

# Bioremediation of polycyclic aromatic hydrocarbons (PAH) in an aged coal-tar-contaminated soil using different in-vessel composting approaches

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## Abstract

The biodegradation of 16 USEPA-listed PAHs ( $\Sigma$ PAHs) during simulated in-vessel composting-bioremediation of an aged coal-tar-contaminated soil amended with fresh green waste compost (FGWC) collected from two landfill sites in the United Kingdom (UK) were studied over 56 days. The experimental design compared three constant temperature profiles (TC = 38, 55 and 70 °C) with one variable temperature profile including treatment at 70 °C to comply with regulatory requirements (TP1). The highest disappearance of  $\Sigma$ PAHs was observed in the soil amended with FGWC (53.2% and 48.1%  $\Sigma$ PAHs disappearance in soil amended with FGWC-Site 1 and FGWC-Site 2, respectively) containing lower initial organic matter (TOM) (Initial TOM<sub>FGWC-Site 1</sub> = 25.6 ± 0.6% < Initial TOM<sub>FGWC-Site 2</sub> = 32.5 ± 0.1%). Compared with previous investigations, higher disappearance of  $\Sigma$ PAHs occurred when the same coal-tar soil was amended with fresh green waste (56.0%  $\Sigma$ PAHs disappearance in soil amended with fresh green waste) instead of FGWC. Using a constant TC1 = 38 °C resulted in a higher disappearance of  $\Sigma$ PAHs when compared to other temperature treatments. However, using a variable temperature profile TP1 during in-vessel composting of a soil amended with fresh green waste is required to promote contaminant degradation and pathogen control.

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## 1. Introduction

The use of conventional composting processes to degrade soil contaminants including polycyclic aromatic hydrocarbons (PAHs), chlorophenols, explosives and aliphatic hydrocarbons has been demonstrated in a number of laboratory-based projects and research field trials. Composting has been demonstrated to be effective in biodegrading PAHs using both conventional windrow composting systems [1–4] and in-vessel composting systems [4–6], at both the laboratory and field-scale.

In-vessel composting techniques differ from conventional composting systems in that they are closed systems, like large incubators, providing the opportunity to ensure the use of high temperatures (>70 °C) when needed to meet regulatory require-

ments for pathogen control [7,8]. Use of such high temperatures with in-vessel composting systems inhibits the development of microbial diversity associated with conventional composting systems, which may reduce the effectiveness of the former when used for the composting-bioremediation of contaminated soils [9]. However, in contrast to conventional composting systems, the use of in-vessel systems provides operators with more control, enabling them to select suitable operating parameters (e.g. temperature, moisture content, mix ratios) to promote both microbial activity and contaminant degradation [8].

In previous studies, we investigated the degradation of PAHs in an aged coal-tar-contaminated soil amended with green waste by in-vessel composting, and the influence of temperature ( $T=38, 55$  or  $70$  °C), soil to green waste ratio (S:GW = 0.6:1, 0.7:1, 0.8:1 or 0.9:1 on a dry weight basis) and moisture content (MC = 40%, 60% or 80%) following 98 days of continuous treatment. A factor analysis of the different operational conditions investigated indicated that optimal PAH degradation occurred at  $T=38$  °C, S:GW = 0.8:1 and MC = 60% [5]. At high temperatures (>70 °C) most of the microorganisms would be rendered

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inactive and thus, the disappearance of PAHs occurred mainly due to volatilization [8,12]. We also investigated the impact of using a temperature profile (TP1) on the degradation of PAHs in the same soil amended with green waste following 56 days of continuous treatment compared to the optimal biodegradation conditions, reporting a lower disappearance of PAHs observed for the temperature profile treatment [5].

One important question yet to be answered for the in-vessel composting approach is how a fresh green waste compost instead of green waste, used as composting amendment will impact on the disappearance of the contaminant(s) [8], when the same composting mixture is treated at constant or variable temperature profiles using higher temperatures (up to 70 °C). Thus, the present study was designed to assess the fate of 16 USEPA-listed PAHs during 56 days of continuous laboratory simulated in-vessel composting-bioremediation using (a) two fresh green waste composts collected from two landfill sites in the UK at (b) four temperature profiles. The results obtained in this investigation were compared with our previous results at the same four temperature profiles using the same aged coal-tar soil [5].

