IMPACT OF BIOSTIMULATION AND BIOAUGMENTATION ON DIESEL CONTAMINATED SOILS AS BIOREMEDIATION SYSTEMS

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Abstract

This paper analyses and compares the effects of bioremediation on total petroleum hydrocarbon (TPH) degradation with composting techniques and following biostimulation and bioaugmentation approaches. Compost and sludge were added as organic amendments with a double mission, providing both nutrients and microorganisms to the contaminated soil. In addition the effect of inoculating white-rot fungus *Trametes versicolor* was assessed. Two different types of soils were considered: a poor soil with low organic matter content and an enriched organic soil. The use of compost and sludge for soil bioremediation through composting techniques was effective for TPH removal. The amount of organic matter present in soil played an important role in TPH removal due to the adsorption phenomenon of the pollutants in the organic fraction of the solid material. When the contaminated soil was rich in organic matter, the use of sludge provided better results than compost (22% of degradation in the first fifteen days front 5%) but no differences between compost and sludge were observed in poor soil. The inoculation of the ligninolytic fungus *T. versicolor* enhanced the removal process of TPH, thus increasing the degradation rate and reducing the process time. However, periodical reinoculation was required.

Keywords: bioaugmentation, bioremediation, contaminated soil, total petroleum hydrocarbons, *Trametes versicolor*.

1. Introduction

As a consequence of massive and widespread use, petroleum hydrocarbon compounds have become common organic pollutants of soil surfaces and have eventually been considered a major environmental and health problem. Amongst hydrocarbon pollutants, fuel and diesel oil are a

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complex mixture of n-alkanes, branched alkanes, cycloalkanes and monoaromatic compounds. All of these pollutants are frequently reported as soil contaminants leaking from storage tanks and pipelines or released in accidental spills during industrial and commercial operations (Gallego et al., 2001).

Today, bioremediation is the most common treatment used for these soils and is an environmentally friendly alternative with respect to physico-chemical treatments. Bioremediation involves turning pollutants into nontoxic forms by using organisms under aerobic or anaerobic conditions to remove the contaminants from soil, water and gases (Riser-Roberts, 1998). Diesel oil bioremediation in soil can be promoted by the stimulation of indigenous microorganisms by introducing nutrients and oxygen into the soil (biostimulation) (Seklemova et al., 2001; Sayara et al., 2009) or through the inoculation of an enriched microbial consortium, whether indigenous or exogenous (bioaugmentation) (Vogel, 1996; Karamalidis et al., 2010; Kauppi et al., 2011).

Composting techniques have long been applied and established as an area of research to degrade organic solid residues (Haug, 1993; Ruggieri et al., 2008). These techniques have also been demonstrated to be effective in biodegrading total petroleum hydrocarbon (TPH) at the laboratory (Namkoong et al., 2002), reactor (Van Gestel et al., 2003) and field (Ros et al., 2010) scales. Diverse nutrient sources, such as inorganic fertilizer, compost, manure and sludge, have been used in bioremediation. Amongst them, sludge seems to be a promising nutrient source for microbes in bioremediation (Namkoong et al., 2002). The primary benefits of sludge include their low (or non-existent) cost, slow release of nutrients (similar to animal manures) and easy availability. In addition, their use gives purpose to what would otherwise be residues. Another organic source with enormous potential for bioremediation are composts, not only because of their provision of nutrients, but also because of their mesophilic and thermophilic bacterial content and their ligninolytic fungi, which are endowed with the ability to degrade some pollutants (Antizar-Ladislao et al., 2004; Anastasi et al., 2008). Also, the presence of biopolymers (cellulose, hemicellulose and lignin) in compost may pave the way to the degradation of some pollutants. In fact, the transformation of biopolymers requires a set of enzymes (peroxidases and phenoloxidases) that degrade cellulose and lignin (Criquet et al., 1999). Filamentous fungi such as white-rot basidiomycetes are amongst the major decomposers of biopolymers, lignin in particular. These organisms have developed non-specific, radical-based degradation mechanisms occurring in the extracellular environment (Singh et al., 2006). It has been probed that their ligninolytic enzyme machinery (including laccases and peroxidases) can reach and deplete petroleum hydrocarbons in contaminated soils even when the pollutants have low availability (Pointing, 2001). However, basidiomycetes are rarely isolated from compost because many of them cannot withstand the

higher-than-50 ºC temperatures generated during the thermophilic stage in the composting process (Ryckeboer et al., 2003).

The main goal of this study was to demonstrate that compost and raw sludge could introduce nutrients and microorganisms which would favor the degradation of TPH in contaminated soils. Both organic materials may have a double impact in the bioremediation system, potentially providing nutrients for endogenous microorganisms present in the soil as well as complex microbiota as additional inoculums. Moreover, bioaugmentation with a specific compound degrader, the white-rot fungus *Trametes versicolor*, was also studied to evaluate whether this organism could improve the degradation or accelerate the time of remediation. This approach employed composting techniques that can be applied both ex-situ and on-site.

Two different types of soils were used to investigate the effects of biostimulation (compost, sludge) and bioaugmentation (microorganisms present in compost and sludge, and *T. versicolor*) on TPH degradation by bioremediation with composting techniques: a poor soil with low organic matter content and an enriched organic soil. Preliminary Petri dish trial experiments were necessary to determine further 4.5 L reactor study conditions. Analyses with the 4.5 L thermally isolated reactors were undertaken with the objective of emulating the environmental conditions found at the field scale regarding heat transfer and temperature changes.

