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Optimization and enhancement of soil bioremediation by composting using the experimental design technique

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Abstract The objective of this study was the application of the experimental design technique to optimize the conditions for the bioremediation of contaminated soil by means of composting. A low-cost material such as compost from the Organic Fraction of Municipal Solid Waste as amendment and pyrene as model pollutant were used. The effect of three factors was considered: pollutant concentration (0.1–2 g/kg), soil:compost mixing ratio (1:0.5–1:2 w/w) and compost stability measured as respiration index (0.78, 2.69 and 4.52 mg O_2 g^{-1} Organic Matter h^{-1}). Stable compost permitted to achieve an almost complete degradation of pyrene in a short time (10 days). Results indicated that compost stability is a key parameter to optimize PAHs biodegradation. A factor analysis indicated that the optimal conditions for bioremediation after 10, 20 and 30 days of process were (1.4, 0.78, 1:1.4), (1.4, 2.18. 1:1.3) and (1.3, 2.18, 1:1.3) for concentration (g/kg), compost stability (mg O_2 g^{-1} Organic Matter h^{-1}) and soil:compost mixing ratio, respectively.

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Introduction

In recent years, the development of chemical processes has contributed to the increase of environmental pollution (Harrison 2001). Simultaneously, the widespread of society's environmental concern and the publication of strict environmental regulations have helped in reducing the levels of pollutants in the environment. However, many pollutants are still released and some of them are eventually deposited in soil, which has received less attention than other media such as water resources or atmosphere. Consequently, contaminated soils constitute one of the major environmental issues that needs to be resolved (Boopathy 2000; Ohura et al. 2004).

Polycyclic aromatic hydrocarbons (PAHs) constitute an important group among the different pollutants that are introduced to the environment either by natural or anthropogenic sources (Johnson et al. 2005; Ohura et al. 2004). Their composition of fused aromatic rings and their specific physical properties such as low aqueous solubility and high solid–water distribution ratios stand against their straightforward microbial utilization and promote their accumulation in the solid phases of the terrestrial environment

(Johnson et al. 2005). PAHs with low molecular weigh such as naphthalene, phenanthrene and anthracene are relatively easy to degrade. However, PAHs with four or more fused aromatic rings are more recalcitrant to microbial attack and are not easily degraded (Cerniglia 1992; Wilson and Jones 1993; Serrano Silva et al. 2009).

Bioremediation techniques have been developed and improved to remediate soils polluted with hazardous chemicals (Romantschuk et al. 2000; Bento et al. 2005). In this framework, composting appears to be particularly useful in the bioremediation of petroleum hydrocarbons, especially the PAH fraction (Antizar-Ladislao et al. 2004, 2006; Haderlein et al. 2006).

Although the concept of treating PAH-contaminated soil by means of co-composting with organic materials or by mixing soil with finished compost has been reviewed (Semple et al. 2001; Antizar-Ladislao et al. 2004) and has proved to be effective in the degradation of PAHs (Beaudin et al. 1996), composting of contaminated soil can be still considered an emerging ex situ biotreatment (Antizar-Ladislao et al. 2006). Specifically, there is a lack of knowledge about the optimal conditions for PAH biodegradation and the properties that a given compost should present to enhance a rapid pollutant removal. In addition, it is necessary to develop a systematic approach that permits to determine the optimal conditions for PAH bioremediation in soil.

According to previous studies, several organic amendments have been used in the bioremediation of contaminated soil, and different biodegradation rates have been obtained. Clearly, when the organic amendment is preferred over the target compound, microbial activity for degrading the target pollutants may be inhibited (Cookson 1995). Moreover, the application of bioremediation for soil decontamination may be further limited by a large number of environmental parameters such as temperature, pH, oxygen availability, nutrients and salinity. Thus, the microbial activity that is necessary for the transformation of organic contaminants in soil (Marín et al. 2005) should be maintained at adequate levels during the bioremediation process to degrade the target contaminant. Accordingly, the addition of compost can facilitate the degradation of organic contaminants because compost adds supplement nutrients and carbon into the contaminated soil (Namkoong et al.

2002; Oleszczuk 2007; Anastasi et al. 2009). Nevertheless, little or no research has been conducted regarding the effect of compost stability on the composting of PAH-contaminated soils, a point that is crucial to understand the real role of organic amendments in soil, as stability is directly related to organic matter composition and biological activity (Said-Pullicino and Gigliotti 2007; Barrena et al. 2009).

Finally, the ratio of the contaminated soil to compost should be also determined since an inappropriate ratio may retard or inhibit microbial activity (Thomas et al. 1992; Namkoong et al. 2002). On the other hand, the economics of a composting technology is based on the amount of the compost to be added to soil. Thus, the overall cost of the system is decreased upon increasing the amount of the contaminated soil ratio to the compost where an equivalent microbial activity and efficiency may be maintained (In et al. 2007).

Since biological and biochemical models are not currently available for a detailed description of soil bioremediation processes, statistical experimental design techniques appears as a suitable and feasible method that can be used to obtain relevant and reproducible information for process optimization, data processing, calibration, quality control and organization of the analytical process. These techniques have been largely applied to other research areas (Deming and Morgan 1987; Sánchez et al. 2000; Delgado-Moreno et al. 2009). However, its application to soil bioremediation is scarce, and often the factors affecting biodegradation in soils are studied separately, which adds an important uncertainty to the conclusions presented and difficulties for results interpretation.

