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Growth profile of *Penicillium chermesinum* Biourge in function of total petroleum hydrocarbon and polycyclic aromatic hydrocarbons (PAH) compounds in oily sludge

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ABSTRACT

Oily sludge is categorized as one of the hazardous wastes that needs to be remediated in order to convert it into a non-toxic compound. Bioremediation involves microorganisms, such as fungi. In the present study, the indigenous oily sludge fungi, *Penicillium chermesinum* was used to assess the bioremediation. The aim of this study was to obtain the growth profile of *Penicillium chermesinum* Biourge in reducing the Total Petroleum Hydrocarbon (TPH) and Polycyclic Aromatic Hydrocarbons (PAH) compounds in oily sludge. A descriptive method was applied in this study that consists of the preparation stage and the biodegradation stage of oily sludge. Biodegradation process was performed by using the Solid State Fermentation methods during 15 days with the addition of 10% of fungi inoculum from the total of fermentation medium (100 g). The observed parameters are the TPH, PAH content, and fungi growth profile that were measured using the Gravimetric, GC/MS and TPC analysis. Our results showed that the TPH content was reduced 29.16% from the control. We identified two degraded PAH compounds, namely Azulene (C10) and Fluoranthene (C16).

***Keywords*:** growth profile, *Penicillium chermesinum,* TPH, PAH, oily sludge

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1. INTRODUCTION

Petroleum is the main energy source in the world. This can lead to an increase in oil exploration, exploitation, processing, and transportation activities that may cause pollution to the environment. The oil pollution often comes from oil refinery waste, a by-product of the production, distribution and transportation processes (ESDM, 2010).

Petroleum waste contains aliphatic and aromatic hydrocarbons, which have a high molecular weight (Sudrajat, 1996, in Yudono *et al*., 2013). These hydrocarbon compounds, which are included in oily sludge waste, have been considered as toxic waste that may endanger human health and the environment. Therefore, a bioremediation effort is needed in order to degrade oily sludge components or other oil waste into environmentally friendly compounds, such as CO_2 and H_2O (Santosa *et al*., 2004). The bioremediation of hydrocarbon can be performed using indigenous microorganisms, such as fungi, yeast, bacteria, and actinomycetes. These organisms can reduce TPH and PAH levels from the oil sludge produced from each oil treatment (Stapleton *et al*., 1998; Gofar, 2011). Therefore, the present study aims to identify the growth profile of *P. chermesinum* in function of the reduction of TPH content, and PAH compounds.

2. MATERIALS AND METHODS

The experiment was conducted using descriptive method that consists of 2 stages: 1) preparation, and 2) biodegradation stage of oily sludge. Biodegradation process was performed using the Solid State Fermentation (SSF) medium. This fermentation medium was prepared by mixing 5% oily sludge, 0.4% $NaNO_3$, soils and sands (volume ratio 2:1) with pH according to the condition of the medium. Moreover, 10% of fungi starter was added into the fermentation medium.

2. 1. Inoculum and Solid State Fermentation medium preparation

The starter medium was prepared by adding the fungal isolates that have been suspended with physiological NaCl as much as 10% of total weight (100 g) into the fermentation medium. This fermentation medium contains oily sludge with a concentration of 2% carbon source, 0.4% $NaNO_3$ as a source of nitrogen, as well as a mixture of sand and soil (with the ratio 2:1).

2. 2. Inoculation of fungal inoculum into Starter Medium

Fungal inoculum was prepared by mixing sterile physiological NaCl into *P. chermesinum* fungal isolates in the agar slant tubes. The fungal that grows on the surface was then separated from the medium using the sterile swab. Afterwards the suspension was transferred to another test tube and homogenized using vortex. Furthermore, 10% from the homogenized suspension (100 g) was placed into the starter medium.

