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Remediation trials for hydrocarbon-contaminated sludge from a soil washing process: Evaluation of bioremediation technologies

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ABSTRACT

The usual fate of highly contaminated fine products (silt-clay fractions) from soil washing plants is disposal in a dump or thermal destruction (organic contaminants), with consequent environmental impacts. Alternative treatments for these fractions with the aim of on-site reuse are needed. Therefore, the feasibility of two technologies, slurry bioremediation and landfarming, has been studied for the treatment of sludge samples with a total petroleum hydrocarbon (TPH) content of 2243 mg/kg collected from a soil washing plant. The treatability studies were performed at the laboratory and pilot-real scales. The bioslurry assays yielded a TPH reduction efficiency of 57% and 65% in 28 days at the laboratory and pilot scale, respectively. In the landfarming assays, a TPH reduction of 85% in six months was obtained at laboratory scale and 42% in three months for the bioremediation performed in the full-scale. The efficiency of these processes was evaluated by ecotoxicity assessments. The toxic effects in the initial sludge sample were very low for most measured parameters. After the remediation treatments, a decrease in toxic effects was observed in earthworm survival and in carbon mineralisation. The results showed the applicability of two well known bioremediation technologies on these residues, this being a novelty.

1. Introduction

A particular contaminated site may require a combination of procedures to achieve optimum remediation. Biological, physical and chemical technologies may be used in conjunction to reduce the contamination to a safe and acceptable level. The selection of appropriate technologies depends on the nature of the contaminant(s) and site characteristics, regulatory requirements, costs, and time constraints. The successful treatment of a contaminated site depends on the proper selection, design and adjustment of remediation technology operations based on the properties of the contaminants and soils and on the performance of the system [1].

Soil washing uses liquids (usually water, occasionally combined with solvents) and physical processes to scrub soils. This process separates fine soil (clay and silt) from coarse soil (sand and gravel). Because hydrocarbon contaminants tend to bind and sorb to smaller particles, separating the smaller soil particles from the larger ones reduces the volume of contaminated soil. Soil washing is cost effective because it reduces the quantity of material that would require further treatment by another technology. This smaller volume of soil, which contains the majority of clay and silt particles, can be further treated by other methods. The usual destination of these fractions is disposal in a dump or thermal destruction (in cases of organic contaminants), with consequential environmental impacts. The development of alternative, environmentally friendly treatments for these fractions allowing for their on-site reuse is therefore needed.

Bioremediation is an attractive approach for cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost effective and leads to the complete destruction of the contaminants. These treatments have emerged as a "green" alternative for treating these environmental contaminants [1]. Conventionally, on-site technologies such as landfarming, composting and soil piles have been employed; the most advanced ex situ methods such the use of bioreactors provide better control to enhance the hydrocarbon degradation process.

One of the difficulties of developing bioremediation strategies lies in achieving results in the field that are as good as those in the laboratory. To date, most of the reported experiments on soil bioremediation have been performed in the laboratory (in

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well-controlled conditions), whereas field-scale experiments have remained scarce [2]. In this context, treatability or feasibility studies are used to determine whether remediation will be effective in a given situation. The extent of the study varies depending on the nature of the contaminants and the characteristics of the site. For sites contaminated with common petroleum hydrocarbons (e.g., gasoline and/or other readily degradable compounds), it is usually sufficient to examine representative samples for the presence and level of an indigenous population of microbes, nutrient levels, the presence of microbial toxicants, and sample characteristics such as pH, porosity, and moisture. Hydrocarbon bioremediation can be promoted by stimulation of the indigenous microorganisms by introducing nutrients and oxygen (biostimulation) [3].

The objectives of remediation processes are usually based on threshold levels of soil contaminants. Bioremediation has proven to be successful in numerous applications for petroleum-contaminated soils. However, during bioremediation processes, changes in bioavailability and metabolite yield can occur. Therefore, questions remain as to the efficiency of bioremediation in lowering soil toxicity. Consequently, it is necessary to incorporate ecotoxicity assessments to evaluate the treatment efficiency.

Due to the scarcity of studies on biological treatment of these classes of residues [4,5], the possibilities for the treatment of sludge (fine fraction) from a soil washing plant at a site contaminated with hydrocarbons using bioremediation technologies were studied in this paper. Chemical and biological assays were combined to evaluate the efficiency of the sludge remediation technologies.

2. Materials and methods

2.1. Sample characterisation

The studied sample was the sludge fraction from a soil washing plant at a site contaminated by hydrocarbons. The site was previously contaminated due to hydrocarbons storage tanks, mainly diesel hydrocarbons. The physical properties of the sample were determined according to the standard procedures [6]. The grain size characterisation was performed by elutriation using Cyclosizer equipment.

The hydrocarbon content was determined by gas chromatography–flame ionisation detection (GC-FID), measuring aliphatic and aromatic hydrocarbon fractions in the range of C_{10}–C_{40} according to the ISO 16703:2004 norm [7]. The total petroleum hydrocarbon (TPH) content was also determined with an infrared (IR) method using the portable InfraCal model HATR-T2 analyser in accordance with EPA method 1664 [8].

In selected samples, gas chromatography coupled to mass spectrometry, GC-MS, was performed to determine the major fractions affected by biodegradation. Samples of 1 g were extracted with hexane. Analyses by GC/MS were performed on the hexane extracts, conducted in scan mode with two columns, CP-Sil 8CB and HP-5MS, to evaluate organic compounds between n-C_6 and n-C_{35}. An Agilent 5890/5972 A GC/MS chromatograph was used for this process.

