

DEGRADATION OF POLYCYCLIC AROMATIC HYDROCARBONS USING BACTERIAL ISOLATE FROM THE CONTAMINATED SOIL AND WHITE ROT FUNGUS *PLEUROTUS OSTREATUS*

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Abstract. The use of microbial consortia composed of bacteria and fungi presents higher rates of polycyclic aromatic hydrocarbon (PAH) degradation and is, therefore, essential for the remediation of contaminated soils. A microbial consortium compound comprising *Ochrobactrum intermedium* and white rot fungus *Pleurotus ostreatus* was found to be capable of degrading PAHs of crude oil in soil. This research studied the degradation capacity of a consortium composed of *O. intermedium* and *P. ostreatus* and the degradation capacity of both the bacteria and the fungus individually. The soil was artificially contaminated using two crude oil concentrations corresponding to 86,000 and 172,000 mg kg⁻¹. The concentrations of PAHs extracted from the two concentrations of crude oil were 138.16 and 268.03 mg kg⁻¹, respectively, with respective extraction resins of 78.3 and 73.5%. In total, the biodegradation of 10 PAHs were studied. Fluoranthene, indene[1,2,3-cd]pyrene and benzo[g,h,i]perylene were completely removed by the consortium after 50, 80 and 50 d of incubation, respectively. The rate at which anthracene, pyrene, chrysene and benzo[a]anthracene biodegraded in the presence of the microbial consortium were 96%, 86%, 98% and 98%, respectively, after 110 d of incubation. In this study, higher rates of degradation were obtained with the consortium than when the components were used individually, showing that consortia composed of fungi and bacteria are an efficient technology for degrading xenobiotics, as is the case with PAHs.

Keywords: PAHs, soil microcosm, microbial consortium, biodegradation, white-rot fungi

Introduction

The most resistant components of petroleum, polycyclic aromatic hydrocarbons (PAHs) are environmentally problematic due to their toxic, mutagenic and carcinogenic properties (Sahoo et al., 2012; Sinha et al., 2012). Low molecular weight (LMW) PAHs exhibit highly toxic characteristics and affect reproduction rates and mortality in aquatic biota, while high molecular weight (HMW) PAHs have higher mutagenic and carcinogenic effects in living beings (Boonchan et al., 2000). There are different transformation pathways for PAHs in nature, such as volatilization, adsorption, photooxidation, chemical oxidation, bioaccumulation and microbial degradation (Yuan et al., 2002). Soil is one of the abiotic resources most affected by PAH pollution. Physical-chemical technologies are generally used to reduce PAH soil contamination, although they have drawbacks, among which are their high cost, difficulty of operation and generation of secondary contaminants (Mamma et al., 2004). Bioremediation is an

economical and efficient method for both eliminating PAHs from the soil and reducing the toxicity of the medium (Frenzel et al., 2010).

The bioremediation technology is based on the use of the catabolic ability of microorganisms using xenobiotics as energy sources. Successful bioremediation requires microorganisms with the capacity to degrade the contaminant (Venosa and Zhu, 2003). The application of isolated pure microorganisms or microbial consortia that have the ability to degrade PAHs and which have been taken from contaminated sites represents a possible alternative for the bioremediation of contaminated sites with similar environmental characteristics if the soil lacks the microbial flora capable of degrading the contaminant (Liu et al., 2011). Numerous studies have focused on degrading PAHs to deal with environmental pollution, reporting the use of a large number of species, with the most studied genera being *pseudomonas*, *mycobacterium*, *alcanivorax*, *sphingomonas*, *microbulbifer*, *shingomonas*, *micrococcus*, *cellulomonas*, *gordonia*, and *penicillium*, among others (Brito et al., 2006; Vila et al., 2010). Fungi genera such as *Chrysosporium*, *Bjerkandera*, *Irpex*, *Agrocybe*, *Lentinus*, *Pleurotus* and *Trametes* are among those that are reported to have been used (Valentín et al., 2006; Chupungars et al., 2009).

Although various studies have been able to degrade PAHs using pure microorganisms, these results can be improved through the use of microbial consortia (Bacosa et al., 2010; Colombo et al., 2011) and bacteria-fungus consortia (Boonchan et al., 2000; Li et al., 2008). One of the advantages of using microbial consortia is their multiple metabolic capacities, which increase the efficiency of the degradation process (Ghazali et al., 2004), the degradation of PAHs with consortia formed by using bacteria-fungus is a very promising technique because of the beneficial characteristics of both kingdoms. Thus, the aim of this study was to evaluate the capacity of the consortium formed by *Ochrobactrum intermedium* and *Pleurotus ostreatus* to degrade PAHs in a microcosm of soil contaminated with oil, with the objective of achieving superior remediation results than those achieved with individual microorganisms.

Materials and methods

Chemicals

The crude oil used in this study was obtained from the Lázaro Cardenas refinery in Minatitlán, Veracruz, Mexico. The chemicals used in the extraction and cleaning of the PAHs were of analytical grade, while the chemicals used in the quantification of the PAHs were of an HPLC grade, with both procured from Sigma-Aldrich (USA).

Microorganisms and culture medium

The bacterium was isolated from sediments in a hydrocarbon contaminated creek in Poza Rica, Veracruz, Mexico, at a depth of 40 cm from a site constantly polluted by oil, located at coordinates 20°32'28.77"N and 97°28'02.60"O. Bushnell Haas medium (BHM) was used as the culture medium in this study, with the following composition: (g l⁻¹): 2 NH₄NO₃; 2 KH₂PO₄; 2 K₂HPO₄; 1 NaCl; and, 0.2 MgSO₄.7H₂O. The micronutrients used were (mg l⁻¹): 1 CaCl₂.2H₂O; 1 MnSO₄.H₂O; 1 FeCl₃; 0.5 ZnSO₄.7H₂O; and, 2 CuSO₄. The pH was adjusted to 7.0 using 10.5 M NaOH. The BHM was sterilized by autoclaving at 121 °C for 15 min. *P. ostreatus* (ATCC38540) was grown in darkness on a potato dextrose agar (PDA) plate at 27 °C for 3 d.

Enrichment and isolation of the PAH-degrading bacterium

The pre-enrichment medium used to isolate the PAH-degrading bacterium was BHM with dextrose (1 g) as a carbon source. The inoculated flasks were incubated in darkness for seven days at 30 °C at 150 rpm, after which 10 ml of said culture was transferred to a flask containing 90 ml of BHM supplemented with 1 g of dextrose and 1 g of oil as carbon sources. The flasks were then incubated under the same conditions described above, with the procedure then undertaken five further times using 1 g of petroleum as a unique carbon source. The PAH-degrading bacterium was isolated via enrichment, using Maya crude oil, while the isolation of the strain was carried out on nutrient agar plates by means of conventional spread-plate techniques, with the plates incubated at 30 °C for 48 h. The isolated strain was stored at -20 °C in liquid culture containing 20% glycerol (v/v). The PAH-degradation capacity of the isolated strain was determined by means of a qualitative evaluation. Petri dishes with BHM agar and oil as a carbon source were inoculated with 1 ml of isolated strain culture and incubated in darkness at 30 °C for 72 h. A Petri dish without inoculate served as a control to determine the loss of hydrocarbons due to abiotic factors (Bacosa et al., 2013). The emulsification capacity was evaluated by adding 10 ml of the bacterial culture after 48 h of incubation to an Erlenmeyer flask containing 90 ml of BHM supplemented with 2 ml of oil and 1 ml of Tween 80. An uninoculated control flask was also used. All flasks were incubated in darkness at 30 °C in an orbital shaker set to 150 rpm. Each assay was performed in triplicate.