The objective of this work is to systematically study the effect of contaminant concentration, soil:compost mixing ratio and compost stability on the optimization of soil bioremediation using a low cost material for the composting process such as compost from the Organic Fraction of Municipal Solid Waste (OFMSW). To the best of our knowledge, no reports are available in literature regarding the influence of the compost properties on the bioremediation of PAH contaminated soil, and the relation among compost stability, contaminant concentration and soil:compost mixing ratio. Pyrene was used as PAH model and composting experiments

were conducted at laboratory-scale reproducing the conditions found at full scale.

Materials and methods

Soil

The soil used in this study was an uncontaminated soil classified as sandy loam soil obtained from Prades (Tarragona, Spain). The soil was collected from the surfaces horizon (0–30 cm). The soil was air-dried, sieved to 2 mm and kept at 4°C until use. The soil consists of 73% sand, 19% silt and 8% clay. Other characteristics of the soil are shown in Table 1. PAHs concentrations were below the detection limit in soil.

Contaminant

Pyrene (98% purity, Sigma–Aldrich, Spain) was selected as model PAH to be monitored during soil composting experiments. This contaminant was dissolved (5 g/l) in a volatile organic solvent (dichloromethane) and homogenously spiked into the soil to obtain the desired concentration according to the experimental design values (range of 0.1–2 g/kg).

Compost

Three types of compost coming from OFMSW with different levels of stability were used during the experimental design to evaluate the effect of these composts on the degradation of pyrene-contaminated soil. Compost A was obtained from a home composter in the University Autònoma of Barcelona. Compost B

and Compost C were obtained from two composting plants located in Barcelona (Spain). Their levels of stability can be considered as full-stable for Compost A, moderately stable for Compost B and unstable for Compost C. Their levels of stability are selected to cover the full range of organic matter stability that can be found in OFMSW compost and related materials, whose range is from 0.5 to 1 mg O_2 g^{-1} Organic Matter h^{-1} for stable OFMSW compost to 7–8 mg O_2 g^{-1} Organic Matter h^{-1} for raw OFMSW (Ponsá et al. 2008; Ruggieri et al. 2008). The main characteristics of the three composts used are also presented in Table 1. Preliminary analysis showed that they are essentially free of PAHs.

Laboratory-scale composting system

Dewar® vessels (4.5 l) were modified and conditioned to operate as batch-mode reactors for the composting experiments. These reactors were thermally isolated, so the influence of ambient temperature could be ignored, besides being perfectly closed. These conditions were similar to that of composting at full-scale, where the isolating properties of organic matter make the composting process to occur under quasi-adiabatic conditions (Barrena et al. 2006).

The air was provided through a pipeline connected to the bottom of the reactor were a plastic mesh is placed to insure a correct distribution of air through the composting mixture. The exhaust air exits the composter through a hole in the reactor cover and then oxygen concentration is monitored. Oxygen concentration was measured by means of an oxygen sensor (Crowcon's Xgard, United Kingdom). Aeration was sporadically provided to the reactors according to the

Table 1 Characteristics of the soil and composts used in the experimental design

Parameter	Compost A	Compost B	Compost C	Soil
Respiration index (mg O_2 g^{-1} OM h^{-1})	0.78	2.69	4.52	–
Organic matter content (%, db)	53.9	66.7	52.7	2.7
Moisture content (%, wb)	30.0	30.0	40.6	11.4
Kjeldahl nitrogen (mg N g^{-1} DM)	27.3	40.0	19.7	6.45
Total carbon (mg C g^{-1} DM)	255.6	315.9	205.4	12.4
Electrical conductivity (ms/cm)	6.46	7.36	7.13	0.2
pH	8.63	8.41	7.61	6.7

OM organic matter, *DM* dry matter, *db* dry basis, *wb* wet basis

process performance, when oxygen concentration was considered limiting for aerobic conditions (lower than 10% in exhaust air). 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 permitted to monitor both the temperature and oxygen content in the reactors.

Soil-compost composting system

Mixtures to be composted were prepared as follows. First, the soil was artificially contaminated by spiking the contaminant (pyrene) at different concentrations ranging between 0.1 and 2.0 g/kg according to the experimental design explained later. Then the soil was mixed with composts A, B or C at different mixing ratios (1:0.5–1:2 w/w) and finally the resulting mixture was mixed with bulking agent at a ratio of 1:1 v/v to provide a proper porosity to maintain aerobic conditions. This ratio can be considered typical and non-limiting for the composting of OFMSW (Ruggieri et al. 2009). The bulking agent

used consisted of wood chips and pruning wastes that were not biodegraded under laboratory composting conditions. If necessary, water content of the composting mixture was adjusted to be within the recommended value (40–60%) by adding tap water at the beginning of each composting experiment.

Composting mixtures were manually prepared according to the values proposed from the experimental design technique (Table 2) as explained below.

Experimental design methodology

The initial conditions for pyrene degradation in composting experiments were selected according to a Central Composite Design (CCD). This approach is adequate since it implies a reduced number of experiments in comparison to a full factorial design.

