433 30 m × 0.25 mm × 0.25 µm). The temperature program comprised 40^0C for 3 min, 10 centigrade degrees per minute to 150^0C, 3 centigrade degrees per minute to 230^0C, and 30 centigrade degrees per minute to 300^0C, which was maintained for 5 min to allow late eluting peaks to exit the column. The MS transfer line was held at 310^0C, thus providing conductive heating of the MS source to about 230^0C. The MS was operated in selective ion monitoring (SIM) mode, using m/z = 74 as the common fragment ion of FAME. To identify the fatty acids, the retention times were compared with those obtained for standard bacterial acid methyl esters (Supelco, UK). The amount of microbial signature acids was calculated using the ^{13}C-16:0 internal standard, which gives a characteristic fragment ion m/z = 75 that can be quantified separately from the bulk ^{12}C-FAME in the sample.

The sum of the following fatty acids was used to represent total bacteria: i15:0, a15:0, i16:0, i17:0, cy17:0, 18:1ω7c and cy19:0 [1,80,81]. Gram-positive bacteria were represented by i15:0, a15:0 and i17:0 [82] and Gram-negative bacteria by cy17:0, 18:1ω7c and cy19:0 [81,83]. Thermophilic bacteria (mainly bacilli) were represented by i15:0 and i17:0 [84]. Fungi were represented by 18:2ω6,9 [80].

Data Analysis

Comparison of ΣPAHs disappearance under different composting conditions was investigated using paired *t*-tests. Factor analysis was used to simplify the complex and diverse relationships that exist among the operational parameters and to investigate the structure in the relationship between the operational parameters during in-vessel composting of PAH-contaminated solid waste. Factor analysis was executed, with factors extracted by the principal component method from the correlation matrix and varimax rotated factor loadings. The effect of different operational parameters on suggested indicators was investigated using

a two-way multivariable ANOVA analysis and post hoc Tukey test. All statistical tests were performed using the latest version of *StatistiXL* Version 1.5.

B. PAHs Disappearance in an Aged Coal-Tar Contaminated Soil During in-Vessel Composting

Eighteen experimental conditions were tested in triplicate using 360 laboratory-scale composting reactors. The standard composting reactors comprised three temperature levels (T, 38^0C, 55^0C and 70^0C) four soil to green waste ratios (S:GW, 0.6:1, 0.7:1, 0.8:1 and 0.9:1 on a dry weight:weight basis) and three moisture contents (MC, 40%, 60% and 80%). Control reactors consisted of 1:0 S:GW ratio. The logistic approach to pinpoint the optimal operational conditions for maximum degradation of PAHs was, first to investigate the influence of S:GW 0.6-0.9:1 at three temperature levels and MC 60%, then to investigate the influence of MC 40-80% at three temperature levels and optimal S:GW ratio. Using this logistic approach allowed us to investigate eighteen experimental conditions at laboratory-scale rather than thirty six possible combinations.

The initial ΣPAH concentration in the investigated soil was 100.3 mg PAHs·kg^{-1} soil, which is lower than those concentrations of about 450 mg PAH·kg^{-1} soil/sediment reported in a typical manufacturing gas plant site by Erickson *et al.* [85]. However, they were above the Dutch List action level of 40 mg PAH·kg^{-1} air dried soil. The 16 US EPA-listed priority pollutant PAHs were grouped as 2- and 3-ring PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene), 4-ring PAHs (fluoranthene, pyrene, benzo[a]anthracene, chrysene) and 5- and 6-ring PAHs (benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, indeno[1,2,3-c,d]pyrene, benzo[g,h,i]perylene) and thus defined as small, medium and large PAHs respectively for ease of discussion. The concentrations of the 16 US EPA-listed PAHs investigated in the present study before treatment and after 98 days at three different temperatures (T, 38^0C, 55^0C and 70^0C), four soil to green-waste ratio (S:GW, 0.6:1, 0.7:1, 0.8:1 and 0.9:1) and three different moisture contents (MC,40%, 60% and 80%) as well as the control reactors PAH content are presented in Table 2. The PAH concentration in all reactors remained consistently distributed in the composting mixtures due to an initial extensive blending of soil and green waste. A mechanistic and fundamental investigation was undertaken to optimize the operational parameters during in-vessel composting of PAH-contaminated solid wastes.

Effect of Temperature

The effect of temperature on PAH-contaminated waste degradation was initially investigated at 38, 55 and 70^0C for as S:GW 0.6:1 and MC 60%. Temperature was an important factor affecting the in-vessel composting of this PAH contaminated soil (Table 2). PAH losses were greater at 38^0C (76.4% removed) than at 55^0C or 70^0C, with differences also observed between the two higher temperature treatments (52.2% and 22.3% removed respectively at 55^0C and 77^0C). Small PAHs were reduced by an average of 98.2% at 38^0C as compared to 53.0% and 16.9% at 55 and 70^0C respectively at S:GW 0.6:1 and MC 60%. Medium PAHs were reduced by 76.2%, 53.7% and 22.7% at 38^0C, 55^0C and 70^0C

respectively and large PAHs were reduced by an average of 40% at 38^0C, 55^0C and 70^0C, S:GW 0.6:1 and MC 60%.

Table 2. PAH bioavailability and lost (mg·kg^{-1} dry soil) and PAH disappearance (% removal) after 98 days of continuous in-vessel composting treatment

