Biodegradation of polycyclic aromatic hydrocarbons (PAHs) in crude oil, by a white rot fungus, Pleurotus ostreatus sp., in broth culture was investigated. It was found that the biomass of the organism increased with the increase of PAHs concentration in the cultures. In the cultures with 25.99, 49.01 and 80.05 mg.kg$^{-1}$ PAHs, the degradation increased with the PAHs of low molecular weight concentration, whereas, the degradation was high and constant for high molecular weight. The addition of Tween 80 improved the ability to degrade PAHs high molecular weight. In conclusion, P. ostreatus is a promising white rot fungus to used degrade PAHs of low and high molecular weight in the environment.

Key words: Pleurotus ostreatus, PAHs, biodegradation, White rot fungi.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic lipophilic pollutants generated during incomplete combustion of organic carbons such as burning fossil fuels, wood and municipal solid waste. They are of great environmental concern due to their ubiquitous presence and persistence in air, water and soil, as well as, their toxicity to humans and other living organisms (Keith and Telliard, 1979). Humans may be exposed to these compounds from a wide variety of sources, such as through occupation, natural environment and diets etc (Doyle et al., 2008). Although PAHs may fade away from the environment through adsorption, chemical degradation, photolysis and volatilization, microbial degradation is the major process to remove PAH contamination in the environment (Yuan et al., 2002).

Microbial diversity offers an immense field of friendly environment for mineralization of pollutants or transformation into non-hazardous compounds with less damaging options. There is general interest in the study of the diversity of indigenous micro-organisms capable of degrading different pollutants due to their varying effects on the environment (Watanabe et al., 2002). White rot fungi, such as Phanerochaete chrysosporium, Pleurotus ostreatus, Trametes versicolor and Bjerkandera sp., were reported as being capable of degrading naturally occurring polymer, lignin. Lignin possesses similar structure to that of PAHs and thus, these fungi are plausible candidates for PAH degradation (Hammel, 1995; Hestbjerg et al., 2003). The world of fungi provides a fascinating and almost endless source of biological diversity, which is a rich source of exploitation (Manoharachary et al., 2005). Although fungal flora mainly species Western fungi have been well documented (Steffen, 2003), there is very little research on fungal species in relation to their use as potential remediation (Manoharachary et al., 2005).

In addition, previous studies showed that various fungi are able to mineralize PAHs and PAHs removal rates with P. ostreatus at different concentrations and can be compared with other investigations conducted (Run and Eyini, 2011; Covino et al., 2010). Previous research showed that the enzymes secreted by fungi of white rot to degrade lignin (peroxidase, MNP and laccase) have been recognized to be especially important in the degradation of PAHs (Chang et al., 2001; Wariishi et al., 1992). In this
study, PAHs biodegradation was evaluated by *P. ostreatus* in liquid culture at different concentrations of the pollutant.

**MATERIALS AND METHODS**

**Chemicals**

The crude oil used in the study was managed from the Lázaro Cardenas refinery in Minatitlán Ver. México. Standard PAHs were purchased from Sigma-Aldrich (98 to 99% purity), containing Benzo [b] fluoranthene, Benzo [g,h,i] perylene, Benzo [k] fluoranthene, Indeno [1,2,3-cd] pyrene, acenaphthene, fluoranthene, naphthalene, Benzo [a] anthracene, Benzo [a] pyrene, chrysene, acenaphthylene, anthracene, fluorene, phenanthrene, pyrene and dibenzo [a,h] anthracene; that according the EPA are the most dangerous pollutants. Solvents used in this study were purchased from Sigma-Aldrich (USA) of an analytical and HPLC grade.

**Organism and culture conditions**

*P. ostreatus* ATCC38540 was obtained from Difco, Detroit, MI, USA. Was stored in potato dextrose agar (PDA) plate at 5°C and was then activated at 27°C for 3 days darkness. When the entire plate was covered by mycelia, 10 agar plugs (5 mm in diameter) cut from the PDA culture plate were transferred into 100 ml of a sterilized 3% malt extract liquid culture medium in a 250 ml Erlenmeyer flask and incubated at 27°C on a rotary shaker (150 rpm) in the dark for 5 d before use.

