Biodegradation of Petroleum Oil by Using Isolated *Penicillium sp.*

Nabilah Razak[1], Saiyidah Nafisah Hashim[2] and Chia Chay Tay[3]
[1]School of Biology, Faculty of Applied Science, Universiti Teknologi MARA (Perlis), Malaysia,
Email: nabilahrazak27@gmail.com
[2]Applied Science Department, Faculty of Applied Science, Universiti Teknologi MARA (Perak), Malaysia,
Email: saiyidah394@perak.uitm.edu.my
[3]Environmental Sciences, Technology and Engineering,Faculty of Applied Science, Universiti Teknologi MARA (Perlis), Malaysia
Email: taychiay@gmail.com

Abstract

Oil spills is considered as one of the critical problems which cause a decline in environment health. Currently the biological solutions have become more familiar to remove hazardous substances from the environment. This present investigation was conducted in order to isolate fungi from contaminated soil collected from workshop area near Arau, Perlis. These isolated fungi were then tested on their ability to degrade petroleum oil on PDA medium that was polluted with different concentration of petroleum oil 2%,4% and 10% (v/v) and on the minimal salt solution by measuring changes in optical density (OD) read on spectrophotometer with 540 nm wavelength. *Penicillium sp.* was isolated from the contaminated soil using soil serial dilution method. *Penicillium sp.* was capable of actively degrade in varying degrees. The result showed significant difference ($P<0.05$) between different concentration of oil and the growth of fungi in 5 days of incubation period at 2% and 10% (v/v) petroleum oil concentration. *Penicillium sp*. showed highest growing diameter in 4% concentration of petroleum oil. Based on the optical density result,*Penicillium sp.* showed maximum peak of OD reading in day 20 with 0.66 ± 0.01, indicate that the highest ability of *Penicillium sp.* to degrade petroleum oil were in day 20. Statistical analysis of one-way ANOVA was done. The ability of*Penicillium sp.* to tolerate oil pollutants and grow on them, suggest it can be employed as bioremediation agent and can be used in restoring contaminated ecosystem with oil.

*Keywords:*Biodegradation, fungi species, petroleum hydrocarbon, oil pollution, optical density

1.0 Introduction

Environment pollution is one of the major problems that faced by many countries in this world. Pollution can be defined as undesirable change in the physical, chemical and biological characteristics of all components of an environment (Aboriba, 2001; Fatuyi *et al.,* 2012). Environment pollution due to oil spill is one of the frequent problems faced by country that has petroleum source like Malaysia, Nigeria and Iraq. According to Okpokwasili (1996) and Fatuyi *et al*. (2012) the petroleum or crude oil that accidentally discharge or flow into the environment lead to oil spillage pollution. The spill has caused most significant pollutant in the environment as it capable of causing serious damages to humans and the ecosystem. It was found from previous studies, biodegradation is the best way in term of cost and efficiency to treat this oil spillage pollution where it uses the microorganism to remove the contaminants into harmless end product. According to Atlas, (1995) and Fatuyi *et al.*(2012) through bioremediation or biodegradation there will be reduction of potential risks to be reduced contaminants to acceptable level. Thus a remediation achieved when the contaminants is converted into less toxic form (Onifade *et al*., 2007; Fatuyi *et al.,* 2012). The key players in bioremediation are microorganisms that live virtually everywhere. This is due the microorganisms posses enzymes that allow them to use environmental contaminants as food and able to contact with contaminants so easily (Efeovbokhan *et al.,* 2012). Fungi frequently grow on wide variety of organic materials, survive under environmental conditions of stress like nutrient deficiency and high salinity and can spread their mycelium wider and deeper than others (Potin *et al.,* 2004 ; Dhar *et al.,* 2014). According to the previous studies, some of the mold species have been recognized in capable as bioremediating agent such as *Aspergillus, Penicilium, Fusarium, Armophoteca, Paecilomyces, and Taloromyces*, and yeast species of *Candida, Yarrowia* and *Pichia* (Dhar *et al.,* 2014). In addition, fungi have ability to synthesize relatively unspecific enzymes involved in cellulose and lignin degradation, which are capable of degrading high molecular weight, complex or recalcitrant compounds, including aromatic structures (Potin *et al.,* 2004 ; Al-Jawhari, 2014).*Penicillium sp.* is well known and one of the most common fungi occurring in a diverse range of habitats. Its main function in nature is to decompose organic materials (Visagie *et al*., 2014). According to Fariba *et al.* (2012*),* petroleum polluted areas have high variation of fungal strains