Briefly, the percentage of pyrene degradation was statistically modeled as a function of three fundamental operating variables, which are the factors of the CCD, that is: initial pyrene concentration (x_1), compost stability measured as respiration index (x_2) and soil:compost mixing ratio (x_3). The factor levels

Table 2 Experimental design matrix with the corresponding normalized coded levels for each factor considered

Run	Concentration (g/kg)	Stability (mg O_2 g^{-1} OM h^{-1})	Mixing ratio (soil:compost)	Degradation (%)
R01	1	1	1:1	89.80
R02	1	−1	1:1	92.80
R03	1	1	1:−1	90.27
R04	1	−1	1:−1	86.72
R05	−1	1	1:1	69.05
R06	−1	−1	1:1	81.99
R07	−1	1	1:−1	65.00
R08	−1	−1	1:−1	76.63
R09	0	0	1:0	98.05
R10	0	1	1:0	91.90
R11	0	−1	1:0	99.31
R12	0	0	1:α	97.02
R13	0	0	1:−α	94.34
R14	α	0	1:0	96.60
R15	−α	0	1:0	87.32
R16[a]	0	0	1:0	98.86
R17	0	0	1:0	98.70
R18	0	0	1:0	98.01
R19	0	0	1:0	98.53
R20	0	0	1:0	98.32

[a] R16–R20 are the replicates carried out at the central point to validate statistically the experimental design

Table 3 Natural values of the levels of the factors considered in the experimental design

Factor	$-\alpha$	-1	0	1	α
Concentration (g/kg)	0.1	0.5	1.05	1.6	2
Stability (mg O_2 g^{-1} OM h^{-1})	–	0.78	2.70	4.52	–
Mixing ratio (soil:compost)	0.5	0.83	1.24	1.66	2

were normalized and coded with the notations ($-\alpha$, -1, 0, 1, α). The value of $\alpha = 1.68$ is determined according to the number of the factors and levels to be studied. A detailed description of the CCD experimental design and its application can be found elsewhere (Deming and Morgan 1987; Rigas et al. 2005).

Table 3 shows the normalized coded levels and the natural values of these factors set in the statistical experiment, whereas Table 2 presents the experimental design matrix and the factor combinations along with the replicates which were run at the central point for statistical validation. A total number of 20 experiments were carried out to represent the system, where a full second-order polynomial model was selected to fit each response function (Y, representing pyrene biodegradation) to the values of the factors considered according to Eq. 1:

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 \qquad (1)$$

For each sampling day (10, 20 and 30 days of process), the model parameters b_i were estimated from experimental values using a multi regression software (Sigmaplot® 8.0, Systat Software Inc, San Jose, USA).

Beside the 20 experiments, control reactor which has only contaminated soil (1 g/kg) and bulking agent was used to follow the degradation of the contaminant by indigenous microorganisms already exist in the soil to be compared with the other amended reactors.

Sampling

Initial concentration of the contaminated soil was measured in three reactors where two grab fresh samples (20–30 g) were taken to be sure that the concentrations were homogeneous and within the required values. The differences among theoretical and actual values were less than 3%.

Samples were collected after 10, 20 and 30 days of composting. The reactors were opened and the reactor contents were mixed well to get homogenous and representative samples. Then two grab fresh samples (20–30 g) were taken, manually grinded, dried, sieved (2 mm) for analysis. During each sampling, moisture content was adjusted, if necessary.

Analytical procedures

Compost stability defined as the rate of organic matter degradation as a result of microbiological activity was determined using the dynamic respirometric index (DRI), determined according to Barrena et al. (2009). Briefly, this index represents the oxygen consumption of a known sample of organic matter incubated under optimal conditions and with a continuous air supply. Moisture content, organic matter content (OM), total carbon content, Kjeldahl nitrogen, pH and electrical conductivity were determined according to standard methods (The US Department of Agriculture and the US Composting Council 2001).

Pyrene in the composting mixture was extracted using a Soxhlet extraction process, then it was determined by gas chromatography. Duplicate 10 g samples were extracted using acetone/dichloromethane (1:1 v/v) as solvent during 2 h. After extraction 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 (GC8690 N, 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, 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 pyrene was determined after the calibration of the method with standard pyrene samples. The recovery of pyrene using this method was higher than 95%. Blank abiotic experiments showed no significant volatilization of pyrene by directly analyzing gas samples from composting exhaust gases.

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 (Deming and Morgan 1987). The optimization of the proposed polynomial function to obtain the optimal conditions corresponding to the three factors considered was solved by using a self-made program using C-language.

Results and discussion

Characterization of materials

Table 1 summarizes the characteristics of the raw materials used in the experimental design. The soil low content of organic matter (2.7%), which is typical of Mediterranean soils (Cayuela et al. 2009), is a good indication of the weakness of the soil by itself to support the activity of microorganisms for any bioremediation process, which is typical of Mediterranean soils (Cayuela et al. 2009). Contrarily, the three composts have an important percentage of organic matter that can be a suitable source for the carbon and nutrients needed for the remediation process to stimulate the microbial activity and consequently the contaminant degradation (Haderlein et al. 2006). The most significant difference found in the three composts used in this study is the degree of stability, whereas pH and electrical conductivity are relatively similar. Compost stability is an important factor related to organic matter composition (Said-Pullicino and Gigliotti 2007). In addition, other authors have pointed that, although stable compost inherently implies low microbial activity, it contents a high percent of humic matter (Gourlay et al. 2003; Plaza et al. 2009), which is strongly related to the possibilities of solubilize PAHs (Quadri et al. 2008). Therefore, the study of compost stability as a controlling factor for the bioremediation of contaminated soil can be of great interest since a waste treatment plant can easily produce compost of different stability by adjusting the conditions and duration of the biological process (Ponsá et al. 2008; Barrena et al. 2009).

Response surface analysis and interpretation

Experimental design methodology was applied to investigate the response of composting process of pyrene-contaminated soil under the effect of the previously mentioned factors. The percentage of pyrene degradation (Y) in the process was measured at 10, 20 and 30 days. The second-order polynomial model was able to fit the obtained results and to statistically represent the process response. The values of the b_i coefficients of the fitted model and the correlation coefficients (R^2) corresponding to each time (10, 20 and 30 days) are presented in Table 4. As can be observed, the values of R^2 and p demonstrate the suitability of the model to fit the experimental results. Specifically, and according to R^2 and p values, the second-order model was adequate to represent the results after 20 and 30 days of composting process; however, experimental values obtained at 10 days were not completely adjusted even when other more complex polynomial models were tested, which means that, at short process times, the studied factors only explain partially the response of the system. Other authors have found similar results when using second-order models to represent the biodegradation of petroleum hydrocarbons (Vieira et al. 2009), although in that case a composting process was not used.

