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Preliminary screening of co-substrates for bioremediation of pyrene-contaminated soil through composting

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Abstract

The feasibility of using different organic amendments of different origin and properties in the bioremediation of pyrene-contaminated soil by means of composting has been tested. The selected pyrene concentration was 1 g of pyrene per kg of dry soil. The organic amendments used include: raw organic fraction of municipal solid wastes (OFMSW), industrial compost from OFMSW composting (COFMSW), compost derived from home composting of OFMSW (HCOFMSW), anaerobically digested sludge (ADS), non-digested activated sludge (NDS) and centrifuged non-digested activated sludge (CNDS). The degradation rate was related to the amendment properties that directly affected the composting process. Raw OFMSW was not capable to enhance pyrene degradation in comparison to control, but stable HCOFMSW exhibited the highest removal rate (69%). The amendments stability and the temperatures reached as a consequence influenced the process, and thermophilic temperatures showed an inhibition effect on the microbial activity related to pyrene degradation. Some of the tested wastes need to be further investigated to find inexpensive organic amendments for soil bioremediation.

Keywords: Composting, Organic wastes, Pyrene, Soil, Stability.

1. Introduction

At present, soil contamination by polycyclic aromatic hydrocarbons (PAHs) is of environmental concern. Many of these compounds have been shown to be acutely toxic, mutagenic and carcinogenic, and are of great concern with respect to both the environment and human health [1]. PAHs are introduced in the environment through accidental spillage, misguided disposal of petroleum and creosote wastes, combustion of fossil fuels, coal, wood preserving products and leaking from underground tanks [2].

Due to their hydrophobic nature, low aqueous solubility, low volatility and resistance to biological degradation, most PAHs bind to soil particles and sediments, which make them less available for biological uptake [3]. Thus, they finally accumulate in the environment. The treatment of PAH-contaminated sites is imperative according to new stringent regulations [4].

Since several microorganisms are capable of metabolizing PAHs [5-8], bioremediation may be a viable option for PAH-contaminated sites remediation. It has been demonstrated that bioremediation or composting can remediate soils contaminated with hydrocarbons, PAHs, chlorophenols, polychlorinated biphenyls and explosives [6-9].

Composting can be used for bioremediation purposes. However, there is still a need for more investigation in the bioremediation of hazardous wastes like PAHs using highly-available and low-cost co-substrates [6-10]. The effectiveness of the composting process offers the potential for substantial cost saving over other chemical and physical methods; also it is a relatively simple process to implement and operate, as raw materials for composting are often organic wastes available everywhere.

Composting is a biological process in which microorganisms are responsible for mineralization and humification of organic matter under optimal conditions of temperature, oxygen availability and moisture, which can increase the enzyme kinetics involved in the degradation of PAHs, solubility and mass transfer rates of the contaminants [7,11]. In the process of composting a contaminated soil, organic amendments are often added to increase the amount of nutrients and the readily biodegradable organic matter present in soil [12]. Also, the application of organic wastes in the bioremediation of PAH-contaminated soil may provide ways for recycling and reutilization of these organic wastes. The resulting end-product of the composting process is of great interest in soil application as fertilizer or organic amendment. Therefore, it seems very important to examine the effect of different organic wastes on the degradation of PAH-contaminated soil.

Recent studies have demonstrated the efficiency of soil bioremediation by composting, but usually these studies only focus on one type of organic amendment, where nowadays various sources of these amendments are available. Carrying out a screening study to have a general idea about applicability of various organic wastes to PAHs composting should help to select future approaches to be adopted for the treatment of PAHs contaminated soils.

2. Materials and methods

2.1 Soil

An uncontaminated agricultural soil classified as sandy loam soil was collected from the surface horizon (0-30 cm) in Prades (Tarragona, Spain). 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. No PAHs were detected in the soil. The main properties of the soil are presented in Table 1.

2.2 Contaminant

Pyrene (98% purity, Sigma-Aldrich, Spain), composed of four benzene rings fused together was selected as PAH contaminant to be monitored during soil composting treatments. Pyrene is a toxic, recalcitrant PAH commonly found in soil and considered as an indicator of monitoring PAH-contaminated wastes, and it is listed among the 16 USEPA priority PAHs pollutants [13] . Normally PAHs with four or more fused aromatic rings are recalcitrant to microbial attack and are not easily degraded [5]; therefore it can be considered a good alternative for organic amendments screening studies on bioremediation. This contaminant was spiked into the soil to obtain the desired concentration (1g of pyrene per dry kg of soil). This concentration can be considered relatively high according to previous studies [5-7] although it has been previously used in bioremediation processes.

