Scientific Bulletin. Series F. Biotechnologies, Vol. XVII, 2013
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

IN VITRO EVALUATION OF CRUDE OIL DEGRADATION POTENTIAL OF SOME *PLEUROTUS OSTREATUS* ISOLATES

Gabriela POPA, Mihai Bogdan NICOLCIOIU, Calina Petruta CORNEA

University of Agronomical Sciences and Veterinary Medicine, Faculty of Biotechnologies,
59 Marasti Blvd., District 1, 011464, Bucharest, Romania,
Phone: +40 (21) 318 22 66, Fax: +40 (21) 318 28 88, e-mail: popagabiro@yahoo.com
Corresponding author e-mail: popagabiro@yahoo.com

Abstract

In the last years degradation of aromatic compounds by white-rot fungi has been intensively studied. Several studies revealed the abilities of the white-rot fungus Pleurotus ostreatus to degrade a variety of polycyclic aromatic hydrocarbons in liquid culture as well as in a semi-natural substrate. In the present study, two isolates of P. ostreatus (P50, P421) and a sample of P. ostreatus collected from Chitila woods were evaluated for their ability to use crude-oil as a source of carbon. The P. ostreatus samples were inoculated in plates with crude oil (1ml) spread on the entire carbon free mineral salt medium (MSM) surface and in plates with pieces of filter paper soaked in crude oil and placed around the inoculum. Two weeks after, the culture media were completely covered by mycelium. These results indicated that both isolates of P. ostreatus as well as P. ostreatus originated from Chitila forest were able to utilize the crude oil as a source of carbon and energy in their metabolism.

Keywords: *crude-oil, Pleurotus ostreatus, mycoremediation.*

INTRODUCTION

Polycyclic aromatic hydrocarbons, relatively abundant, may persist in the environment affecting animals and aquatic organisms due to their carcinogenic and mutagenic properties (Clemente et al, 2001; Cerniglia and Sutherland 2001). Bioremediations is a natural process that can accelerate degradation of hydrocarbons-contaminated waste into non-toxic residues by bacteria and fungi. During bioremediation, microorganisms utilize chemical contaminants in the soil as an energy source.
This process is based on microbial enzyme activities (Philip et al, 2005). Biological method provides the best solution for oil polluted environment remediation using the ability of indigenous microorganisms from soil to convert oil into harmless substances (Perelo, 2010). Different strains of bacteria in bioremediation such as *Bacillus* sp., *Pseudomonas* sp., *Micrococcus* sp., *Vibrio* sp. (Ijah and Antai, 2003) or fungal isolates of *Aspergillus* sp*., Alternaria* sp*., Penicillium* sp. and *Fusarium* sp. displayed highest ability for biodegradation of aromatic hydrocarbon-contaminated soil (Chaudhry et al., 2012). Mycoremediation, which is the use of the mushroom in the remediation of various polluted media, has demonstrated positive results, verified by scientists. *Pleurotus ostreatus, Pleurotus tuber-regium, Irpex lecteus* or *Lentinus* sp. have been used in the bioremediation of engine-oil polluted soil, crude oil contaminated soil and chemically polluted soil (Bezalel et al., 1996a; Adenipekun and Fasidi, 2005; Adenipekun, 2008; Ogbo and Okhuaya, 2008; Okparanma et al., 2011). Particularly, the white-rot fungus *Pleurotus ostreatus* have the potentials to degrade polycyclic aromatic hydrocarbons (Sack and Gunther, 1993; Vyas et al., 1994; Okparanma et al., 2011). Therefore, the goal of this study was to evaluate the potency of *Pleurotus ostreatus* for the growth on minimal salt media (MSM) with crude oil as carbon source.

MATERIALS AND METHODS

Inocula preparations. A sample of *P. ostreatus* collected in 2012 from Chitila woods and two isolates of *P. ostreatus*, P50 and P421 kindly provided by dr. Ioana Tudor were used in experiments. For mycelia production a well

grown mushroom from the Chitila forest was selected and a gill portion was taken using a sterile forceps and inoculated on Potato Dextrose Agar (PDA) or 2 % malt extract broth media plates under aseptic conditions. In the case of the two isolates of *P. ostreatus*, segments of 5 mm from the culture media were taken and placed on the same media as those described above. The samples were incubated at 25^0C for a week. The mycelia grown on the media surface were used for the following experiments.

