Effects of compost stability and contaminant concentration on the bioremediation of PAHs contaminated soil through composting

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Abstract

The objective of this study was to investigate the effect of two factors: the stability degree (0.37 - 4.55 mg O$_2$ g$^{-1}$ Organic Matter h$^{-1}$) of different composts derived from the organic fraction of municipal solid wastes and the concentration of a complex mixture of PAHs including Flourene, Phenanthrene, Anthracene, Flouranthene, Pyrene and Benzo(a)anthracene in the bioremediation of soil. The two factors were systematically studied applying central composite design methodology. The obtained results demonstrated that compost stability degree was particularly important during the first stage of the process. Stable composts enhanced the levels of degradation in soil-compost mixture and a degradation rate of 92% was achieved in this period, but only 40% was degraded with the least stable compost. The PAHs concentration was also important during the process, since the degradation rates increased with the increase in the PAHs concentration. Moreover, all the individual PAHs demonstrated a notable decrease in their concentrations after the incubation period, but pyrene was degraded to lower levels in some treatments compared to others PAHs.

Keywords: Compost; Polycyclic aromatic hydrocarbons (PAHs); Soil; Stability; Experimental design.
1. Introduction

The large range of hazardous chemicals with different structures and different toxicity levels that are continuously released from several anthropogenic sources are continuously causing environmental pollution [1]. Polycyclic aromatic hydrocarbons (PAHs) are one of the most encountered pollutants in the ecosystems; as a consequence, soil contamination with these contaminants is a matter of major concern as they can be introduced to the soil by various sources, where these compounds are categorized as toxic for both humans and environment [2]. Indeed, with more concern regarding the ecosystem and more strict regulation like the EU landfill directive (1999/31/EC), which tries to reduce the amount of wastes that can be sent to the landfill, transforming the contaminated soil to such landfills has become limited. Furthermore, the restoration of many contaminated sites is preferable as the available agricultural areas are gradually degraded with time [3]. Accordingly, there is a critical need to develop and implement an effective remediation technology to reduce the threats caused by such contaminants and create a sustainable reuse of soil.

Bioremediation can be regarded as an attractive technology that results in the partial or complete biotransformation of many organic contaminants to microbial biomass and stable innocuous end-product. Moreover, this technology is believed to be cost-effective and environmentally accepted [4,5]. The contaminated soil are normally deficient in nutrients that are necessary to support the indigenous microorganisms to develop themselves, or sometimes the microorganisms are only available at low levels that makes the bioremediation process progress at very slow rates [6]. To overcome these conditions, normally the bioremediation of hydrocarbon contaminated soils often rely upon the addition of nutrients or microorganisms (biostimulation and bioaugmentation) [7,8]. Composting as a remediation tool has been considered a
suitable technology in the bioremediation of contaminated soils, and it has been used to
mitigate these limiting factors, as it also improves the soil properties [9]. On the other
hand, applying composting technology provides a sustainable reuse of the organic
biodegradable fraction of wastes, which is both microbial and nutrient rich. However,
contaminants bioavailability is an important factor affecting microbial degradation rates
in soil and sediments as the microorganism are able to attack the target contaminant
only when it is dissolved within the materials [10]. Thereby, the selected organic
amendment for the bioremediation process should serve to improve and overcome any
deficiencies or limitations that influence the process efficiency. One challenge with this
type of research is that composting feedstock composition can vary widely from one
facility to another; this can affect the chemical and microbial conditions in the
amendments [11]. Bioremediation of contaminated soils using composting process
depends on a number of physical, chemical, and biological factors that determine the
microbial accessibility to the target contaminants [10,12,13], where the amendment
properties are of great role in determining the process behaviour. Although various
amendments have been applied during the composting of contaminated soil, still much
specialized research is needed. Compost stability is one of these factors as this
parameter is related to microbial activity within the organic material and it also can be
related to the compost composition like humic matter [6]. Until now, no studies were
reported that explore this important parameter and its influence on the degradation of
PAHs in the soil. For an efficient treatment process, the microbial activity, which is
considered the main factor in the bioremediation process, should be maintained at
adequate levels [14]. The contaminants concentration is one of the factors that influence
the microbial activity as these contaminants may exhibit some toxic or inhibition
influence when they exist at high levels. However, low concentration could be below
the required level needed to provoke the microbial enrichment to attack this contaminant [15].

In this research, we tried to investigate the bioremediation process of soil contaminated by a complex mixture of PAHs using the composting technology, where the mixture of PAHs simulated a real creosote sample. Different composts derived from the organic fraction of municipal solid wastes were suggested as organic amendments considering the effect of their different degrees of stability as an objective to be clarified. We believe that although the development and widespread application of bioremediation process are in a good research position, it still is limited by a lack of full understanding of such important factors (compost stability and pollutant concentration). For this reason, this two factors were studied systematically using central composite design methodology. All the experiments were scheduled and carried out under laboratory-scale level that is representative of real composting conditions.