The 16S rRNA sequence analysis of isolated strain

The cells of the strain were grown in 50 ml Luria Bertani medium at 30 °C in darkness for one night. DNA was extracted according to Sambrook and Roussel (2001). To amplify the 16S rDNA gene, a polymerase chain reaction (PCR) was performed using two universal primers, the forward primer Eu 530F (5'-TGA CTG ACT GAG TGC CAG GAC CCG CGG-3') and the reverse primer Eu 1449R (5'-TGA CTG ACT GAG GCT ACC TTG TTA CGA CTT-3'), described by Borneman et al. (1996). Each 50 µl reaction contained the following: 3 µl of DNA template; 0.25 µl of Eu 530F; 0.25 µl of Eu 1449R (final concentration 20 pmol); 25 µl of master mix (Vivantis); 0.5 µl of MgCl₂ (final concentration 2.0 mM/L); and, 21 µl of water. The PCR mixtures were preheated in Eppendorf Mastercycler EP Gradient thermal cycler at 95 °C for 5 min prior to running the following cycles: 95 °C - 1 min; 55 °C - 1 min; and, 72 °C - 1 min. The reactions were conducted for 30 cycles in a DNA thermal cycle. After the last cycle had been completed, a chain-elongation step was carried out at 72 °C for 5 min. The amplified DNA was sequenced by the Macrogen Company (Korea). The sequence was subjected to a BLAST similarity search on the NCBI-USA website (<http://www.ncbi.nlm.nih.gov>) and deposited into GenBank. Many relevant 16S rDNA gene sequences with authenticated and published names were selected as references from Gen-Bank, while the phylogenetic tree was constructed using the MEGA 6.0 software.

Experimental design

The degradation of PAH in a soil microcosm by the microbial consortium consisting of *P. ostreatus* and isolated bacteria was evaluated via the application of a 3 × 3 × 2 factorial design, using controls comprising contaminated soil and soil contaminated with substrate, both without inoculum, while each treatment was performed in triplicate. The bacterial degradation was determined using 50 g of sieved soil (2 mm) placed into

glass bottles (150 ml), while the fungal and consortium degradation were assessed in 40 g of sieved soil and 10 g of substrate (corn stubble and coffee pulp 1: 1, w: w) to obtain a final weight of 50 g. The moisture content was adjusted to 70%, while the molar ratio C: N: P was 100: 10: 1. All soil samples were autoclaved for 60 minutes at 121 °C. 4.3 with 8.6 g of crude oil added dropwise to each bottle to achieve uniform distribution and final concentrations of 86,000 and 172,000 mg of total petroleum hydrocarbons kg⁻¹ soil. 30 ml of acetone was then added, after which a homogeneous mixture of the soil was obtained. 30 ml of acetone was added to homogenize the soil with the oil of the soil, after which the acetone was completely evaporated from the glass bottles. Then, 3 ml of bacterial inoculum (3.2 x 10⁸ CFU/ml) was added to a set of bottles containing 50 g of soil, while another set of bottles contained 40 g of soil and 10 g of substrate invaded with the consortium, and the third set of bottles contained only 40 g of soil and 10 g of substrate invaded with mycelium of *P. ostreatus*. All bottles were incubated at 30 °C in the dark, under three different incubation times (50, 80 and 110 d).

PAH extraction and analytical methods and data analysis

The extraction of PAHs was undertaken in accordance with Li et al. (2008), with the soil dried and passed through a 60-mesh sieve, and approximately 2 g of dry soil placed in a 50 mL test tube, to which 15 mL of acetone:dichloromethane (1:5) mixture was added. The PAHs were extracted via sonication for 40 min in an ultrasonic bath (Brand Branson, MH Series, MOD 1800), after which two extractions were taken from each sample and the supernatants then mixed. The extracts were concentrated in a rotary evaporator and resuspended in 2 ml of cyclohexane. For the cleaning, 2 ml of the sample resuspended in cyclohexane was eluted with 15 ml of a dichloromethane:hexane mixture (2:3) through a C-18 cartridge. The eluate was concentrated and resuspended in 2 ml of acetonitrile (Tian et al., 2008), while the characterization of residual PAH in the soil after the completed incubation times was performed using an Agilent 1260 HPLC System with a diode array and fluorescence detectors. The separation of the 16 PAHs was carried out on a ZORBAX Eclipse PAH (4.6 mm x 50 mm, 1.8 µm) column. The mobile phase comprised an acetonitrile:water mixture (60:40), a flow rate of 0.8 mL/min, a sample volume of 20 µl, and a run time of 16 min. The data was recorded as means ± standard deviation. One-way analysis of variance (ANOVA) was conducted to determine the significant differences in the degradation of the PAHs by microorganisms at different time periods. The differences between treatments were analyzed via a Tukey (HSD) test using the SAS 9.0 software. Differences between means at a level of 5% (P < 0.05) were considered significant.

Results and discussion

Degradation of PAHs by *O. intermedium*

Figure 1 shows the biodegradation of PAHs by *O. intermedium*, *P. ostreatus* and the consortium of both microorganisms. When the PAH concentration was 138 mg kg⁻¹, during the 80 d of incubation, a linear degradation was observed, while an increase in the rate of degradation was observed after 80 d of incubation. During the incubation of Control A, soil contaminated with oil without inoculum, a slight decrease of non-significant PAHs was observed. A trend of increasing degradation was observed at

268 mg kg⁻¹ PAH. As with Control A, Control B did not present a significant decrease of PAHs. This study found that *O. intermedium* has a higher rate of degradation when exposed to a concentration of 268 mg kg⁻¹ of PAHs, and offers higher degradation potential the longer the incubation time, with the degradation trend presenting a constant drop along with falling PAH concentrations (Tirado-Torres et al., 2017). Cerqueira et al. (2011) observed that the most challenging method of PAH elimination is that which uses microorganisms.