2.2. Soil sample

Control soil for ecotoxicity testing was collected from the surface layer of a field located near Madrid (Spain). Soil was air-dried and sieved (2 mm mesh). The main physicochemical characteristics of this soil were as follows: clay, 7.8%; silt, 18.8%; sand, 73.4%; pH, 7.3 and organic C, 1.09%. This soil fulfilled the conditions outlined by the OECD [9] for use as a control soil in microbial assays (sand >70%, pH 5.5–7.5 and organic carbon content ranging from 0.5 to 1.5%).

Table 1
Test program of treatability study.

Treatability test	Level of treatment	
	Laboratory	Pilot/Demonstration
Bioslurry	• Microcosms (Respirometry) • Stirred reactors	Air-lift pilot plant
Landfarming	• Microcosms (Respirometry) • Mesocosms (Columns + Trays)	Field (landfarming cell)

2.3. Treatability study

Two different treatment scales were studied: a laboratory scale and a pilot or demonstration scale (Table 1).

2.3.1. Bioslurry

For the bioslurry treatability tests, the optimal conditions were established at the laboratory scale by respirometry, first in microcosms and then in reactors. The experimental design covered different pulp densities and C:N:P ratios, i.e., natural (control), 100:10:1 and 100:10:0.5, in accord with previous work reported in the literature [10,11], and different treatment times.

Bioreactor simulation tests were performed by respirometry at different conditions in the microcosms, following the oxygen consumption and carbon dioxide production with a Micro-Oxymax respirometer (Columbus Instruments) equipped with an IR sensor for CO_2 and a paramagnetic sensor for O_2.

The respirometer houses 20 independent chambers that allow for the simultaneous measurement of oxygen consumption and carbon dioxide production in each chamber. The chambers are 250 mL ISO flasks with magnetic stirring and contain 20 g of sample for each study condition.

The different treatment conditions studied were mainly pulp density (from 1% to 20% w/v) and nutrient addition compared with the control assay (without nutrients). All tests were performed in triplicate. Tests lasted 31 days, and samples were harvested periodically for the analysis of hydrocarbon content to evaluate contaminant biodegradability and biodegradation rate.

Biotreatment simulated with bioreactors was conducted at the laboratory scale using 2 L mechanically stirred reactors at 20% (w/v) pulp density. The addition of nutrients and treatment time were tested. Based on the results obtained at the laboratory scale, the optimal conditions were applied at the pilot scale in a BiOEIMCO air-lift plant equipped with three 60 L reactors.

2.3.2. Landfarming

The optimal landfarming conditions were established by treatability tests in microcosms monitored by respirometry and were applied to mesocosms in packed columns and trays. The experimental design covered different C:N:P ratios and humidities with respect to the water holding capacity (WHC). The microcosm assays were performed as described above with the following differences. The humidity of the samples was adjusted to 60%, 70% or 80% of WHC, different C:N:P ratios were tested by nutrient addition, and the duration of the tests was 61 days.

Due to the fine texture of the initial sample, the material was amended with barley straw to facilitate its aeration and handling. Amended assays (with nutrients) were compared with the control assay (without nutrients). All samples were turned over weekly to facilitate aeration. Samples were harvested periodically, and the hydrocarbon content was analysed.

Landfarming mesocosms assays were performed at the optimal conditions obtained at the microcosm scale, using a two-step treatment simulation at field temperature. The first step was performed in packed columns with 4 kg of sludge, monitored daily and

maintained with respect to moisture control and nutrient levels, and the evolution of temperature and oxygen and carbon dioxide concentrations was measured at several sampling ports along the columns (deep, at 20 cm, medium, at 10 cm and in the headspace). Samples were taken monthly, and the TPH contents were analysed. The aliphatic and aromatic hydrocarbons fractions in the range of C_{10}–C_{40} were determined in selected samples. This step lasted 4 months.

The second, less intensive step was performed in trays in which samples from the columns were laid out in a bed 5 cm wide. During this phase, the soil was aerated by tilling the bed weekly, and bed moisture was adjusted at the same time. This step lasted 2 months.

Finally, based on the results obtained at the laboratory scale, the optimal conditions were applied at full scale. This entailed the construction of a landfarming cell for the treatment of 4000 kg of sludge.

The landfarming experiment was performed over three months. Two treatment areas were conditioned, each 5 × 21 m. The sludge (fine fraction) produced by the soil washing plant was piled into heaps 0.5 m high in these areas. The sludge was adjusted to an organic matter (OM) content of 4% by the addition of straw. The sludge was then amended with fertiliser to yield a C:N:P ratio of 100:10:0.5. The sludge in both cells was irrigated from the surface to maintain a moisture content close to 70% of WHC. The sludge was turned over weekly, and it was mixed intensively with a rotobator every two weeks.

Samples of sludge were taken at 0, 7, 14, 21, 30, 45, 60 and 90 days. Four simple samples were taken from each cell at each sampling time. These subsamples were mixed to form a composite sample for each cell for the analysis of TPH according to the ISO 16703:2004 norm [7] and other parameters. The samples were analysed in triplicate.

2.4. Ecotoxicity assays

Ecotoxicity assays were performed on the initial sample and optimally remediated samples using the different treatments studied, designated as:

- BIOSLU, optimally remediated sample using the bioslurry in the pilot plant.
- LABLF, optimally remediated sample using laboratory-scale landfarming (packed column plus tray tests).
- REALLF, optimally remediated sample using full-scale landfarming on site.