		Temperature								
		38 ^{0}C			55 ^{0}C			70 ^{0}C		
Treatment MC/S:GW	Time (days)	PAH bioavailable	PAH lost	PAH removal	PAH bioavailable	PAH lost	PAH removal	PAH bioavailable	PAH lost	PAH removal
60%/ 0.6:1	21	50 ± 0	48 ± 2		54 ± 0	14 ± 4		46 ± 3	20 ± ±5	
	56	23 ± 2	13 ± 2		36 ± 5	39 ± 3		38 ± 1	2 ± 3	
	98	22 ± 2	15 ± 2	76.4	22 ± 9	0 ± 2	52.2	21 ± 7	0 ± 2	22.3
60%/ 0.7:1	21	52 ± 1	41 ± 11		62 ± 11	45 ± 8		52 ± 8	12 ± 8	
	56	26 ± 13	26 ± 16		66 ± 20	6 ± 11		34 ± 6	11 ± 5	
	98	18 ± 8	16 ± 4	82.0	41 ± 7	17 ± 2	67.7	24 ± 8	23 ± 3	46.0
60%/ 0.8:1	21	32 ± 2	36 ± 16		70 ± 7	37 ± 11		33 ± 2	4 ± 1	
	56	35 ± 8	40 ± 3		74 ± 11	27 ± 5		23 ± 7	19 ± 0	
	98	9 ± 0	2 ± 3	76.7	68 ± 9	0 ± 10	60.6	17 ± 1	33 ± 2	55.7
60%/ 0.9:1	21	32 ± 9	50 ± 15		29 ± 6	37 ± 6		42 ± 10	19 ± 2	
	56	17 ± 0	23 ± 2		25 ± 1	18 ± 2		23 ± 2	0 ± 2	
	98	8 ± 1	0 ± 2	69.1	30 ± 1	0 ± 2	62.4	22 ± 3	0 ± 1	54.0
40%/ 0.8:1	21	47 ± 1	18 ± 14		53 ± 6	10 ± 15		60 ± 1	11 ± 11	
	56	63 ± 2	22 ± 17		72 ± 3	9 ± 2		62 ± 6	2 ± 11	
	98	66 ± 1	0 ± 13	39.2	56 ± 13	0 ± 6	21.9	30 ± 18	0 ± 8	40.4
80%/ 0.8:1	21	28 ± 2	72 ± 7		33 ± 3	45 ± 13		50 ± 0	0 ± 9	
	56	34 ± 1	0 ± 4		48 ±	0 ± 0		43 ± 0	0 ± 12	
	98	24 ± 2	0 ± 8	68.4	65 ± 21	0 ± 7	51.2	46 ± 5	0 ± 10	34.4

PAH concentration at start: 100.3±3.2 mg·kg^{-1} dry soil.

Regarding concentrations of individual PAHs, chrysene, benzo[k]fluoranthene and benzo[a]pyrene were not removed at a S:GW ratio 0.6:1 and MC 60%, thus proving their recalcitrant nature. The final concentration of benzo[g,h,i]perylene was ~55% of the initial concentration following the three temperature levels treatments, which may indicate that the mechanisms of removal of this particular PAH may be due to mechanisms other than biological degradation. Benzo[g,h,i]perylene is expected to be strongly bound to the organic composting matrix due to its high hydrophobicity (log K_{ow} = 7.23) [6] and volatilisation is not expected to be an important fate process, given its Henry constant value ($2\cdot10^{-7}$ atm·m^3·mol^{-1}) [6]. In addition, adsorption to the organic composting matrix is expected to attenuate its potential volatilization. However, under the investigated composting conditions, benzo[g,h,i]perylene may partially exist in particulate phase (vapour pressure 6×10^{-8} Pa at 25^0C) [6] or adsorbed onto fine particles, and it may have been transferred to the atmosphere.

Large PAHs are often difficult to biodegrade and different strategies have been investigated in an attempt to find the best conditions to remove these recalcitrant

contaminants. Most of these strategies have been developed to overcome the low bioavailability of large PAHs, such as an increase in temperature [86] or use of surfactants [34,87] combined with the use of specialized microorganisms which degrade aromatic hydrocarbons[18,21,51]. Current composting approaches and technologies tend to emphasize the use of high temperatures (> 70^0C) in order to meet regulatory requirements for pathogen control [15]. Such temperatures severely inhibit the microbial diversity and hence enzymatic potential of the system [26]. What little research has been done suggests that the range between 40 and 45^0C allows for such maximum diversity to be expressed for both, bacteria, actinomycetes, and fungi [13,26]. It is of interest to note that this range represents an overlap point at which both mesophilic and thermophilic microorganisms will be viable and active.

Effect of Soil to Green Waste Ratio

The effect of soil to green waste ratio on PAH-contaminated waste degradation was investigated at 38, 55 and 70^0C (Table 2). A temperature of 38^0C proved to be the optimal for ΣPAHs removal. Of the 12 experimental conditions (S:GW 0.6-0.9:1, MC 60, T 38-70^0C), ΣPAHs removal at 38^0C was significantly higher than ΣPAHs removal at 55-70^0C ($p < 0.01$) over time; and ΣPAHs removal at 55^0C was as well higher than ΣPAHs removal at 70^0C ($p < 0.1$). Higher removal rates were apparent at S:GW 0.7-0.8:1, although no significant (P>0.1)differences in ΣPAH removal were found for different S:GW at MC 60%. Small PAHs and medium PAHs were removed by an average of 91.3% and 79.4% respectively at 38^0C, S:GW 0.7:1, 0.8:1, 0.9:1 and MC 60%. Average removal of large PAHs was 58.0% and 52.3% at S:GW 0.7:1 and 0.8:1 respectively as compared to 25.0% at S:GW 0.9:1, both at 38^0C and MC 60%. Increasing the S:GW ratio to 0.9:1 resulted in a lower removal of benzo[b]fluoranthene, benzo[k]fluoranthene and indeno[1,2,3-c,d]pyrene. Crawford *et al.* [45] reported a pilot study to assess the feasibility of treating 2-, 3- and 4-ring PAH-contaminated soils (about 100 $mg \cdot kg^{-1}$) by composting leaves in small windrows (about 19 m^3 each). They found that the amendment ratio did not affect the extent of degradation of the PAHs. Our study corroborates the results of Crawford *et al.* [45] within the S:GW ratios investigated, but the S:GW ratio may influence the rate of removal of PAHs.

Effect of Moisture Content

The effect of moisture content on PAH-contaminated waste degradation was investigated at 40, 60 and 80% for a S:GW 0.8:1 and T 38, 55 and 70^0C (Table 2). The removal of ΣPAHs was significantly lower ($p < 0.05$) at a MC 40% as compared to 60% within the investigated temperature range, although no significant difference was found between MC 60 and 80%. As aforementioned, while removal of PAHs was also observed at 70^0C and MC 40-80%, this occurred to a lesser extend than at 38^0C or 55^0C. Small PAHs were removed by an average of 88.1% at 38^0C within the MC range under investigation. Medium PAHs were removed by an average of 75.8% at MC 60% and 80% as compared to 49.5% at MC 40% and 38^0C, and large PAHs were removed by 52.3% and 24.9% at MC 60% and 80% as compared to no removal at MC 40% and 38^0C. Potter *et al.* [42] investigated the degradation of 19 PAHs from a soil during in-vessel composting at the laboratory scale, using 70% soil and 30% corn-cob mixture on a dry weight basis, amended with cow manure, a modified OECD fertilizer, or activated sludge to adjust the nutrient content (CNP 100:5:1). They maintained a MC of 30-35%, aerobic conditions, and temperature range 41-53^0C. Potter *et al.* [42] reported that 2-

and 3-ring PAHs were removed by an average of 87% in all composters after 84 days of treatment, and 4-ring were reduced by an average of 61%; however, none of the amendments conditions appeared effective in degrading 5- and 6-ring PAHs. This investigation is in agreement with Potter *et al.* [42] results, and emphasizes the importance of MC optimisation during in-vessel composting-remediation of contaminated wastes.