2. Materials and Methods

2.1. Materials

The two soils used were collected in the surroundings of Lugo composting plant (Galicia, NW Spain) and were contaminated with 3 % v/v of a mixture of gasoline and diesel (ratio 1:1) one week before the experiments were undertaken, reaching 36 g TPH/ kg of soil. The soils were selected because of their different organic matter content. The mineral composition of soils A and B was coarse sand 50.5 %; fine sand 27.9 %; loam 13 % and clay 8.6 %. In fact, soil B was collected in an area where soil A had been periodically amended with compost for several years. The main properties are presented in Table 1.

Raw sludge from a wastewater treatment plant and compost obtained from sludge composting piles (four weeks treatment time) were used as organic amendments. Wood chips were used as a bulking agent. All three materials were collected from the Jorba treatment plant (Barcelona, Spain). The characterization of the amendments is shown in Table 1.

The white-rot basidiomycete fungus *T. versicolor* ATCC # 42530 was used in the bioaugmentation experiments (Font et al., 1993). The assays were inoculated with 1.3 mg of

triturated mycelium fungus per g of soil (dry matter). The fungal colonization and effect of inoculation dose and procedure were previously analyzed in Petri dish experimental trials (data not shown).

Tween 80 (polysorbate 80, Sigma Aldrich Co, Spain), was used as a non-ionic surfactant and an emulsifier of hydrocarbons.

Table 1. Characterization of soils, organic amendments and bulking agent. Properties were analyzed according to methods described in Section 2.3.1.

Materials	**Bulk density** g/L	**Water content** (%)	**Organic Matter** (% dw)	**Water holding capacity** (% dw)
Soil A	1539	12.4	5	15
Soil B	834	9.1	38	34
Sludge	891	88.7	64	n.a.
Compost	525	27.3	65	155
Bulking agent	178	13.0	83	111

dw: dry weight

2.2 Removal of TPH

2.2.1 Evaluation of the amendment dose and inoculation procedure

Preliminary TPH degradation experiments were assayed in Petri dishes for thirty days at 25ºC to find a suitable dose of compost and sludge and the best inoculation procedure for later 4.5 L reactor experiments. *T. versicolor* was inoculated following two different inoculation strategies: i) inoculating right after mixing the materials, and ii) inoculating the bulking agent and incubating at 25ºC for two weeks prior to its mixture with soils and amendments. Additionally, four different amendment:soil ratio (0.02:1; 0.06:1; 0.155:1 and 1:1 on wet weight) were tested. The assay was prepared by mixing 15 g of the different soils, 3 g of the bulking agent and the different doses of the amendments. The experiments were performed in triplicate. The samples were analyzed at the end of incubation (thirty days).

2.2.2 Evaluation at the 4.5 L reactor scale

The experiments were conducted for sixty days in 4.5 L air-tight reactors that were thermally isolated and equipped with on-line temperature monitoring by Pt-100 sensors (Sensotran,

Spain) connected to a data acquisition system (MAC-3580, Desin, Spain) and to a personal computer. An intermittent aeration was provided to the reactors according to the process performance to ensure a high oxygen level (over 10 %) and to avoid anaerobic conditions. The oxygen concentration in the exhaust gases was measured by means of an oxygen sensor (Crowcon's Xgard, United Kingdom).

The mixtures were prepared by mixing spiked soil, amendment and bulking agent together at a weight ratio of 1:0.15:0.20. The water content of the mixture was adjusted to within the recommended value (75 % of the water holding capacity) (Haug, 1993) by adding water before and during the experiments when necessary. A percentage of water content is necessary in order to promote an adequate biomass growth. The different mixtures are described below and are summarized in Table 2. The main properties of the mixtures are presented in Table 3. The experiments were undertaken in duplicate. The results are presented as the average of duplicates (differences between duplicates were always below 15%). The reactors were filled to their maximum capacity, thus containing a total mass of 2.50-3.00 kg. 120 g samples were collected at zero, fifteen, thirty and sixty days of treatment after the homogenization of the mass in the reactors. TPH removal was calculated as the difference in TPH content at a certain day compared to the initial TPH content, and expressed as a fraction of the initial content. This was calculated for each TPH fraction and for the total TPH content.

2.2.2a Soils A and B: natural attenuation and bioaugmentation

Experiments were undertaken in soils with (AI, BI) and without (A, B) *T. versicolor* (Table 2) to evaluate the removal of the hydrocarbons without the addition of nutrients and microbiota from compost or sludge. Moreover, emissions of volatile organic compounds (VOCs) were analyzed along the process to determine whether losses by volatilization were significant when using a forced-aeration system.

2.2.2b Bioremediation treatments: composting and bioaugmentation

The composting bioremediation treatments were tested for each mixture. Compost and sludge were used as amendments because of their different organic matter content and degree of stability as well as the different microorganisms that can be found in both materials (experiments AC, AS, BC and BS, Table 2).

The same mixtures were used in the bioaugmentation studies with the inoculation of *T. versicolor* at the initial time (experiments ACI, ASI, BCI and BSI, Table 2). The experimental design included a second inoculation on day 21st for two reasons. On one hand, previous studies had shown that *T. versicolor* activity significantly reduces in bioprocesses on day 21 (Blánquez et al.,

2006; Rodriguez-Rodriguez et al., 2012). On the other hand, high temperatures expected in the initial decomposition phase may negatively affect *T. versicolor*.