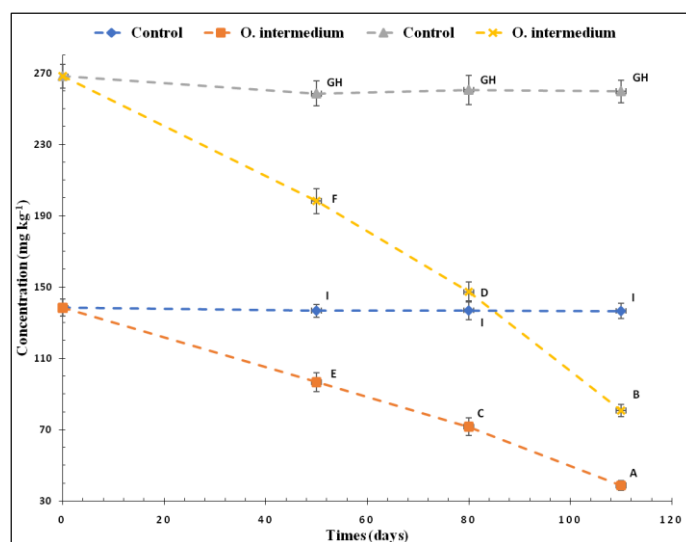


Figure 1. Changes in the concentrations of PAHs during biodegradation in crude oil PAH-contaminated soils. Control: Sterile soil without inocula. Letters represent significant differences among treatments (Tukey, $P < 0.05$)

Limited by the low bioavailability of high molecular weight compounds, native microbial flora has a low capacity to remove PAHs from contaminated soils. Therefore, soil remediation processes are extremely complex in the case of hydrocarbon contamination (Janbandhu and Fukelar, 2011). The isolation of certain microorganisms with the ability to degrade PAHs has enabled significant progress in the development of bioaugmentation techniques and soil remediation (Mao et al., 2012). While few studies of *O. intermedium* have been conducted and especially research seeking to verify its potential to degrade PAHs, it is well known that microorganisms producing biosurfactants present a better option for the bioremediation of contaminants with hydrophobic characteristics, such as hydrocarbons, given that they increase their bioavailability (Wang et al., 2008).

In this study, *O. intermedium* was isolated and adapted to degrade oil PAHs in contaminated soils. Previous studies were reported to use *O. intermedium* isolated from the rhizosphere in soils contaminated by heavy metals and that it has the ability to reduce metal contaminants and subsist in contaminated soils (Sultan and Hasnain, 2007; Waranusantigul et al., 2011). Recently, a study carried out in a liquid medium proved that the species produces biosurfactants with a high level of thermal stability and tolerance to extreme levels of salinity and, thus, able to degrade up to 40% of PAHs in sludge contaminated with oil, while, in soil, the microcosms are able to degrade 70% of the most hydrophobic compounds in only three weeks (Bezza et al., 2015).

Degradation of PAHs by *P. ostreatus*

Figure 2 shows the action of *P. ostreatus* on PAHs in soils contaminated with oil during incubation at 50, 80 and 110 d in this study. Compared to control assays, *P. ostreatus* presented significant degradation rates at concentrations of 138 mg kg⁻¹, at which a constant degradation between 80 and 110 d of incubation was observed. *P. ostreatus* was observed to significantly degrade PAHs at concentrations of 268 mg kg⁻¹. An adaptation stage was observed to occur during the first 50 d, while, at 80 and 110 d, a linear decreasing trend was observed, signifying a higher rate of degradation.

Initially, the study of the degradation potential of microorganisms was given on a laboratory scale and mainly comprised degradation tests on liquid culture. Earlier studies found that the *P. ostreatus* strain ATCC38540 is highly efficient in degrading PAHs in liquid culture (Tirado-Torres et al., 2016). Therefore, this study inoculated *P. ostreatus* in soil contaminated with petroleum to study its capacity to degrade the PAHs present in the crude oil.

P. ostreatus was able to degrade up to 115.2 mg kg⁻¹ of a PAH concentration of 138.3 mg kg⁻¹ and 186.2 of 268.2 mg kg⁻¹ after 110 d of incubation compared to the respective control. During the first 50 d of remediation, the PAH elimination efficiency was attributed to the sorption of the contaminant in the co-substrate added to the treatment, plus the soil particles (Johnsen et al., 2005). At 80 and 110 d, the elimination of PAHs was attributed to the release of ligninolytic enzymes produced by *P. ostreatus* to break the cellulose of the co-substrate, since at those it was observe traces of co-substrate and an increase of the biomass of *P. ostreatus*. This suggests that the addition of the co-substrate enabled the fungus to execute an efficient cometabolic process (Bouchez et al., 1999).

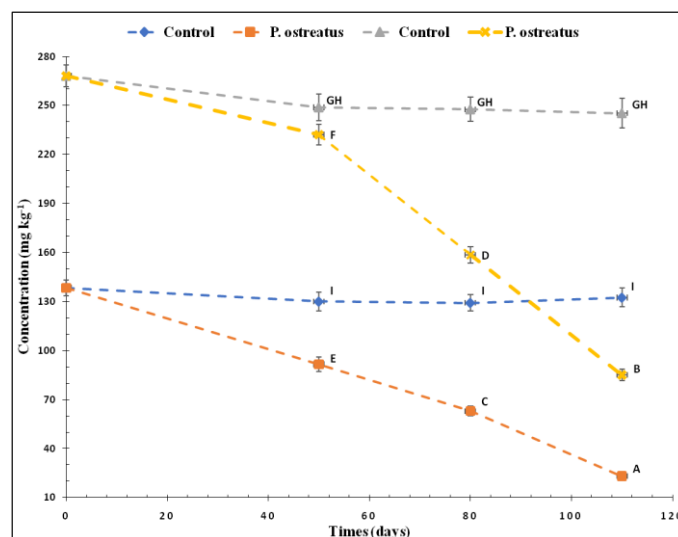


Figure 2. Concentrations of PAHs during biodegradation in crude oil PAH-contaminated soils for fungus. Control: Sterile soil without inocula. Letters represent significant differences among treatments (Tukey, $P < 0.05$)

Basidiomycetes act by means of extracellular enzymes in the co-metabolism process, during which these extracellular compounds act on the contaminant (Namhyun et al., 2000). White-rot fungi are basidiomycetes with the capacity to generate extracellular

enzymes such as laccase, lignin peroxidase and manganese peroxidase, which are found to obtain good results in the degradation of xenobiotic agents (Hatakka, 1994). Acevedo et al. (2011), studied the efficacy of eliminating PAHs with a Chilean white-rot fungus (*Anthracoophyllum discolor*) in contaminated soil, finding that *A. discolor* showed a high PAH removal capacity associated with the production of the extracellular enzyme MnP, results which are comparable with the ability of *P. ostreatus* to degrade PAHs as they are both white-rot fungi. In previous studies conducted in liquid culture, the presence of PAHs has been shown to stimulate MnP production in *P. ostreatus* (Bezalel et al., 1996). The production of ligninolytic enzymes by white-rot fungi, as well as other biochemical molecules, is known to facilitate the removal of xenobiotic agents such as PAHs (Baldrian et al., 2008).

Degradation of PAHs by *O. intermedium*-*P. ostreatus* consortium

This section presents the residual concentrations of PAHs in contaminated soils during the biodegradation induced by the consortium formed by *O. intermedium* and *P. ostreatus* (Fig. 3). As observed, after the inoculation of the consortium, the PAHs were efficiently degraded by 110 d, attaining a degradation efficiency of 86.7 and 75.5% for the PAH concentrations of 138 and 268 mg kg⁻¹, respectively.