Optimal Conditions for PAH Disappearance during in-Vessel Composting

The factor analysis result using 18 operational conditions indicated that 3 factors could explain 99.8% of the total variance [43]. Thus, for a simpler and easier interpretation, factor rotation was performed using varimax rotation after factor extraction, and rotation was normalized. On the basis of these analyses, optimal operational conditions for degradation of PAHs would occur at MC 60%, S:GW 0.8:1 and T 38^0C. In this study it appears that 38^0C enhances the biological removal of PAHs, which might occur due to promotion of the native microbial population and activity [2,26]. Temperature thus is the factor that more strongly affects the disappearance of PAH under in-vessel composting conditions. In addition, higher temperatures may facilitate PAHs volatilization [51,88] and result in a combination of various removal mechanisms, thus higher temperatures should be avoided to minimize the transfer of PAHs from the solid phase to the atmospheric phase. Moisture content is the second most important factor, and thus water amendments may be normally necessary during composing processes. It has been previously reported that the amendment of PAHs contaminated soils may enhance PAH removal [64]. For an elevated operational yield (i.e. higher amount of solid waste treated), a higher S:GW ratio will be advantageous, although a S:GW ratio that is too high may have inhibitory effects on the resident microbial population.

Kinetics of PAH Removal during in-Vessel Composting

Most of the PAH losses occurred within the first 45 days of treatment with a plateau forming by 98 days. These temporal profiles suggested typical biphasic kinetics indicating relatively rapid removal of PAHs during the initial phase of treatment followed by slower removal during the later phase. The rates of removal of PAHs were estimated by linear regression of the temporal profiles of PAH concentration over 98 days of continuous treatment. First order constants of ΣPAH reached a maximum of about 0.014 day^{-1} during optimal composting conditions at S:GW 0.8:1, MC 60% and T 38^0C. The removal rates reported in this investigation are in agreement with those reported by McFarland and Qiu [51] and Potter *et al.* [42] during composting of PAH-contaminated wastes. The reduction in degradation over time in the kinetic study can be explained by reduced bioavailability of PAHs due to immobilization in micropores or changes in binding forms [27,50,89].

C. Organic Matter Dynamics in an Aged Coal-Tar Contaminated Soil During in-Vessel Composting

The application of composting as a bioremediation technology of contaminated wastes has two goals: firstly, to maximize disappearance of the contaminant(s); secondly, to produce mature compost that could be used in land restoration for industrial, municipal or housing developments depending on final quality. By definition, composting accelerates the processes

involved in the biological transformation of organic matter under controlled aerobic conditions to produce a stabilised product, compost, which implies the formation of humic-like substances (HS) [4]. The temporal dynamics of HS during composting may be used to determine the state of composting and the stability or maturity of the compost [4,90,91]. Although maturity of compost is an important factor, with agricultural and environmental implications [92-94], the majority of the reported studies on the use of composting approaches to bioremediate PAH contaminated wastes fail to present results on the assessment of maturity of the final compost [6]. This might be due to various reasons, but the lack of so-called "easy-to–perform" methodologies to characterize the maturity of composting/compost mixtures is certainly one of the most important. Among many available techniques [91,93,95,96], fluorescence spectroscopy has been applied to the characterization and differentiation of humic and fulvic acids of different origins and natures [97,98]. Further fluorescence spectroscopy in the excitation, emission, and synchronous scan modes has been recently applied to the characterization of whole compost from domestic solid wastes sampled at 1, 7, 15 and 30 days of windrow-composting treatment [91]. In this section, the dynamics of organic matter of a coal-tar contaminated soil during in-vessel composting-bioremediation will be assessed by using excitation-emission matrix (EEM) fluorescence spectroscopy.

Total Organic Matter Evolution During Composting

The TOM levels were high (approx. 62%) at the beginning of the composting process and then decreased, reaching a value of approx. 40% after 98 days treatment at 38^0C, thus indicating the occurrence of mineralization. At higher temperatures the TOM decrease was less, which may indicate that mineralization occurred to a lesser extent because higher temperatures constrained microbial growth [26]. That the TOC and ΣPAH concentration decreased during composting is consistent with this interpretation, and indicated that T, S:GW ratio and MC have an important influence on the behaviour of PAHs during composting.

To test whether bioavailability might be a limiting factor on the kinetics of in-vessel composting, relationships between the first order constant of losses and the total organic carbon content following 98 days of continuous treatment were sought. A linear correlation of k_1 (day^{-1}) *vs.* TOM (%) was found (R = 0.88), indicating that lower disappearance rates corresponded with higher organic content in the composting mixtures (Figure 2). Outliers occurred at operational conditions where MC was 40% and also at MC 60%, S:GW 0.6:1 and 70 ^{0}C where, in spite of the periodical water amendment, the reactors were probably under water stress conditions during long periods within the treatment process.

Correlations between microbial degradation of sequestered hydrophobic contaminants and the TOM of the soil in which the contaminant is present have been found [99,100], although TOM is not the sole contributor to the sequestration and desorption processes [72,100,101]. Table 2 summarizes the amount of ΣPAH bioavailable *vs*. the amount of ΣPAH lost after 21, 56 and 98 days of continuous composting treatment under the eighteen different operational conditions investigated. At 38 ^{0}C the majority of the ΣPAH bioavailable was lost. An increase in temperature treatment resulted in a decrease in the ΣPAH bioavailable and a decrease in the ΣPAH lost. At 70 ^{0}C a lower amount of ΣPAH was bioavailable and also a lower amount of ΣPAH was lost as compared to 55 ^{0}C for the identical MC and S:GW conditions. This indicates that different physico-chemical conditions in the composting mixtures due to different operational conditions may have changed the organic matter

structure in a way that it has entrapped higher amounts of PAHs at higher temperatures, making them less available.