Table 4 Values of b_i coefficients, R^2 and p obtained after 10, 20 and 30 days according to the fit of experimental values to Eq. 1

Coefficient	Time		
	10 days	20 days	30 days
b_0	26.3	16.3	38.35
b_1	69.4	49.96	29.03
b_2	0.05	6.15	13.79
b_3	16.3	60.02	35.8
b_{11}	−20.1	−16.18	−11.09
b_{22}	0.13	−2.99	−3.2
b_{33}	−2.61	−17.06	−10.6
b_{12}	−3.59	4.45	3.0
b_{13}	−5.8	−10.70	−2.4
b_{23}	−0.36	0.26	−1.31
R^2	0.69	0.96	0.85
P	0.086	<0.0001	0.0038

Pyrene degradation

In Figs. 1, 2, and 3, the responses obtained for pyrene degradation according to the fitted model are presented as contour lines as a function of both the concentration and mixing ratio where stability is fixed for Composts A, B and C, which represents a different degree of stability from highly stable (A) to unstable (C). With this methodology the responses shape and the optimal conditions of the studied factors for bioremediation can be precisely described.

Figures 1, 2, and 3 shows that the three types of compost enhanced pyrene removal although the degradation rate was different. The rate of degradation is strongly correlated to the stability degree, especially in the first stage of composting (10 days, Fig. 1a). These results are in agreement with other research studies on PAHs degradation (Laor et al. 1999; Haderlein et al. 2006; Tejada et al. 2008).

During the entire composting process that took place in 30 days, a high degradation rate was observed during the first 10 days in comparison to the final period that exhibited a low rate of degradation. These observations have been previously reported in other studies (Namkoong et al. 2002; Wan et al. 2003), where a rapid degradation of total petroleum hydrocarbons (TPH) was observed in the early stage (within 15 days) of reaction in all experiments. Following the rapid degradation of TPH within 15 days, residual TPH was slowly degraded. Also, Lee et al. (2008) observed two differentiated phase in compost-amended soil during the degradation process, where about the 82% of the overall degradation occurred in the first stage.

In this study, the degradation rate in the last days decreased, which might be explained by the low content of contaminant and nutrients. Anyway, it is worthwhile to mention that a practically complete pyrene removal was observed by the end of the process with both Compost A and Compost B (Fig. 3). Since, to our knowledge, no such complete removal has been previously reported for a wide range of pollutant concentrations, this fact confirms the suitability of the composting technology for the bioremediation of contaminated soil when the controlling factors are systematically studied and suitable amendments are provided.
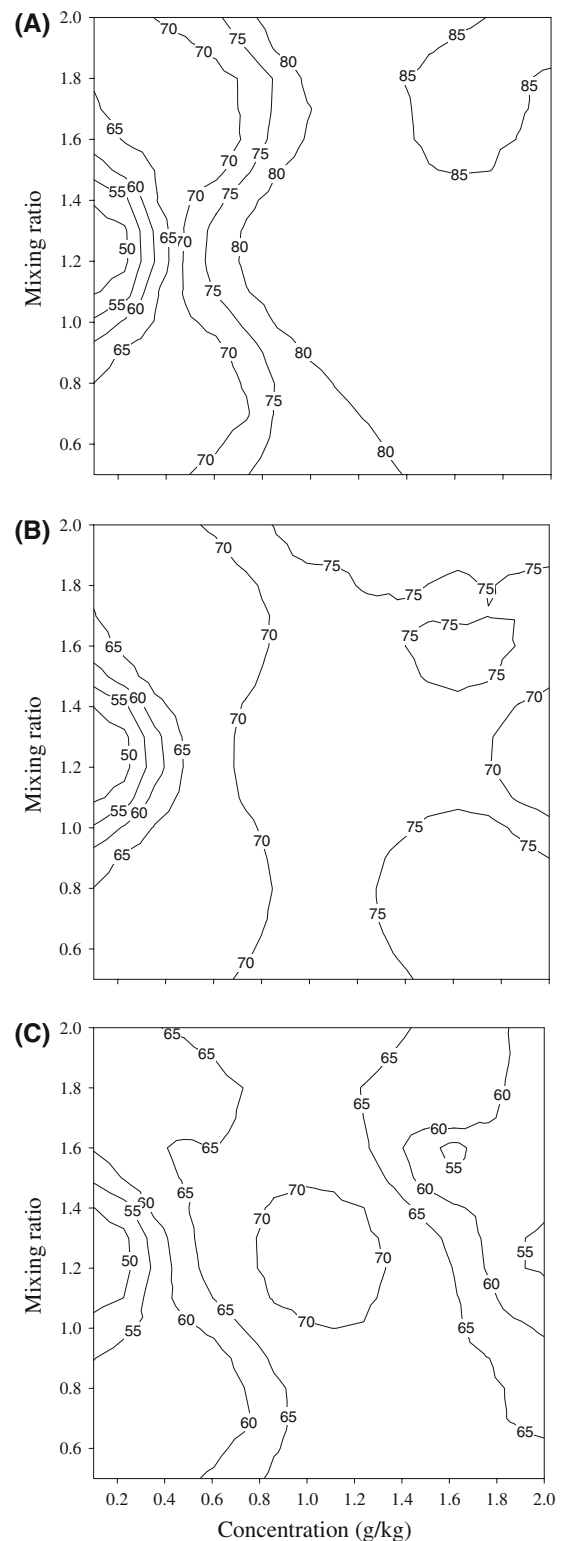