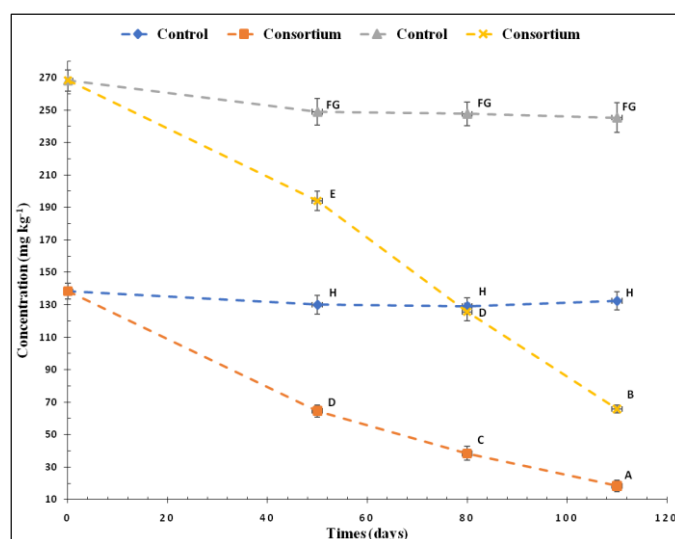


Figure 3. Concentrations of PAHs during biodegradation in crude oil PAH-contaminated soils for consortium. Control: Sterile soil without inocula. Letters represent significant differences among treatments (Tukey, $P < 0.05$)

While the consortium was observed to present a high rate of degradation at 138 mg kg⁻¹ of PAHs after 50 d of incubation, a decrease in degradation efficiency occurs between 80 and 110 d. However, the consortium requires less time to adapt to the contaminated environment than the organisms used separately. At PAH concentrations of 268 mg kg⁻¹, the consortium presented similar behavior to that obtained at 50 d of incubation with 138 mg kg⁻¹ of PAHs. An increase in the degradation response is observed between 50 and 80 d of incubation, while a decrease in the rate of degradation is observed between 80 and 110 d due to the decreasing concentration of PAHs. These results demonstrate that the consortium presents a greater degradation capacity in the

first 80 d of remediation than the microorganisms in their isolated form. This part of the study reveals that *O. intermedium* and *P. ostreatus* act in synergy to efficiently metabolize PAHs from contaminated soil.

In this study, the consortium proved to have a greater capacity to degrade soil PAHs due to the synergism between the two microorganisms, which facilitates the degradation of PAHs. The consortium removed 119.9 and 202.4 mg kg⁻¹ of PAHs at respective concentrations of 138.3 and 268.2 mg kg⁻¹ in soil at 110 d of incubation. This is attributed to the enzymatic activity of the fungus, which formed a habitat that was less toxic to the bacteria (Chagas-Spinelli et al., 2012). Basidiomycetes normally synthesize enzymes that, to an extent, degrade complex PAHs (Sack et al., 1997; Aranda et al., 2010). Bacteria that generate enzymes such as laccase can contribute to an improved degradation of PAHs (Zeng et al., 2010). At 110 d of incubation at the two concentrations handled in this study, it was observed that the consortium achieved the highest level of PAH removal, while the other two treatments achieved levels close to the degradation rate of the consortium. This can be attributed to the limitation of nutrients necessary for the consortium (Hollender et al., 2003), while limiting the source of energy can also affect the efficiency of microorganisms (Kim et al., 2009). The consortium's efficiency in degrading PAHs is due to the qualities of the co-metabolism generated by *P. ostreatus*, as initiated by the addition of co-substrate, which, when decomposed by the extracellular enzymes expelled by the fungus, releases nutrients outside the cell that are available both for their products and for the bacteria (Šnajdr et al., 2011).

In addition, bacteria are able to form biofilms around fungal hyphae in order to bridge pores and penetrate soil aggregates (Kohlmeier et al., 2005). Fungi can also actively take and transfer PAHs through their hyphae via the cytoplasmic stream (Furuno et al., 2012). The primary degradation strategy carried out by fungi and bacteria comprises the release of biosurfactants in order to obtain greater contaminant bioavailability (Johnnsen and Karlson, 2004; Bento et al., 2005). The strong competitive capacity of a particular fungal species is considered a premise of successful soil colonization (Baldrian, 2008). Although, in this study, *P. ostreatus* has been shown to coexist with *O. intermedium*, other studies have reported decreasing *P. aeruginosa* biomass when forming a consortium with *P. ostreatus* (Tornberg et al., 2003). In contrast, Federici et al. (2007) observed an increase in bacterial counts of *mycobacterium*, *nocardia* and *rhodococcus* when used in consortium with *P. ostreatus* (DeBruyn et al., 2007). It should be noted that interactions between fungi and bacteria can positively affect biodegradation applied metabolically in conjunction with a fungal co-metabolism (Mueller et al., 1989), as well as negatively affecting the degradation of PAHs in soil (Borras et al., 2010). In some cases, the use of consortia has not obtained results showing the potential to degrade xenobiotics (Tornberg et al., 2003), while, in other cases, the positive cohabitation of consortium has been obtained with high rates of PAH degradation (Federici et al., 2007).

In this study, the consortium degraded 75.5 to 86.7% of PAHs, while Gallego et al. (2007) observed that a microbial consortium degraded from 31 to 55% of PAHs. It should be reiterated that, as Toledo et al. (2006) state, the comparison of results varies greatly due to the concentrations of hydrocarbons and the incubation times obtained. In another successful study of consortia, Díaz-Ramírez et al. (2008) formed a microbial consortium of six strains which, after 18 d of incubation, degraded 31% of PAHs. Ruberto et al. (2006) found that a combination of bioaugmentation and biostimulation in

a microbial consortium caused a 46.6% elimination of 1,744 mg kg⁻¹ of phenanthrene in soil after 56 days of incubation in Antarctic soil. The results obtained in this study show that fungal and bacterial consortia can be an efficient technology for degrading xenobiotics, as is the case for PAHs.

Bioremediation treatments: individual PAH removal

Previous studies have reported that microbial consortia obtain higher degradation efficiencies in liquid culture with the addition of surfactants in order to increase the bioavailability of xenobiotics (Bautista et al., 2009; Molina et al., 2009; González et al., 2011). The present study was carried out in soil without the addition of surfactants in order to observe the capacity of the consortium without needing to apply biostimulation. Sixteen PAHs were used in this study, of which six were not detected in the initial treatment period: acenaphthene (ACE); Acenaphthylene (ACY); benzo [b] fluoranthene (BBF); benzo [k] fluoranthene (BKF); Benzo [a] pyrene (BaP); and, dibenzo [a, h] anthracene (DahA). *Table 1* shows the 10 compounds detected in the chromatographic analysis and the degradation efficiencies obtained by the treatments at 110 days of incubation under a constant temperature of 30 °C. It can be seen that the compounds which were completely removed by both the consortium and *P. ostreatus* are FLT, IND and BgP. The compounds that showed significant degradation are ANT and CHY, with removal efficiencies ranging from 96 to 98%.