2.3 Co-substrates (organic amendments)

Several organic amendments were tested to identify their effect as composting co-substrates in the bioremediation of pyrene-contaminated soil. These organic co-substrates include: raw organic fraction of municipal solid wastes (OFMSW), industrial compost from the OFMSW composting (COFMSW), compost derived from home composting process of the OFMSW (HCOFMSW), anaerobically digested sludge (ADS), non-digested activated sludge (NDS) and centrifuged non-digested activated sludge (CNDS). OFMSW and COFMSW were obtained from a composting plant (Barcelona, Spain) and HCOFMSW was obtained from a home composter in the University Autònoma of Barcelona, with wastes of the same origin and constituents than those used in industrial composting. ADS, NDS and CNDS were obtained from different wastewater treatment plants (Barcelona, Spain) with different operation technologies. Main characteristics of soil and organic amendments are presented in Table 1. Heavy metal contents are of Class A (the lowest content) for all the organic amendments according to National Spanish Ministry of Agriculture (*http://www.mapa.es/agricultura/pags/fertilizantes/documentos/RD824_2005.pdf*).

2.4 Preparation of composting experiments

Firstly, the contaminated soil was manually mixed with the organic amendment at a ratio of 1:1 (w/w, wet weight). The mixture was then mixed with bulking agent at a ratio of 1:1 v/v to provide a proper porosity to maintain aerobic conditions. The bulking agent consists of wood chips and pruning wastes and it can be considered not biodegradable under laboratory composting conditions. Water content of the composting matrix was adjusted to be within the recommended value (40-60 %) by adding tap water before composting. The final wet weight ratio of soil to amendment was dependent on the biodegradation of organic matter, but as a general rule, it was within 1:1 to 1:0.5, once bulking agent was sieved. All the composting experiments were carried out in duplicates.

2.5 Laboratory reactors

4.5-L Dewar vessels were modified and conditioned to operate in a batch operation mode in the composting process [14]. Temperature was monitored by Pt-100 sensors (Sensotran, Spain) connected to a data acquisition system (DAS-8000, Desin, Spain). Aeration was provided sporadically to the reactors according to the process performance, where oxygen concentration was maintained between 15-18 % to ensure aerobic conditions. Oxygen concentration was measured by means of an oxygen sensor (Crowcon's Xgard, United Kingdom). Composting time was set at 25 days because in this type of reactors this is a sufficient time to cover completely the active decomposition stage of the composting process [14]. The volatilization of pyrene during the composting process was considered negligible since blank experiments with sterilized soil showed that the contaminant concentration remained constant during more than 25 days.

2.6 Analytical procedures

Stability of organic amendments was determined using the static respirometric index determined according to Barrena et al. [15]. This index is equivalent to the oxygen uptake rate of the material. Moisture content, organic matter content (OM), total Kjeldahl nitrogen, total phosphorous, total potassium, total carbon content, pH and electrical conductivity were determined according to standard methods [16].

Sampling was performed by opening the reactor and mixing well its content to get two representative grab samples (about 20-30 g). Afterwards, 10 g from each sample were extracted using acetone/dichloromethane (1:1 v/v) as solvent during two hours. After this extraction the solvent was left to evaporate during 24 hours 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 splitless injector. A Zebron ZB-5HT Inferno column (Agilent, Spain) was used for pyrene identification. 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 (0.010-2 g/kg, correlation coefficient R^2=0.9983). Quality assurance and quality control data indicated that both the extraction system and gas chromatography are within the acceptable level according to international methods. Results are presented as average of two samples with standard deviation.

2.7 Statistics

Anova test was performed to compare different degradation values obtained from duplicate experiments. If Anova test resulted in statistically significant differences, Tukey test was performed in pairwise comparisons. 95% confidence level was selected for all statistical comparisons. Statistical tests were conducted with SPSS 15.0.1 (SPSS Inc., USA).

3. Results and discussion

Table 1 summarizes the characteristics of the materials used. All the organic wastes proposed as amendments were characterized by a high content of organic matter in comparison to soil. This makes them good candidates for composting purposes as nutrient sources to encourage the growth of the populations already present in the soil. Also these amendments are suggested to provide valuable populations of microorganisms which along with the indigenous ones can degrade the contaminant. In terms of nutrient composition, it is evident that both composts from OFMSW presented higher levels of P and K, which could enhance the biological activity. Moreover, values of pH and electrical conductivity are different to some extent among them, which can also affect the process. Normally, microorganisms function within a narrow optimal range of pH and their activity is inhibited in more acidic or alkaline conditions. As expected, the main difference observed was the respiration index that presented high values for non-stabilized wastes (NDS, CNDS and specially raw OFMSW) and lower values for biologically-treated materials (ADS and HCOFMSW), as reported in previous studies [17]. The only abnormal value of stability was found for industrial COFMSW, which was probably due to an incomplete curing process. However, no data about the composting process at full-scale was available. Although the identification of microorganisms was not performed, the respiration index is considered a suitable index to measure the overall aerobic microbial activity of the used amendments [15,18].