## 2. Materials and methods

Four experimental conditions were tested in triplicate using 102 laboratory-scale bioreactors. The bioreactors comprised soil and fresh green waste compost (FGWC, derived from fresh green waste) at a soil to amendment ratio 0.8:1 on a dry weight basis, moisture content MC 60% and four temperature profiles: TC1 = 38 °C, TC2 = 55 °C, TC3 = 70 °C and TP1 = days 0–14 at 38 °C, days 15–35 at 55 °C, days 36–42 at 70 °C and days 43–56 at 38 °C. Batches of 51 reactors were placed in different incubators at TC1-3 and TP1.

### 2.1. 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 [10]. 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 soil contained a post-dilution concentration of 100.3 mg kg<sup>-1</sup> PAHs in soil. Soil organic content was 4.79 ± 0.16% (wt/dry wt).

### 2.2. Fresh green waste compost

The soil was mixed with FGWC and was subjected to simulated in-vessel composting at various temperature conditions. The FGWCs were collected from two Cleanaway landfill sites (Site 1 and in Site 2) in the UK (Table 1). FGWCs was brought to the laboratory, passed through a 2 mm sieve and stored in the laboratory at room temperature before mixing with aged coal-tar-contaminated soil. The composition of the FGWCs satisfied

Table 1

Total organic matter (TOM) and moisture content (MC) of the fresh green waste composts (FGWC) from Site 1 and Site 2, and of green waste (GW) used in previous investigations for composting-bioremediation of a coal-tar contaminated soil

	TOM <sup>a</sup> (%)	MC <sup>a</sup> (%)
FGWC-Site 1	34.44 ± 0.87	38.33 ± 0.75
FGWC-Site 2	44.35 ± 0.18	49.74 ± 0.04
GW <sup>b</sup>	80.46 ± 0.02	47.65 ± 3.29

<sup>a</sup> Values are means in percentage (%) ± S.D. of data from triplicate bioreactors.

<sup>b</sup> Ref. [2].

the nutrient requirement (C:N 40–50) according to the calculations using Cornell's system [11].

### 2.3. Bioreactor's design

One hundred and two glass bioreactors (200 ml) were constructed, which allowed conditions to be easily monitored and controlled and (importantly) enabled us to successfully manage the inherent variability in coal-tar-contaminated soil and 'compost' mixtures allowing us to make meaningful measurements and discriminate changes between investigated time intervals with confidence [5]. A diagram of the bioreactors has been reported by Antizar-Ladislao et al. [12]. Bioreactors were placed in triplicates in temperature controlled incubators at TC1-3 and TP1 to simulate representative mesophilic and thermophilic microbiological stages during in-vessel composting processes [12,13] which simulate full-scale in-vessel composting. For each glass bioreactor, the composting mixture was thoroughly mixed in a glass beaker (500 ml), and then introduced into the reactor. Initial moisture content of the composting mixture was measured (see Section 2.4.1) and double distilled water (DDW) was added when needed to reach the required moisture content for the experimental conditions under investigation (60%). Moisture content was measured after 0, 7, 21, 42, and 56 days to ensure that it was maintained at the required level, and amended with DDW when necessary. The reactor units stood vertically with air flowing continuously, to avoid oxygen content limitation, and vented outdoors to avoid volatiles accumulation in the bioreactors. Air flow up through the soil/compost mixture by means of a stainless steel air-delivery tube inserted into the bottom of the bioreactors 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.

### 2.4. Sample analysis

Destructive sampling was carried out in triplicate for each experimental treatment after 7, 21, 42, and 56 days. The contents of each bioreactor were thoroughly mixed in a 500 ml beaker, and sub-samples were collected for analyses.

#### 2.4.1. Total organic matter (TOM) of soil/compost mixtures

The TOM of composting mixtures was determined by ashing using a loss-on-ignition procedure [14]. Triplicate 5 g

samples were dried at 110 °C for 24 h (moisture content) and then transferred to a muffle furnace held at 550 °C for 12 h. Ash content was calculated from the difference 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 °C).