Also, the effect of the addition of surfactant was analyzed in bioaugmentation experiments in both soils using sludge as amendment (ASTI and BSTI). The dose used was 5 g of Tween 80 for every 100 g of the studied mixture, as determined in previous studies (Rodriguez-Escales et al., 2012).

Table 2. Mixtures and nomenclature for 4.5L reactor scale experiments

Experiments nomenclature	***Amendments and bioaugmentation***				
	Bulking agent	***Compost***	***Sludge***	***T. versicolor***	***Surfactant***
Soil A					
A	-	-	-	-	-
AI	-	-	-	+	-
AC	+	+	-	-	-
ACI	+	+	-	+	-
AS	+	-	+	-	-
ASI	+	-	+	+	-
ASTI	+	-	+	+	+
Soil B					
B	-	-	-	-	-
BI	-	-	-	+	-
BC	+	+	-	-	-
BCI	+	+	-	+	-
BS	-	-	+	-	-
BSI	-	-	+	+	-
BSTI	-	-	+	+	+

2.3 Analytical Methods

2.3.1 Physicochemical analyses

Moisture and dry matter were determined by gravimetric analyses after drying at a maximum temperature of 105°C until constant weight. The organic matter content was determined from mass loss after heating at 550 ºC for four hours (US Department of Agriculture, 2001).

The total organic carbon (TOC) was determined using O.I. Analytical Solid TOC Analyzer/Win TOC Solids v3.0, and the total nitrogen Kjeldahl (TNK) was determined by standard procedures (US Department of Agriculture, 2001). For the TOC and TNK analyses, the samples were previously dried up and sieved at 0.5 mm. The bulk density and free air spaced (FAS) defined as ratio of air volume to total volume of the sample were measured by picnometry (Ruggieri et al., 2009), and the water holding capacity was also measured (US Department of Agriculture, 2001).

The soil samples for petrol hydrocarbon analyses were extracted with Acetone/Petroleum Ether, cleaned with Florisil® and then analyzed by GC-FID and DB-1 column as described in Van Gestel et al. (2003).

The exhaust gases were collected daily in Tedlar bags, and the VOCs content was analysed by GC, as described in Pagans et al. (2005). Thus, the total VOCs emission could be calculated.

2.3.2 Laccase activity

The extracellular ligninolytic laccase enzyme activity was determined. The laccase enzyme was extracted by adding 30 ml of acetate buffer (0.16 M, pH 5) and 3 g of mixture sample to each flask, shaking at 130 rpm at 4 ºC for 30 min and centrifuging at 10.000 rpm at 4 ºC for 15 min, a procedure adapted from Snajdr and Boldrian (2006). The supernatants were collected, and the laccase activity was assayed spectrophotometrically according to Kaal et al. (1993).

2.3.3. Respiration Index

A dynamic respirometer was used as described by Ponsá et al. (2010). Briefly, a sample of 150 g of the mixture was placed in a 500 mL Erlenmeyer flask and incubated in a water bath at 37 ºC. The starting organic material moisture was adjusted to a range of 50-60 %, if necessary. Air was continuously supplied to the samples using a mass flowmeter (Bronkhorst Hitec, The Netherlands) to ensure aerobic conditions during the experiment (oxygen concentration higher than 10 %). The oxygen content in the exhaust gas from the flask was measured using a specific probe (Xgard Crowcon, UK) and was recorded on a personal computer equipped with commercial software (Indusoft Web Studio, version 2008, USA). From the curve of oxygen concentration vs. time, two respiration indices can be calculated:

A) Dynamic Respiration Index (DRI): This index represents the average oxygen uptake rate during the twenty-four hours of maximum activity observed during the respiration assay. The DRI is expressed in mg of oxygen consumed per g of dry matter and per hour.

B) Cumulative Respiration Index (CRI): This index represents the cumulative oxygen consumption during the four days of maximum respiration activity without considering the initial lag phase. The CRI is expressed in mg of oxygen consumed per g of dry matter.

Table 3. Characterization of initial mixtures

Initial samples*	***A***	***B***	***AC***	***ACI***	***BC***	***BCI***	***AS***	***ASI***	***BS***	***BSI***
Water content (%)	11.6	28	20.3	23.8	35.6	34.4	28.8	29.1	30.9	37.3
Organic Matter (% dw)	4.1	16.2	26.5	18.9	41.6	38.9	24	22.7	28.4	44.6
Total Organic Carbon (% dw)	2.3	10.2	15.9	n.a.	15.1	n.a.	10.3	n.a.	10.7	n.a.
Total Kjeldhal Nitrogen (% dw)	0.5	0.9	0.8	n.a.	0.8	n.a.	0.6	n.a.	0.7	n.a.
C/N ratio	5.1	13.3	18.7	n.a.	18.1	n.a.	16	n.a.	15	n.a.
Respiration Index mg O_2 kg^{-1} dw h^{-1}	n.a.	n.a.	69	86	133	169	257	304	336	149
Cumulative oxygen consumption mg O_2 g^{-1} dw	n.a.	n.a.	30.8	39.6	79.0	53.3	63.6	65.1	101.4	43.2
Bulk Density (g/L)	1539	834	757	n.a.	670	n.a.	784	n.a.	918	n.a.
Free Air Space (%)	n.a.	n.a.	65	n.a.	59	n.a.	54	n.a.	55	n.a.