Fig. 1 Percentage of pyrene degradation after 10 days of process. **a** Compost A; **b** Compost B; **c** Compost C
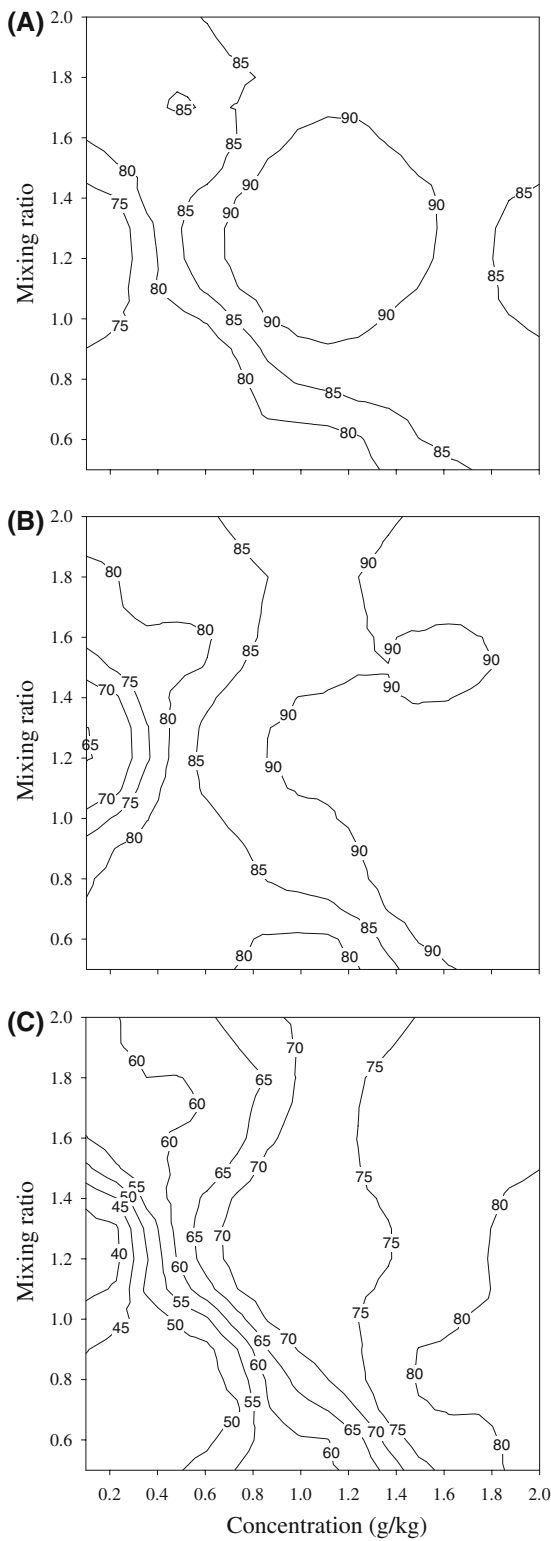
Fig. 2 Percentage of pyrene degradation after 20 days of process. **a** Compost A; **b** Compost B; **c** Compost C
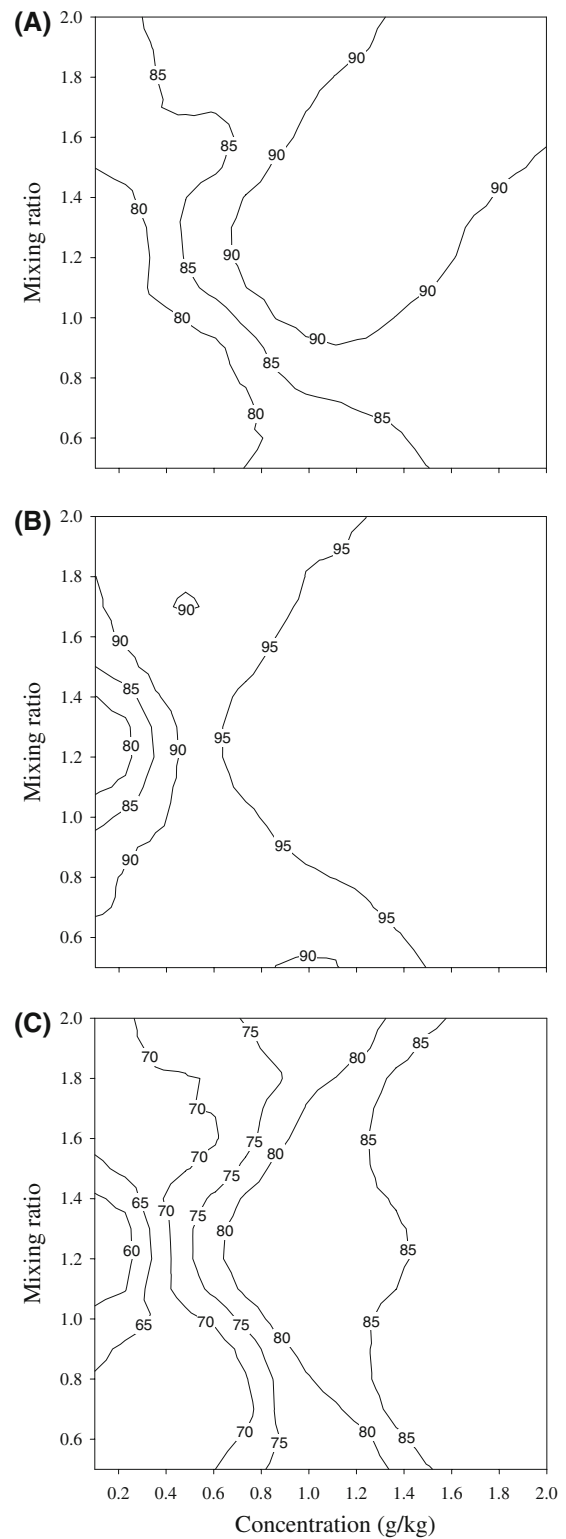
Fig. 3 Percentage of pyrene degradation after 30 days of process. **a** Compost A; **b** Compost B; **c** Compost C