2. Materials and methods

2.1. Soil

The soil used in this study was collected from the surface horizon (0-30 cm) in Prades (Tarragona, Spain). The zone where the soil was collected is an agricultural one; hence it is free from any PAHs contamination and preliminary analysis confirmed that there are no PAHs in the used soil or their concentration are below the detection limits. This soil is classified as sandy loam soil and consists of 73% sand, 19% silt and 8% clay. For the experimental purpose, the soil was air-dried, sieved to 2 mm and kept at 4°C until use. Some characteristics of the soil were determined and are presented in Table 1.
2.2. Chemicals

Different compounds of PAHs were purchased from (Sigma-Aldrich, Spain). These PAHs include: Flourene, Phenanthrene, Anthracene, Flouranthene, Pyrene and Benzo(a)anthracene with 98-99% purity. According to the US Environmental Protection Agency (USEPA), these PAHs are classified as priority pollutants as they are toxic and carcinogens. As a group of contaminants to be followed during the experiment course, the abovementioned six PAHs were mixed together in a stock solution that was spiked into the mixture soil-compost with a concentration based on total PAHs. The percentage of each individual PAH compound as a part of the total PAHs concentration ($\sum$PAHs) was 30%, 29%, 9%, 20%, 3.5%, 8.5% respectively. These percentages were determined according to the results of fractionation process of a creosote sample (Creosote lot: 42-13B, Chem Service, SUGELABOR S.A, Spain) in our laboratory using the method 3611B of the USEPA, where the volatile part was ignored. A stock solution of the used PAHs was prepared using the previous percentages. Afterwards, they were spiked into the soil to obtain the desired concentrations according to the experimental design matrix (0.1-2 g/kg) as total PAHs. For instance, the low to high concentrations were applied to understand the performance of the process under such conditions considering PAHs concentration as an important factor in a remediation technology. Also the same stock solution was used to calibrate the instrumentation for the PAHs determination.

2.3. Organic co-substrates

As an organic co-substrate (amendment) to be added for stimulating the composting process, composts derived from the organic fraction of municipal wastes (OFMSW) were applied during the bioremediation experiments. In this study, five types of OFMSW composts were used during the experimental treatments. The main
difference between these composts was the degree of stability “the rate of organic matter decomposition as a result of the microbiological activity” and it is mainly related to the availability of readily degradable substrates. The different levels of stability were determined using the Dynamic Respirometric Index (DRI). Only compost B was obtained from a home composter in the University Autònoma of Barcelona, where the others were obtained from composting plants located in the Barcelona area (Spain). These composts were selected to be characterized by a different degree of stability ranging from full-stable to unstable compost [16, 17], which would provide the ability to examine their effects on the degradation of the used PAHs and to relate and predict the effect of their major components as a consequence on the bioremediation process. The main characteristics of the used composts are also presented in Table 1.

2.4. Composting reactors and monitoring instruments

The used reactors were Dewar® vessels (4.5-L), which were modified and conditioned to operate in a batch-mode way for the composting experiments. These reactors are thermally isolated, so the process can be kept under the natural composting temperatures, and the influence of ambient temperature can be minimized. Aeration was provided through a pipeline connected to the bottom of the reactor where a plastic mesh is placed to insure a correct distribution of the air through the composting mixture, and the exhausted air exits the reactor through an outlet in the reactor cover. Oxygen concentration was measured by means of an oxygen sensor (Crowcon’s Xgard, United Kingdom), where the inlet of the sensor is connected to the reactor outlet and consequently the oxygen percentage in air was determined. Aeration rate and frequency were adjusted to prevent any limitation or excess in the oxygen percentage in the
reactors, consequently, sporadically aeration mode was used and the oxygen concentration was well maintained to insure aerobic conditions (more than 10%). Temperature was monitored by Pt-100 sensors (Sensotran, Spain) connected to a data acquisition system (DAS-8000, Desin, Spain) that was connected to a personal computer. The software used (Proasis®Das-Win 2.1, Desin, Spain) also permits to monitor both the temperature and oxygen content in the reactors. These two parameters (temperature and oxygen concentration) are useful to control and follow the process during the different phases.

2.5. Experiments set-up

The PAHs were mixed together according to their percentages to be introduced as the target contaminants during the composting process. These contaminants were spiked into soil to have the initial concentration expressed as total of PAHs according to the values determined by the experimental design technique that were decided to be from 0.1g/kg to 2g/kg (dry matter). After this, the contaminated soil was mixed with the organic amendment at ratio of 1:1 (w:w, dry weight). The mixture was then mixed with bulking agent at a ratio 1:1 (V:V) in an attempt to provide proper porosity to maintain aerobic conditions. All the components of the composting treatments were manually mixed according to the aforementioned ratios, resulting in about 3.5 kg that were used in each reactor. The used bulking agent consisted of wood chips and pruning wastes that were not biodegraded under laboratory composting conditions. Water content of the composting mixtures was adjusted to be within the recommended values (50-60%) by adding tap water before incubation. The composting matrix was left under natural composting temperatures. Aeration flow rate and frequency were monitored and adjusted during the process to avoid any limitation or excess in the oxygen
concentration that may affect the process. All the composting mixtures were manually prepared according to the proposed values of the experimental design technique (Table 2) and were incubated for 30 days.