*dw: dry weight

3. Results and discussion

3.1 Results at the laboratory scale

Preliminary TPH degradation experiments were undertaken at the laboratory scale as described in section 2.2.1 and results are summarized below (data not shown). The use of the bulking agent inoculated prior to the remediation trials did not offer any advantage, thus it was decided to inoculate at the starting moment of the bioaugmentation experiments. In general, fungus growth was favored at increasing dose of amendment, as observed by the higher visible colonization of dishes by the white filamentous fungus (data not shown). A higher growth of *T.*

versicolor was observed when using sludge with soil A and when using compost with soil B. Also, TPH degradation (Fig. 1) was enhanced when increasing the amendment dose in the tested range (0.02, 0.06, 0.155 and 1 gram of amendment for 1 gram of soil) with soil B, but no effect of amendment dose was observed for soil A. No differences were observed among amendments regarding TPH degradation. From these previous trials, the dose 3 (0.155:1g amendment / g contaminated soil on wet basis) was chosen for the following experiments at the 4.5 L reactor scale. This dose was selected as a compromise solution to obtain good degradation levels and to avoid using large doses of amendment which would make the treatment more expensive (amendment transport and mixing and overall treatment surface needed).

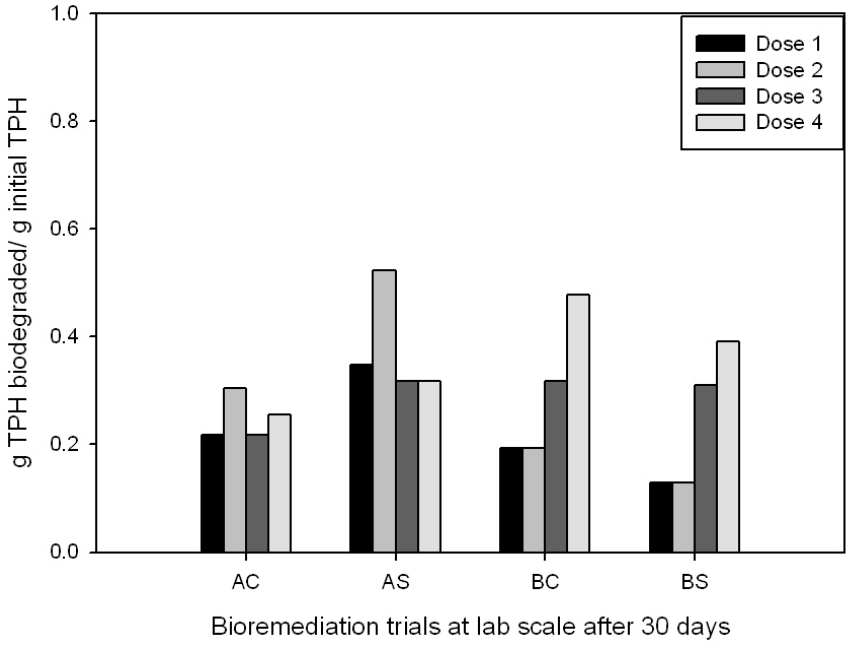


Fig. 1. TPH degradation in the different treatments at laboratory scale using different doses of amendments. AC: soil A, compost; AS: soil A, sludge; BC: soil B, compost; BS: soil B, sludge. Doses g amendment:g contaminated soil on wet basis: Dose 1 **0.02:1**; Dose 2.**0.06:1**; Dose 3.**0.155:1**; Dose 4.**1:1**

3.2. Overall performance of bioremediation trials in 4.5 L reactors

Bioremediation trials in 4.5 L reactors were carried out with different mixtures of soil A and B using compost and sludge as amendment and inoculating with the white-rot fungus *T. versicolor* (Tables 2 and 3). A respirometric study was undertaken with all mixtures intended for study to evaluate both the effect of amendments (sludge and compost) and bioaugmentation with *T.*

versicolor. The results are presented in Table 3 as a respiration rate DRI and cumulative oxygen consumption CRI. Respiration rate and cumulative oxygen consumption are indicative of the amount of biodegradable organic matter in a solid organic waste and its biodegradability (Ponsá et al., 2010). The soil B mixtures presented a higher respiration activity than the soil A mixtures probably due to their greater organic matter content. Also the use of sludge as amendment provoked higher biological activity than the compost, measured as a higher oxygen consumption rate and higher total oxygen consumption for both soils A and B (Puyuelo et al., 2011). The mixtures inoculated with *T. versicolor* showed a higher oxygen consumption rate, but no differences were found in the final oxygen consumption at the end of the respiration study among inoculated and non-inoculated mixtures.

These experiments were undertaken in 4.5 L adiabatic reactors to emulate the energy transfer conditions at the industrial scale in composting processes. A rise in temperature was observed at the beginning of the process from room temperature (20ºC) to maximum temperatures ranging from 30 to 40ºC (Fig. 2 is presented as an example). This rise was a common factor in all cases except for the natural attenuation experiments. The temperature rise was due to heat released in the biodegradation of the organic matter present in the compost and sludge. A secondary temperature rise was usually observed after homogenizing the reactor contents on sampling days fifteen and thirty. After an initial decomposition phase, the temperature fell and stabilized at approximately room temperature before day thirty and until the end of the process. In general, the inoculated reactors showed higher temperatures than the non-inoculated trials. Table 4 shows the maximum temperature achieved and the area below temperature curve, calculated for the first 14 days (until the first sampling), as the average of the two replicates for each trial. This area and average maximum temperature was 3.6% and 3.4% higher in inoculated trials. This reflects a higher biological activity and confirms the observations from the respirometric analysis. However, because the temperatures remained in the mesophilic range in all cases, the survival of *T. versicolor* should not be affected by thermal conditions.