Effect of compost stability on the composting process

As expected, compost stability (Table 1) strongly influenced the composting process, where the most active compost (C) followed by moderately stable compost (B) presented a high microbial activity during the first 10 days. The increase in the temperatures observed and the high rate of aeration needed (data not shown) are evidences of such activity. After a lag period of about 2 days the temperature began to increase to reach thermophilic ranges (>45°C), which were maintained for 1 week. Contrarily, experiments with Compost A were always in the mesophilic range of temperature. It is important to note that, although Compost C presented a high microbial activity, this was not favorable for pyrene degradation.

In relation to process temperature, it is clear that mesophilic range is preferable for microorganisms responsible for PAHs biodegradation, although high temperatures are supposed to increase the desorption and mass transformation of the contaminant and to enhance the enzyme kinetics (Pignatello and Xing 1996). The effect of temperature is in agreement with other studies that show the inactivation of bioremediation of PAHs in contaminated soil by thermophlic temperatures (Potter et al. 1999; Antizar-Ladislao et al. 2004; Haderlein et al. 2006; Hesnawi and McCartney 2006). In addition, stable compost are believed to have a significant portion of humic acids, which were found to enhance the desorption of hydrophobic organic components from soil (Janzen et al. 1996; Quadri et al. 2008), which increases the contaminant bioavailability. Furthermore, it has been suggested that the sorption of both the microorganisms and the PAHs to the colloidal surfaces of humic matter stimulate their biodegradation (Laor et al. 1999).

In our study, it is evident that stable compost (A) resulted in high removal percentages at short process times, but it is also important to note that with more time, Compost B achieves similar results. It can be hypothesized that the stabilization of organic matter that is inherent to any composting process finally results in the same bioremediation results, if enough time is given. These observations are supported by other studies (Plaza et al. 2009), which suggest that the changes undergone by the humic acids fraction during composting may be expected to contribute to

facilitate microbial accessibility to PAHs, whereas other works point out that the application of compost to PAH-contaminated soil is expected to increase the affinity of soil humic acids for PAHs (Senesi et al. 2007; Plaza et al. 2009).

Effect of mixing ratio

Figures 1, 2, and 3 show the effect of the different mixing ratios on the process performance. This factor is especially important since the determination of the minimum quantity of the amendment that could support and maintain the desired activity to have a high degradation rate is directly related to the process economics. In this study, the minimum mixing ratio soil:compost used (1:0.5) was able to enhance the microbial activity to degrade the contaminant up to 80% with Compost A, but the maximum rate was found when a mixing ratio of 1:1.5 was used during the first 10 days for both Compost A and B. In the remaining incubation period the mixing ratio effect was almost the same for Compost A and B, whereas a lower ratio of Compost C showed good results when compost progressively stabilize (Fig. 3c). In fact, in the case of active compost (C), high mixing ratio showed low degradation rate, which may be caused by a preferential degradation of easily degradable materials. Namkoong et al. (2002) and Chang et al. (2009) reported that while the addition of organic supplements increased the rate of contaminant degradation, excessive supplementation could eventually inhibit degradation.

Another important aspect shown in Figs. 1, 2, and 3 is the relationship between the mixing ratio and the pollutant concentration, since it is especially important to observe that when low concentrations were tested, more amount of compost was needed to accelerate the degradation rate (Figs. 1a, 2a, 3a).

Effect of pollutant concentration

In this study, high degradation rate was achieved when high pollutant concentration is available in both Compost A and Compost B in the first 10 days (Fig. 1). In Compost C, concentration within 0.8–1.3 g/kg was found to be suitable for biodegradation. Concentration above this value inhibits the activity, while the low concentration may be not sufficient to support the microbial activity. According to JØrgensen

et al. (2000), usually the degradation of hydrocarbons is governed by first-order kinetics, where the degradation rate of a compound is proportional to its concentration. In this study, a complete kinetic analysis of degradation rate showed that both first and second order kinetics can represent the experimental data. First-order rate constant was within 0.07–0.15 day^{-1}, whereas second-order rate constant was within 0.10–0.30 (g/kg)$^{-1}$ day^{-1}. These rate constants are significantly higher than those found in the bioremediation of total petroleum hydrocarbons using a composting strategy by adding grass clippings and sheep manure to soil (Mihial et al. 2006). Moreover, a clear trend was observed between the value of rate constant and the compost stability (Fig. 4). This fact again empathizes the influence of stability on PAH biodegradation rate.

Process optimization

The obtained function Y from the experimental design after 10, 20 and 30 days of composting process was used to determine the optimal combination of initial conditions for the implementation of the composting process. Table 5 shows the optimal values of the three studied factors. The results obtained confirmed again that Compost A gives better results than the other used compost at short times. With this compost, 86% of pyrene removal was reached for experiment runs R01 and R11. The same results were obtained using the second-order model function. At longer process times (20 and 30 days of composting), Compost A and Compost B approximately presented the same result with a practically complete removal of pyrene. In fact, the optimized values showed that if compost has a degree of stability of about 2.18 mg O$_2$ g^{-1} OM h^{-1}, a 100% degradation can be achieved in 30 days. From this point of view, it is essential to study the stability of any organic amendment that it is intended to be used for soil bioremediation.