**Experimental design**

Experiments were performed in 250-ml Erlenmeyer flasks containing 100 ml of fungal culture in minimum medium for *basidiomycetes* plus 1 ml tween 80. The experimental design was a randomized completely block design (RCBD) where three treatments at concentrations of 17400, 32700 and 53400 mg.L⁻¹ of crude oil were handled to study their effects on the degradation of PAHs. Mycelia plugs of selected fungi were cut from the outer edge of an actively growing culture on an inoculums plate. Ten 5-mm disks obtained by punching out with a cork-borer from the outer edge of an actively growing culture of a particular fungus were inoculated in a flask containing 100 ml of liquid medium. The flasks were incubated at 27°C on a rotary shaker (150 rpm) in the dark. Growth and substrate consumption were determined at 15 days. All media were sterilized by autoclaving at 121°C for 20 min. Control experiments were performed by incubating crude oil in autoclaved liquid medium (121°C for 20 min) without inoculums. All assays were conducted in triplicate.

**Analytical methods**

After the incubation, the culture broth was filtered and residue (fungal body) separated by filtration. Briefly, PAHs extract was prepared by mixing 10 ml culture sample and 30 ml hexane in a separating funnel and each sample vigorously shaken for 3 min. The hexane extract HAPs was collected in vials amber and the extraction procedure repeated. The final extract was concentrated in a rotary evaporator and each concentrated sample was resuspended in cyclohexane and cleaned C-18 cartridge packed with silica gel SUPELCO mark. Each sample was eluted with 30 ml of the solvent mixture dichloromethane: hexane (2:3). The eluate was concentrated on Rotavapor and resuspended in acetonitrile. Samples were stored in amber vials at -4°C until analysis.

The mycelium of each culture incubated at 15 days was cleaned with acetone and dried at 55°C for 24 h. The dry weight of each mycelium was measured. Approximately 1 g of mycelium (previously milled in a mortar) dry weight was mixed in 15 ml of acetone: dichloromethane (1:5) in a test tube for 1 min using a vortex mixer. The sample was incubated at 15°C for 40 min in an ultrasonic cleaner Branson 2510 and centrifuged for 15 min at 8000 rpm. The supernatant was transferred to a vial and the extraction procedure repeated for the same sample earlier mentioned. The two sample extractions were combined and concentrated in a rotary evaporator; the concentrate also was resuspended in cyclohexane and cleaned in a C-18 cartridge. The resuspended sample in cyclohexane was eluted with 15 ml of dichloromethane: hexane (2:3, V/V). Finally, the eluate was concentrated again on a rotary evaporator and resuspended in acetonitrile. Each sample was analyzed in a chromatograph high performance liquid (HPLC) 1260 equipped with a fluorescence detector, quaternary pump, autosampler and Chemstation software (all from Agilent technologies, Germany). Separation of the PAHs was Carried out on a ZORBAX Eclipse PAH (4.6 mm × 50 mm, 1.8 um) column. The mobile phase was water mixture (60:40), flow rate 0.8 ml.min⁻¹, the sample volume of 20 µl and a run time of 16 min. Fluorescence levels excitation and emission were set at 260/352, 260/420 and 260/460 respectively.

**Data analysis**

The data was recorded as means ± standard deviations. One-way analysis of variance (ANOVA) and Tukey’s multiple comparisons were carried out to find the best treatment using the software SAS 9.0. Differences between means at 5% (P <0.05) level were considered significant.

**RESULTS AND DISCUSSION**

The growth of the organism in cultures with different PAH
concentrations were compared at the end of 15 days incubation (Table 1). It was found that the higher the PAH concentration, the higher the biomass. These data are contrary to those reported by Ting et al. (2011) where with an incubation time of 10 days it was observed that the micellar biomass decreased with increasing concentration of PAHs using G. lucidum. This result suggested that PAH did not inhibit the growth of P. ostreatus. When the concentrations of PAHs in the cultures were elevated to 49.01 and 80.05 mg.L⁻¹, the degradation activities were enhanced to 91.9 and 98.2% respectively. The increment of degradation activities in the cultures with high concentrations of PAHs was probably due to no growth inhibition. This is because basidiomycetes are able to act through mechanisms based on degradation unspecific radicals produced in the extracellular environment (Chung et al., 2000). Previous studies showed that several fungi are able to mineralize PAHs and removal rates of PAHs with P. ostreatus at different concentrations can be compared with studies already reported and varying degradations of 150 to 1500 mg.kg⁻¹ PAHs (Run and Eyini, 2011; Covino et al, 2010).