The initial mixtures presented a FAS over 50% (Table 3), which is the recommended value for solid bioconversion processes to ensure aerobic conditions and the proper air circulation through the organic matrix (Ruggieri et al., 2009). Aeration was adjusted to slightly higher values for the first days of the process during the decomposition phase and was reduced at the end of the experiment (equivalent air flow ranging from 0.17 to 0.05 L/min). Oxygen was maintained over 5% in all cases. The water content was also kept at approximately 75% of the water holding capacity of the mixtures in all trials.

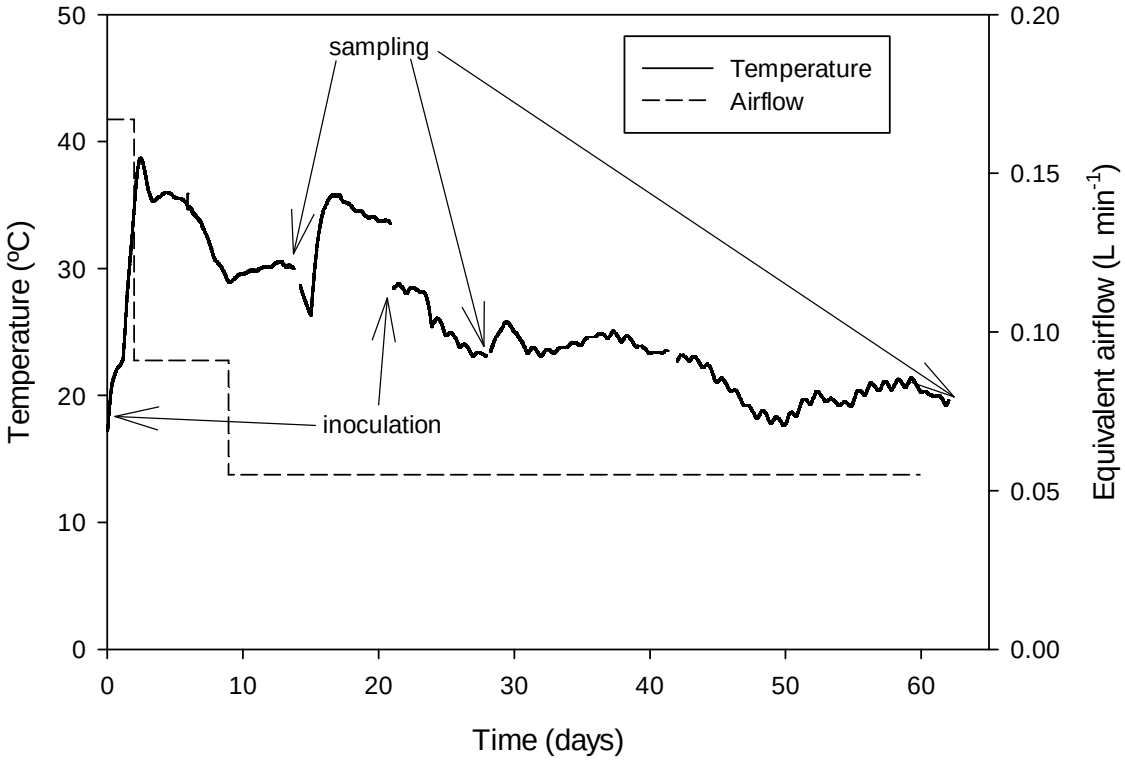


Fig. 2. Temperature profile and aeration requirements for experiment ASII. Arrows indicate both sampling and inoculation moments

Laccase activity was analyzed to monitor the activity and viability of *T. versicolor*. Decreasing levels of activity (Table 5) were detected at days fifteen, thirty and sixty in both the composting and bioaugmentation experiments when using compost as amendment. Detecting laccase activity in not inoculated (with *T. versicolor*) trials indicated the presence of other laccase-producer microorganisms in the initial composting mixtures because compost is a material enriched with a diverse microbial population, including bacteria, fungi and actinomycetes. However, laccase levels were higher in the inoculated mixtures, pointing to a higher fungal activity, but they were negligible after sixty days of processing in any case, indicating the inactivation of the fungus. No laccase was detected when using sludge as amendment and in the natural attenuation trials, whether inoculated with *T. versicolor* or not.

Table 4. Maximum temperature achieved and area below temperature curve for the different experiments considered.

Experiment	Area below temperature curve (ºC·day)	Maximum temperature (ºC)
AC	$7.66 \cdot 10^6$	30.2
ACI	$8.24 \cdot 10^6$	33.6
BC	$8.60 \cdot 10^6$	37.5
BCI	$8.51 \cdot 10^6$	36.4
AS	$8.58 \cdot 10^6$	38.1
ASI	$8.64 \cdot 10^6$	37.5
BS	$6.36 \cdot 10^6$	31.2
BSI	$6.94 \cdot 10^6$	34.2

Table 5. Laccase activity detected at 15, 30 and 60 days in both composting and bioaugmentation experiments with compost (average of two replicates).