2.6. Sampling

During the incubation period, the performance of the process was monitored and samples were collected after 10, 20 and 30 days of composting in order to measure the degradation rate in these periods. For sampling, the reactors were opened and the reactor contents were manually well mixed. Then duplicate grab samples (20-30 g) were taken. During each sampling, moisture content was adjusted, if necessary. Thus, the composting mixture was moistened with tap water and remixed well to maintain water content within the optimum values (50-60%).

2.7. Analytical procedures

2.7.1. Co-substrates stability degree

Compost stability was determined using the Dynamic Respirometric Index (DRI), determined according to Barrena et al. [18]. Briefly, this index represents the oxygen consumption by microorganisms to degrade the easily degradable organic matter of a sample within a unit time (h) when it is incubated under optimal and controlled conditions and with a continuous air supply. About 150 g duplicated samples were incubated in 500 ml Erlenmeyer glass flasks provided with a plastic mesh placed in the bottom to support the incubated sample and to ensure equally distributed air. The flasks are perfectly sealed with a rubber pieces where the aeration and exhausted air tubes passing through. The samples are incubated in a thermostatic water bath adjusted at 37˚C where the respirometer system is supplied with an oxygen sensor, a control
cabinet and air supply system based on mass flow-meters and personal computer unit. A constant air flow was supplied to ensure that the oxygen concentration in the exhausted air is greater than 10%. As a result DRI provides an accurate measure of the biological stability of the organic matter contained in the biomass in form of the maximum respiration activity (Oxygen Uptake Rate) of the samples. The complete details of this analysis can be found elsewhere [19,20].

2.7.2 PAHs analysis

The content of the PAHs in the composting mixture was determined after extraction using a Soxhlet extraction process. Duplicated 10 g samples were extracted using acetone/dichloromethane (1:1 v/v) as solvent during two hours. Afterwards, the solvent was left to evaporate and then the remaining residue (extract) was dissolved in 10 ml of dichloromethane. A 1-µl extract of this solution was injected in a gas chromatograph (GC8690N, Agilent, Spain) equipped with flame ionization detector (FID) and a splitless injector. A Zebron ZB-5HT Inferno column (Agilent, Spain) was used. Initial temperature was maintained at 50˚C for 1 min, and then it was increased at a rate of 7˚C/min until 320˚C, then another rate of 20˚C/min until 400˚C was applied and maintained at this final temperature for 5 min. The concentration of the PAHs was determined after the calibration of the method with standard PAHs samples.

To investigate the volatilization of the PAHs during the composting process, samples from the exhausted air were collected using Tedlar bag of known volume [21], and then samples of 1 ml of that air were analyzed using the same GC methodology. However, this test was simply used to check if part of the PAHs decrease is caused by volatilization, but the actual amount of the volatilized PAHs could not be determined as only small amounts of some low molecular weight PAHs were detected.
2.7.3. Other characteristics

The other characteristics of both soil and organic co-substrates including: moisture content, organic matter content (OM), pH, electrical conductivity, organic carbon, Kjeldahl nitrogen and humic matter fraction were determined on collected samples according to standard methods [22].

2.8. Experimental design methodology

For bioremediation of PAHs-contaminated soil through composting, the influence of PAHs concentration \( (x_1) \) and compost stability \( (x_2) \) were processed and statistically validated through setting up a Central Composite Design (CCD) technique and a second-order model was produced to correlate the studied factors. A series of experiments were carried out at different points as follow: four experiments using the extreme points \((\alpha=1.414)\), four experiments corresponding to a two factors complete factorial design and two experiments at central points. For the validation of the design, two more experiments were conducted at the central points and so 12 experiments as total were carried out and five values for each independent variable were tested.

To simplify the recording of the conditions and processing of the experimental data, the factor levels were coded with the notations \((-\alpha, -1, 0, +1, \alpha)\). The value of \(\alpha=1.414\) was determined according to the number of the studied factors (2). The experiment design technique was carried out as it is explained in the literature [23,24]. Table 2 presents the design matrix, where the coded and the actual values of the studied factors and their combination are described, also the actual response of the process \((Y)\) after 10, 20 and 30 days is also shown.
2.9. Data statistical analysis

Statistical analysis was performed for all variables using the Sigmaplot® 8.0 software package (Systat Software Inc, San Jose, USA). The replication of experiments at central point permits the statistical validation of results according to CCD experimental design [23].