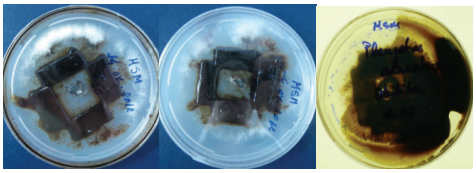
Evaluation of *P. ostreatus* growing potential on culture media with crude-oil added. Crude oil used for this study was obtained from the Petroleum Refinery, Ploiesti. To evaluate the crude-oil degradation potential of *P. ostreatus*, mycelia inoculum were taken from the culture media and placed on carbon free mineral salt medium (MSM) prepared according Bhattacharya et al., 2012 (g/l: 0.5 $(NH_4)_2HPO_4$; 0.8 KH_2PO_4; 0.3 $MgSO_4.7H_2O$; 0.055 $CaCl_2.2H_2O$; 0.004 $ZnSO_4.6H_2O$; 0.07 $CuSO_4$; 0.2 yeast extract; 1 ml Thiamine 2mg/ml; pH 6). The crude oil was added to the medium surface in the following ways:

a. 1 ml crude oil spread on the entire MSM medium surface using a sterile glass baguette;
b. Sterile filter paper pieces (20x10 cm) soaked in crude oil and placed around the inoculum.

The control was prepared on 2% malt extract media without pollutant. Three replicates of each experimental variant were made for the *P. ostreatus* samples. All the Petri dishes were incubated at 27 C for 14 days. Every day, the radial growth of mycelia (in cm) was measured and extrapolated as growing percentage to culture plate diameter (9 cm).

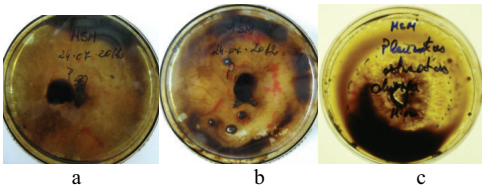
RESULTS AND DISCUSSIONS

In this work, a sample of *P. ostreatus* collected from Chitila woods and two isolates named P50 and P421 were used, in order to evaluate the potency of fungal pure culture samples for the growth on minimal salt media (MSM) with crude oil as carbon source. Two weeks after experiments initiation, in the samples which contained the filter paper soaked in crude oil mycelium growth of both isolates was faster (89% P50; 80% P421) (Figure 1) compared with the treatments which the crude oil was spread on the entire media surface (69% P50 and 75.5% P421 respectively) (Figure 2).



a b c

Figure 1. *P. ostreatus* mycelia growth on MSM medium with filter paper in crude oil P50 (a), P421 (b) and *P. ostreatus* originated from Chitila forest (c)



a b c

Figure 2. *P. ostreatus* mycelia growth on medium covered with oil: P50 (a), P421 (b) and *P. ostreatus* originated from Chitila forest (c)

The same situation was observed at the *P. ostreatus* mushroom collected from Chitila woods (Figures 1 and 2): the rate of mycelium growth was 78% on the filter paper with crude oil treatment and 67% in the plates with the medium surface completely covered with oil.

The observations extended for another two weeks revealed that mycelia covered completely the media surfaces treated with filter paper soaked in oil (Figure 3). Effectively, the mycelium covered the filter paper and has been extended to the plate edge.

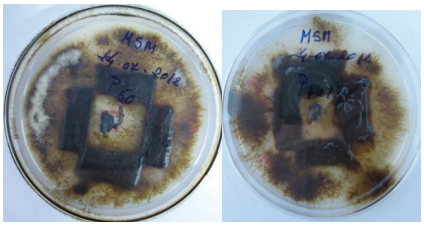


Figure 3. Mycelia growth of P50 (left) and P421 isolates (right) on filter paper with crude oil four weeks after

Assessment of fungal growth a month after, showed that both P421 and P50 mycelia almost covered the medium surface (Figure 4).