Mixture	**Laccase activity (U / g dry weight)**		
	Day 15	Day 30	Day 60
AC	1.3	0.2	n.d
ACI	2.1	0.9	0.2
BC	n.d	1.5	n.d
BCI	2.1	2.5	0.2

3.3 TPH removal in 4.5 L reactors

Fig. 3 shows the degradation of TPH in all of the treatments after fifteen, thirty and sixty days of treatment. No degradation of TPH was detected in the natural attenuation trials (soils A and B without the addition of amendments) or with bioaugmentation. In contrast, the addition of amendment resulted in a considerable TPH degradation in both soils (AS, AC, BS, BC). In soil A, the TPH degradation reached similar values over 60% both using compost (66%) and sludge (68%). In soil B, a similar level of degradation was achieved when using compost (65%). However, only 45% of the TPH was degraded when using sludge as amendment with soil B. These results highlight the contribution to TPH degradation made by the microorganisms present in compost and sludge and are in accordance with previous reports where compost was demonstrated to have a high capacity for enhancing the biodegradation of contaminated soils compared with other amendments (Tejada et al., 2008; Sayara et al., 2009; Gandolfi et al., 2010).

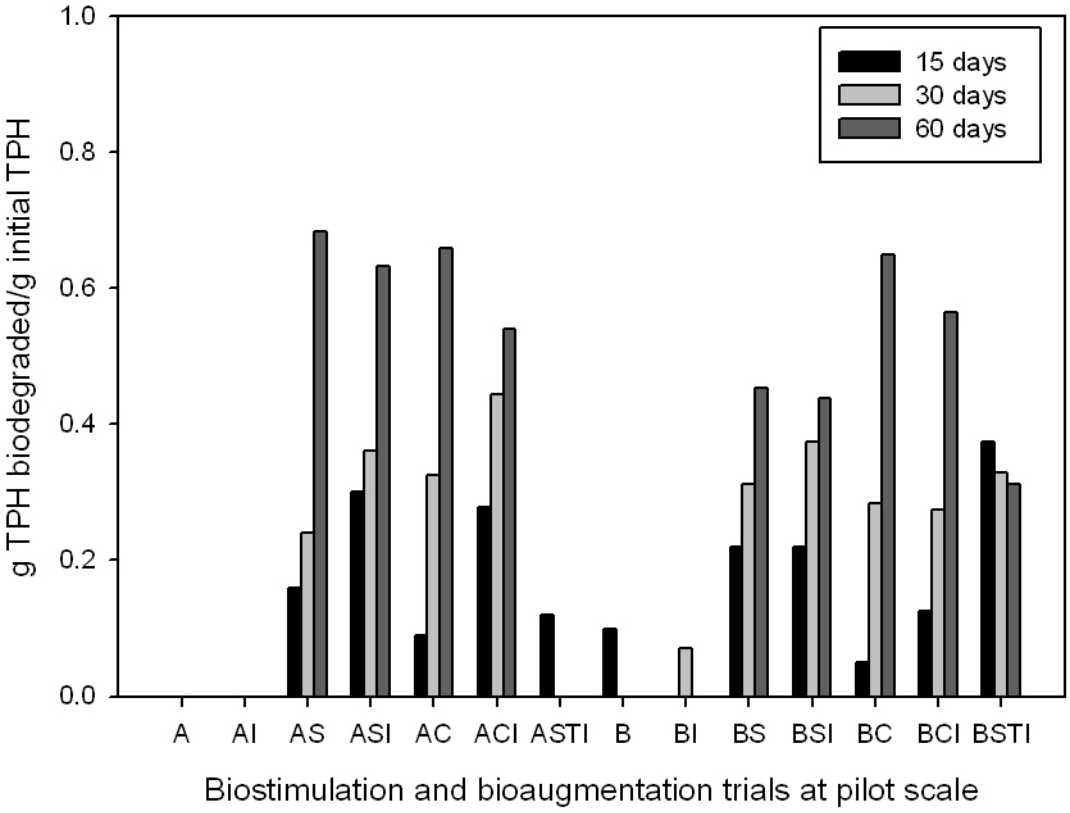


Fig. 3. TPH degradation in the different treatments in 4.5L reactors. A: soil A; AS: soil A, sludge; AC: soil A, compost; I inoculation with *T. versicolor* at 0 and 21 days process; T surfactant. B: same nomenclature than soil A for soil B

When comparing the TPH degradation of the composting trials (AS, AC, BS and BC) with the bioaugmentation experiments (ASI, ACI, BSI and BCI, respectively), a higher TPH removal can be observed in the inoculated trials at days fifteen and thirty, with this effect being more evident in the soil A trials. However, after sixty days of processing, the inoculation of *T. versicolor* did not provide any advantage in TPH degradation. Note that the inoculations were undertaken at days zero and twenty-one. It seems that the addition of this fungus enhances TPH degradation, but this microorganism is not able to survive without periodical reinoculation. This result confirms the previous observations of respiration analysis, as the final CRI was equivalent for the inoculated and non-inoculated samples. Because the temperatures reached were always below 40ºC, the inactivation of *T. versicolor* is attributed to competition with the microorganisms present in the amendment or soils, which are naturally more adapted to the aggressive and successively changing environment in batch processes, such as bioremediation systems. Eventually, these microorganisms

are able to biodegrade the pollutants to the same extent. Competition with autochthonous soil microflora is an important factor in soil bioremediation by white-rot fungi, but the knowledge of their interactions with soil microbiota is poor and sometimes inconsistent (Arun et al., 2008; Borràs et al., 2010; Field et al., 1995; Mougin, 2002; Singh, 2006).