2.4.1. Multispecies-soil system; MS-3

Multispecies-soil systems (MS-3) have been used for the assessment of different types of wastes [12,13]. The initial sludge was tested at four dilutions with control soil: 100%, 50%, 25% and 12.5% (w/w). The dilutions of initial sludge with control soil were prepared on a dry-weight basis by mechanically mixing the soils in a B50 Solid V-mixer (Lleal, S.A.). Sludges obtained after remediation processes were assessed without dilution due to the low toxicity determined in the initial sample.

Test samples were placed in methacrylate columns 15 cm high × 15 cm in diameter (2.0 kg soil dry wt. per column), and ten adult earthworms (*Eisenia fetida*; Oligochaeta: Lumbricidae) from our laboratory cultures were added on day 0 to each soil microcosm to represent soil invertebrates. Seven seeds of three plant species (wheat, *Triticum aestivum*; rape, *Brassica napus*; and red clover, *Trifolium pratense*) were sown in the soil in each microcosm. Certified seeds of these vascular plants were kindly supplied by the Spanish Office of Plant Varieties. The species selected for this study are recommended by the OECD guidelines [14] for the testing of chemicals. Three replicates were performed for each treatment.

Columns were incubated in a climate-controlled room at a temperature of 20 ± 2 °C and illuminated with fluorescent bulbs (18 W) with a photoperiod of 16 h of daylight and 8 h of darkness; the light intensity was 1600–1900 lux. Water was added to the soil to reach 100% of its water-holding capacity. Columns were watered 5 days a week with 50 mL/day of dechlorinated water, simulating 1000 mm of annual rainfall, and the soils were allowed to drain to field capacity. Leachates were collected over 21 days in association with watering events. Successive leachate fractions for each microcosm were mixed and kept refrigerated at 4 °C. At the end of the assay, the leachates were stored at –20 °C for further chemical and biological analysis when immediate analysis was not possible.

After 21 days, the MS-3 columns were opened, and the earthworms were counted for survival assessment, washed with distilled water, kept for 24 h on moist filter paper and weighed. Plant toxicity was determined by the emergence of seedlings and above-ground biomass production, measured as wet mass of shoots. Soil samples from the superficial layer were collected for microbial activity assays. Toxic effects on microorganisms were determined using a soil respiration test induced by glucose and by soil enzymatic activities, specifically, dehydrogenase (DH) and phosphatases (acidic and alkaline). Microbial respiration was determined following the principles of standardised methods [9]. Samples were amended with 4 mg glucose/g soil (dry weight), and carbon dioxide release was measured using a BacTrac 4000 SY-Lab (Microbiological Analysers). Dehydrogenase and phosphatase (acidic and alkaline) activities were measured according to Carbonell et al. [15], Freeman et al. [16] and Tabatabai and Bremner [17], respectively. Treatments and control soil were run in triplicate, and duplicates of each sample were taken for analysis. Microbial activities were determined at 0 and 21 days.

The indirect effects on aquatic organisms due to the leaching of sludge contaminants were assessed through a battery of bioassays on aquatic invertebrates (*Daphia magna*), an acute toxicity test following OECD guideline 202 [18] and an algal (*Chlorella vulgaris*) growth inhibition test using the method described in Ramos et al. [19].

2.4.2. Microbial assays

In addition to the MS-3 assays, microbial activities were measured in a single-species test. Both the initial and remediated sludges were assessed at four dilutions with control soil (100%, 50%, 25% and 12.5%, w/w), prepared as described above. Samples were incubated under aerobic conditions in the dark at room temperature of 20 ± 2 °C. The moisture content of soil samples was maintained between 40% and 60% of the maximum water holding capacity of the soil for 28 days. Three replicates were used for each concentration. The effects on microorganisms were determined at 0 and 28 days using the soil respiration test, as described in the standardised method [9], and enzymatic activities were determined as described previously.

2.4.3. Statistical analysis

The toxicity responses obtained in test soils were compared with those in the control soil by one-way analysis of variance (ANOVA) with Fisher's least significant difference procedure (LSD, $P < 0.05$) using the StatGraphics software package. In assays at different concentrations, toxicity effects were calculated as EC_{50} values using log-probit methods in StatGraphics.

3. Results and discussion

3.1. Characterisation of initial sample

The main physical parameters of the studied sample are shown in Table 2.

Table 2
Physical characteristics of initial sample.

Parameter	Value
pH	8.2
Conductivity (μS/cm)	1002
Moisture content (%)	45.33
WHC (%)	61.02
Total C (%)	2.6
Total inorganic C (%)	1.3
Black C (%)	0.3
Total organic C (%)	1.0
Oxidisable organic carbon (%)	0.8
Total nitrogen (%)	0.09

Table 3
Hydrocarbon composition of initial sample.

Hydrocarbons	Hydrocarbon content (mg/kg)
Aliphatic	
C_{10}–C_{12}	5.4
C_{12}–C_{16}	396.2
C_{16}–C_{21}	837.7
C_{21}–C_{35}	446.0
C_{35}–C_{40}	18.3
Total aliphatic	1703.6
Aromatic	
C_{10}–C_{12}	<3.0
C_{12}–C_{16}	90.3
C_{16}–C_{21}	274.6
C_{21}–C_{35}	163.6
C_{35}–C_{40}	11.3
Total aromatic	539.8
Total C_{10}–C_{40}	2243.4

Table 3 reports the hydrocarbon characterisation of the sample. The TPH content obtained by IR was 2262 mg/kg.

The results of the particle size analysis and the TPH distribution showed that more than 83% of the hydrocarbons were found in the particles under 12 μm.

3.2. Bioreactor trials

In the treatability tests simulating a bioreactor process, it was first studied the influence of pulp density ranging from 1 to 20% (w/v) and nutrient addition. Table 4 summarises the results of these tests.