Table 1. Removal of individual PAH compounds in soil contaminated by crude oil after the 110-day incubation period for the three different treatments and two controls

PAH	Rings	Initial concentration Mg.kg ⁻¹	PAHs removed from treatments (%)				
			C-1	C-2	O.I	P.O	C.O
Naphthalene (NAP)	2	74.16 ± 2.3	1.53 ± 0.8 _d	3.06 ± 0.8 _d	76.24 ± 2.8 _c	84.52 ± 0.8 _b	88.77 ± 0.1 _a
Fluorene (FLU)	2	27.83 ± 0.1	1.70 ± 0.8 _d	5.68 ± 1.7 _c	41.59 ± 1.3 _b	69.77 ± 0.6 _a	69.77 ± 0.9 _a
Fenanthrene (FEN)	3	4.06 ± 0.5	1.31 ± 0.9 _d	0.28 ± 0.1 _d	69.74 ± 0.6 _c	76.27 ± 1.6 _b	85.23 ± 1.7 _a
Anthracene (ANT)	3	7.43 ± 0.1	1.89 ± 0.3 _d	4.02 ± 1.0 _c	93.50 ± 0.4 _b	92.50 ± 1.4 _b	96.27 ± 0.6 _a
Total LMW	2 y 3	113.48 ± 1.8	1.60 ± 1.1_d	3.71 ± 0.9_d	68.64 ± 0.5_c	81.13 ± 1.3_b	84.47 ± 0.6_a
Fluoranthene (FLT)	4	6.75 ± 0.1	5.04 ± 0.5 _c	5.03 ± 0.3 _c	88.72 ± 0.5 _b	100 ± 0.0 _a	100 ± 0.0 _a
Pyrene (PYR)	4	4.77 ± 0.4	2.47 ± 0.8 _e	6.69 ± 0.9 _d	68.03 ± 0.8 _c	78.57 ± 0.8 _b	86.51 ± 1.7 _a
Chrysene (CHY)	4	6.19 ± 0.6	2.83 ± 0.8 _d	1.87 ± 0.5 _d	94.63 ± 0.6 _b	91.60 ± 1.4 _c	98.23 ± 0.9 _a
Benzo[a]anthracene (BaA)	4	3.37 ± 0.2	5.14 ± 0.7 _b	8.59 ± 0.8 _b	98.08 ± 0.8 _a	95.67 ± 1.3 _a	98.18 ± 0.5 _a
Indene[123-cd]pyrene (IND)	5	1.44 ± 0.1	5.86 ± 0.8 _d	13.04 ± 0.9 _c	75.74 ± 2.8 _b	100 ± 0.0 _a	100 ± 0.0 _a
Benzo[g,h,i]perylene (BgP)	6	2.35 ± 0.1	4.69 ± 0.5 _c	11.99 ± 0.5 _b	97.59 ± 1.5 _a	100 ± 0.0 _a	100 ± 0.0 _a
Total HMW	4 a 6	24.87 ± 0.4	4.80 ± 0.9_c	7.04 ± 0.7_c	87.31 ± 1.8_b	93.21 ± 0.8_a	96.73 ± 0.4_a
Total PAHs		138.35 ± 5.42	1.33 ± 0.9_d	4.30 ± 0.53_d	72.04 ± 0.5_c	83.30 ± 0.51_b	86.69 ± 0.21_a

Data are presented as mean ± SD. Means in the same row with a letter in common are not significantly different among treatments (P < 0.05). C-1: Soil without inoculum C-2: Soil and cosubstrate without inoculum O.I: *O. intermedium* P.O: *P. ostreatus* C.O: *O. intermedium-P. ostreatus*

These results can be used for future studies of those compounds which have been totally eliminated. *Figure 4* shows the degradation kinetics generated by the three treatments for each PAH. FLT was completely degraded after 80 d of treatment with both *P. ostreatus* and the consortium. IND was completely degraded after 50 d of treatment with the consortium, whereas treatment with *P. ostreatus* achieved complete degradation after 80 d. BgP, the aromatic compound used in this study with the greatest number of rings, was the PAH that was completely eliminated in less than 50 d of

treatment with the consortium, whereas it was totally degraded after the 50 d of *P. ostreatus* treatment. The time required to obtain total degradation was shortest for BgP, followed by IND and, finally, FLT. Several studies have concluded that petroleum components have different susceptibilities to degradation by microorganisms (Greenwood et al., 2008; Bacosa et al., 2010).

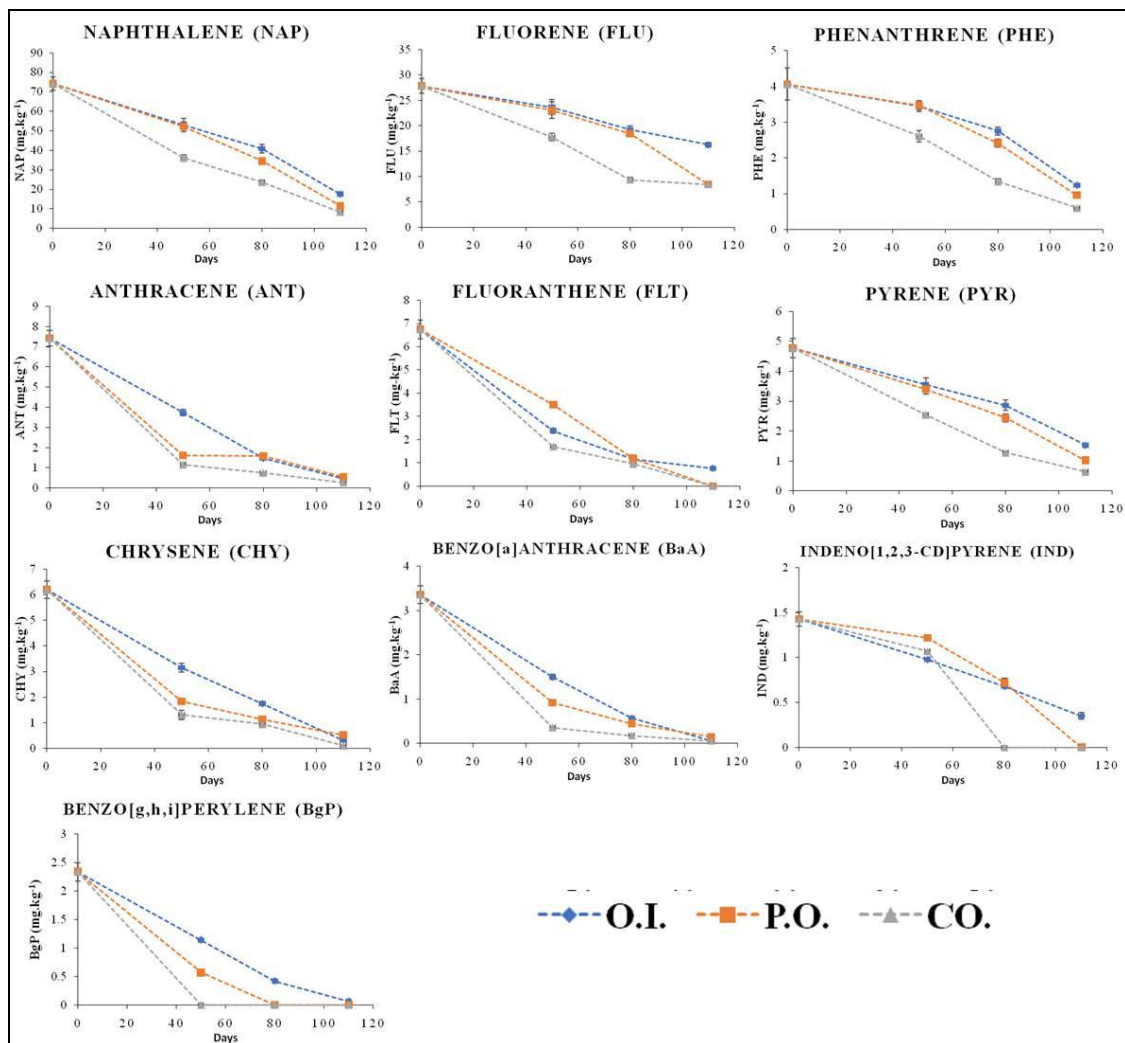


Figure 4. Concentration of individual PAH compounds during the 110-day experimental period for O.I. (*O. intermedium*), P.O. (*P. ostreatus*) and CO. (consortium) treatments