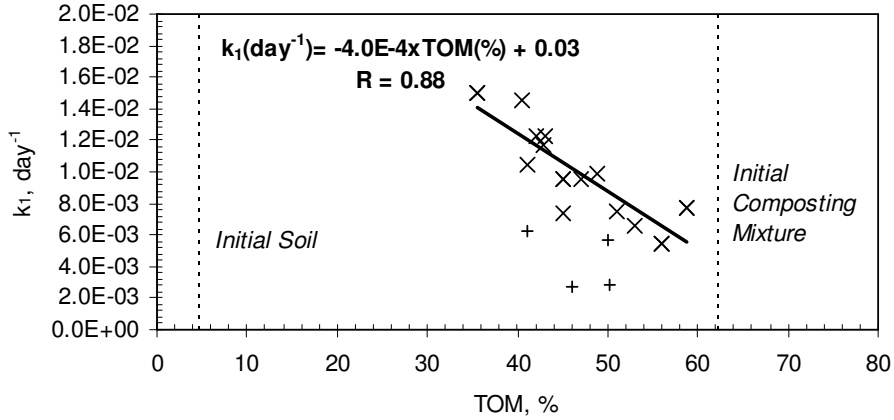

Figure 2. Kinetics of losses of ΣPAH vs. TOM after 98 days of continuous treatment for the eighteen experimental conditions investigated. (Legend: x refers to experimental conditions where MC was 60-80%; + refers to experimental conditions where MC was 40%, and the particular case where T 70 ^{0}C, S:GW 60:1 and MC 60%).

Contour EEM Fluorescence Spectra in Composting Mixtures of a Coal-Tar Contaminated Soil as Compared to other EEM Fluorescence Spectra

Contour EEM spectra of the composting mixtures after 21, 56 and 98 days treatment at three temperature levels (T; 38 ^{0}C, 55 ^{0}C and 70 ^{0}C), four soil to green waste ratios (S:GW; 0.6:1, 0.7:1, 0.8:1 and 0.9:1 soil to green waste mixture ratio on a dry weight basis) and three moisture contents (MC; 40%, 60% and 80%) did not exhibit major differences among them. Figure 3 illustrates the EEM spectra of the composting treatment at S:GW 0.8:1 and MC 60%. The spectra indicate the presence of two different fluorophores, each characterized by an Ex/Em wavelength pair, ~ 338/440 nm and ~ 239/430 nm, designated as primary peak (PP) and secondary peak (SP) respectively. These wavelength pairs have been computed from 54 wavelength pairs identified at eighteen different operational conditions.

The location of EEM peaks in this study was compared with the location of EEM peaks reported in the literature [102]. Excitation and emission boundaries were operationally defined into five regions based on supporting literature [103] to ease the interpretation of the results. Thus, the two different fluorophores observed in this study fell in two different regions defined by the boundaries of Chen *et al.* [103]. The PP fell in the region defined by longer excitation wavelengths (> 280 nm) and longer emission wavelengths (> 380 nm) related to humic acid-like organics. The SP fell in the region defined by shorter excitation wavelengths (< 250 nm) and longer emission wavelengths (> 350 nm) related to fulvic acid-like region.

The maximum Ex/Em wavelength pair ~338/440 nm coincided with peaks typical of humic-like organic substances. Thus, the presence of PP indicates the formation of humic acids during simulated in-vessel composting of an aged coal-tar contaminated soil. Peaks in this region have been previously reported.

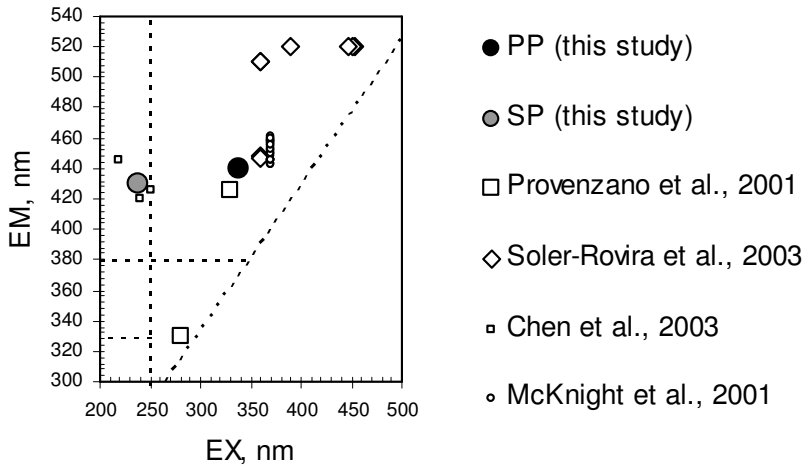

Figure 3. Location of EEM peaks based on literature reports and this study.

Provenzano *et al.* [91] reported peaks characterized by an Ex/Em wavelength pair 330/425 nm related to domestic solid waste following 30 days of composting treatment. Furthermore, Soler-Rovira *et al.* [97] reported peaks characterized by an Ex/Em wavelength pair 330/448 related to humic acids isolated from a composting mixture of a sewage sludge and wood chips and soil amended with this compost at a rate of 80 $Mg \cdot ha^{-1}$ in the presence and absence of barley crop after 180 days. McKnight *et al.* [98] reported peaks characterized by an Ex/Em wavelength pair 370/446 nm related to microbially derived organic matter.

Research in the literature indicated that fulvic acids (FAs) have only recently been investigated to monitor the maturity of composted wastes. Baddi *et al.* [104] reported a significant increase of nitrogen content, a high level of acidic functional groups and an increase of aromatic structures in FAs following 12 months of composting of olive mill wastes, which was related to a high degree of humification or maturation [94]. Molecular components which are potential contributors to the fluorescence of fulvic acids are hydroxycoumarins (Ex/Em wavelength pair 320-343/400-475 nm), chromone derivatives (Ex/Em wavelength pair 320-365/409-490 nm), flavones and isoflavones (Ex/Em wavelength pair 313-365/415-475 nm) and others [105]. These molecular components may derive from lignin and other degraded plant materials and subsequently possibly incorporated into the humic macromolecules during their formation (Senesi *et al.*, 1991). In this study a secondary peak characterized by an Ex/Em wavelength pair ~ 239/430 nm was observed, which additionally coincided with peaks reported by Chen *et al.* [103] typical of fulvic-acid like. Thus, the presence of SP indicates the formation of fulvic acids during simulated in-vessel composting of an aged coal-tar contaminated soil.