and *Penicilliumsp*. were one of the common fungi, with high colony frequency. From the experiment done by Fariba *et al*. (2012), all of the studied fungal strains were able to growth in media with 2% (v/v) oil pollution.Therefore, the purpose of this research is to find the potential fungi with capabilities in reducing oil contamination within polluted area by measuring the growth diameter of isolated fungi in different concentration of petroleum oil and optical density.

2.0 Materials and Method

2.1 Collection of Samples

The 100 g of contaminated soil sample was collected from the workshop area in Arau, Perlis by using hoe at 0-5 cm depth from soil surface. The soil sample was selected based on the visual examination of the soil with dark discolored soil (Merkel *et al.*,2004 ; Mohsenzade *et al.*,2009). The 100 ml of petroleum oil was collected from Shell station.

2.2 Isolation and identification of fungi

The soil dilution method was performed to isolate fungi from the contaminated soil. Five test tubes were used to carry out the dilution method. 90 ml of sterile distilled water waspoured inside the beaker. Then, 10 g of contaminated soil was placed inside the beaker stirred for 10 minutes. Then, the mixture was diluted according to 10 serial fold dilutions up to 10^{-5} by using micropipette. Lastly, 1ml from each dilution of 10^{-1}, 10^{-2} , 10^{-3} and 10^{-4} were plated out into fresh PDA medium that was amended with 150 µg/ml streptomycin. The PDA plate was sealed by using Parafilm^{TM} and incubated at 26 ± 2ºCfor three days (Mubashar *et al.*, 2013; Umi, 2014). The identification was performed at genus level on the basis of macroscopic and microscopic characteristics.

2.3 Determination fungal growth diameter on PDA polluted media

The petroleum from Shell petrol station was added to the warm PDA solution that amended with streptomycin prior pouring the mixture inside the media plate. In order to have uniform concentration of oil in all plates, the solution was thoroughly mixed with magnetic stirrer. Three concentrations of oil/PDA mixture at2%,4% and 10 % (v/v) were prepared. Pure PDA medium was prepared without addition of petroleum oilas control plate. All media plates were plugs with 1cm^2 diameter of fungal mycelia using cork-borer. Next, the plates were incubated at 25 ± 2ºC in an incubator. The diameter of fungi grow in plate was measured by using ruler after 4 days and were compared with the control plates.

2.4 Confirmatory test for hydrocarbon utilization potential of isolated fungi

The estimation of hydrocarbon utilization were tested by following the modified enrichment procedure (Nwachukwu, 2000; Adekunle and Oluyode, 2005). A minimal salt solution (MSS) containing 2.0 g of Na_2HPO_4, 0.17 g of K_2SO_4, 4.0 g of NH_4HPO_4, 0.53 g of KH_2PO_4 and 0.10 g of $MgSO_4.7H_2O$ were dissolved in 1000 ml of distilled water and further autoclaved. The test was conducted in 12 ml of final reactions volume, containing 10 ml of (MSS) and 2 ml of petroleum from Shell petrol station in the 12 boiling tubes except for the last boiling tubes which serves as controls. Isolated fungi from the soil were added into boiling tubes. Each of the boiling tubes was plugged with sterile cotton wool followed by wooden cork, and was wrapped with aluminum foil to ensure maximum aeration and cross contamination. Finally, the boiling tube was incubated at room temperature (28 -31ºC) for 20 days. The boiling tubes were shaken constantly using incubator shaker at 120 rpm throughout duration of experiment to facilitate oil-cell phase contract. The ability to degrade the petroleum product (base on growth rate of the organism in MSS solution) were measured every 5 days using visual method based on the turbidity or optical density. This was measured using spectrophotometer with 540 nm wavelength.