Conclusions

The results obtained in this research can provide important indications for the improvement of bioremediation processes using the composting technology. The general conclusions are summarized in the following points:

– A systematic approach is necessary to optimize the biodegradation of PAHs in soil if the composting technology has to be applied, since a large number of factors can severely affect the process performance.
– The experimental design technique seems a suitable approach to study the factors affecting the bioremediation process. A CCD technique seems a good compromise for statistical validation with an assumable number of experiments.
– CCD permits to obtain reliable values of the optimal conditions for PAH biodegradation, with the aim to minimize the cost of the process.
– Stability is the most important property when an organic waste or compost is used to enhance soil bioremediation. As this property can be easily
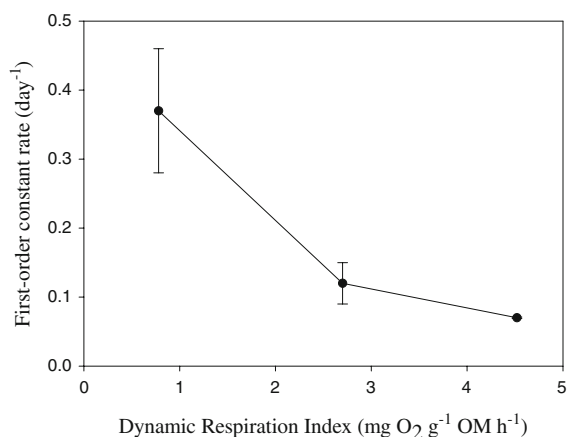

Fig. 4 Influence of compost stability on first-order rate constant

Table 5 Natural values of the experimental design factors for the optimal responses after 10, 20 and 30 days of process

Day	Concentration (g/kg)	Stability (mg O$_2$ g^{-1} OM h^{-1})	Mixing ratio (soil:compost)	Pyrene degradation (%)
10	1.4	0.78	1.4	86.1
20	1.4	2.18	1.3	97.7
30	1.3	2.18	1.3	100

changed when compost is produced, it seems a good parameter to consider in bioremediation planning.

– Further work is needed in the bioremediation with more recalcitrant PAHs or when mixtures of several PAHs are to be biodegraded.

References

Anastasi A, Coppola T, Prigione V, Varese GG (2009) Pyrene degradation and detoxification in soil by a consortium of basidiomycetes isolated from compost: role of laccases and peroxidases. J Hazard Mater 165:1229–1233

Antizar-Ladislao B, Lopez-Real JM, Beck AJ (2004) Bioremediation of polycyclic aromatic hydrocarbon (PAH)-contaminated waste using composting approaches. Crit Rev Environ Sci Technol 34:249–289

Antizar-Ladislao B, Lopez-Real J, Beck AJ (2006) Degradation of polycyclic aromatic hydrocarbons (PAHs) in an aged coal-tar contaminated soil under in-vessel composting conditions. Environ Pollut 141:459–468

Barrena R, Cánovas C, Sánchez A (2006) Prediction of temperature and thermal inertia effect in the maturation stage and stockpiling of a large composting mass. Waste Manage 26:953–959

Barrena R, d'Imporzano G, Ponsá S, Gea T, Artola A, Vázquez F, Sánchez A, Adani F (2009) 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 162:1065–1072

Beaudin N, Caron RF, Legros R, Ramsay J, Lalor L, Ramsay B (1996) Composting of weathered hydrocarbon-contaminated soil. Compost Sci Util 4:37–45

Bento FM, Camargo FAO, Okeke BC, Frankenberger WT (2005) Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresour Technol 96:1049–1055

Boopathy R (2000) Factors limiting bioremediation technologies. Bioresour Technol 74:63–67

Cayuela ML, Sinicco T, Mondini C (2009) Mineralization dynamics and biochemical properties during initial decomposition of plant and animal residues in soil. Appl Soil Ecol 41:118–127

Cerniglia CE (1992) Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3:351–368

Chang BV, Lu YS, Yuan SY, Tsao TM, Wang MK (2009) Biodegradation of phthalate esters in compost-amended soil. Chemosphere 74:873–877

Cookson JT (1995) Bioremediation engineering design and application. McGraw-Hill, New York, USA

Delgado-Moreno L, Peña A, Mingorance MD (2009) Design of experiments in environmental chemistry studies: example of the extraction of triazine from soil after olive cake amendment. J Hazard Mater 162:1121–1128

Deming SN, Morgan SL (1987) Experimental design: a chemometric approach. Data handling in science and technology, vol 3. Elsevier, Amsterdam

Gourlay C, Tusseau-Vuillemin MH, Garric J, Mouchel JM (2003) Effect of dissolved organic matter of various origins and biodegradability on the bioaccumulation of polycyclic aromatic hydrocarbons in *Daphnia magna*. Environ Toxicol Chem 22:288–1294

Haderlein A, Legros R, Ramsay BA (2006) Pyrene mineralization capacity increased with compost maturity. Biodegradation 17:293–302

Harrison RM (2001) Pollution: causes, effects and control, 4th edn. The Royal Society of Chemistry, Birmingham

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

In BH, Park JS, Namkoong W, Kim JD, Ko BI (2007) Effect of sewage sludge mixing ratio on composting of TNT-contaminated soil. Ind Eng Chem Res 13:190–197

Janzen RA, Xing B, Gomez CC, Salloum MJ, Drijber RA, McGill WB (1996) Compost extract enhances desorption of α-naphtol and naphthalene from pristine and contaminated soil. Soil Biol Biochem 28:1089–1098