Figure 1 presents the results obtained for the pyrene removal when the composting experiments were carried out. Although a certain removal was achieved in the control experiment (non-amended soil), it was evident that the percentage of pyrene degradation significantly increased in the presence of some organic amendments. Specifically, degradation using OFMSW of several stability degrees (OFMSW, COFMSW, HCOFMSW) or several types of sludge (ADS, NDS, CNDS) were statistically different among them and different from control with soil. According to this, the stimulation of the indigenous microorganisms by adding suitable organic amendments could be a viable option, although the selection of the organic amendment is crucial to enhance PAHs biodegradation.

For instance, raw OFMSW was inefficient to enhance pyrene removal although most factors related to a successful composting process were in the optimal ranges [19]. In this case, temperature rapidly reached the thermophilic range and it was maintained in this range for six days (Figure 2). In fact, is it observed that a there is a positive correlation between the maximum temperature achieved in the composting experiments and the level of respiration index (Table 1). Accordingly, respiration index can be determined for predicting the temperature to carry out a bioremediation process (thermophilic or mesophilic). This is of special relevance for PAH biodegradation. Although it is reported that high temperatures are supposed to increase the desorption of hydrophobic contaminant, to improve the mass transfer rates and to enhance the enzymatic kinetics involved in the biodegradation process [20], the results obtained in this study demonstrated that these conditions were not adequate for microorganisms responsible for pyrene biodegradation. This could be explained by the preferential degradation of easily degradable material observed in some organic amendments rather than PAHs as more decrease in the organic matter (11%) was observed in less stable amendments when comparing to those presenting a high degree of stability (Table 1). Another hypothesis can be the negative effect of high temperatures on specific microorganisms responsible for PAHs degradation, as reported in other studies [21]. A similar behaviour was observed with COFMSW, which is biologically active according to respiration index (Table 1). In fact, when considering only wastes whose source is OFMSW; it seemed to be an exponential positive correlation between waste stability and pyrene removal (Figure 1) in the sense that, the more stable a waste is, the more pyrene removal is observed. Although only three stability values were available, for this waste this correlation presented a correlation coefficient of 0.95 (Eq. 1):

$$\text{Pyrene removal (\%)} = 621 \cdot \exp(-1.24 \cdot \text{RI}) \quad (1)$$

where RI is the static respiration index expressed in mg O_2 g^{-1} OM h^{-1}.

Other studies have shown that the fraction of humic acids present in stable compost enhance the desorption process of hydrophobic organic components from soil [22], which increases the contaminant bioavailability, thus increasing the rate of the degradation. Furthermore, it has been suggested that the sorption of both microorganisms and PAHs to the colloidal surfaces of humic matter stimulate their biodegradation [23]. Unfortunately, the humic characterization of the organic amendments used in this study was not carried out, although it can be the focus of future studies.

Experiments with several typologies of wastewater sludge as organic amendment showed different results. Pyrene removal was similar to that of soil for ADS and CNDS, and higher for NDS. In this case, no clear correlation could be found among stability and pyrene degradation since stability values were similar (especially NDS and CNDS) and the temperature reached was in the mesophilic range during all the composting process. Although sewage sludge has been reported to enhance the degradation of hydrocarbons in soil-compost mixtures [24,25], no results have been found on the use of different typologies of sludge to enhance PAHs biodegradation. In this case, the bioavailability of the contaminants can be affected by the sludge type and, consequently, the degradation rate. Oleszczuk [25] noted a great difference in the bioavailable fraction of PAH depending on the stage of the experiment and the sludge type.

4. Conclusions

The preliminary results presented in this work show that low-cost and easily available organic wastes can be used as organic amendments to enhance the biodegradation of pyrene contaminated-soils. In particular, stable home compost from OFMSW was found to improve significantly the pyrene removal. Further studies should be focused on the importance of organic amendments’ stability for PAHs biodegradation in composting processes and in the knowledge of whether stability influence is related to chemical composition (for instance, presence of humic acids) or the microbiological communities that are active at each stability level. Nevertheless, it is important to mention that the use of stable amendments for soil bioremediation will inherently produce an end product of high stability, which can be used as organic amendment or fertilizer.

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Tables

Table 1: Characteristics of the soil and organic amendments used.