Impact of Temperature, Soil to Greenwaste Ratio and Moisture Content on Contour EEM Fluorescence Spectra

Following 98 days of continuous in-vessel composting treatment, the localization of the PP migrated to higher excitation wavelengths. When favourable operational conditions for degradation of PAHs were applied to the in-vessel composting reactors, the excitation wavelength increased from ~ 335 nm after 21 days of treatment to ~ 340 nm after 98 days of

treatment. Similar shifts were noticed at MC 60% and S:GW 0.6:1-0.8:1 and MC 80% and S:GW 0.8:1. No shift of the emission peak position was observed at the mentioned operational conditions. No shift in the PP excitation wavelength was observed at MC 60% and S:GW 0.9:1 and MC 40% and S:GW 0.8:1. Differently, the localization of the SP did not migrate in the EEM fluorescence spectra at different operational conditions.

The optimal operational conditions for disappearance of PAHs from a contaminated aged coal-tar soil during in-vessel composting treatment occur at MC 60%, S:GW 0.8:1 and T 38 ^{0}C. By contrast, the poorest operational conditions for PAH disappearance was observed at MC 40%, S:GW 0.8:1 and MC 60%, S:GW 0.9:1. The EEM spectra in this investigation indicated that the treatment at optimal operational conditions for maximum degradation of PAHs coincide with the highest shift (~ 5 nm) in the excitation wavelength of the PP, while the worst operational conditions coincide with no shift in the excitation wavelength of the PP. The shift of PP toward higher excitation wavelengths may be indicative of the formation of increasing molecular size components, which is consistent with the formation of humic acid-like substances during composting treatment [106].

Fluorescence Intensity Evolution during in-Vessel Composting of an Aged Coal-Tar Contaminated Soil

An increase of fluorescence intensity may result from an increased molecular complexity associated with aromatic structures and conjugated double bonds. On the contrary, a decrease of fluorescence intensity suggests a lower molecular weight and lower degree of aromatic polycondensation [105], which may be related to mineralization of OM. In this study, an overall decrease of the fluorescence intensity of the PP and SP was observed under favourable operational conditions for loss of PAHs during simulated in-vessel composting ($p < 0.01$). Under these favourable conditions, the EEM spectra become simpler in appearance (less contour lines) with in-vessel composting time, which may be related to the mineralization or humification of organic matter that occur during composting [102]. However, the highest fluorescence intensities were observed at the worst operational conditions for biotic disappearance of PAHs, i.e. MC 40% - S:GW 0.8:1 and MC 60% - S:GW 0.9:1, regardless the temperature level, and the EEM spectra did not become simpler in appearance. The results from the multivariable statistical analysis indicated a significant influence of MC on the fluorescence intensity of PP ($p < 0.0001$) and the Tukey test indicated a significant higher fluorescence intensity of PP at MC 40% as compared to MC 60% and 80% ($p < 0.0001$). S:GW presented a less significant influence on the fluorescence intensity ($p < 0.05$) and T presented no significant influence on the fluorescence intensity. Complex EEM spectra may result due to the complex nature of the organic matter present in the coal-tar contaminated soil that under unfavourable conditions have not been metabolized and cause fluorescence in the composting mixture.

Fluorescence Index Evolution during in-Vessel Composting of an Aged Coal-Tar Contaminated Soil

McKnight *et al.* [98] suggested the use of a fluorescence index (ratio of fluorescence emission intensity at wavelength 450 nm to that at 500 nm, or E_{450}/E_{500}) to differentiate the microbially derived FAs (~ 1.9) from those of the terrestrially derived FAs (~ 1.4). In this study the fluorescence index measured at an excitation wavelength of 340 nm (instead of 370

nm used by McKnight *et al.* [98]) at the eighteen operational in-vessel composting conditions after 21, 56 and 98 days of continuous in-vessel composting treatment were recorded [102]. Fluorescence index ranged from ~ 1.5 to ~ 1.9 at 70 ^{0}C and 38 ^{0}C respectively, finding in general higher fluorescence indexes under favourable operational conditions for loss of PAHs during simulated in-vessel composting ($p < 0.01$). Additionally fluorescence indexes were correlated to TOC values of the composting mixture following 98days of continuous in-vessel treatment ($p < 0.01$). The results from the multivariable statistical analysis indicated a significant influence of temperature on the fluorescence index ($p < 0.01$). Fluorescence indexes at 38 ^{0}C were significantly different from those at 55 ^{0}C (Tukey-test, $p < 0.05$) and 70 ^{0}C (Tukey-test, $p < 0.005$). However, a dependence of fluorescence indexes on S:GW ratio or MC was not observed. The fluorescence index computed in this study during in-vessel composting of an aged coal-tar indicates the presence of microbially derived FAs, or microbially mediated mineralization. Additionally, these results indicate that although the fluorescence index was initially developed to elucidate the nature of fulvic acids in water media [98], this index may be also applied to elucidate the nature of humic substances in soil (composting) media.

D. Microbial Community Structure Changes in an Aged Coal-Tar Contaminated Soil During in-Vessel Composting

Active compost has a high level of microbial activity and a rapidly changing microbial community. Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media designed to maximize the recovery of diverse microbial populations. However, only a small fraction (possibly < 0.1%) of the soil microbial community is amenable to investigation using this approach. To overcome these problems, other methods such as the analysis of phospholipid fatty acids (PLFA) have been used in efforts to study a greater proportion of the soil microbial community.