Degradation efficiencies of 69.7 to 85.2% PHE were obtained, proving that this compound can be degraded by actions associated with the microorganisms used in this study. Other studies using microorganisms have reported the biodegradation of PHE by such microorganisms as *Sphingobacterium* sp., *Achromobacter* sp., and *Bacillus* sp. (Janbandhu and Fulekar, 2011), while, in consortia, they have been able to degrade PHE at rapid rates without any additional carbon source (Arulazhagan and Vasudevan, 2009). Chagas-Spinelli et al. (2012), studied the degradation of PAHs via three treatments, including BSBA consortium, obtaining, after 126 d of incubation, degradation efficiencies similar to those found in this study. PAHs comprising two aromatic rings,

such as NAP and FLU, achieved high degradation efficiencies, of 100 and 98.3%, at concentrations of 2.1 and 12.3 mg kg⁻¹, respectively. Although the concentrations used in the present study were much higher, the high degradation can be attributed to the fact that LMW-PAHs degrade more easily due to their lower hydrophobicity than those of HMW-PAHs (Lors et al., 2012).

The total degradation of FLT and IND was found to be possible in this research, results which are superior to those reported by Chagas-Spinelli et al. (2012), who obtained 86.6 and 93.9% degradation in their most effective treatment, with the FLT concentration 2.4 times lower and the IND concentration 4.4 higher than the concentrations used in this study. The efficiency of *O. intermedium* was found to be similar to that obtained by the research undertaken with the BSBA microbial consortium, whereas the consortium used in this study managed to eliminate both FLT and IND completely, meaning that the use of consortia formed by fungi and bacteria is more efficient than the microbial consortia. This is attributed to the ability of *P. ostreatus* to both generate extracellular enzymes and obtain greater xenobiotic bioavailability for microorganisms (Namhyun et al., 2000). As PYR is classified as an HMW, its degradation process is more difficult than that of LMWs. This compound has been previously studied in liquid culture using a microbial consortium, in which it was degraded by up to 80%, although the increased PAH concentration decreased the degradation capacity of the consortium (Arulazhagan and Vasudevan, 2009). It is known that HMW PAHs, due to their high hydrophobicity, require longer degradation time and sometimes different microorganisms to degrade them (Luo et al., 2009; Boonchan et al., 2000). In this study, the concentrations of PYR were low so that its high degradation efficiencies had to be obtained by all three treatments. These results show that consortia composed of a bacteria-fungus mixture can more efficiently degrade existing LMW and HMW PAHs in soil (Li et al., 2008).

Conclusions

The consortium formed by certified *P. ostreatus* strain and *O. intermedium* strain isolated from soil contaminated with hydrocarbons were able to degrade PAHs in soil. The highest rate of degradation occurred using the consortium during the first 80 days of incubation, suggesting synergistic effects. In particular, the total degradation of HWM indene [1,2,3-cd] pyrene and benzo [g, h, i] perylene Haps was achieved using the consortium. The results of this study show that the consortium is a promising technology for the elimination of PAHs from soils contaminated with hydrocarbons; however, further research on the consortium must be undertaken through a field study as well as toxicity testing of the treated soils.

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REFERENCES

- [1] Acevedo, F., Pizzul, L., Castillo, M. P., Cuevas, R., Diez, M. C. (2011): Degradation of polycyclic aromatic hydrocarbons by the Chilean White-rot fungus *Anthraco-phyl-lum discolor*. – Journal of Hazardous Materials 185: 212-219.

- [2] Aranda, E., Ullrich, R., Hofrichter, R. (2010): Conversion of polycyclic aromatic hydrocarbons, methyl naphthalenes and dibenzofuran by two fungal peroxygenases. – *Biodegradation* 21: 267-281.
- [3] Arulazhagan, P., Vasudevan, N. (2009): Role of a moderately halophilic bacterial consortium in the biodegradation of polyaromatic hydrocarbons. – *Marine Pollution Bulletin* 58: 256-262.
- [4] Bacosa, H. P., Suto, K., Inoue, C. (2010): Preferential degradation of aromatic hydrocarbons in kerosene by a microbial consortium. – *International Biodeterioration and Biodegradation* 64: 702-710.
- [5] Bacosa, H. P., Suto, K., Inoue, C. (2013): Degradation potential and microbial community structure of heavy oil-enriched microbial consortia from mangrove sediments in Okinawa, Japan. – *Journal Environmental Science Health A* 48: 1-12.
- [6] Baldrian, P. (2008): Mini-review: wood-inhabiting ligninolytic basidiomycetes in soils: ecology and constraints for applicability in bioremediation. – *Fungal Ecology* 1: 4-12.
- [7] Bautista, L. F., Sanz, R., Molina, M. C., González, N., Sánchez, D. (2009): Effect of different non-ionic surfactants on the biodegradation of PAH by diverse aerobic bacteria. – *International Biodeterioration and Biodegradation* 30: 1-10.
- [8] Bento, F. M., Camargo, F. A., de, O., Okeke, B. C., Frankenberger Jr, W. T. (2005): Diversity of biosurfactant producing microorganisms isolated from soils contaminated with diesel oil. – *Microbiological Research* 160(3): 249-255.
- [9] Bezalel, L., Hadar, Y., Fu, P. P., Freeman, J. P., Cerniglia, C. E. (1996): Initial oxidation products in the metabolism of pyrene, anthracene, fluorene, and dibenzothiophene by the White rot fungus *Pleurotus ostreatus*. – *Applied of Environmental Microbiology* 62(7): 2554-2559.
- [10] Bezza, F., Beukes, M., Nkhalambayausi-Chirwa, E. (2015): Application of biosurfactant produced by *Ochrobactrum intermedium* CN3 for enhancing petroleum sludge bioremediation. – *Process Biochemistry* 50: 1911-1922.
- [11] Boonchan, S., Britz, M. L., Stanley, G. A. (2000): Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial cocultures. – *Applied Environmental Microbiology* 66: 1007-1019.
- [12] Borneman, J., Skroch, P. W., O'sullivan, K. M., Palus, J. A., Rumjanek, N. G., Jansen, J. L., Nienhuis, J., Triplett, E. W. (1996): Molecular Microbial Diversity of an Agricultural Soil in Wisconsin. – *Applied and Environmental Microbiology* 62(2): 1935-1943.
- [13] Borrás, E., Camanial, G., Sarra, M., Novotny, C. (2010): Effect of soil bacteria on the ability of polycyclic aromatic hydrocarbons (PAHs) removal by *Trametes versicolor* and *Irpex lacteus* from contaminated soil. – *Soil Biology and Biochemistry* 42: 2087-2093.
- [14] Bouchez, M., Blanchet, D., Bardin, V., Haeseler, F., Vandecasteele, P. (1999): Efficiency of defined strains and of soil consortia in the biodegradation of polycyclic aromatic hydrocarbon (PAH) mixtures. – *Biodegradation* 10: 429-435.
- [15] Brito, J. C., Santos, X., Pleguezuelos, J. M., Fahd, S., Llorente, G. A., Parella, X. (2006): Morphological variability of the Lataste's viper (*Vipera latastei*) and the Atlas dwarf viper (*Vipera monticola*): patterns of biogeographical distribution and taxonomy. – *Amphibia-Reptilia* 27: 219-240.
- [16] Cerqueira, V. S., Hollenbach, E. B., Maboni, F., Vainstein, M. H., Camargo, F. A. O., Peralba, M. C. R., Bento, F. M. (2011): Biodegradation potential of oily sludge by pure and mixed bacterial cultures. – *Bioresource Technology* 102: 11003-11010.
- [17] Chagas-Spinelli, A. C. O., Kato, M. T., de Lima, E. S., Gavazza, S. (2012): Bioremediation of a tropical clay soil contaminated with diesel oil. – *Journal of Environmental Management* 113: 510-516.
- [18] Chupungars, K., Rerngsamran, P., Thaniyavarn, S. (2009): Polycyclic aromatic hydrocarbons degradation by *Agroclybe* sp. CU-43 and its fluorene transformation. – *International Biodeterioration and Biodegradation* 63: 93-99.