2.5 Statistical analysis

The statistical analysis of one way analysis of variance (ANOVA) was done to determine the significant difference between the growth rates of fungal species isolated and biodegradation of rate of fungal species petroleum sample from Shell petrol station tested by using IBM SPSS Statistics 20.

3.0 Results and Discussion

Penicillium sp. had successfully isolated from the contaminated soil.The growth ability of the isolated fungi strains were carried out under 2%, 4% and 10% (v/v) concentration of petroleum oil and were expressed as diameter of colony in Table 1. Table 1 shows that isolated *Penicillium sp.* were able to grow and tolerate under different concentration of petroleum pollution

Table 1: Average growth diameter of fungi on PDA medium culture after 5 days (cm ± sd).

Note: Means that do not share a letter are significantly different

Concentrations of petroleum oil / Fungal isolates	0%	2%	4%	10%
***Penicillium* sp.**	**7.80 ± 0.0**	**4.53 ± 0.57[a]**	**6.63 ± 0.55[b]**	**2.67 ± 2.84[a]**

Penicillium sp. was recorded as the highest growth of fungi in 4% concentration of petroleum with diameter 6.63 ± 0.55 cm of colony after 5 days of incubation period. However, the growth diameter of *Penicillium sp.* was suppressed in 10% concentration of petroleum with diameter only 2.67 ± 2.84 cm and at 2% concentration of petroleum shows growth diameter with 4.53 ± 0.57 cm of colony after 5 days.*Penicillium sp*., *Aspergillus sp*. and *Rhizopus sp.* were commonly isolated fungi from total hydrocarbon contaminated soil (Chaillan *et al*., 2004; Mancera-López *et al*., 2007 and Singh *et al*., 2012).

Results found concluded thatthe fungal could be useful for the remediation of light soil pollution.From observation, the fungi sp. grew with high numbers of colonies (11.2) with small range of diameter in 10% (v/v) media compared to 2 % and 4 % (v/v) media. The small diameter of the colonies that the fungi is less resistance on high concentration of petroleum.

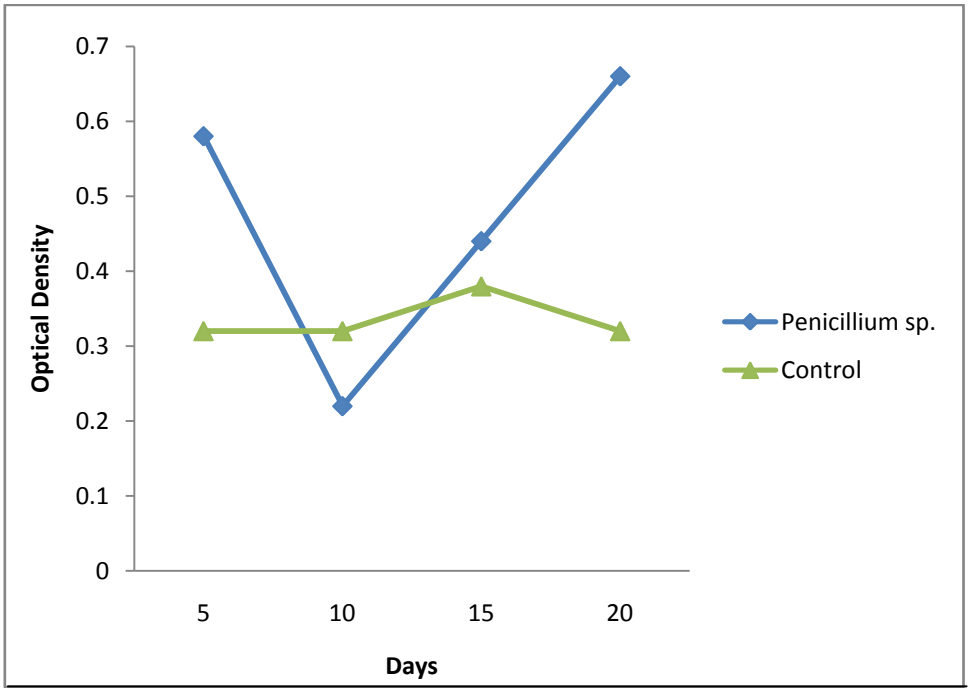

Figure 1: The growth pattern of fungi in petroleum oil and minimal salt solution (MSS)