Table 4
Tests performed with different pulp densities and C/N/P ratios.

Pulp density (%) (w/v)	C/N/P ratio	TPH Reduction (%)
1%	Control	38
	100/10/0.5	72
5%	Control	36
	100/10/0.5	52
10%	Control	29
	100/10/0.5	57
	100/10/1	51
15%	Control	26
	100/10/0.5	50
20%	Control	25
	100/10/0.5	52
	100/10/1	54

The best results were obtained at a pulp density of 1% with nutrient addition, yielding a reduction efficiency of 72% in 28 days, but this is clearly not feasible. However, promising results were obtained at higher pulp densities at a C/N/P ratio of 100/10/0.5, with reductions very similar to that at the C/N/P ratio of 100/10/1 (see Fig. 1).

Table 5 compares the hydrocarbon contents and reduction efficiencies with respect to the initial sample obtained at 10 and 20% pulp densities with a C/N/P ratio of 100/10/0.5.

A total TPH reduction of 57% (48% of the aliphatics and 84% of the aromatics) was obtained in 31 days of treatment at a 20% pulp density with a C/N/P ratio of 100/10/0.5, which slightly less than that obtained at a 10% pulp density.

From these results, the next test was conducted in 2 L mechanically agitated reactors at 20% pulp density and a C/N/P ratio of 100/10/0.5 and compared with the control (Fig. 2) prior to conducting the pilot plant tests. The TPH reductions obtained were 26% and 47% for the control and nutrient addition, respectively.

3.3. Pilot plant tests

These tests were performed using two of the three 60 L airlift bioreactors of the pilot plant. The TPH reduction with the addition of nutrients was from 2243 mg/kg to 803 mg/kg in 28 days, which is a reduction efficiency of 65%; the control efficiency was 44% (Table 6).

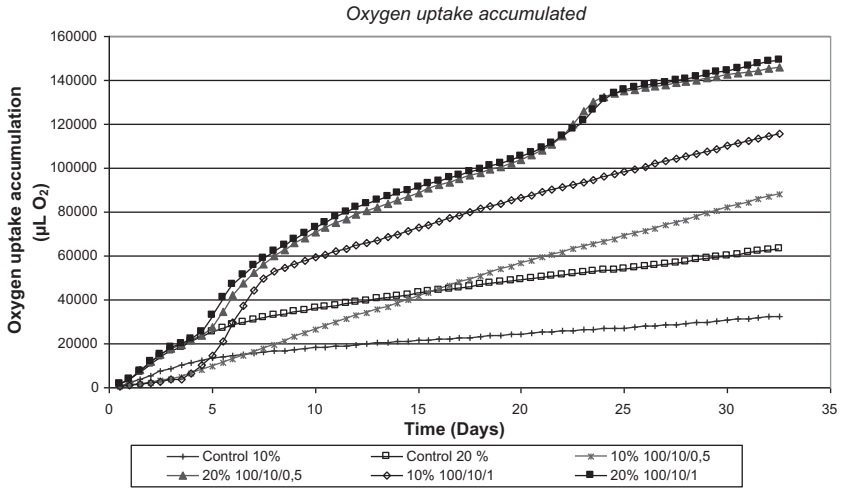

Fig. 1. Oxygen uptake evolution in tests performed at 10 and 20% pulp densities with different nutrient amendments.

Table 5
Hydrocarbon content and reduction efficiency with respect to the initial sample obtained at 10 and 20% pulp densities with a C/N/P ratio of 100/10/0.5.

Hydrocarbons	Hydrocarbon content (mg/kg) Initial sample	Hydrocarbon content (mg/kg) 10% 100/10/0.5	Hydrocarbon reduction efficiency (%) 10% 100/10/0.5	Hydrocarbon content (mg/kg) 20% 100/10/0.5	Hydrocarbon reduction efficiency (%) 20% 100/10/0.5
Aliphatic					
C_{10}–C_{12}	5.4	3.6	33	3.7	31
C_{12}–C_{16}	396.2	45.2	89	66.8	83
C_{16}–C_{21}	837.7	316.2	62	431.2	49
C_{21}–C_{35}	446.0	333.4	25	374.0	16
C_{35}–C_{40}	18.3	11.9	35	14.2	22
Total aliphatic	1703.6	710.3	58	889.9	48
Aromatic					
C_{10}–C_{12}	<3.0	<3.0	–	<3.0	–
C_{12}–C_{16}	90.3	<3.0	–	<3.0	–
C_{16}–C_{21}	274.6	23.3	91	18.1	93
C_{21}–C_{35}	163.6	73.0	55	60.4	63
C_{35}–C_{40}	11.3	6.8	40	5.8	49
Total aromatic	539.8	103.1	81	84.3	84
Total C_{10}–C_{40}	2243.4	813.4	64	974.2	57

Table 6
Hydrocarbon content and reduction efficiency with respect to the initial sample obtained at the optimal conditions in the pilot plant for different hydrocarbon ranges.