#### 2.4.2. PAHs analysis

PAH extraction from soil/compost mixtures and soil was by Accelerated Solvent Extraction (ASE™) 200, with 22 ml stainless steel extraction cells meeting the requirements for the extraction of PAHs from solid waste as described in the USEPA Method 3545 [15]. The extracts were purified on chromatographic columns packed with 1 g of activated-florisil (SiO<sub>2</sub>, 84.0%; MgO, 15.5%; Na<sub>2</sub>SO<sub>4</sub>, 0.5%; 60/100 mesh; 130 °C; 12 h) and 2 g of Na<sub>2</sub>SO<sub>4</sub>. 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. Data acquisition and processing was achieved using a Hewlett Packard MS Chemstation (G1034C Version C.02.00). The GC inlet was operated in pulsed (0.90 min, 30.0 psi) splitless mode at 270 °C with helium as carrier gas. The injection volume was 1 µl and the inlet purged at 50 ml min<sup>-1</sup> 1 min after injection; inlet pressure was varied by electronic pneumatics control (EPC) to maintain a constant column flow of 1 ml min<sup>-1</sup>. Separation was achieved using an HP-5MS column (19091S-433 30 m × 0.25 mm × 0.25 µm). The temperature program comprised 70 °C for 2 min, 10 °C min<sup>-1</sup> to 300 °C, which was maintained for 10 min to allow late eluting peaks to exit the column. The MS transfer line was 280 °C providing conductive heating of the MS source to about 230 °C. The MS was operated in

selective ion monitoring (SIM) mode. More detail is given in Antizar-Ladislao et al. [12].

#### 2.5. Data analysis

The effect of different temperature profiles and amendment during in-vessel composting of a coal-tar contaminated soil on the evaluated indicators was investigated using a two way multivariable ANOVA analysis and post hoc Tukey test. All the statistical tests were executed with Statistix Version 1.5.

### 3. Results and discussion

Losses of PAHs in the aged coal-tar-contaminated soil under in-vessel conditions and the influence of temperature (TC1 = 38 °C, TC2 = 55 °C, TC3 = 70 °C and TP1 = days 0–14 at 38 °C, days 15–35 at 55 °C, days 36–42 at 70 °C and days 43–56 at 38 °C), contaminated soil to FGWC (S:FGWC = 0.8:1 on a dry weight basis) and moisture content (MC = 60%) were investigated. The temperature profile TP1 was designed to maximize disappearance of PAHs as well as pathogen control according to the current regulatory requirements [7]. The 16 USEPA PAHs (ΣPAHs) under investigation were grouped as two- and three-ring PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene), four-ring PAHs (fluoranthene, pyrene, benzo[*a*]anthracene, chrysene) and five- and six-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 initial ΣPAH concentration level in the investigated soil