3. Results and discussion

3.1. Soil and co-substrates characterizations

In this study several types of OFMSW compost were evaluated as an organic amendment during the composting of PAHs-contaminated soil. Obviously, the available high content of organic matter in these composts in comparison with the available amount in the soil (Table 1) is thought to be needed to support and develop the microbial activity during the bioremediation process. Meanwhile, these composts are suggested to provide valuable populations of microorganisms that presumably can degrade the contaminant [6]. One of the most important characteristics of the applied amendments that play a major role in the remediation process is the humic matter portion [24,25]. Analysis of the composts showed that humic matters are available among them, but their portions are different according to their stability degree. Indeed, humic matter as part of the compost organic matter was found to be increased with stability degree in the sense that, the more stable the compost was the higher humic matter content was observed. However, compost C deviated from this fact to a small extent, which might be attributed to its high content of organic matter (Table 1). The other characteristics are almost considered within the acceptable levels for such process. Generally, the growth factors in the used compost are better than those in the soil;
consequently, these organic amendments may have a major role in improving the
degradation of the contaminants.

3.2. Response surface and statistical analysis

The obtained percentage of PAHs degradation (Y) after 10, 20 and 30 days were
used as functions to correlate the studied factors (x₁ and x₂), where second-order
polynomial model was used to fit these values as shown in the following equations:

\[ Y_{10} = 75.2 + 3.36x_2 + 2.16x_1^2 - 2x_2^2 - 1.59x_1x_2 \]  
\[ Y_{20} = 82.33 + 6.57x_1 - 2.3x_1x_2 \]  
\[ Y_{30} = 106.33 - 24.59x_1 - 4.52x_2 + 8.5x_1^2 + 2.4x_1x_2 \]

Depending on these equations the regression coefficients (R) of Y₁₀, Y₂₀ and Y₃₀ were
0.83, 0.53 and 0.6 respectively. The obtained regression coefficient after 10 days
represents a good regression model, whereas those of 20 and 30 day represent a non
perfect regression. In all cases the P values describing the significance levels were not
concluding (P>0.05). However, although these statistical values are not within the
preferable values to describe the process, the constant variance test indicated that these
can be used to predict the degradation rates within ±10%.

3.3. The composting process

Temperature variations of the composting materials with time in some
experimental runs are illustrated in Figure 1. A lag phase was observed even though its
duration varied among the treatments, but it was clear in run 9 where about two days
were needed to stimulate the microorganisms. As a result of the microbial activity,
temperature began to rise to thermophilic ranges that were achieved during the first
week in the treatments when less stable composts were used, but it was always in the
mesophilic ranges when more stable composts. It is believed that the achieved
thermophilic temperatures in the beginning of composting were attributed to the
sufficient amounts of easily degradable materials [19,26]. This assumption agreed with
results obtained regarding the organic matter degradation (Figure 2) that showed a
notable decrease in this stage for the less stable composts. Moreover, high aeration and
frequencies were needed during the first stage of the process especially in the less stable
composts (data not shown), which implied that the microbial activity was intense.
However, as the materials became more stable and the process entered to the cooling
phase, less amount of air was needed. The temperature increases as well as the reduction
in the organic matter fraction during the whole process are the most important evidences
of the process [17,26].

3.4. PAHs degradation

The degradation of the PAHs was assessed during the entire incubation period
(30 days). Figure 3 presents the remaining PAHs throughout the different runs. By the
end of the incubation period, high rates of degradation (76.11%-96.53% as total PAHs)
were achieved among all the experiments except the run 6 where only 45.8% was
achieved. For instance, during the first 10 days the highest rate of degradation (92%)
was observed in run 2, whereas the lowest rate (18%) was in run 6 during this period,
and in the other runs it was within 40%-80%. During the remaining period, a low rate of
degradation was observed. The contrast among the different treatments could be clearly
visualized during the first 10 days of incubation as different degradation rates were
obtained. Thus, the compost stability appeared as an effective factor especially when
treatments with the same concentration are to be compared (run 5, 8 and 9). In run 9,
which had the most active compost, almost 40% of the PAHs were degraded during that
period (10 days). However, almost the double degradation rates were obtained when more stable compost was used. It is worthy to indicate that less stable composts got stabilized with more incubation time. Consequently, degradation rates with these composts improved with time, for instance, degradation rate of 76% after 30 days was obtained in run 9. Nevertheless, this rate of degradation was still less than those obtained under the same conditions when more stable compost was supplied. On the other hand, the rates of degradation were found to be varied under the different concentrations, which imply that the degradation process is also influenced by this factor. In general, the PAHs degradation was influenced by the two factors, where the used amendments were able to enhance the degradation process to a great extent.