- [19] Colombo, M., Cavalca, L., Bernasconi, S., Andreoni, V. (2011): Bioremediation of polyaromatic hydrocarbon contaminated soils by native microflora and bioaugmentation with *Sphingobium chlorophenicum* strain C3R: A feasibility study in solid and slurry-phase microcosms. – *International Biodeterioration and Biodegradation* 65: 191-197.
- [20] DeBruyn, J. M., Chewing, C. S., Sayler, G. S. (2007): Comparative quantitative prevalence of Mycobacteria and functionally abundant *nidA*, *nahAc*, and *nagAc* dioxygenase genes in coal tar contaminated sediments. – *Environmental Science Technology* 41: 5426-5432.
- [21] Díaz-Ramírez, I. J., Escalante-Espinosa, E., Favela-Torres, E., Gutiérrez-Rojas, M., Ramírez-Saad, H. (2008): Design of bacterial defined mixture cultures for biodegradation of specific crude oil fractions, using population dynamics analysis by DGGE. – *International Biodeterioration and Biodegradation* 62: 21-30.
- [22] Federici, E., Leonardi, V., Giubilei, M. A., Quarantino, D., Spaccapelo, R., D'Annibale, A., Petruccioli, M. (2007): Addition of allochthonous fungi to a historically contaminated soil affects both remediation efficiency and bacterial diversity. – *Applied Microbiology and Biotechnology* 77(1): 203-211. DOI: 10.1007/s00253-007-1143-1.
- [23] Frenzel, M., Scarlett, A., Rowland, S. J., Galloway, T. S., Burton, S. K., Lappin-Scott, H. M., Booth, A. M. (2010): Complications with remediation strategies involving the biodegradation and detoxification of recalcitrant contaminant aromatic hydrocarbons. – *Science of the Total Environment* 408: 4093-4101.
- [24] Furuno, S., Foss, S., Wild, E., Jones, K. J., Semple, K. T., Harms, H., Wick, L. Y. (2012): Mycelia promote active transport and spatial dispersion of polycyclic aromatic hydrocarbons. – *Environmental Science Technology* 46: 5463-5470.
- [25] Gallego, J. L. R., García-Martínez, M. J., Llamas, J. F., Belloch, C., Peláez, A. I., Sánchez, J. (2007): Biodegradation of oil tank bottom sludge using microbial consortia. – *Biodegradation* 18: 269-281.
- [26] Ghazali, F. M., Rahman, R. N. Z. A., Salleh, A. B., Basri, M. (2004): Biodegradation of hydrocarbons in soil by microbial consortium. – *International Biodeterioration and Biodegradation* 54: 61-67.
- [27] González, N., Simarro, R., Molina, M. C., Bautista, L. F., Delgado, L., Villa, J. A. (2011): Effect of surfactants on PAH biodegradation by a bacterial consortium and on the dynamics of the bacterial community during the process. – *Bioresource Technology* 102: 9438-9446.
- [28] Greenwood, P. F., Wibrow, S., George, S. J., Tibbett, M. (2008): Sequential hydrocarbon biodegradation in a soil from arid coastal Australia, treated with oil under laboratory controlled conditions. – *Organic Geochemistry* 39: 1336-1346.
- [29] Hatakka, A., 1994. Lignin-modifying enzymes from selected white-rot fungi: production and role in lignin degradation. – *FEMS Microbiology Reviews* 13: 125-135.
- [30] Hollender, J., Althoff, K., Mundt, M., Dott, W. (2003): Assessing the microbial activity of soil samples, its nutrient limitation and toxic effects of contaminants using a simple respiration test. – *Chemosphere* 53: 269-275.
- [31] Janbandhu, A., Fukelar, M. H. (2011): Biodegradation of phenanthrene using adapted microbial consortium isolated from petrochemical contaminated environment. – *Journal of Hazardous Materials* 187: 333-340.
- [32] Johnsen, A. R., Karlson, U. (2004): Evaluation bacterial strategies to promote the bioavailability of polycyclic aromatic hydrocarbons. – *Applied Microbiology and Biotechnology* 63(4): 452-459.
- [33] Johnsen, A. R., Wick, L. Y., Harms, H. (2005): Principles of microbial PAH-degradation in soil. – *Environmental Pollution* 133: 71-84.
- [34] Kim, Y. M., Ahn, C. K., Woo, S. H., Jung, G. Y., Park, J. M. (2009): Synergic degradation of phenanthrene by consortia of newly isolated bacterial strains. – *Journal of Biotechnology* 144: 293-298.