Johnson AR, Wick LY, Harms H (2005) Principles of microbial PAH-degradation in soil. Environ Pollut 133:71–84

Jørgensen KS, Puustinen J, Suortti AM (2000) Bioremediation of petroleum hydrocarbon-contaminated soil by composting in biopiles. Environ Pollut 107:245–254

Laor Y, Strom PF, Farmer WJ (1999) Bioavailability of phenanthrene sorbed to mineral-associated humic acid. Water Resour 33:1719–1729

Lee S, Oh B, Kim J (2008) Effect of various amendments on heavy mineral oil bioremediation and soil microbial activity. Bioresour Technol 99:2578–2587

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

Mihial DJ, Viraraghavan T, Jin Y-C (2006) Bioremediation of petroleum-contaminated soil using composting. Pract Period Hazard Toxic Radioact Waste Manag 10:108–115

Namkoong W, Hwang EY, Park JS, Choi JY (2002) Bioremediation of diesel-contaminated soil with composting. Environ Pollut 119:23–31

Ohura T, Amagai T, Fusaya M, Matsushita H (2004) Polycyclic aromatic hydrocarbons in indoor and outdoor environments and factors affecting their concentrations. Environ Sci Technol 38:77–83

Oleszczuk P (2007) Investigation of potentially bioavailable and sequestrated forms of polycyclic aromatic hydrocarbons during sewage sludge composting. Chemosphere 70:288–297

Pignatello JJ, Xing B (1996) Mechanics of slow sorption of organic chemicals to natural particles. Environ Sci Technol 30:1–11

Plaza C, Xing B, Fernández JM, Senesi N, Polo A (2009) Binding of polycyclic aromatic hydrocarbons by humic

acids formed during composting. Environ Pollut 157:257–263

Ponsá S, Gea T, Alerm L, Cerezo J, Sánchez A (2008) Comparison of aerobic and anaerobic stability indices through a MSW biological treatment process. Waste Manage 28:2735–2742

Potter CL, Glaser JA, Hermann R, Dosani MA (1999) Remediation of contaminated east river sediment by composting technology. In: Leeson A, Alleman BC (eds) Bioremediation technologies for polycyclic aromatic hydrocarbon compounds. The fifth international in situ and on-site bioremediation symposium. Battelle Press, Columbus, pp 31–36

Quadri G, Chen X, Jawitz JW, Tambone F, Genevini P, Faoro F, Adani F (2008) Biobased surfactant-like molecules from organic wastes: the effect of waste composition and composting process on surfactant properties and on the ability to solubilize Tetrachloroethene (PCE). Environ Sci Technol 42:2618–2623

Rigas F, Dritsa V, Marchan R, Papdopoulou K, Avramids EJ, Hatzianestis I (2005) Biodegradation of lindane by *Pleurotus Ostreatus* via central composite design. Environ Int 31:191–196

Romantschuk M, Sarand I, Petänen T, Peltola R, Jonsson-Vihanne M, Koivula T, Yrjälä K, Haahtela K (2000) Means to improve the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. Environ Pollut 107:179–185

Ruggieri L, Gea T, Mompeó M, Sayara T, Sánchez A (2008) Performance of different systems for the composting of the source-selected organic fraction of municipal solid waste. Biosyst Eng 101:78–86

Ruggieri L, Gea T, Artola A, Sánchez A (2009) Air filled porosity measurements by air pycnometry in the composting process: a review and a correlation analysis. Bioresour Technol 100:2655–2666

Said-Pullicino D, Gigliotti G (2007) Oxidative biodegradation of dissolved organic matter during composting. Chemosphere 68:1030–1040

Sánchez A, Valero F, Lafuente J, Solà C (2000) Highly enantioselective esterification of racemic ibuprofen in a packed bed reactor using immobilized *Rhizomucor miehei* lipase. Enzyme Microb Technol 27:157–166

Semple KT, Reid BJ, Fermor TR (2001) Impact of composting strategies on the treatment of soils contaminated with organic pollutants. Environ Pollut 112:269–283

Senesi N, Plaza C, Brunetti G, Polo A (2007) A comparative survey of recent results on humic-like fractions in organic amendments and effects on native soil humic substances. Soil Biol Biochem 39:1244–1262

Serrano Silva I, dos Santos E, de Menezes CR, Fonseca de Faria A, Franciscon E, Grossman M, Durrant LR (2009) Bioremediation of a polyaromatic hydrocarbon contaminated soil by native soil microbiota and bioaugmentation with isolated microbial consortia. Bioresour Technol 100:4669–4675

Tejada M, González JL, Hernández MT, García C (2008) Application of different organic amendments in a gasoline contaminated soil: effect on soil microbial properties. Bioresour Technol 99:2872–2880

The US Department of Agriculture, The US Composting Council (2001) Test methods for the examination of composting and compost. Edaphos International, Houston

Thomas JM, Ward CH, Raymond RL, Wilson JT, Loehr RC (1992) Bioremediation. Encyclopaedia of microbiology. Academic Press, San Diego

Vieira P, Faria S, Vieira R, França F, Cardoso V (2009) Statistical analysis and optimization of nitrogen, phosphorus, and inoculum concentrations for the biodegradation of petroleum hydrocarbons by response surface methodology. World J Microbiol Biotechnol 25:427–438

Wan CK, Wong JWC, Fang M, Ye DY (2003) Effect of organic waste amendments on degradation of PAHs in soil using thermophilic composting. Environ Technol 24:23–30

Wilson SC, Jones KJ (1993) Bioremediation of soil contaminated with polycyclic aromatic hydrocarbons (PAHs): a review. Environ Pollut 81:229–249