Hydrocarbons	Hydrocarbon content (mg/kg) Initial sample	Hydrocarbon content (mg/kg) Final sample pilot plant BIOSLU 20% 100/10/0.5	Hydrocarbon reduction efficiency (%)
Aliphatic			
C_{10}–C_{12}	5.4	<3.0	–
C_{12}–C_{16}	396.2	77.5	80
C_{16}–C_{21}	837.7	263.4	69
C_{21}–C_{35}	446.0	374.7	16
C_{35}–C_{40}	18.3	<3.0	–
Total aliphatic	1703.6	715.6	58
Aromatic			
C_{10}–C_{12}	<3.0	<3.0	–
C_{12}–C_{16}	90.3	<3.0	
C_{16}–C_{21}	274.6	19.6	93
C_{21}–C_{35}	163.6	35.5	78
C_{35}–C_{40}	11.3	<3.0	–
Total aromatic	539.8	62.7	88
Total C_{10}–C_{40}	2243.4	778.3	65

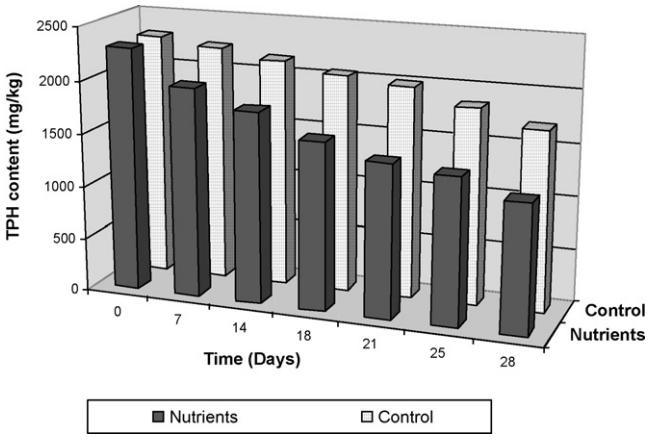

Fig. 2. The evolution of TPH content in tests performed at 20% pulp density in 2 L reactors.

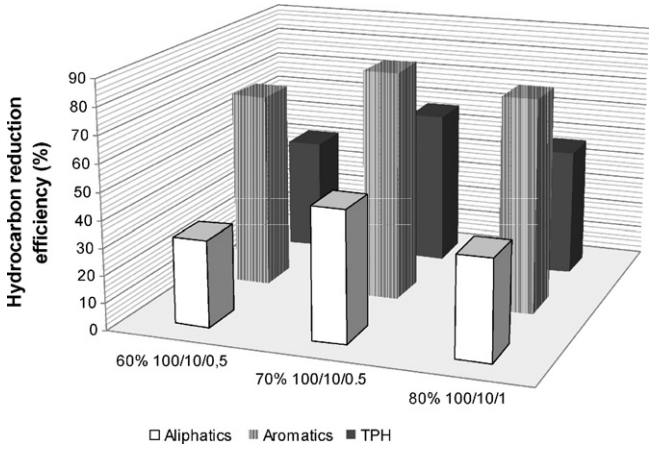

Fig. 3. Comparison of TPH reductions and total aromatic and aliphatic hydrocarbons at optimal conditions depending on humidity as a percentage of WHC.

Note that not all hydrocarbons are affected in the same way. Usually, aromatic hydrocarbons show a degradation rate over an order of magnitude less than that of n-alkanes. In this case, however, it has been observed a 58% elimination rate for aliphatic hydrocarbons and an 88% rate for aromatic hydrocarbons.

3.4. Landfarming laboratory-scale trials

Table 7 presents the optimal results obtained in microcosm tests with different nutrient additions and humidities with respect to WHC. Fig. 3 shows the TPH reductions obtained in these tests.

Table 7
Hydrocarbon content and reduction efficiency with respect to the initial sample obtained at optimal conditions in landfarming microcosm tests for different hydrocarbon ranges.

Hydrocarbons	Hydrocarbon content (mg/kg)	Humidity (%) with respect to WHC					
		70% WHC		80% WHC		60% WHC	
	Initial	Hydrocarbon content (mg/kg) 100/10/0.5	TPH reduction (%)	Hydrocarbon content (mg/kg) 100/10/1	TPH reduction (%)	Hydrocarbon content (mg/kg) 100/10/0.5	TPH reduction (%)
Aliphatic							
C_{10}–C_{12}	5.4	<3.0		<3.0		<3.0	
C_{12}–C_{16}	396.2	70.1	82	147.2	63	120.1	70
C_{16}–C_{21}	837.7	461.7	45	607.5	27	637.2	24
C_{21}–C_{35}	446.0	348.2	22	322.3	28	398.7	11
C_{35}–C_{40}	18.3	4.9	73	4.4	76	3.1	83
Total aliphatic	1703.6	884.9	48	1081.4	37	1159.1	32
Aromatic							
C_{10}–C_{12}	<3.0	<3.0		<3.0		<3.0	
C_{12}–C_{16}	90.3	<3.0		4.3	95	4.4	95
C_{16}–C_{21}	274.6	32.9	88	54.5	80	72.5	74
C_{21}–C_{35}	163.6	50.6	69	55	66	75.5	54
C_{35}–C_{40}	11.3	<3.0		<3.0		<3.0	
Total aromatic	539.8	83.5	85	113.8	79	152.4	72

The best results were obtained at 70% WHC and a C/N/P ratio of 100/10/0.5. Based on these results, mesocosms tests were then conducted in columns under these conditions.

Figs. 4 and 5 show the evolution of oxygen and CO_2 concentrations, respectively, at the deeper sampling ports of the columns for the control and nutrient columns over the first four months. As can be seen here, only slight differences were observed between the control and nutrient columns in the first two months. This was also observed when comparing the evolution of TPH in the samples taken during the test (Fig. 6). At the end of the test, the columns with nutrients had a TPH content of 1121 mg/kg versus 1209 mg/kg in the control columns (Table 8).