Parameter/Material*	Soil	OFMSW	COFMSW	HCOFMSW	ADS	NDS	CNDS
Moisture content (%)	11.4	54.4	49.7	48.2	83.4	79.5	68.1
Organic matter content (%, db)**	2.7	67.7	52.4	47.3	41.2	76.4	83.2
Total Carbon (mg C g^{-1}) (db)	12.4	315.5	201.3	251.8	280.7	451.3	483.5
Total Nitrogen Kjeldahl (mg N g^{-1}) (db)	6.5	19.4	19.0	26.1	21.3	44.6	42.8
C/N ratio	1.9	16.3	10.6	9.6	13.2	10.1	11.3
Phosphorous (%) (db)	-	0.5	1.4	1.6	1.0	0.8	1.0
Potassium (%) (db)	-	0.8	2.0	2.5	0.17	0.33	0.12
pH	6.7	5.8	7.4	8.7	8.1	6.5	8.7
Elec. conductivity (mS/cm)	0.2	2.8	2.9	3.0	2.0	2.2	1.5
Static respiration index (mg O_2 g^{-1} OM h^{-1})***	0	5.0	2.6	1.7	1.4	2.9	2.8

* OFMSW: raw organic fraction of municipal solid wastes, COFMSW: industrial compost from OFMSW composting, HCOFMSW: compost derived from home composting of OFMSW, ADS: anaerobically digested sludge, NDS: non-digested activated sludge, CNDS: centrifuged non-digested activated sludge.

** db: dry basis

*** OM: organic matter

Figure 1: Removal of pyrene in the soil after composting with organic wastes following 25 days of treatment. Results are presented as average of two samples with standard deviation. OFMSW: raw organic fraction of municipal solid wastes, COFMSW: industrial compost from OFMSW composting, HCOFMSW: compost derived from home composting of OFMSW, ADS: anaerobically digested sludge, NDS: non-digested activated sludge, CNDS: centrifuged non-digested activated sludge.

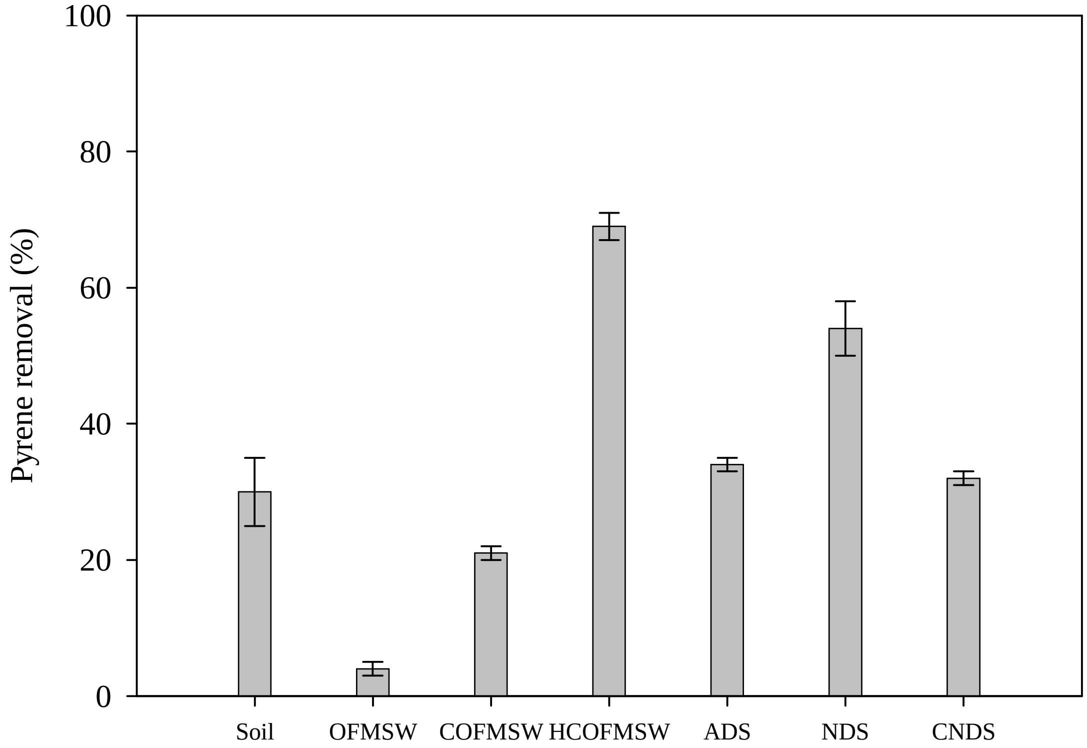

Figure 2: Temperature profiles of the composting process with organic wastes. Temperature differences between duplicate experiments were lower than 5ºC. OFMSW: raw organic fraction of municipal solid wastes, COFMSW: industrial compost from OFMSW composting, HCOFMSW: compost derived from home composting, ADS: anaerobically digested sludge, NDS: non-digested activated sludge, CNDS: centrifuged non-digested activated sludge.