Regarding the degradation pattern of the individual PAHs, they demonstrated a notable rate of degradation in the treatments except in the case of pyrene, which had a recalcitrant behaviour especially in the first 10 days where a low rate of degradation was observed in almost all the experiments. However, with more incubation time, its concentrations was decreased but not as the other PAHs. The PAHs degradation order was the same among the experiments and it was, in general: Flourene > phenanthrene > anthracene > flouranthene > benzo(a)anthracene > pyrene. However, in run 6 it was different and was: flouranthene > phenanthrene > flourene > benzo(a)anthracene > anthracene > pyrene. Clearly, neither the solubility nor the octanol-water partition coefficient (K_{ow}) order of these PAHs controlled the degradation. According to [27], the molecular conformation prevails over other parameters in controlling the degradation when the degradation trends for each hydrocarbon are similar under all conditions.

In general, the rate of degradation during the first 10 days was faster compared to the rest incubation period where less degradation occurred. In this sense, the organic contaminants could be sequestrated into the matrix of the soil as this process is
generally a function of time [28]. Analysis of exhausted air samples indicated that very small amounts of some low molecular weight PAHs were volatilized during the thermophilic stages (especially for temperatures over 50˚C), thus a portion of these PAHs reduction is due to volatilization but this amount is so small compared to that resulting from the biodegradation as these elevated temperatures remained for about one week in the reactors with less stable compost. However, the majority of the experiments were in the mesophilic ranges (Figure 1) where no or negligible volatilization occurred. It was also reported that although a portion of the total petroleum hydrocarbons reduction is due to volatilization, abiotic loss has been reported to be generally less than 10% at 25˚C in the first 30 days [29].

3.5. Effect of compost stability on PAHs degradation

The response of the PAHs degradation percentages under different composts stability degrees denoted by DRI (0.37-4.55 mg O$_2$ g$^{-1}$ OM h$^{-1}$) are illustrated in Figure 4. As shown, the process response is changed when different composts were used as well as during the different composting stages indicating that the compost stability is one of the factors that influence the process performance. This effect was significant during the first stage of the composting process (10 days) as the applied composts had to pass different stages according to their composition and dominant microbial enrichment. With compost E (run 9), which is the most active one, only 40.13% of the total PAHs was degraded comparing to the other composts (A and C) under the same conditions (run 5 and 8) where 70.4% and 73.79% of degradation were achieved respectively. The same fact was also observed in run 1 and 3 which demonstrated more degradation as more stable compost was used. Without doubt, these observations are of great interest when the efficiency of the composting technology is to be evaluated with
other technologies. However, the highest rate of degradation (92.2%) was observed in run 2 indicating that the PAHs concentration has its influence in this case. For instance, Oleszczuk [30], observed that the influence of the composting process on the contribution of the potentially bioavailable fraction of the PAH depended on the stage of the experiment.

Regarding the composting process and as commented before, the thermophilic temperatures are thought to be not suitable for the microorganisms needed to attack the PAHs contaminants as the lowest degradation rate was observed (run 9). These temperatures were reported to inhibit the degradation process [31-33]. Contrarily, the mesophilic temperatures obtained under the same conditions gave higher rates of degradation indicating that these temperatures and the dominant microorganisms under these conditions are preferable for degradation of such compounds. However, other studies are not coincident with these observations [34].

The organic matter decrease was proportional with the stability degree, where more reduction occurred in compost E. This reduction shows that these types of composts still have a considerable amount of easily degradable matter which was preferable by the microorganisms rather than other sources of nutrients like PAHs. Accordingly, the mass loss after composting is a suitable evidence indicating the bio-oxidation of the composting matrix.

Among the most important properties correlated with the compost stability is the available amount of the humic matter as part of the organic matter. This matter was found to increase with stable compost (Table 1) where more stable composts had more humic matter. Sorption of the organic contaminants with the soil particles usually decreases the degradation rates as these contaminants become less accessible to the microorganism. However, it was found that the humic matter increases the
bioavailability of the organic compounds and it can behave as surfactant during the
remediation process, which reduces the bond between the soil and PAHs. The more
degradation rates with more stable compost confirm this hypothesis and the PAHs were
easily desorbed from the soil particles thereby the degradation was stimulated. Plaza et
al. [25], demonstrated that during the composting process, the changed underwent by
the humic matter are expected to facilitate the microbial accessibility to PAHs. These
experiments concurred well and confirm such suggestions. It was clear in the remaining
composting period that the degradation process continued among all the experiments
and better results had been obtained with more incubation time as the organic matter got
more stabilized and the microorganisms were more acclimatized especially with less
stable compost. The humic matter found to be more effective to increase the degradation
rates than the high temperatures although it is well-known that the produced high
temperatures usually increase the kinetics and desorption of such compounds.