Analysis of Phospholipids Fatty Acids

In total, twenty three different microbial PLFA were identified during the composting process, although only eight were used as biomarkers in this study. The major shifts in the microbial community during the simulated in-vessel composting treatment could be ascertained using fungal to bacterial, and Gram-positive to Gram-negative bacterial PLFA ratios. The initial PAH-contaminated soil contained small amounts of PLFA that were indicative of fungi, giving a low value of the fungal to bacterial ratio (≈ 0.03); there were approximately equal proportions of Gram-positive to Gram-negative bacteria on the basis of signature PLFA content. When this PAH-contaminated soil was mixed with artificial green waste, the mixture contained PLFA indicative of fungi in a higher proportion than did the initial soil (fungal to bacterial PLFA ratio ≈ 1.29), with a dominance of Gram-positive bacteria (Gram-positive to Gram-negative ratio ≈ 1.97). Frostegard and Bääth [80] found that the fungal to bacterial PLFA ratio varied from 0.02-0.04 in different agricultural soils that were low in organic matter to a ratio of 0.3-0.5 in forest soils that were dominated by fungal biomass. Bolta *et al*. [1] found that the fungal to bacterial PLFA ratio in a composted household organic waste with shredded wood varied from 0.12 to 0.15. Andersson *et al*. [107]

reported a fungal to bacterial PLFA ratio of 0.5 in an aged PAH-contaminated soil mixed with birch wood. Fungal to bacterial PLFA ratios calculated in the present study for the initial PAH-contaminated soil and a mixture of the same soil with green waste correlate with the fungal to bacterial PLFA ratios reported in the literature. Thus, the initial composting mixture was dominated by fungal biomass.

Fungal to Bacterial Ratio Changes during Composting

Analysis of the structure of major microbial groups during composting revealed a high proportion of bacterial biomass over fungal biomass (fungal to bacterial PLFA ratio < 1) for the first three weeks that was maintained to the end of the experiment after 98 days (Figure 4). The relative proportion of fungi in the in-vessel composting reactors was lowest at the highest in-vessel composting temperature investigated, namely 70ºC ($p < 0.05$).

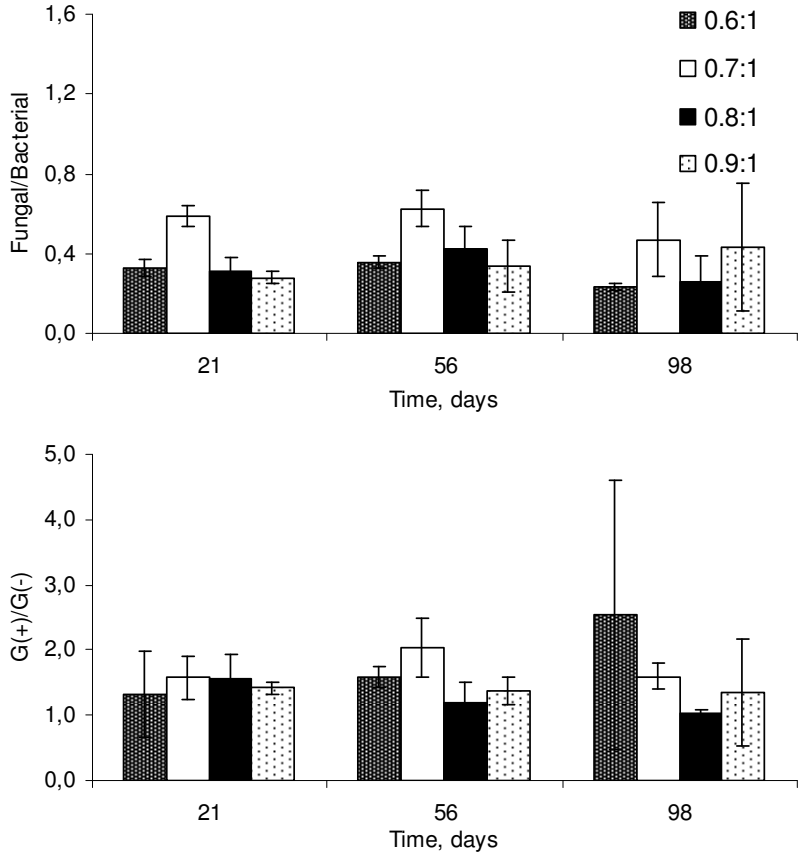

Figure 4. Temporal profiles of (a) fungal to bacterial PLFA ratio and (b) Gram-positive to Gram-negative bacterial biomass ratio at MC 60% and T 38^0C.

These results are comparable with the findings of Klamer and Bääth [83], who reported a rapid decrease in the fungal to bacterial PLFA ratio from 0.37 to 0.007 during the heating phase reaching 69^0C in the composting mixtures of straw materials. They observed a posterior increase in the fungal to bacterial PLFA ratio which never exceeded 0.1 after 100 days of

treatment. However, Carpenter-Boggs *et al.* [84] reported that the PLFA markers for fungi (18:2ω6c, 18:3ω6c) did not change significantly over 60 days following a conventional composting temperature profile that reached a maximum temperature of 60ºC, although they suggested that the types of fungi that were present and active at any stage may have varied significantly. Bolta *et al.* [1] reported a temporal decrease in the relative proportion of fungi during the course of in-vessel composting of a mixture of shredded wood plus organic household waste, in which the average temperature during the first 17 days was close to 60^0C, decreasing to 15^0C after 30 days. No significant influence of the S:GW ratio or MC on the fungal to bacterial ratio was observed in the composting reactors.

Fungal to bacterial PLFA ratios were within the range 0.02 to 0.56 after 98 days of continuous composting treatment, showing that fungi are an important microbial group for in-vessel composting of a contaminated soil, and that drastic changes in microbial community structure occur during in-vessel composting at different operational conditions.

Gram-Positive to Gram-Negative Bacterial Ratio Changes during Composting

Temperature, S:GW ratio and MC all had a significant influence on the Gram-positive to Gram-negative bacterial ratios ($p < 0.05$) during the in-vessel composting treatment (Fig. 4). Thermophilic organic composting systems are largely comprised of bacilli and actinomycetes, and thus a higher relative proportion of Gram-positive bacterial PLFA would be expected at higher temperatures . This was observed at 38 and 55^0C, but not at 70^0C following 98 days of continuous in-vessel composting treatment ($p < 0.001$), probably because 70^0C is above the upper growth limits of such bacteria. The proportions of Gram-positive bacteria at 38^0C and 55^0C were similar, rising during the early stages of composting to a plateau after 21 days of treatment that was maintained to the end of the experiment after 98 days. A similar observation was made by Carpenter-Boggs *et al.* [84], who used an initial temperature of 60^0C decreasing to near 42^0C and 22^0C after an average 28 and 56 days, respectively, in a conventional composting treatment. A high ratio of Gram-positive (which include thermophiles) to Gram-negative bacteria, corresponded to the presence of a large amount of branched-chain fatty acids such as i15:0 and i17:0 that are common in species of *Bacillus*, a genus well known to be dominant in compost at high temperatures [108,109].