2. 3. Biodegradation with SSF medium

The fermentation process was carried out by adding the fungal starter (10%) to the fermentation medium (100 g). Chemical and biological parameters were observed during the fermentation process, such as TPH content and PAH compounds by using the Gravimetric and

GC / MS analysis, respectively. Parameter observations were carried out by calculating the number of fungal colonies using TPC. This was conducted from the start (day 0) until the end of experiment (day 15).

2. 4. Calculation of fungal colonies with Total Plate Count (TPC)

Fungal colonies were calculated using the Total Plate Count (TPC) method, as described in Cappuccino and Sherman (1987):

$$\text{Colony number (cfu/g)} = \frac{(a \times 10^{d}) + (b \times 10^{e}) + (c \times 10^{f})}{3}$$

2. 5. PAH analysis with Gas Chromatography/Mass Spectrometry (GC/MS)

PAH compounds in the sample was analyzed by using Gas Chromatography/Mass Spectrometry **(**GC/MS) method, following Ikechukwu *et al*. (2012). Briefly, soil samples were dissolved into hexane. A total of 1 µL of sample was then injected into GC/MS apparatus under the following conditions: injector temperature 280 °C, split mode injector, 1 min sampling time, column temperature 40-270 °C (initial temperature setting 40 °C), detector temperature 280 °C, interval temperature 250 °C.

2. 6. TPH analysis

TPH content was measured using Gravimetric method as described in EPA 1664 (2004) with modification. Briefly, soil samples were dried for 2 h in an oven with a temperature of 55 °C. Afterwards, 3 g of dried soil samples were taken and placed into the bags made from filter paper. The samples were then extracted using Soxhlet apparatus for 7-8 h, using n-hexane solvents. After the extraction, the flask was distilled to separate the oil from the solvent. Then, the flask was heated in the oven at 105 °C:

$$\text{Oil content (\%)} = \frac{Difference\ between\ flask\ mass\ (g)}{Sampel\ mass\ (g)}\ x\ 100\%$$

3. RESULTS AND DISCUSSION

3. 1. Growth profile of *P. chermesinum* in the function of TPH

The growth of fungal colonies of *P. chermesinum* and TPH content in the function of fermentation time is presented in Figure 1.

Lag phase can be identified during the first week of fermentation, where the fungal isolates were adapted with the medium environment. The exponential phase started from the day 6 until day 12, where the colony number was exponentially increased. Exponential phase is a multiplication phase of cell numbers due to increased cell activity and is the most important phase for fungal growth (Gandjar *et al*., 2006). On the last day of fermentation, the growth of fungal colonies decreased. This can be defined as the death phase. TPH content was nearly constant during the first 6 days of experiment, suggesting no significant difference in TPH

content in the sample. On the other hand, TPH content decreased on the 6th day of experiment. This decrease continued until the end of experiment (day 15).