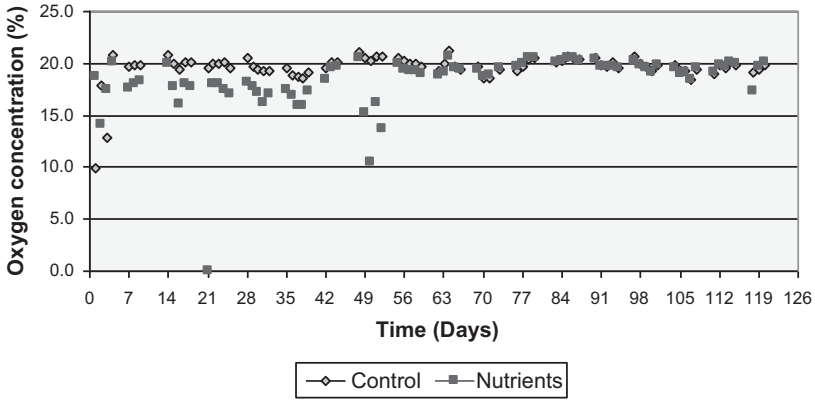

Fig. 4. Oxygen concentrations in control and nutrient columns.

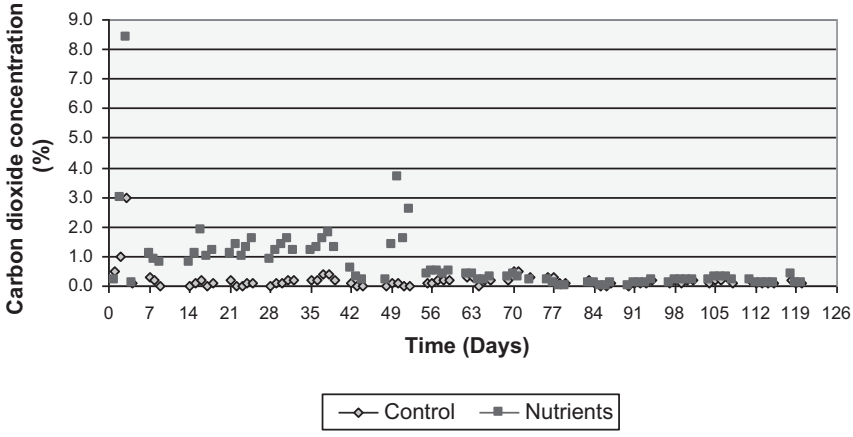

Fig. 5. Carbon dioxide concentrations in control and nutrient columns.

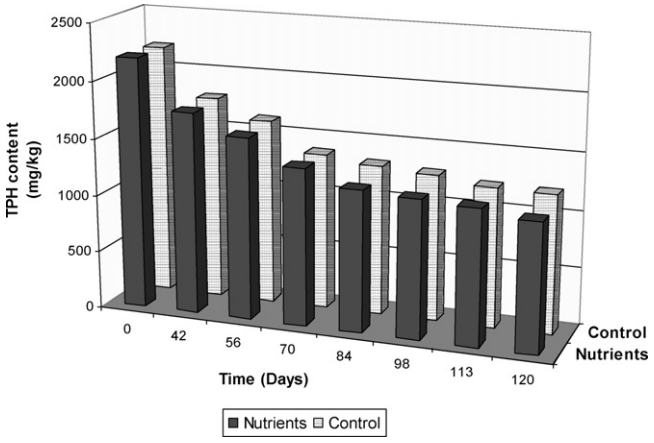

Fig. 6. TPH evolution during column tests.

Table 8
Hydrocarbon content obtained at the end of the column tests (4 months).

Hydrocarbons	Hydrocarbon content (mg/kg) Control column	Hydrocarbon content (mg/kg) Nutrient column
Aliphatic		
C_{10}–C_{12}	3.5	<3.0
C_{12}–C_{16}	106.6	108.1
C_{16}–C_{21}	620.0	585.8
C_{21}–C_{35}	441.3	390.3
C_{35}–C_{40}	<3.0	<3.0
Total aliphatic	1171.4	1084.2
Aromatic		
C_{10}–C_{12}	<3.0	<3.0
C_{12}–C_{16}	<3.0	<3.0
C_{16}–C_{21}	14.0	13.0
C_{21}–C_{35}	24.3	23.9
C_{35}–C_{40}	<3.0	<3.0
Total aromatic	38.0	36.9
Total C_{10}–C_{40}	1209.4	1121.0

Taking into account these unsatisfactory data, it was undertaken a second, less intensive remediation step performed in trays with samples from the columns tests previously described. Table 9 shows the results obtained in this step.

Over the two months of treatment, the hydrocarbon reduction efficiencies obtained were higher than 70% in both tests (control and amended with nutrients). The best results were obtained in samples amended with nutrients, with an aromatic hydrocarbon reduction of 97% and an aliphatic hydrocarbon reduction of 82%. These results are very successful compared with other works reported in the literature. Line et al. [20], reported a landfarming remediation of hydrocarbon-contaminated soil in which TPH declined from a mean of 4644 mg/kg to near 100 mg/kg over 12 months, with the greatest losses in the chain lengths C_{10}–C_{28}. Gallego et al. [21] reported the landfarming treatment of a soil affected by diverse and very old crude oil spills; after 5 months, the TPH was reduced by 50% from an initial TPH content of 3025 mg/kg under the best treatment conditions.

Fig. 7 shows the chromatographic profiles of mesocosms landfarming final samples and the initial sample for comparison. We observed the degradation suffered by hydrocarbons due to bioremediation process. It is significant that, in these samples, the major recalcitrance was observed for aliphatic hydrocarbons in the range of C_{16}–C_{35}. Normally, light-chain aliphatics are eliminated faster during the biodegradation processes because of their higher bioavailability [22,23]. However, in our case, the biodegradation affected aromatic hydrocarbons to more extent.