In general, the Gram-positive to Gram-negative bacterial PLFA ratio increased while PAH concentration in the composting mixtures decreased, which indicated that Gram-positive bacteria were inhibited in the presence of a high concentration of PAHs. Accordingly, the Gram-positive to Gram-negative bacterial ratio decreased from 21 to 98 days of in-vessel composting, but only in those bioreactors having the larger populations. These results are similar to the findings of Carpenter-Boggs *et al*. [84], who reported a decrease of indicators of general bacteria (15:0 and 17:0) and aerobic bacteria (16:1ω7c) over time. A different trend was observed in the composting reactors treated at 70^0C following 98 days of treatment, which showed a slight decrease of Gram-positive to Gram-negative bacterial ratio at lower PAH concentrations in the composting reactors. The effects of soil to green waste ratio and moisture content on the Gram-positive to Gram-negative bacterial ratio were also investigated. The relative proportion of PLFA indicative of Gram-negative bacteria increased with respect to Gram-positive bacteria at 60% moisture content and a soil to green waste ratio of 0.8:1 to 0.9:1 ($p < 0.01$). The Gram-positive to Gram-negative bacterial ratio changed significantly following the first 21 days of in-vessel composting treatment ($p < 0.05$) but

thereafter no significant changes were observed when the moisture content varied from 40 to 80%.

E. Different in-Vessel Composting Approaches to Remove PAHs in an Aged Coal-Tar Contaminated

One important question yet to be answered for the in-vessel composting approach is how fresh green waste compost (FWGC) instead of green waste (GW), used as composting amendment will impact on the disappearance of the contaminant(s). Thus, the fate of 16 US EPA-listed PAHs during in-vessel composting of the same coal-tar contaminated soil amended with either green waste or fresh green waste composts collected from two landfill sites in the UK were compared during 56 days of continues treatment. Soil to amendment ratio was 0.8:1 on a dry weight basis, MC 60% and T 38^0C, 55^0C and 70^0C [110].

The TOM levels at the beginning of the composting process in the soil amended with the FGWC from Site 1 and Site 2 were 25.60±0.61% and 32.49±0.06% respectively. A decrease in TOM levels was observed in the soil/compost mixture of the soil amended with FGWC from Site 1 treated at all temperature regimes, to 21.48±0.16% within the first 21 days at 38^0C. The soil/compost mixture from Site 2 also presented a significant decrease in TOM level of 23.64±1.04% ($p < 0.005$) treated at 55^0C. Using FGWC instead of GW resulted in a lower disappearance of ΣPAHs during in-vessel composting of the same coal-tar contaminated soil (Figure 5). Both GW and FGWC sustain populations of microorganisms with the potential to degrade a variety of organic contaminants and they can improve the contaminated soil environment for indigenous or introduced microorganisms by changing the soil pH, nutrient status, aeration, and moisture retention characteristics [58]. Amendment with GW facilitated a higher oxygen transfer than FGWC amendment and this may explain why a higher disappearance of ΣPAHs was observed in the soil amended with GW than in the same soil amended with FGWC.

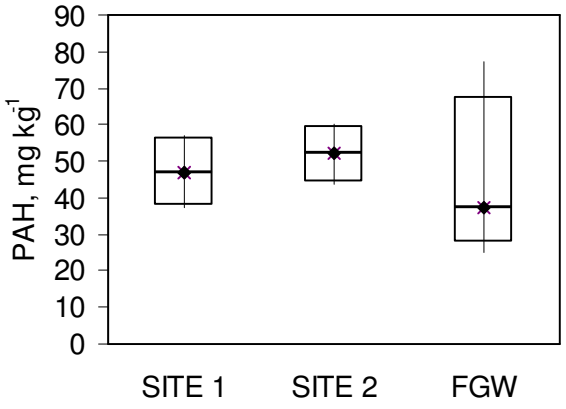

Figure 5. Concentration of PAHs at different temperature profiles after 56 days of in-vessel composting-bioremediation of a coal-tar contaminated soil amended with FGWC from Site 1, Site 2 and GW.

One major concern of using compost as a bioremediation approach is the problem of mixing non-contaminated material with contaminated soil, resulting in a greater quantity of contaminated material if the treatment does not succeed [6]. Thus, although the use of FGWC may offer important operational advantages during the application of the technology at a field scale (i.e. homogenization), this investigation indicated that in order to obtain a higher disappearance of ΣPAHs during in-vessel composting of an aged coal-tar contaminated soil, the use of GW as amendment resulted in a higher disappearance of ΣPAHs than using FGWC as amendment.

V. Regulatory and Sustainable Aspects of in-Vessel Composting as a Bioremediation Technology

Building a more sustainable society by using more sustainable waste management strategies to achieve recycling and landfill diversion, is not only concerned with technical aspects and optimization of physico-chemical and biological parameters, but is also very connected with regulatory and sustainable aspects.

A. Regulatory Aspects

In Europe, the Dutch List provides criteria relating to the clean up of PAH-contaminated soils, although regulatory objectives and priorities vary by country [6]. In the United Kingdom there is an action level of 500 mg PAH·kg^{-1} air-dried soil for land used for recreation and 10,000 mg PAH·kg^{-1} air dried soil for land with a hard covering. According to Italian regulatory guidelines, the maximum PAHs content in soils is 10 mg PAH·kg^{-1} for residential use and 100 mg PAH·kg^{-1} for industrial or commercial use. The United Kingdom and Italy take the view that land should be cleaned to make it fit for its intended use. By contrast, The Netherlands adopt a multifunctional approach whereby land must be fit for any use. Consequently, soil quality limits are much lower than in the United Kingdom, *i.e.* The Netherlands has an action level of 40 mg PAH·kg^{-1} air-dried soil [71,111].

Regulatory objectives and priorities relating to composting of biodegradable waste also vary by country. In the United Kingdom, composting is classified as a waste recovery operation under the Waste Framework Directive. In addition, composting of waste is a vital component of meeting the Waste Strategy 2000 [16] targets for recycling and composting set at 30% by 2010 and 33% by 2015. In Europe, the EC Landfill Directive 1999 [112] sets a target for reduction of biodegradable waste to landfill of 25% by 2010, 50% by 2013 and 65% by 2020. In relation to the longer-term requirements of the EC Landfill Directive, composting of kitchen waste, green waste, and general biodegradable organic waste, which might include contaminated soil, will probably have an important role to play [113]. Additionally, the EU Animal By-Products Regulation [15] requires that the composting of catering waste containing meat (or meat-derived products) must take place in a “closed composting reactor”, operated at least 70^{0}C for 1 hour. This means that catering waste containing meat cannot be treated in an open windrow, except as a second stage after it has first been treated in a closed reactor.