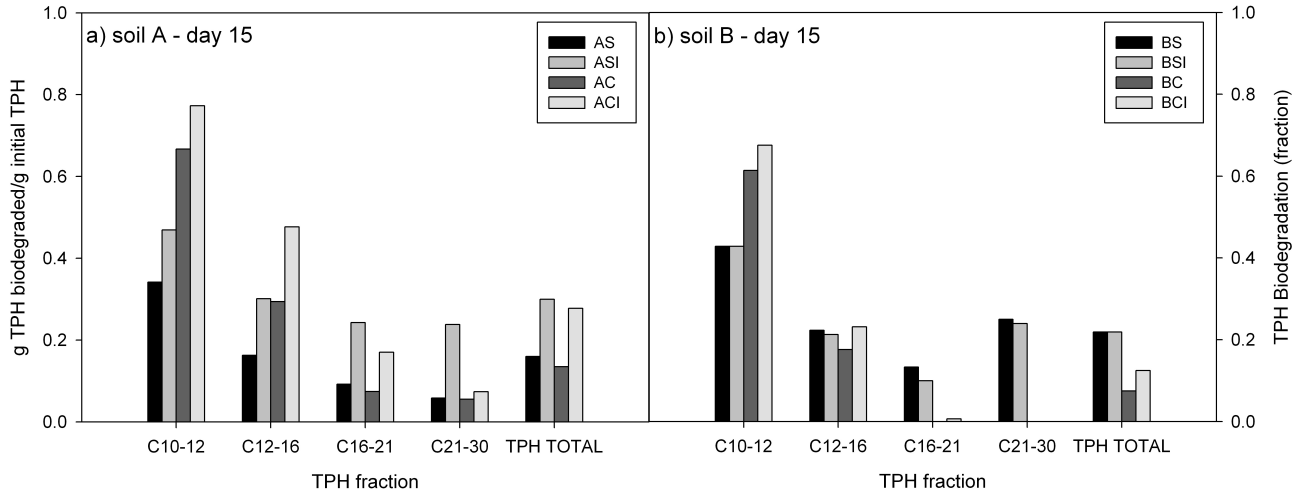
These results indicate that the addition of amendments is an interesting strategy to increasing both available nutrients and the amount and biodiversity of biodegrading microorganisms in soils, especially in poor inorganic soils such as soil A. Bioaugmentation with ligninolytic fungi enhances the TPH biodegradation rate, and thus, this strategy can reduce total processing time. These results are in agreement with previous microcosmos studies that demonstrated fungi suitability as TPH degraders in soils (Mancera-López et al., 2008; Yateem et al., 1998). Lladó et al. (2012) reported 50% TPH removal in 200 days with *T. versicolor* in microcosmos assays and established that the inoculation with *T. versicolor* promoted autochthonous hydrocarbon-degraders. However, despite these promising results with white-rot fungus bioaugmentation, periodical reinoculations are necessary. Consequently, the minimum inoculation dose and the fungus production cost would determine whether the bioaugmentation strategy is economically viable.

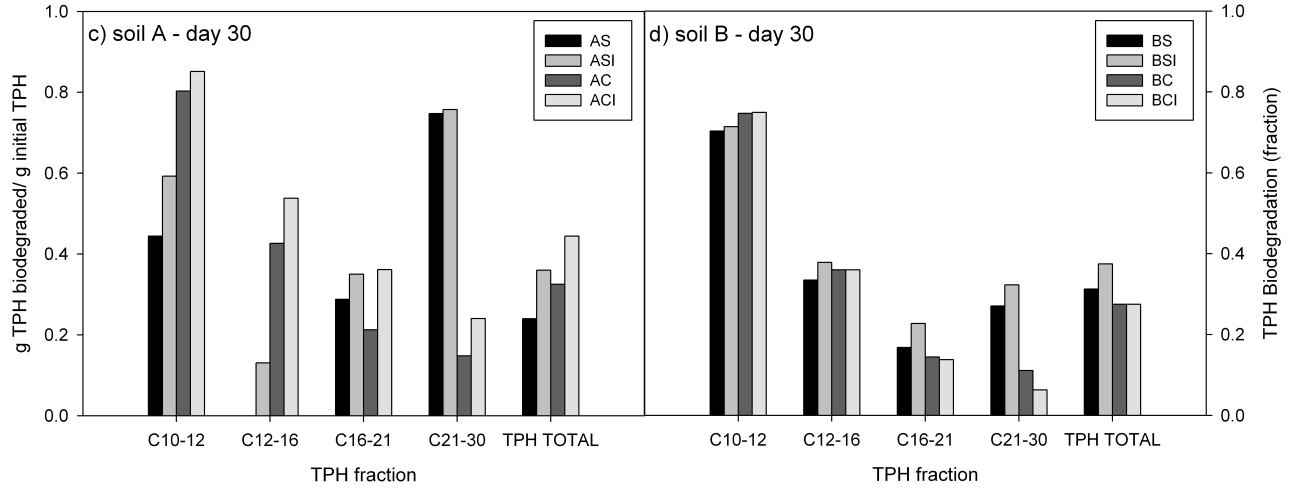
The use of surfactant enhanced the removal in the experiments with soil B rich in organic matter, especially in the first fifteen days. The surfactant probably assists hydrocarbon desorption from organic matter and makes pollutants more bioavailable (Rodriguez-Escales et al., 2012). In contrast, no effect of surfactant addition was observed in soil A with low organic matter content. Interactions between surfactant and the presence of dissolved organic matter have been observed to increase pollutant availability in contaminated soils (Cheng and Wong, 2006).