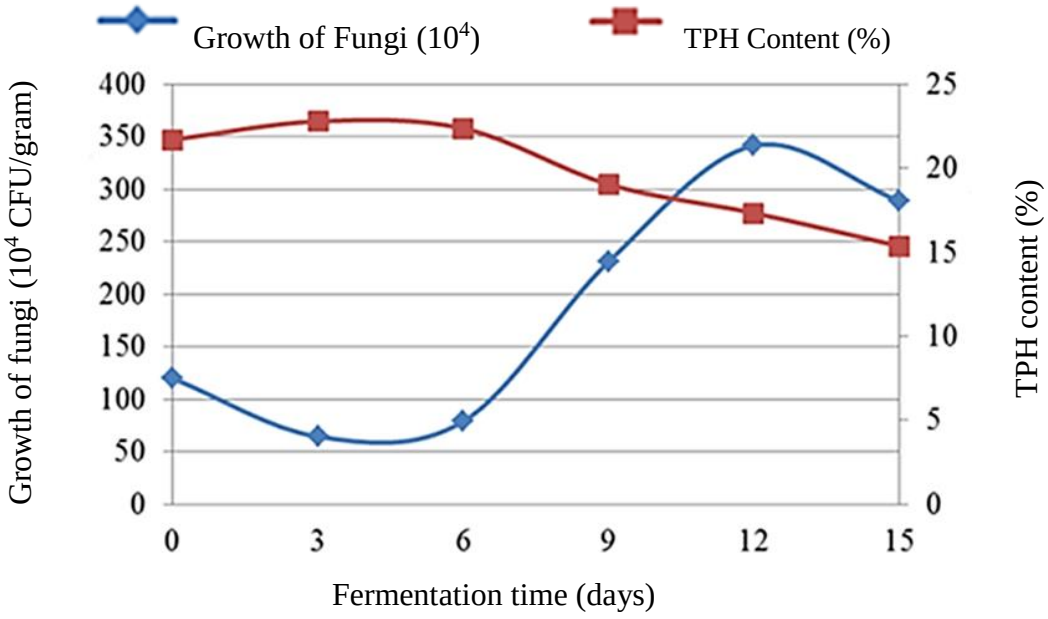

Figure 1. TPH content and fungal colony growth during fermentation

The lag phase that occurs on *Chimesinum* was probably due to differences in the concentration of oil sludge, i.e. from a concentration of 2% (starter) to a concentration of 10% (fermentation medium) so that it required adjustment. The exponential phase that occurs on the day 6 to day 12 causes a decrease in TPH levels. This reduction was probably due to an increase in the number of cells of microorganisms that show metabolic processes by degrading existing hydrocarbon components (Lee *et al*., 2006), which are used as energy sources. At the end of the experiment (day 15), the growth profile tends to decrease that indicates the death phase, followed by a decrease in the percentage of TPH. The decrease in TPH content with the number of colonies less than on the 12th day indicates that in the death phase the fungal colonies are still able to degrade hydrocarbons.

The acceleration of TPH reduction appeared to be in line with the increase of maximum fungal biomass on day 12 until the end of observation (day 15). This is possibly due to the presence of extracellular biosurfactants secreted by fungi, which can increase the solubility of hydrocarbons in water (Helmy *et al*., 2010). Additionally, our result is in accordance with the study demonstrated by Rossiana *et al*. (2015) that *Penicillium* sp. produces biosurfactants. To our knowledge, the surfactant is suspected to be produced by *P. mesinum*, with an increase in solubility and degeneration of this organism on that day.

3. 2. Percentage of TPH production during hydrocarbon reduction

Penicillium chermesinum was able to reduce TPH content as much as 29.16% from the initial TPH content. There was no change in the control treatment from the first day of observation (day 0) to the last of observation (day 15). This result implies that the degradation

of oily sludge was not affected by the microorganisms of indigenous sample. Thus, the change in TPH content from the sample treatment was due to enzymatic activities from *P. chermesinum* and probably influenced by surfactant produced by this microorganism.