3.6. Effect of PAHs concentration

PAHs concentration influenced the degradation rate as observed in the different
experiments. Figure 3 presents the percentages of the remaining PAHs after 30 days of
incubation, where Figure 4 presents the response of the process under different
concentrations. The lowest degradation rate (18%) after 10 day of incubation was
observed in 6th run that has the lowest concentration (0.1g/kg), where the highest rate
(92.21%) was obtained in the 2nd run that has a concentration of 1.7g/kg, where
degradation rate of 66.5% was achieved with the highest used concentration (2g/kg)
during the same incubation period. By the end of the process (30 days), the degradation
rate in the 6th run (0.1g/kg) still maintained its order as the lowest achieved rate
(45.8%), where it was able to achieve 80.9% with the highest applied concentration
(2g/kg). However, when comparing the rate obtained with highest concentration, it was less than the other obtained rates where less concentration were used, therefore, the concentration levels are considered crucial when composting process is to be used. For this reason, when low concentrations are present, these concentrations are thought to be below the levels that are assumed to begin the degradation process as the microorganisms start with easily available materials and as these materials depleted quickly before the degradation take place, it will be difficult to keep the required activity. These results agreed with those obtained in [15], where concentration of low PAH did not degrade even when the system was supplanted with additional carbon sources. Furthermore, Jørgensen et al. [35] argued that the degradation of hydrocarbons is governed by first-order kinetics, where the degradation rate of a compound is proportional to its concentration. However, this argument may be validated to some limits as the microbial activity could be affected (retardation or inhibition) when high concentration is available. In this study when the results of high concentrations are compared to other presenting lower concentrations, it is better to assume that retardation conditions were noted.

4. Conclusions

During the composting process, it was clear that the potentially available PAHs are influenced by the compost stability and the composting stages as a consequence. Accordingly, the following conclusions were deduced:

1. The observed different behaviors during the first 10 days of composting demonstrate that less stable compost is not adequate for this type of remediation, but more stable ones can promote the degradation quickly when the process is well controlled.
2. Humic matter was assumed to facilitate the desorption of the PAHs to be more available for degradation by the microbial activity. Indeed, humic matter was more effective to accelerate the degradation rates than the high temperatures.

3. By the end of the process, experiments with the less stable compost were able to improve their behavior as the composted materials were more stable, but their results were still less favorable than those obtained with more stable compost.

4. PAHs concentration was found to influence the process mainly when low concentrations are available, where the lowest degradation rate was obtained.

5. Both of the studied factors (compost stability and PAHs concentration) had a direct effect on the process behavior; therefore, before carrying out the composting process, initial knowledge about the available conditions may help to have an estimation about the process performance and consequently the expected degradation rates.

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References

[1] L. Gianfreda, M.A. Rao, Potential of extra cellular enzymes in remediation of polluted soils: a review. Enzyme Microb. Technol. 35 (2004) 339-354.

[2] C.E. Boström, P. Gerde, A. Hanberg, B. Jernström, Ch. Johansson, T. Kyrklund, A. Rannug, M. Törnqvist, K. Victorin, R. Westrholm, Cancer risk assessment, indicators and guidelines for polycyclic Aromatic Hydrocarbons in the ambient air. Environ. Health Perspect. 110 (2002) 451-488.

[3] P.F. Hudson, I. Alcántara-Ayala, Ancient and modern perspectives on land degradation. Catena 65 (2006) 102-106.

[4] V. Šašek, J.A. Glaser, Ph. Baveye, The Utilization of bioremediation to reduce soil contamination: Problems and Solution. Nato Science Series, IV. Earth and Environmental Sciences. Kluwer Academic Publisher, Dordrecht (2003).

[5] E.E. Diplock, D.P. Mardlin, K.S. Killham, G.I. Paton, Predicting bioremediation of hydrocarbons: Laboratory to field scale. Environ. Pollut. 157 (2009) 1831-1840.

[6] T. Sayara, M. Sarrà, A. Sánchez, Preliminary screening of co-substrates for bioremediation of pyrene-contaminated soil through composting. J. Hazard. Mater. 117 (2009) 1695-1698.

[7] R.M. Atlas, Bioremediation of petroleum pollutants. Int. Biodeterior. Biodegrad. 35 (1995) 317-327.

[8] M. Romantschuk, I. Sarand, T. Petänen, R. Peltola, M. Jonsson-Vihanne, T. Koivula, K. Yrjälä, K. Haahleta, Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. Environ. Pollut. 107 (2000) 179-185.
K.T. Semple, B.J. Reid, T.R. Fermor, Impact of composting strategies on the treatment of soils contaminated with organic pollutants. Environ. Pollut. 112 (2001) 269-283.

R.J. Grosser, M. Friedrich, D.M. Ward, W.P. Inskeep, Effect of model sorptive phases on phenanthrene biodegradation: different enrichment conditions influence bioavailability and selection of phenanthrene-degrading isolates. Appl. Environ. Microbiol. 66 (2000) 2695-2702.

R.M. Hesnawi, D. McCartney, Impact of compost amendments and operating temperature on diesel fuel bioremediation. Environ. Eng. Sci. 5 (2006) 37-45.