Fig. 4 presents the removal levels obtained for the different fractions of TPH (C10-12, C12-16, C16-21 and C21-30) at fifteen, thirty and sixty days for the bioremediation and bioaugmentation trials with soils A and B. The shorter TPH fractions were more easily biodegraded, reaching 90% removal for the C10-12 fraction, while only 50% of C21-30 was biodegraded after sixty days. However, a biodegradation yield over 90% in all fractions can be expected for longer process times, as deduced from the overall performance of these experiments, reaching removal percentages comparable to those reported in the literature (Sarkar et al., 2005).

The effect of inoculation with *T. versicolor* is reflected in Fig. 4. All fractions present a higher percentage of removal in the bioaugmentation experiments for days fifteen and thirty, but similar levels were observed in the final values. The lower removal levels reached in the experiments with soil B using compost as a amendment are also evident in Fig. 4. In the first fifteen days, the degradation of fractions C16-21 and C21-30 was negligible. This behavior can be associated with the adsorption of TPH in the organic matter fraction of the soil and compost.

Margesin et al. (2000) and Riffaldi et al. (2006) reported that biodegradation is thought to be the main TPH removal process during bioremediation, but volatilization can also play an important role. Volatile organic compounds were analyzed in the exhaust gases for experiments A, B, AI and BI to evaluate whether forced aeration could enhance TPH volatilization causing atmospheric pollution. The total emissions in the first thirty days of the process ranged from 1 to 1.6 g C per kg of initial mixture. This level of emission is below the reported emissions found in composting plants (3.7 – 7.8 g C per kg of treated waste, Cadena et al., 2009), and it was considered negligible compared to the initial TPH concentration (36 g per kg of soil). Thus, TPH removal could be attributed mainly to biodegradation undertaken by microorganisms.





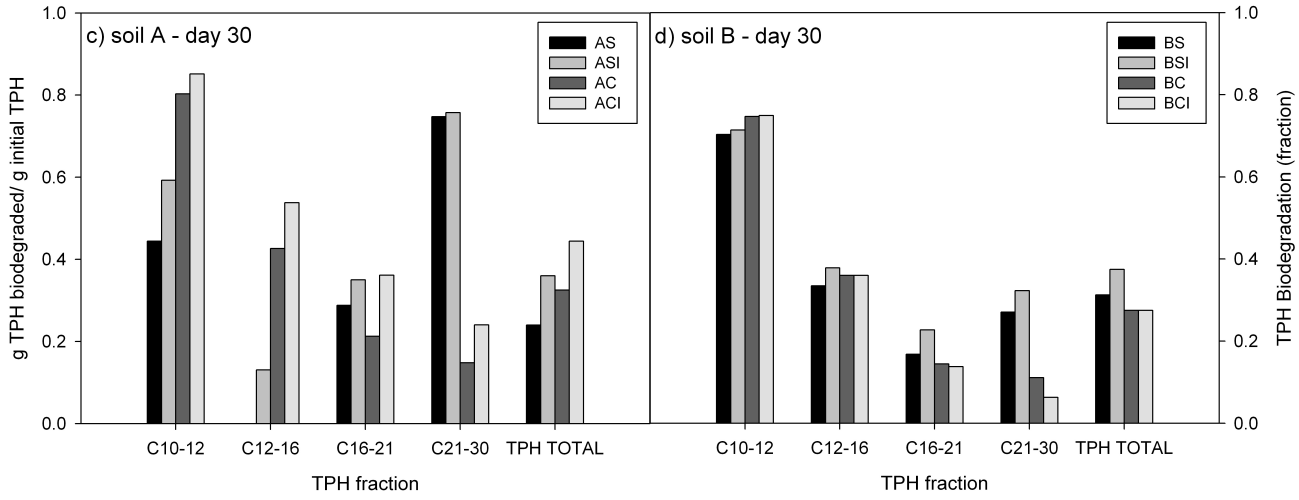


Fig. 4. Removal of different TPH fractions and total TPH at days 15, 30 and 60 for bioremediation trials with (ASI, ACI, BSI, BCI) and without (AS, AC, BS, BC) bioaugmentation

4. Conclusions

The use of compost and sludge for soil bioremediation appears as an effective technique although the effect depends on the type of soil, the amendment and probably the interaction among them. For the poor soil tested, no differences are observed when using compost or sludge. Inoculation with *T. versicolor* enhances the removal process of TPH, increasing the degradation rate and reducing the process time. However, periodical reinoculation is required. Thus, further research is needed to define whether the process is economically viable when faster processes are required. When time is not a limiting factor, the use of amendments provides enough nutrients and microorganisms for efficient TPH removal. For the rich soil tested, the use of sludge provides better results than compost. Also in this case, bioaugmentation offers reduced advantages.

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