It was also shown that enzymes secreted by fungi of white rot degraded lignin (peroxidase MnP and laccase) and are responsible for the degradation of PAHs (Chang et al., 2001). At concentrations of 80.051 mg.L⁻¹, P. ostreatus was capable of removing 98.2% (Table 2; high rates of degradation were observed in each HAP) in these concentrations. The HAP presented a complete removal of naphthalene. PAHs achieved low molecular weight (LMW) of 97.7%, while 98.9% of high molecular weight (HMW) PAHs. In the case of P. ostreatus at concentrations of 80.051 mg.L⁻¹ high rates of degradation of individuals PAHs were observed and this may be due to the concentration of tween 80 added to them. In cultures of P. ostreatus, the benzenanthrene, pyrene and benzo[a]anthracene degraded evenly over a period of 15 days culture. In addition to the stimulating effects caused by the type of inoculums, support could be derived from its ability to slowly release phenolic compounds capable of acting as mediators in the degradation of PAHs (Cañas et al, 2007) and stimulating of ligninolytic fungal system (Crestini et al., 1996).

A study with P. ostreatus and addition of tween 80 facilitated degradation of benzenanthrene, pyrene and benzo[a]pyrene in a reactor rotating biological contact where the results showed that the tween 80 increased the solubility of PAHs so as to make available to ligninolytic enzyme system of P. ostreatus, (Akdogan and Pazarlioglu, 2011). Moreover, the high removal hap at different concentrations is because it proved to be more susceptible to oxidative attack by fungi of white rot (Novotny et al.,

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Table 1. Effect of the initial PAH concentration on biomass and PAH degradation in the culture of P. ostreatus ATCC38540 at 27°C.

<table>
<thead>
<tr>
<th>PAHs conc. (mg.L⁻¹)</th>
<th>Biomass (g)</th>
<th>% Degradation of PAHs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LMW</td>
</tr>
<tr>
<td>25.99 ± 1.28</td>
<td>1.253 ± 0.052</td>
<td>77.7 ± 1.59</td>
</tr>
<tr>
<td>49.01 ± 2.18</td>
<td>1.421 ± 0.061</td>
<td>88.5b ± 1.33</td>
</tr>
<tr>
<td>80.05 ± 3.85</td>
<td>1.671 ± 0.073</td>
<td>97.7 ± 1.13</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Means in the same column with a letter in common are not significantly different among treatments (P < 0.05).