3.5. Landfarming full-scale trials

Based on the results obtained at the laboratory scale, the optimal conditions selected were applied at full scale. The results obtained for the landfarming, performed in cells as described above, are presented in Fig. 8.

Under these conditions, the TPH reduction observed for the final sample (REALLF) was 42%. At the end of the test, no stabilisation was observed. The TPH reduction efficiencies were lower than those in the laboratory tests. In the full-scale landfarming test, there were a number of problems encountered in maintaining the humidity at the optimum level, and the periodic handling of samples in the cells was very problematic due mainly to the clay texture of the treated sludge. These facts explain the lower rates obtained compared with laboratory trials.

3.6. Ecotoxicity evaluation of proposed treatments

The toxicity data for soil organisms obtained in the microcosm assays are shown in Table 10. The initial sludge sample showed toxic effects on the three taxonomic groups of soil organisms tested. Earthworm survival was slightly but significantly affected (10 ± 0) in the initial sample. However, earthworm mortality was not observed in the treated sludges, although a decrease in earthworm weight was measured in these samples. The effect on weight

Table 9
Hydrocarbon content and reduction efficiency with respect to the initial sample obtained in landfarming tray tests for different hydrocarbon ranges.

Hydrocarbons	Hydrocarbon content (mg/kg) Initial sample	Hydrocarbon content (mg/kg) Final sample Control	Hydrocarbon reduction efficiency (%) Control	Hydrocarbon content (mg/kg) Final sample Nutrient LABLF	Hydrocarbon reduction efficiency (%) Nutrient
Aliphatic					
C_{10}–C_{12}	5.4	<3.0	–	<3.0	
C_{12}–C_{16}	396.2	9.6	98	4.7	99
C_{16}–C_{21}	837.7	273.2	67	137.3	84
C_{21}–C_{35}	446.0	255.0	43	165.2	63
C_{35}–C_{40}	18.3	<3.0	–	<3.0	–
Total aliphatic	1703.6	537.8	68.	307.2	82
Aromatic					
C_{10}–C_{12}	<3.0	<3.0	–	<3.0	–
C_{12}–C_{16}	90.3	<3.0	–	<3.0	–
C_{16}–C_{21}	274.6	10.0	96	3.6	99
C_{21}–C_{35}	163.6	26.5	84	11.7	93
C_{35}–C_{40}	11.3	<3.0	–	<3.0	–
Total aromatic	539.8	36.5	93	15.3	97
Total C_{10}–C_{40}	2,243.4	574.3	74	322.5	86

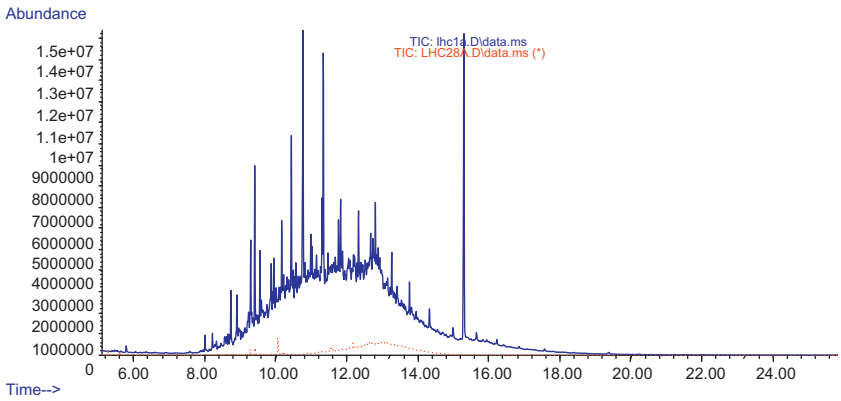

Fig. 7. Total ion chromatograms (GC/MS) of initial sample and optimal final samples (dotted line) obtained in mesocosms landfarming tests.

Table 10
Toxicity data for soil organisms exposed to initial sludge and treated sludge samples in the MS-3 microcosm system.

			Percentage of inhibition compared with control soil (%)			
			Initial sample	BIOSLU	REALLF	LABLF
Earthworm		Mortality	10 ± 0	ns	ns	ns
		Weight	ns	27 ± 2a	31 ± 2a	nd
Plants	*Triticum aestivum*	Seedling emergence	57 ± 9	ns	ns	62 ± 15
		Wet Weight	63 ± 6	64 ± 10a	82 ± 8a	81 ± 10
	Brassica napus	Seedling emergence	56 ± 17	89 ± 4a	62 ± 6b	100 ± 0
		Wet Weight	72 ± 32	73 ± 20a	73 ± 10a	*
	Trifolium pratense	Seedling emergence	90 ± 11	79 ± 12	76 ± 6	100 ± 0
		Wet Weight	66 ± 13	35 ± 5a	61 ± 6ab	*
Microorganisms		C transformation	47 ± 3	74 ± 6a	51 ± 4b	55 ± 10b
		Dehydrogenase	*−110 ± 25*	54 ± 18	*−27 ± 8*	nd
		Alkaline phosphatase	nd	88 ± 6	81 ± 5	nd
		Acid phosphatase	*−213 ± 28*	*−226 ± 20*	*−548 ± 44*	nd

*Effects on growth cannot be determined because of the seedling emergence inhibition was 100% in these species; ns: Significant differences with the control were not observed ($P < 0.05$) by the LSD procedure; nd: Not determined; italics indicate increase effects compared with the control. Different letters indicate significantly different values using one-way ANOVA (LSD; $P < 0.05$).