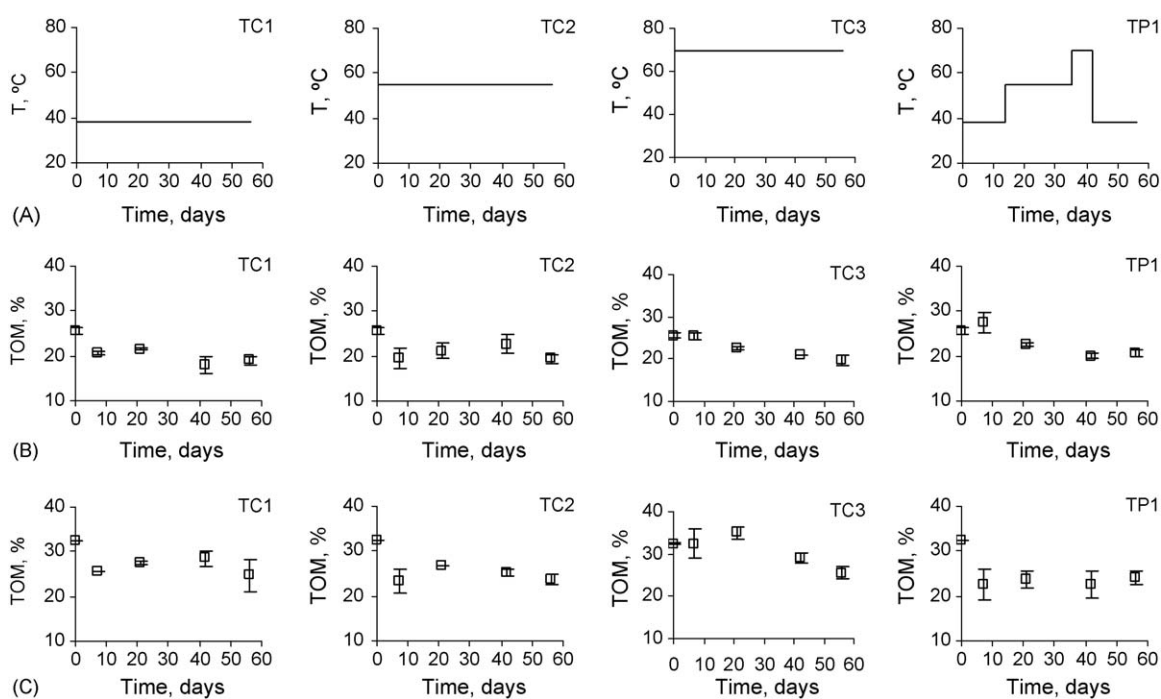


Fig. 1. Temporal profiles in the bioreactors at different temperature profiles (as shown in the top row) during 56 days of in-vessel composting-bioremediation. Row A, temperature treatments; row B, total organic matter in the bioreactors treated with FGWC-Site 1; row C, total organic matter in the bioreactors treated with FGWC-Site 2.

was  $100.3 \text{ mg PAH kg}^{-1}$  air dried soil, above the Dutch List action level of  $40 \text{ mg PAH kg}^{-1}$  air dried soil and thus required to be treated.

The TOM levels at the beginning of the composting process in the soil amended with the FGWC-Site 1 and FGWC-Site 2 were  $25.6 \pm 0.6\%$  and  $32.5 \pm 0.1\%$ , respectively. A decrease in TOM levels was observed in the soil/compost mixture of the soil amended with FGWC-Site 1 treated at all temperature regimes, to  $21.5 \pm 0.2\%$  within the first 21 days and  $20.0 \pm 0.4\%$  within the first 42 days ( $p < 0.05$ ) for treatments TC1 and TP1, respectively. The soil/compost mixture from Site 2 also presented a significant ( $p < 0.005$ ) decrease in TOM levels of  $23.6 \pm 1.0\%$  and  $23.9 \pm 1.5\%$  for treatments TC2 and TP1, respectively (Fig. 1). Additionally, previous work in our laboratory indicated that disappearance of PAHs from composting mixtures was governed by biological mechanisms [12].

### 3.1. Constant temperature versus temperature profile

The versatility of the designed in-vessel composting simulation system was used to investigate the disappearance of PAHs in a coal-tar contaminated soil amended with FGWC during 56 days. The concentrations of the 16 USEPA-listed priority pollutant PAHs investigated before and after 56 days treatment at TC1, TC2, TC3 and TP1, respectively (as  $\text{mg PAH kg}^{-1}$  dry soil) are presented (Fig. 2, Table 2).

The use of different temperature profiles resulted in different final concentration of  $\Sigma$ PAHs. In the bioreactors amended with FGWC-Site 1 a temporal temperature profile resulted in a lower disappearance of  $\Sigma$ PAHs when compared to treatments at a single temperature: after 56 days of continuous in-vessel composting treatment,  $\Sigma$ PAHs concentration in the bioreactors amended with FGWC-Site 1 was  $37.9 \pm 15.3$  and  $57.1 \pm 0.6 \text{ mg kg}^{-1}$  at temperatures TC1 and TP1, respectively. Similar results were observed in the bioreactors amended with FGWC-Site 2:  $\Sigma$ PAHs concentration in the bioreactors amended with FGWC-Site 2 was  $46.0 \pm 6.8$  and  $60.3 \pm 4.2 \text{ mg kg}^{-1}$  at temperatures TC1 and TP1, respectively.

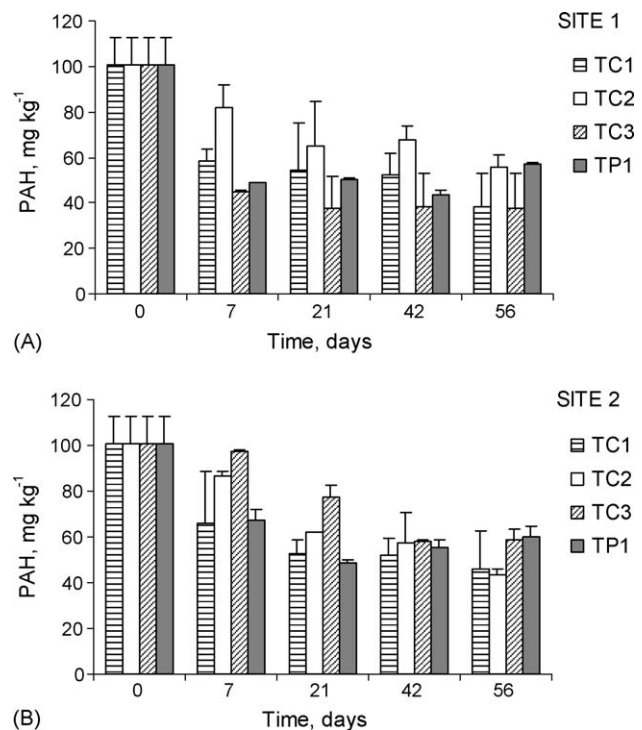


Fig. 2. Concentration of  $\Sigma$ PAH at different temperature profiles during 56 days of in-vessel composting-bioremediation. Row A, in the bioreactors treated with FGWC-Site 1; row B, in the bioreactors treated with FGWC-Site 2.