W. Namkoong, E.Y. Hwang, J.S. Park, J.Y. Choi, Bioremediation of diesel-contaminated soil with composting. Environ. Pollut. 119 (2002) 23-31.

L.Y. Wick, R. Remer, B. Wurz, J. Reichenbach, S. Braun, F. Schafer, H. Harms, Effect of fungal hyphae on the access of bacteria to phenanthrene in soil. Environ. Sci. Technol. 41 (2007) 500-505.

J.A. Marín, T. Hernández, C. García, Bioremediation of oil refinery sludge by landfarming in semiarid conditions: influence on soil microbial activity. Environ. Res. 98 (2005) 185-195.

M.E. Zappi, B.A. Rogers, C.L. Teeter, D. Gunnison, R. Bajpai, Bioslurry treatment of a soil contaminated with low concentrations of total petroleum hydrocarbons. J. Hazard. Mater. 46 (1996) 1-12.

S. Ponsá, T. Gea, L. Alerm, J. Cerezo, A. Sánchez, Comparison of aerobic and anaerobic stability indices through a MSW biological treatment process. Waste Manage. 28 (2008) 2735-2742.
[17] L. Ruggieri, T. Gea, M. Mompeó, T. Sayara, A. Sánchez, Performance of
different systems for the composting of the source-selected organic fraction of
municipal solid waste. Biosys. Eng. 101 (2008) 78-86.
[18] R. Barrena, G. d’Imporzano, S. Ponsá, T. Gea, A. Artola, F. Vázquez, A. Sánchez,
F. Adani, In search of a reliable technique for the determination of the biological
stability of the organic matter in the mechanical-biological treated waste. J.
Hazard. Mater. 162 (2009) 1065-1072.
[19] F. Adani, C. Ubbiali, P. Genevini, The determination of biological stability of
composts using the Dynamic Respiration Index: The results of experience after
two years. Waste Manage. 26 (2006) 41-48.
[20] S. Ponsà, T. Gea, A. Sánchez, Different indices to express biodegradability in
organic solid wastes. J. Environ. Qual. 39 (2010) 1-8.
[21] A. Torkian, R. Dehghanzadeh, M. Hakimjavadi, Biodegradation of aromatic
hydrocarbons in a compost biofilter. J. Chem. Technol. Biotechnol. 78 (2003)
795-801.
[22] The US Department of Agriculture and The US Composting Council, Test
methods for the examination of composting and compost. Edaphos International,
Houston, (2001).
[23] S.N. Deming, S.L. Morgan, Experimental design: a chemometric approach. Data
handling in science and technology-Volume 3. Elsevier, Amsterdam, The
Netherlands (1987).
[24] F. Rigas, V. Dritsa, R. Marchan, K. Papdopoulou, E.J. Avramids, I. Hatzianestis,
Biodegradation of lindane by *Pleurotus Ostreatus* via central composite design.
Environ. Int. 31 (2005) 191-196.
[25] C. Plaza, B. Xing, J.M. Fernández, N. Senesi, A. Polo, Binding of polycyclic aromatic hydrocarbons by humic acids formed during composting. Environ. Pollut. 157 (2009) 257-263.

[26] R.T. Haug, The Practical Handbook of Compost Engineering. Lewis Publishers, Boca Raton (1993).

[27] R. Ambrosoli, L. Petruzzelli, J.L. Minati, F.A. Marsan, Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. Chemosphere 60 (2005) 1231-1236.

[28] P.B. Hatzinger, M. Alexander, Effect of aging of chemicals in soil on their biodegradability and extractability. Environ. Sci. Technol. 29 (1995) 537-545.

[29] R. Margesin, F. Schinner, Laboratory bioremediation experiments with soil from a diesel-oil contaminated site- significant role of cold-adapted microorganisms and fertilizers. J. Chem. Technol. Biotechnol. 70 (1997) 92-98.

[30] P. Oleszczuk, Investigation of potentially bioavailable and sequestrated forms of polycyclic aromatic hydrocarbons during sewage sludge composting, Chemosphere 70 (2007) 288-297.

[31] C.L. Potter, J.A. Glaser, R. Hermann, M.A. Dosani, Remediation of Contaminated East River sediment by composting technology. In: A. Leeson, B.C. Alleman (Eds.) Bioremediation Technologies for Polycyclic Aromatic Hydrocarbon Compounds. The Fifth International In-situ and On-site Bioremediation Symposium. Battelle Press: Columbus (1999), pp 31-36.

[32] B. Antizar-Ladislao, J.M. Lopez-Real, A.J. Beck, Bioremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated waste using composting approaches. Crit. Rev. Environ. Sci. Technol. 34 (2004) 249-289.
[33] A. Haderlein, R. Legros, B.A. Ramsay, Pyrene mineralization capacity increased with compost maturity. Biodegradation 17 (2006) 293-302.