Table 2. Degradation and bioaccumulation of PAHs at a concentration of 80.051 mg.L⁻¹, P. ostreatus as degrading agent.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Rings</th>
<th>Control (mg.L⁻¹)</th>
<th>Biodegradation (mg.L⁻¹)</th>
<th>Intracellular accumulation (mg.L⁻¹)</th>
<th>Total removal (mg.L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>2</td>
<td>15.712 ± 0.07</td>
<td>14.661 ± 0.02 (93.3)</td>
<td>1.051 ± 0.00 (6.7)</td>
<td>15.712 ± 0.83 (100)</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>3</td>
<td>9.459 ± 0.09</td>
<td>7.954 ± 0.07 (84.1)</td>
<td>0.547 ± 0.01 (5.8)</td>
<td>8.501 ± 0.27 (89.9)</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>3</td>
<td>3.694 ± 0.04</td>
<td>3.594 ± 0.01 (97.3)</td>
<td>0.037 ± 0.01 (1.0)</td>
<td>3.631 ± 0.10 (98.3)</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>3</td>
<td>16.680 ± 0.10</td>
<td>16.140 ± 0.01 (96.8)</td>
<td>0.527 ± 0.01 (3.2)</td>
<td>16.667 ± 1.00 (99.9)</td>
</tr>
<tr>
<td>Total LMW PAHs</td>
<td>2 y 3</td>
<td>45.545 ± 0.15</td>
<td>42.349 ± 0.07 (93.0)</td>
<td>2.162 ± 0.01 (4.7)</td>
<td>44.511 ± 1.13 (97.7)</td>
</tr>
<tr>
<td>Chrysene</td>
<td>4</td>
<td>6.783 ± 0.05</td>
<td>6.645 ± 0.01 (98.0)</td>
<td>0.055 ± 0.00 (0.8)</td>
<td>6.700 ± 0.21 (98.1)</td>
</tr>
<tr>
<td>Pyrene</td>
<td>4</td>
<td>2.853 ± 0.06</td>
<td>2.795 ± 0.00 (98.0)</td>
<td>0.000 ± 0.00 (0.0)</td>
<td>2.795 ± 0.10 (98.0)</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>4</td>
<td>3.060 ± 0.01</td>
<td>2.990 ± 0.01 (97.7)</td>
<td>0.025 ± 0.00 (0.0)</td>
<td>3.085 ± 0.21 (98.5)</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>4</td>
<td>2.358 ± 0.01</td>
<td>2.338 ± 0.00 (99.2)</td>
<td>0.000 ± 0.00 (0.0)</td>
<td>2.338 ± 0.00 (99.2)</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>5</td>
<td>0.742 ± 0.01</td>
<td>0.737 ± 0.00 (99.3)</td>
<td>0.000 ± 0.00 (0.0)</td>
<td>0.737 ± 0.00 (99.3)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>5</td>
<td>4.269 ± 0.02</td>
<td>4.248 ± 0.00 (99.5)</td>
<td>0.000 ± 0.00 (0.0)</td>
<td>4.248 ± 0.00 (99.5)</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>6</td>
<td>7.400 ± 0.08</td>
<td>6.588 ± 0.02 (89.0)</td>
<td>0.660 ± 0.03 (8.9)</td>
<td>7.268 ± 0.13 (97.9)</td>
</tr>
<tr>
<td>Indene[1,2,3-cd] pyrene</td>
<td>6</td>
<td>7.041 ± 0.04</td>
<td>6.567 ± 0.02 (93.3)</td>
<td>0.455 ± 0.02 (6.5)</td>
<td>7.022 ± 0.22 (99.8)</td>
</tr>
<tr>
<td>Total HMW PAHs</td>
<td>4 a 6</td>
<td>34.506 ± 0.05</td>
<td>32.908 ± 0.04 (95.4)</td>
<td>1.195 ± 0.01 (3.5)</td>
<td>34.103 ± 1.69 (98.9)</td>
</tr>
<tr>
<td>Total PAHs</td>
<td>80.051 ± 0.55</td>
<td>75.257 ± 0.02 (94.0)</td>
<td>3.357 ± 0.02 (4.2)</td>
<td>78.614 ± 1.34 (98.2)</td>
<td></td>
</tr>
</tbody>
</table>

Numbers in () are % degradation defined by the following equation: (PAH removed*100)/control). Data are presented as means ± SD.
1999) and purified preparations of degradative enzymes lignin (Johannes and Majchercyz, 2000). Bioavailability of the compound increases by the effect of the surfactant used to emulsify the medium. Fluoranthene high surfactant is comparable to that of other fungi such as Trametes versicolor, Trametes trogii, Ganoderma carnasum and P. ostreatus, while T. versicolor, G. carnasum and T. trogii degraded the fluoranthene 30% and P. ostreatus and metabolized 85% approximately a concentration of 30 mg.L\(^{-1}\) within six weeks (Akdogan and Pazarlioglu, 2011).

Degradation of chrysene was more efficient in other studies with fungi such as Nematoloma frowardi, Bjerkandera, Irpex lacteus and Lentinus tigrinus where it degrades up to 35% of 10 mg.L\(^{-1}\) in four weeks (Valentin et al., 2006; Sack et al., 2006; Leonardi et al., 2008). The enzymes released by P. ostreatus were oxidized by this compound bioavailability tween 80 that produced the observed increased intracellular degradation. In cultures of P. ostreatus, fenanthrene, pyrene and benzo[a]anthracene its degradation percentages were similar though each PAH concentrations are different. In addition, P. ostreatus can produce an emulsifying agent Pseudomonas capable of solubilizing as PAHs (Diaz De Rienzo et al., 2016).

**Conclusion**

The results showed that PAHs were degraded by P. ostreatus ATCC38540 in liquid culture. As the concentration of PAHs increased it was observed that P. ostreatus was able to increase activity removal and increase their biomass. Furthermore, addition of tween 80 achieved a better bioavailability HMW PAHs which led to a high degradation of the compounds.

**ACKNOWLEDGMENT**

The authors wish to thank CONACyT (Consejo Nacional de Ciencia y Tecnologia) from Mexico for PhD scholarship granted.

**REFERENCES**


Yuan SY, Shing LC, Chang BV (2002). Biodegradation of polycyclic aromatic hydrocarbons by a mixed culture. C.