Figure 6 summarizes the regulations to consider at the different stages of the application of composting-bioremediation to PAH-contaminated waste. The 'A' indicates regulations setting up an action concentration of PAHs present in soil, i.e. the New Dutch List, whilst 'B' indicates regulations dealing with organic wastes to compost, i.e. EC Landfill Directive.

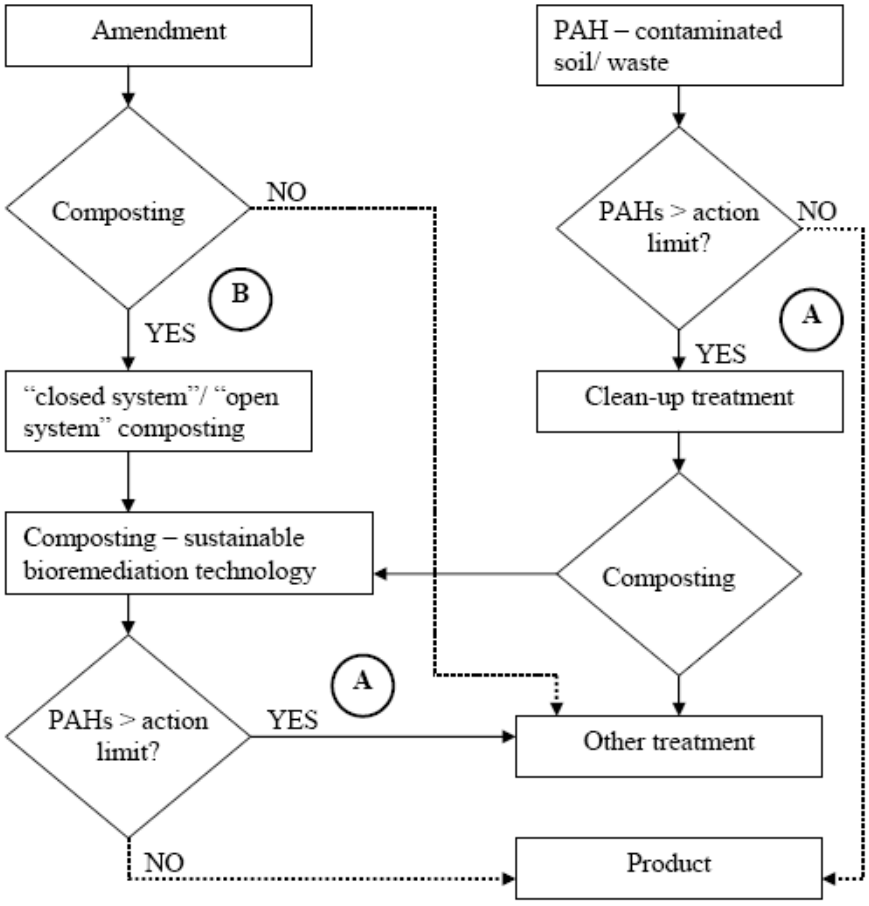

Figure 6. Regulatory considerations for bioremediation of PAH-contaminated soil using composting approaches.

B. Sustainable Aspects

Traditionally biodegradable waste has not been source-segregated and it has been buried in landfills, decomposed via a complex series of microbial and abiotic reactions, producing mainly methane (CH_4) and carbon dioxide (CO_2). The EC Landfill Directive 1999 [112] imposes strict engineering requirements on landfills, requiring landfill CH_4 to be captured and used. In the United Kingdom (UK), there are more than 1,000 landfill sites [114], only ca. 150 engineered landfills [115], thus the largest source of CH_4 emission is from landfill sites where CH_4 escapes through the landfill cover into the atmosphere. A low-cost approach such

as increasing microbial activity to increase CH_4 oxidation using a suitable cover layer can help reducing CH_4 emissions from landfills.

This chapter has indicated that an aged PAH-contaminated coal-tar soil mixed with a biodegradable amendment can be treated by applying composting as a bioremediation technology, and accomplish a final concentration of PAHs below the regulatory limits. Thus, the final compost may be applied to land. It is here suggested that in order to abate CH_4 emission from landfill sites, compost obtained from the treatment of a PAH-contaminated soil/waste using composting as a bioremediation technology would be applied as a landfill cover. By doing so, global CH_4 emissions will be reduced since during composting there are no CH_4 emissions, and posterior application of compost as a landfill cover will reduce possible CH_4 emissions and consequently global warming.

Compost produced from municipal solid waste used as a fertilizer has been demonstrated to improve the quality of soil [116,117]. The application of compost produced following composting of a PAH-contaminated soil or waste may raise public concern if applied to food crops. However, the compost produced following composting of a PAH-contaminated soil or waste may be applied to energy crops. The use of compost as a fertilizer will not only reduce the energy requirements to synthesize inorganic fertilizers, but will also contribute to the production of biofuels. The use of biofuels for transport is becoming increasingly important for a number of reasons such as environmental concerns relating to climate change, depletion of fossil fuel reserves, and reduction of reliance on imports [118,119].

Therefore, the use of in-vessel composting as a sustainable bioremediation technology may contribute to reducing the amount of biodegradable waste buried in landfills and additionally lower CH_4 greenhouse gas emission from traditional waste management practice. Additionally, compost may contribute to energy production. This approach agrees with the recent European Sustainable Development Strategy, which addresses seven key challenges as priorities until 2010, being climate change and clean energy, better management and natural resources among them [108].

VI. CONCLUSIONS

Whilst more research on comparisons of composting/compost bioremediation technologies vs. conventional technologies remain limited, the investigations presented in this chapter have proved that composting, regardless the approach used, is a good sustainable environmental technology that may be used to remove PAH from contaminated soils and wastes. These bioremediation technologies have proved to be beneficial for the amelioration of contaminated soils by reducing the concentration of PAHs, which promotes soil sustainability and soil re-use in contrast to landfill or incineration approaches. Additionally, the final compost may be applied as a landfill cover to abate CH_4 emission from non-engineered landfills and contribute to sustainable development.

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