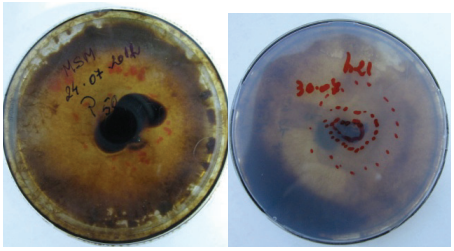


Figure 4. Mycelia growth of P50 (left) and P421(right) on crude oil covered culture media, four weeks after

These results show that the tested *P. ostreatus* samples had about the same growth potential on media supplemented with crude-oil (Figure 5). All *P. ostreatus* tested grew on carbon free minimal salt media treated with oil but the mycelium growth was considerably reduced when the oil was applied on entire surface on the medium compared to growth on media partially treated with oil.

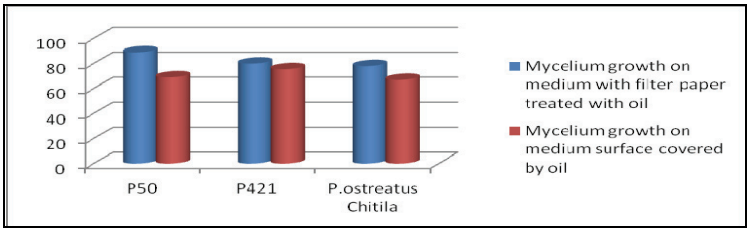


Figure 5. Mycelium growth of *P. ostreatus* on MSM medium treated with crude-oil

Apparently, in the first phase, the radial growth of mycelium was inhibited at higher concentration of oil. It has been demonstrated that the tolerance of mycelia of various species of *Pleurotus* of the pollutant which served as treatments in some studies, varied (Adedokun and Ataga, 2006). Ogbo et al. (2006) investigated the effect of different levels of spent lubricating oil (from 5 to 160%) on the growth of *P. tuber-regium*. They found that the fungus grew optimally at 98% level of contamination. However, in our experimental variants with crude oil treated culture media has been noted that developed mycelium had a dark brown colour compared with the typical white- gray colour of the *P. ostreatus.* Moeder et al. (2005) mentioned that *P. ostreatus* are the ability to eliminate the hydrocarbon pollutants accumulated from a contaminated soil to the fruit bodies. Moreover, some authors shown that *Pleurotus ostreatus* produce lacasse which it seems to be implicated in hydrocarbons degradation (Pozdnyakova et al, 2011). In mycoremediation process the fungi can degrade or transform organic contaminants to less toxic or non toxic compounds (Sasek et al, 2003). White-rot and waste –decomposing fungi are the potential candidates for the treatment of contaminated soils because of their high ability to degrade a wide range of xenobiotics in culture media or in contaminated soil (Hou et al., 2004) as well as due the hyphal penetration and the excretion of some oxidative enzymes in the polluted sites.

CONCLUSIONS

We investigated the effect of crude oil on the growth of two isolates of *Pleurotus ostreatus*, P50 and P421 respectively and a *P. ostreatus* mushroom originated from Chitila woods for their capability to use crude-oil as a source of carbon and energy. For this purpose, the *P. ostreatus* samples were inoculated in Petri plates with crude oil applied in two different ways: spread on the entire MSM medium surface as well as in plates with pieces of filter paper soaked in oil and placed around the inocula. In the first phase, the radial growth of each isolates mycelium was inhibited on culture media treated with oil spread on the entire surface. Four weeks later, the culture media were covered by mycelium. These results indicated that samples of *P. ostreatus* tested were able to utilize the crude oil as a source of carbon in their metabolism.

REFERENCES

Adedokun, O.M., Ataga, A.E., 2006. Effects of crude oil and oil products on growth of some edible mushrooms. J. Appl. Sci. Environ., 10 (2) : 91-93.

Adenipekun, C.O., Fasidi, I.O., 2005. Bioremediation of oil-polluted soil by *Lentinus subnudus*, a Nigerian white-rot fungus. African Journal of Biotechnology, 4 (8) : 796-798.

Adenipekun, C.O., 2008. Bioremediation of engine-oil polluted soil by *Pleurotus tuber-regium* Singer, a Nigerian white-rot fungus. African Journal of Biotechnology, 7 (1) : 055-058.