Based on the Figure 1, *Penicillium sp.* showed fluctuation in growth pattern. At day 5, the fungi recorded a high optical density reading (OD) at 0.58 ± 0.01. On the day 10, *Penicillium sp.* growth was depleted (0.22 ± 0.01). This indicated that *Penicillium sp.* required more time in order to adapt with the new environment. This could be factorized by the changes on Minimal Salt Solution (MSS) pH and temperature. On the day 15, the OD reading of*Penicillium sp.* increased up to 0.44 ± 0.01. As indicated in Figure 1, it was observed that *Penicillium sp.*have maximum peak (0.6 ± 0.01) of OD reading on day 20th. This explained that the fungi had better degradation rate on that day number. Statistical analysis results showed significant different ($P<0.05$) between the optical density reading and the incubation period in 5 day intervals along 20 day of incubation period.

In the present study, increased in the optical density during the treatment period indicated degradative ability of the fungal growth towards the petrol as a source of hydrocarbon as substrates for growth. As cited by Adekunle and Adebambo (2007), the degradation is done by releasing the extra cellular enzymes and acids to dismantle the long chains of hydrogen and carbon which convert petroleum into simpler forms of products (Fatuyi *et.al.*, 2012).

Within 20 days, the growth of fungus was observed to increase. This indicates the ability of fungal to grow in media with limited sources of nutrient in longer period time. It was observed that the fungi had successfully breaks the petrol into small oil droplets. The layer of the petrol was also changed as it become thinner compared to control within the experimental period. The data proven that the fungi capable to break the petrol as additional food sources for growth.

4.0 Conclusion

In conclusions, isolated *Penicillium sp.* from the contaminated soil near workshop area had the capability to become bioremediating agent as there were significant difference ($P<0.05$) between the different concentration of petroleum oil and the growth diameter of fungi. Itwas also concluded that *Penicillium sp.* can be exploited in biodegradation of petroleum oil spill and bioremediation of the environment. For further study, it is recommended to increase the parameter used such as the pH of the contaminated soil and soil moisture in order to quatify what is the best environment conditions of isolated fungi need in order to perform well in degradative ability.

5.0 References

Aboriba, R.I. (2001). Oil politics and Niger Delta development commission. The tussle for Control and Domination. *African Journal of Environmental Studies* , 2,168-175.

Adekunle, A.A. and Oluyode, T.F. (2005). Biodegradation of crude petroleum and petroleum products by fungi isolated from two oil seeds(melon and soybean). *Journal of Environmental Biology* , 26(1) 37-42.

Adekunle, A.A., and Adebambo, O.A. (2007). Petroleum hyrdrocarbon utilization by fungi isolated from detarium snegalense (J. F Gmelin) seeds. *Journal of America Science* , 3(1)-76.

Al-Jawhari, I. (2014). Ability of some soil fungi in biodegradation of petroleum hydrocarbon. *Journal of Applied & Environmental Microbiology* , (2),46-52.

Atlas, R.M. (1995). Bioremediation of petroleum pollutants. *International Bioremediation and Biodegradation* , 317-327.

Chaillan, F., Fleche, A.L., Phantavong, Y., Crimount, P., Saliot, A. and Oudot, J. (2004). Identification and biodegradation potential of tropic aerobic hydrocarbon-degrading microorganisms. *Research in Microbiology* , 155, 587-595.

Dhar, K., Dutta, S. and Anwar, M.N. (2014). Biodegradation of petroleum hydrocarbon by indigenous fungi isolated from ship breaking yards of Bangladesh. *International Research Journal of Biological Science* , 3(9),23-30.