The disappearance of  $\Sigma$ PAHs was higher in the bioreactors amended with FGWC-Site 1 than in those amended with FGWC-Site 2 (Fig. 3). Previous studies have reported that significant correlations between biodegradability of sequestered hydrophobic contaminants and the TOM [16] or humic acid contents [17] of the solid matrix in which the contaminants are present exists. This may explain higher disappearance of  $\Sigma$ PAHs in soils amended with FGWC containing lower initial TOM levels in this study (Initial  $\text{TOM}_{\text{FGWC-Site 1}} = 25.6 \pm 0.6\% < \text{Initial } \text{TOM}_{\text{FGWC-Site 2}} = 32.5 \pm 0.1\%$ ).

Table 2  
Small, medium and large PAH concentrations at the beginning and after 56 days of in-vessel composting-bioremediation treatment using green waste amendment

	Time (days)		Small <sup>a</sup> ( $\text{mg kg}^{-1}$ )	Medium <sup>a</sup> ( $\text{mg kg}^{-1}$ )	Large <sup>a</sup> ( $\text{mg kg}^{-1}$ )	$\Sigma$ PAH <sup>a</sup> ( $\text{mg kg}^{-1}$ )	Disappearance (%)
Initial	0		$32.5 \pm 1.3$	$46.5 \pm 3.9$	$21.4 \pm 1.8$	$100.3 \pm 3.2$	–
FGWC-Site 1	56	TC1	$3.3 \pm 0.2$	$14.2 \pm 1.5$	$20.3 \pm 1.4$	$37.9 \pm 15.3$	62.4
	56	TC2	$3.3 \pm 0.1$	$28.9 \pm 0.8$	$23.2 \pm 0.4$	$55.4 \pm 5.4$	44.9
	56	TC3	$1.9 \pm 0.2$	$18.2 \pm 1.6$	$17.4 \pm 1.3$	$37.5 \pm 15.4$	62.8
	56	TP1	$4.0 \pm 0.3$	$33.9 \pm 0.1$	$19.3 \pm 0.3$	$57.1 \pm 0.6$	43.2
FGWC-Site 2	56	TC1	$4.5 \pm 0.4$	$20.0 \pm 2.7$	$20.5 \pm 0.6$	$46.0 \pm 6.8$	54.3
	56	TC2	$1.0 \pm 0.3$	$21.0 \pm 0.3$	$21.5 \pm 0.2$	$43.5 \pm 2.3$	56.8
	56	TC3	$0.8 \pm 0.1$	$36.2 \pm 0.4$	$21.6 \pm 0.6$	$58.6 \pm 4.7$	41.7
	56	TP1	$12.4 \pm 0.4$	$28.6 \pm 0.9$	$19.4 \pm 0.4$	$60.3 \pm 4.2$	40.0
GW <sup>b</sup>	56	TC1	$2.3 \pm 0.1$	$12.9 \pm 0.1$	$9.6 \pm 0.3$	$24.9 \pm 3.0$	75.4
	56	TC2	$14.8 \pm 0.1$	$8.3 \pm 0.1$	$13.1 \pm 0.3$	$36.1 \pm 3.0$	64.2
	56	TC3	$29.1 \pm 0.3$	$28.2 \pm 0.7$	$20.1 \pm 0.5$	$77.4 \pm 4.6$	22.9
	56	TP1	$8.2 \pm 4.5$	$18.5 \pm 4.9$	$11.5 \pm 1.6$	$38.2 \pm 11.0$	62.1

<sup>a</sup> Values are means  $\pm$  S.D. of data from triplicate bioreactors.

<sup>b</sup> Ref. [2].