Bezalel, L., Hadar, Y, Cerniglia, C.E., 1996a. Mineralization of polycyclic aromatic hydrocarbons by white-rot fungus (*Pleurotus ostreatus*). J. Appl. Environ. Microbiol. 62 (1) : 292-295.

Bhattacharya, S., Angayarkanni, J., Arijit Das, Palaniswamy, M., 2012. Mycoremediation of Benzo [a]Pyrene by Pleurotus ostreatus usolated from Wayanad District in Kerala, India. International Journal of Pharmacy and Biological Sciences, 2 (2) : 84-93.

Cerniglia, C.E., Sutherland, J.B., 2001. Bioremediation of polycyclic aromatic hydrocarbons by ligninolytic and non-ligninolytic fungi. In: *Fungi in Bioremediation*. G.M. Gadd. Ed. Cambridge University Press. Cambridge. Pp. 136-187.

Chaudhry, S., Luhach, J., Sharma, V., Sharma, C., 2012. Assessment of Diesel Degrading Potential of Fungal Isolates from Sludge Contaminated Soil of Petroleum Refinery, Haryana. Research Journal of Microbiology, 7: 182-190.

Clemente, A.R., Anazawa, T.A., Durrant, L.R., 2001. Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Brazilian J. Microbiol. 32: 255-261.

Hou, H., Zhou, J., Wang, J., Du, C., Yan, B., 2004. Enhancement oflaccase production by *Pleurotus ostreatus* and its use for the decolorization of anthraquinone dye. Proc. Biochem., 39 (11) : 1415-1419.

Ijah, U.J., Antai, S.P., 2003. Removal of Nigerian light crude oil in soil over a 12 month period. Int. Biodeterior. Biodegradation, 51: 93-99.

Moeder MCajthaml TKoeller GErbanová P Sasek V., 2005. Structure Selectivity In Degradation And Translocation Of Polychlorinated Biphenyls (Delor 103) With *Pleurotus ostreatus* (Oyster Mushroom) Culture. Chemosphere, 61 (9) :1370-8.

Ogbo, M.E, Okhuoya, J.A., Anaziah, O.C., 2006. Effect of different levels of spent lubricating oil on the growth of *Pleurotus tuber-regium* Fr. Singer. Nig. J. Bot., 19 (2) : 266-270.

Ogbo, E.M., Okhuaya, J.A., 2008. Biodegradation of aliphatic resinic and aliphatic fractions of crude oil contaminated soils by *Pleurotus tuber-regium* Fr. Singer a white-rot fungus. African Journal of Biotechnology, 7 (23) : 4291-4297.

Okparanma, R.N., Ayotamuno, J.M., Davis, D.D., Allagoa, M., 2011. Mycoremediation of polycyclic aromatic hydrocarbons (PAH)-contaminated oil-based drill-cuttings. African Journal of Biotechnology, 10 (26) : 5149-5156.

Perelo, L.W., 2010. Review: In situ and bioremediation of organic pollutants in aquatic sediments. Journal of hazardous Materials, 177: 81-89.

Philip, J.C., Bamforth, S.M., Singleton, I., Atlas, R.M., 2005. Environmrntal pollution and restauration: A role for bioremediation. In: *Bioremediation*. R.M. Atlas (Ed.). Washington D.C.: ASM Press. Pp 1-48.

Pozdnyakova, N.; Nikiforova, S.; Makarov, O.; Turkovskaya, O., 2011 Effect of polycyclic aromatic hydrocarbons on laccase production by white rot fungus Pleurotus ostreatus D1 Applied Biochemistry & Microbiology; 47 (5), 543

Sack, U., Gunther, T., 1993. Metabolism of PAH by fungi and correction with extracellular enzymatic activities. J. Basic Microbiol. 33: 269-277.

Sasek, V., Cajthaml, T., Bhatt, M., 2003. Use of fungal technology in soil remediation: a case study. Water Air Soil Pollut. Focus., 3 (3) : 5-14

Vyas, B.R.M, Bakowski, S., Sasek, V., Matucks, M., 1994. Degradation of anthracene by selected white –rot fungi. FEMS. Microbiol. Ecol. 14: 65-70.