[34] S. Viamajala, B.M. Peyton, L.A. Richards, J.N. Petersen, Solubilization, solution equilibria, and biodegradation of PAH’s under thermophilic conditions. Chemosphere 66 (2007) 1094-1106.

[35] K.S. Jørgensen, J. Puustinen, A.M. Suortti, Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. Environ. Pollut. 107 (2000) 245-254.
### Table 1
Characteristics of the used composts and soil.

| Parameter/Material                          | Soil          | Compost A        | Compost B        | Compost C         | Compost D         | Compost E         |
|---------------------------------------------|---------------|------------------|------------------|-------------------|-------------------|-------------------|
| Moisture content (%, wb) *                  | 6.64±0.01     | 38.64±0.22       | 30.1±0.42        | 53.8±0.25         | 32.63±0.08        | 40.55±0.35        |
| Organic matter content (%, db) **           | 3.68±0.35     | 44.48±0.39       | 53.96±0.11       | 61.63±1.83        | 44.59±0.35        | 51.85±1.2         |
| Total Organic Carbon (%, db) **             | 1.26±0.02     | 18.52±1.14       | 24.29±2.79       | 31.75±0.28        | 19.43±1.2         | 20.44±0.28        |
| Total Kjeldahl Nitrogen (%, db) **          | 0.65±0.14     | 2.67±0.45        | 2.62±0.06        | 4.09±0.12         | 3.09±0.15         | 1.94±0.12         |
| pH                                          | 6.7±0.02      | 8.07±0.08        | 8.63±0.04        | 8±0.16            | 8.11±0.01         | 7.61±0.01         |
| Electrical Conductivity (mS/cm)             | 0.2±0.01      | 4.91±0.13        | 6.46±0.18        | 5.27±0.14         | 6.01±0.0          | 7.13±0.04         |
| Humic Acids (%, db) **                       | 1.5           | 10.12            | 11.62            | 14.6              | 8.95              | 4.72              |
| Dynamic Respiration index (mg O₂ g⁻¹ OM h⁻¹)| -             | 0.37±0.02        | 0.58±0.4         | 1.71±0.13         | 3.07±0.3          | 4.55±0.1          |

* wb: wet basis.
** db: dry basis.
Table 2
Design matrix including factor levels (coded and actual) and their response values for the two factors.

| Run | Factor levels | Response* |
|-----|---------------|-----------|
|     | coded Concentration ($x_1$) (g/kg) | Stability ($x_2$) (mg O$_2$ g$^{-1}$ OM h$^{-1}$) | actual Concentration (g/kg) | Stability (mg O$_2$ g$^{-1}$ OM h$^{-1}$) | $Y_{10}$ | $Y_{20}$ | $Y_{30}$ |
| 1   | -1            | -1        | 0.38 | 0.58±0.04 | 81.47 | 88.26 | 96.38 |
| 2   | +1            | -1        | 1.74 | 0.58±0.04 | 92.21 | 96.47 | 96.53 |
| 3   | -1            | +1        | 0.38 | 3.07±0.29 | 67.55 | 79.01 | 89.81 |
| 4   | +1            | +1        | 1.74 | 3.07±0.29 | 75.26 | 89.09 | 96.56 |
| 5   | 0             | 0         | 1.05 | 1.71±0.13 | 73.79 | 81.82 | 84.39 |
| 6   | -$\alpha$     | 0         | 0.1  | 1.71±0.13 | 18.61 | 57.44 | 45.84 |
| 7   | +$\alpha$     | 0         | 2.0  | 1.71±0.13 | 66.51 | 78.26 | 80.98 |
| 8   | 0             | -$\alpha$ | 1.05 | 0.37±0.02 | 70.40 | 90.79 | 86.46 |
| 9   | 0             | +$\alpha$ | 1.05 | 4.55±0.01 | 40.13 | 77.28 | 76.11 |
| 10  | 0             | 0         | 1.05 | 1.71±0.13 | 75.61 | 79.29 | 78.68 |
| 11  | 0             | 0         | 1.05 | 1.71±0.13 | 75.07 | 90.28 | 85.58 |
| 12  | 0             | 0         | 1.05 | 1.71±0.13 | 69.35 | 75.42 | 85.21 |

*The response (Y) represents the degradation percentage (%) after 10, 20 and 30 days of composting
Legends to Figures

**Figure 1.** Temperature profiles of the composting materials over time.

**Figure 2.** Percentage of organic matter degradation after 30 days of composting.

**Figure 3.** Percentage of the remaining PAHs after 30 days of composting.

**Figure 4.** The response of the PAHs degradation (%) under different concentrations and DRI (mg O$_2$ g$^{-1}$ OM h$^{-1}$) during the incubation period where (A) corresponds to 10 days, B (20) days and (C) 30 days.
Figure 1: Sayara et al.
Figure 2: Sayara et al.
Figure 3: Sayara et al.
Figure 4: Sayara et al.