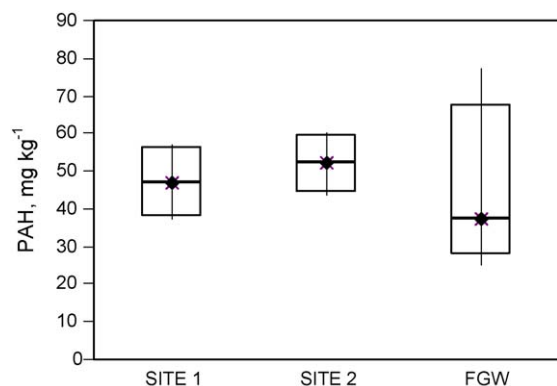


Fig. 3. Concentration of  $\Sigma$ PAH at different temperature profiles after 56 days of in-vessel composting-bioremediation of a coal-tar contaminated soil amended with FGWC from Site 1, Site 2 and FGW.

Results from previous studies [12] indicate that difference in the amount and rate of disappearance of PAHs in these comparative temperature profile studies may be explained due to a suppression of microflora diversity including white-rot fungi [18,19] and growth of microbial communities at higher temperatures ( $>55^{\circ}\text{C}$ ). In addition, native microflora may have been negatively influenced by changing their environmental conditions, i.e. temperature, during the length of the composting treatment reducing bacterial and fungal activity. Previous investigations have recommended that compost mixture moisture content and amendment ratio should be kept constant for optimal disappearance of aged PAHs during composting [20], which is supported by this study.

This investigation indicates that optimal temperatures for maximum disappearance of small, medium and large PAHs are all within the mesophilic range ( $30\text{--}50^{\circ}\text{C}$ ). However; when composting processes using temperature profiles are needed to meet regulatory requirements, it is recommended that they first allow a long mesophilic stage before finishing with thermophilic, cooling and maturation stages to achieve maximum disappearance of the most recalcitrant large PAHs (e.g. TP1).

### 3.2. Fresh green waste versus fresh green waste compost

Recent investigations on the bioremediation of PAHs in the same coal-tar soil amended with fresh green waste indicated that most of the PAH losses occurred within the first 45 days of treatment [5]. A factor analysis of the 18 different operational conditions under investigation indicated that the optimal operational conditions for degradation of PAHs in simulated in-vessel composting units occurred at  $\text{TC1} = 38^{\circ}\text{C}$ ,  $\text{S:GW} = 0.8:1$  and  $\text{MC} = 60\%$ , resulting in 75.4% losses of the total PAHs. Additionally, first order constants of losses of  $\Sigma$ PAH estimated by linear regression of the temporal profiles of PAH concentration over 98 days of continuous treatment reached a maximum of about  $0.014\text{ day}^{-1}$  during optimal composting conditions. Using FGWC instead of FGW resulted in a lower disappearance of  $\Sigma$ PAHs during in-vessel composting of the same coal-tar contaminated soil (Fig. 3).

FGW 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 [21]. FGW amendment facilitated a higher oxygen transfer than FGWC amendment and this may explain why a higher disappearance of  $\Sigma$ PAHs was observed in the soil amended with FGW than in the same soil amended with FGWC.

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 [8]. 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  $\Sigma$ PAHs during in-vessel composting of an aged coal-tar contaminated soil, the use of FGW as amendment resulted in a higher disappearance of  $\Sigma$ PAHs than using FGWC as amendment.

## 4. Conclusions

The present closely controlled study indicated that in-vessel composting-bioremediation of an aged coal-tar soil amended with green waste can reduce PAH concentrations by 75% after 56 days of continuous treatment following a constant temperature,  $\text{TC1} = 38^{\circ}\text{C}$ . In-vessel composting-bioremediation of the same coal-tar soil amended with fresh green waste compost can reduce PAH concentrations by 62% (FGWC-Site 1) and 54% (FGWC-Site 2) after 56 days at  $\text{TC1} = 38^{\circ}\text{C}$ . Thus, using green waste as composting amendment instead of fresh green waste compost is required to achieve a higher disappearance of PAHs. Using a constant  $\text{TC1} = 38^{\circ}\text{C}$  resulted in a higher disappearance of PAHs as compared to TP1, which resulted in 43%, 40% and 62% disappearance of PAHs using FGWC-Site 1, FGWC-Site 2 and fresh green waste as composting amendment, respectively. However, the use of TC1 is not a valid option because it does not meet with current UK regulatory requirements for pathogen control. Therefore, using a variable temperature profile during in-vessel composting is required to promote contaminant degradation, microbial activity, mature final compost and in the same time meet regulatory requirements for pathogen control.

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