- [35] Kohlmeier, S., Smits, T. H. M., Ford, R. M., Keel, C., Harms, H., Wick, L. (2005): Taking fungal highway: mobilization of pollutant degrading bacteria by fungi. – *Environmental Science Technology* 39: 4640-4646.
- [36] Li, X. J., Li, P. J., Lin, X., Zhang, C. G., Li, Q., Gong, Z. Q. (2008): Biodegradation of aged polycyclic aromatic hydrocarbons (PAHs) by microbial consortium in soil and slurry phases. – *Journal of Hazardous Materials* 150: 21-26.
- [37] Liu, P. W. G., Chang, T. C., Whang, L. M., Kao, C. H., Pan, P. T., Cheng, S. S. (2011): Bioremediation of petroleum hydrocarbon contaminated soil: effects of strategies and microbial community shift. – *International Biodeterioration and Biodegradation* 65: 1119-1127.
- [38] Lors, C., Damidot, D., Ponge, J. F., Périé, F. (2012): Comparison of a bioremediation process of PAHs in a PAH-contaminated soil at field and laboratory scales. – *Environmental Pollution* 165: 11-17.
- [39] Luo, Y. R., Tian, Y., Huang, X., Yan, C. L., Hong, H. S., Lin, G. H., Zheng, T. L. (2009): Analysis of community structure of a microbial consortium capable of degrading benzo(a)pyrene by DGGE. – *Marine Pollution Bulletin* 58: 1159-1163.
- [40] Mamma, D., Kalogeris, E., Papadopoulos, N., Dimitris, G., Hatzinikolaou, Christrakopoulos, P., Kekos, D. (2004): Biodegradation of phenol by acclimatized *Pseudomonas putida* cells using glucose as an added growth substrate. – *Journal Environmental Scientific Health A* 39(8): 2093-2104.
- [41] Mao, J., Luo, Y., Teng, Y., Li, Z. (2012): Bioremediation of polycyclic aromatic hydrocarbon-contaminated soil by a bacterial consortium and associated microbial community changes. – *International Biodeterioration and Biodegradation* 70: 141-147.
- [42] Molina, M. C., González, N., Bautista, L. F., Sanz, R., Simarro, R., Sánchez, I., Sanz, J. L. (2009): Isolation and genetic identification of PAH degrading bacteria from a microbial consortium. – *Biodegradation* 20: 789-800.
- [43] Mueller, J. G., Chapman, P. J., Pritchard, P. H. (1989): Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. – *Applied and Environmental Microbiology* 55: 3085-3090.
- [44] Namhyum, C., Lee, I. S., Song, H. S., Bang, W. G. (2000): Mechanisms used by white rot fungus to degrade lignin and toxic chemicals. – *Journal of Microbiology and Biotechnology* 10(6): 737-752.
- [45] Ruberto, A. M., Vazquez, S. C., Curtosi, A. (2006): Phenanthrene biodegradation in soils using an Antarctic bacterial consortium. – *Bioremediation Journal* 10: 191-201.
- [46] Sack, U., Hofrichter, M., Fritsche, W. (1997): Degradation of polycyclic aromatic hydrocarbons by manganese peroxidase of *Nemalotonomia frowardii*. – *FEMS Microbiology Letters* 152: 227-234.
- [47] Sahoo, N. K., Ramesh, A., Pakshirajan, K. (2012): Bacterial degradation of aromatic xenobiotic compounds: an overview on metabolic pathways and molecular approaches. – *Microorganisms in Environmental Management* 201-220.
- [48] Sambrook, J., Russell, D. (2001): *Molecular Cloning. A Laboratory manual*. – Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- [49] Sinha, S., Chattopadhyvy, P., Sen, S. K. (2012): *Microbial Degradation of Recalcitrant PAHs-Microbial Degradation of Xenobiotics*. – Springer, Berlin, pp. 395-410. <http://dx.doi.org/10.1007/978-3>.
- [50] Šnajdr, J., Dobiášová, P., Vetrovský, T., Valášková, V., Alawi, A., Boddy, L., Baldrian, P. (2011): Saprotrophic basidiomycete mycelia and their interspecific interactions affect the spatial distribution of extracellular enzymes in soil. – *FEMS Microbiology Ecology* 78: 80-90.
- [51] Sultan, S., Hasnain, S. (2007): Reduction of toxic hexavalent chromium by *Ochrobactrum intermedium* strain SDCr-5 stimulated by heavy metals. – *Bioresource Technology* 98: 340-344.

- [52] Tian, Y., Liu, H. J., Zheng, T. L., Kwon, K. K., Kim, S. J., Yan, C. L. (2008): PAHs contamination and bacterial communities in mangrove Surface sediments of the Jiulong River Estuary, China. – *Marine Pollution Bulletin* 57(1): 707-715.
- [53] Tirado-Torres, D., Gayosso-Canales, M., Marmolejo-Santillán, Y., Romo-Gómez, C., Acevedo-Sandoval, O. (2016): Removal of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* sp. ATCC38540 in liquid medium. – *Academia Journal of Scientific Research* 4(10): 376-379. DOI: 10.15413/ajsr.2016.0406.
- [54] Tirado-Torres, D., Acevedo-Sandoval, O., Rodríguez-Pastrana, B. R., Gayosso-Canales, M. (2017): Phylogeny and polycyclic aromatic hydrocarbons degradation potential of bacteria isolated from crude oil-contaminated site. – *Journal of Environmental Science and Health, Part A* 9(52): 897-904. DOI: 10.15413/ajsr.2016.0406.
- [55] Toledo, F. L., Calvo, C., Rodelas, B., González-Lopez, J. (2006): Selection and identification of bacteria isolated from waste crude oil with polycyclic aromatic hydrocarbons removal capacities. – *Systematic and Applied Microbiology* 29(3): 244-252.
- [56] Tornberg, K., Bååth, E., Olsson, S. (2003): Fungal growth and effects of different wood decomposing fungi on the indigenous bacterial community of polluted and unpolluted soils. – *Biology and Fertility of Soils* 37(3): 190-197. DOI: 10.1007/s00374-002-0574-1.
- [57] Valentín, L., Feijoo, G., Moreira, M. T., Lema, J. M. (2006): Biodegradation of polycyclic aromatic hydrocarbons in forest and salt marsh soils by White-rot fungi. – *International Biodeterioration and Biodegradation* 58: 15-21.
- [58] Venosa, A. D., Zhu, X. (2003): Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. – *Spill Science and Technology Bulletin* 8: 163-178.
- [59] Vila, J., Nieto, J. M., Mertens, J., Springael, D., Grifoll, M. (2010): Microbial community structure of a heavy metal fuel oil-degrading marine consortium: linking microbial dynamics with polycyclic aromatic hydrocarbon utilization. – *FEMS Microbiology Ecology* 73: 249-362.
- [60] Wang, J., Xu, H., Guo, S. (2008): Isolation and characteristics of a microbial consortium for effectively degrading phenanthrene. – *Petroleum Science* 4(3): 68-75.
- [61] Waranusantigul, P., Lee, H., Kruatrachue, M., Pokethitiyook, P., Auesukaree, C. (2011): Isolation and characterization of lead-tolerant *Ochrobactrum intermedium* and its role in enhancing lead accumulation by *Eucaliptus camaldulensis*. – *Chemosphere* 85: 584-590.
- [62] Yuan, S. Y., Wei, S. H., Chang, B. V. (2002): Biodegradation of polycyclic hydrocarbons by mixed culture. – *Chemosphere* 41: 1463-1468.
- [63] Zeng, J., Lin, X. G., Zhang, J., Li, X. Z. (2010): Isolation of polycyclic aromatic hydrocarbons (PAHs)-degrading *Mycobacterium* spp. and the degradation in soil. – *Journal of Hazardous Materials* 183: 718-723.