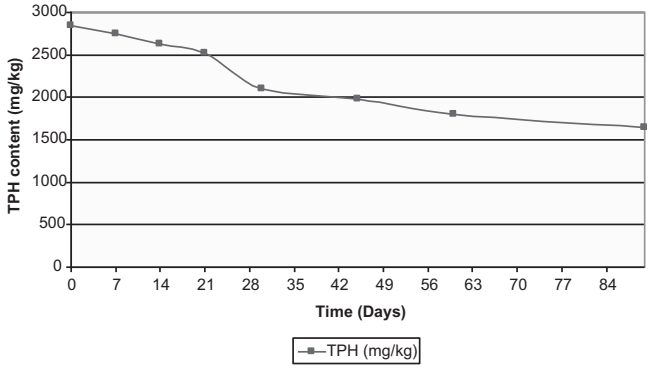

Fig. 8. Evolution of TPH concentration in the landfarming cells.

could be due to the low organic matter content and the high clay content in the residues, which hinders earthworm mobility.

There was no relationship observed between treatments and effects on seedling emergence and plant growth in the initial or treated samples despite the decrease of contaminants in the treated sludge, although an increase in the inhibition of germination was observed in the LABLF sample. These effects did not seem to be due to contaminants in the soil because previous data reported in the literature [24,25] indicated that oil levels <4000 mg/kg dry soil have little effect on plants. The effects were likely due to the physicochemical characteristics of the residue, especially its clay texture, which delays seedling emergence; consequently, plant growth was low at the end of the assay period. In fact, many of the most important phenomena affecting plant growth, such as the ease of root penetration and the storage and movement of water and gases, depend on soil pore size [26]. These effects should be taken into account if the waste is applied to soil, depending on the intended future land use.

The sludge toxicity to soil microorganisms varied with the effect on microbial activity examined (Table 11). The sludge negatively affected carbon transformation induced by glucose and alkaline phosphatase activity. Conversely, the activities of the dehydrogenase and acid phosphatase enzymes showed an increase compared with the control soil, indicating an active microbial population. Serrano et al. [27] reported an increase in dehydrogenase activity after exposure to hydrocarbons; this can be explained as the result of microorganisms using aliphatic hydrocarbons as carbon source. Nevertheless, data on microbial activities in undiluted soil (100%) are only a measure of the characteristics of the microbial populations of the soil that can be adapted to the contaminants. To determine possible toxic effects of the sludge contaminants on unadapted microbial soil populations, it is more appropriate to test samples of contaminated sludge diluted with a control soil, as shown below.

Toxicity to aquatic organisms (algae and daphnia) was not observed in soil leachates, indicating the low mobility of the contaminants in the soil [22,28,29] as a result of strong adsorption onto the soil mineral matter (clay).

Table 11
EC_{50} ecotoxicity values for microorganisms in initial sludge and treated sludge samples obtained in the single-species test. Data are expressed as percentages of sludge concentration (%, w/w).

	EC_{50} sludge (%, w/w)			
	Initial sample	BIOSLU	REALLF	LABLF
C transformation	54 (43–66)	80 (71–89)	103 (79–175)	>100
Dehydrogenase	72 (68–80)	77 (62–95)	111 (87–257)	>100
Alkaline phosphatase	nd	83 (67–112)	92 (74–128)	>100

To study the effect of sludge contaminants in unadapted microbial populations of control soil, a dose-response assay was performed. In this assay, toxicity to soil microorganisms was observed in both initial and treated sludges. The effects on carbon mineralisation were higher in the initial sample than in the treated samples, specifically in the LABLF sample, where a 50% inhibition was not reached even at the highest concentration tested. Treated sludges negatively affected the activity of alkaline phosphatase in all treatments, although the LABLF sample had the lowest toxicity. It is known that hydrocarbons affect the biological properties of the soil by modifying the populations of particular microorganisms [30] and thus affect soil enzymatic activities [26].

Dehydrogenase activity showed the opposite results in both the microcosms and single-species tests. In the microcosm assays, increased activities were found in the initial sludge (-110 ± 25) and in the sample from the landfarming trial (-27 ± 8). Conversely, in the single-species assay, inhibition of dehydrogenase activity was observed in all the samples at the 100% sample concentration, with the exception of the REALLF treatment, where no effects were observed. These differences may be due to different exposure conditions in both systems, which can affect contaminant toxicity. In contrast with the single-species test, the multispecies system accounts for species interactions. Thus, the presence of vascular plants in MS-3 may affect enzymatic activities, as reported by Xu and Jonhson [31]. It is generally acknowledged that microcosm studies better reflect field-relevant situations and enable more reliable predictions of ecosystem responses.

In summary, the toxic effects in the initial sludge sample were very low for most measured parameters. After the remediation treatments, a decrease in toxic effects was observed in earthworm survival and in carbon mineralisation in the assays performed at various sample concentrations. More sensitive assays would be necessary to observe toxicity differences between sludges obtained by applying the different treatments. However, test results indicated that the remediation processes did not produce changes in the bioavailability of the contaminants or metabolite formation and did not increase the toxicity of the samples.

4. Conclusions

The results of the chemical and ecotoxicological tests performed indicate that the bioremediation processes developed in this study are viable alternatives for the recovery of this type of sludge. Although the result in full-scale landfarming of a TPH reduction efficiency of 42% in three months was slightly lower than that obtained at the laboratory scale, landfarming was also efficient for sludge contaminated with hydrocarbons.

This work confirmed the usefulness of the MS-3 system, initially developed for the assessment of soil contaminants, for the study of other types of matrices such as soil washing sludge. The main problem encountered was the high clay content of the samples, which may mask the toxic effects of sludge contaminants. Moreover, differences of toxicity between the initial and treated sludge samples were low, indicating that long-term assays using more sensitive parameters are recommended.

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