Efeovbokhan, V. E., Hymore, F.K., Oyakhire, C. and Taiwo, G. (2012). The effects of Pseudomonas Aeroginosa and Aspergillus Niger on the bioremediation of raw and treated crude oil polluted water. *International Journal of Science and Technology* , 2(6):345-352.

Fariba, M., Abdolkarim, C.R. and Mehrangiz, A. (2012). Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum polluted soil. *Iranian Journal of Environment Health Sciences & Engineering* , 9: 26 pg 1-8.

Fatuyi, O.E., Oluwatoyin, O. and Esther, A.E. (2012). Biodegradation of bonnylight crude oil by locally isolated fungi from oil contaminated soils Akure, Ondo state. *Malaysian Journal of Microbiology* , 8 (1)42-46. 35

Mancera-Lopez, M.E., Rodriguez-Casasola, M.T., Rios-Leal, E., Esparza-Garcia, F., Chaves-Gomes, B., Rodriguez-Vazquez, R., and Narrera-Cortesa, J. (2007). Funig and bacteria isolated from two highly polluted soil for hydrocarbon degradation. *Acta Chimica Sloenica* , 54(1), 201-209.

Merkel, N., Schultez-Kraft, R. and Infante, C. (2004). Phytoremediation of petroleum-contaminated soils in the tropics– preselection of plant species from eastern Venezuela. *Journal of Applied Botany and Food Quality* , 78: 185–92.

Mohsenzade, F., Nasseri, S., Mesdaghinia, A., Nabizadeh, R., Zafari, D. and Chehregani, A. (2009). 'Phytoremediation of petroleum-contaminated soils: pre-screening for suitable plants and rhizospheral fungi. *Toxicological and Environmental Chemistry* , 91(8):1443-1453.

Mubashar, R., Muhammad, U., Ghazanfar, Muzammil, H., Zafar, I. and Yasir, I. (2013). Pathogenic and non-pathogenic fungi isolated from soil and roots of tobacco. *Pakistan Journal of Phytopathology* , 25(1): 95-100.

Nwachukwu, S.C.U. (2000). Enhanced rehabilitation of tropical aquatic environments polluted with crude petroleum using Candida utilis. *Journal of Environmental Biology* , 21(3) :241-250.

Okpokwasili, G. (1996). Microbial degradation of petroleum hydocarbon by brackish water isolates in Nigerian wetlands. In T. V. Akpata, *The Nigerian Man and The Biosphere* (pp. 13-146). (M.AB-5): National Committe.

Onifade, A.K., Abubakar, F.A and EKundayo, F.O. (2007). Bioremediation of crude oil polluted soil in Niger Delta area of Nigeria using enhanced natural attenuation. *Research Journal of Applied Sciences* , 498-504.

Potin, O., Rafin, C. and Veignie, E. (2004). Bioremediation of an aged polycyclic aromatic hydrocarbon (PAHs)- from the contaminated soil. *International Biodeterior., Biodegradation* , 54 : 45-52.

Singh, K., Sharma, R., Sharma, A. and Chandra, S. (2012). Biodegradating fungal flora isolated from petroleum contaminated soil in rural area of Jaipur Distict, Rajasthan (India) . *CIBTech Journal of Biotechnology* , 1 (2,3), 1-7.

Umi, S.Y. (2014). *Diversity and Occurence of Fungal in Oil Palm Area at Uitm (Perlis).* Perlis, Malaysia: Faculty of Plantation and Agrotechnology University Technology Mara.

Vanishree, M., Thatheyus, A.J. and Ramya, D. (2014). Biodegradation of petrol using the fungus Penicilium sp. *Research Article Science International* , 2(1)26-31.

Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.B., Klaassen, C.H.W., Perrone, G., Seifer, K.A., Varga,J., Yaguch, T. and Samson, R.A. (2014). Identification and nomenclature of the genus Penicillium. *Studies in Mycology* , 78, 343-371.