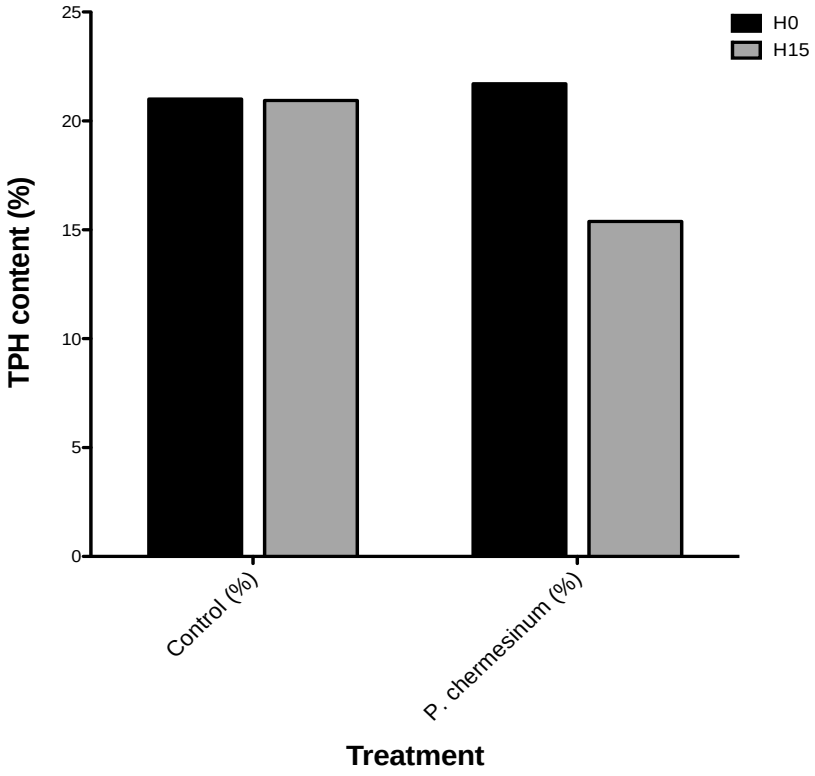

Figure 2. TPH content degradation in control and treatment from day 0 to day 15.

Salleh *et al*. (2003) demonstrated that in general, fungal population is metabolically active in hydrocarbon-polluted areas. This could be possible due to their properties. In fact, fungal organisms have hyphae that can penetrate petroleum-contaminated soil to search for hydrocarbons (Gadd, 2001). Additionally, they can increase the available surface area that can be degraded by other microorganisms. These microorganisms are able to utilize hydrocarbons as their carbon and/or energy source because these microorganisms have enzymes (Salleh *et al*., 2003). Leitao (2009) also showed that *Penicillium* has the ability to produce extracellular enzymes and metabolize hydrocarbons. *Penicillium chermesinum* has the ability to reduce TPH levels by 29.16%, which is higher than *Penicillium* sp7. (19.40%), *Cladosporium* sp. (17.92%), (Rossiana *et al*., 2015), *Aspergillus* sp1. (11.78%) and *Aspergillus* sp2. (10.50%), (Anggraini, 2015), with similar time of experiment (15 days). According to the study from Aisyah (2015), the fungal consortium, namely *Cladosporium* sp. and *Talaromyces* sp. can reduce TPH levels by 71.12% for 18 weeks. These results suggest that the biodegradation of complex hydrocarbons is better by using several fungal species (in the form of consortium). In addition, single microorganism can metabolize the hydrocarbon substrate only at a limited range, hence the fungal consortium is better in biodegradation since it produces more enzymes (Sorkhoh *et al*., 1995; Ghazali *et al*., 2004).

3. 3. PAH analysis

In this study, there were nine main compounds, five of which were not degraded, such as Naphthalene, Anthracene, Phenanthrene, Pyrene, and Chrysene, while the two other compounds such as Azulene and Fluoranthene have been degraded. Two other compounds, such as Benz[a]azulene and Benz[a]anthracene appeared as the new compounds. The lowest carbon chains were identified as Naphthalene (C_{10}) and Azulene (C_{10}), while the highest carbon chains were identified as Benz[a]anthracene (C_{18}) and Chrysene (C_{18}). During the degradation process, there was a change in the proportion of each compound within 15 days of experiment.

Table 1. Proportion of the compound.

No.	Name of compound	Number of C atoms	Day 0	Day 15
1	*Naphthalene*	10	√	√
2	*Azulene*	10	√	-
3	*Benz[a]azulene*	14	-	√
4	*Anthracene*	14	√	√
5	*Phenanthrene*	14	√	√
6	*Fluoranthene*	16	√	-
7	*Pyrene*	16	√	√
8	*Benz[a]anthracene*	18	-	√
9	*Chrysene*	18	√	√

√ : Detected
- : Undetected

In the present study, PAH compounds came from the *oily sludge* and thus they were the mixture of several compounds. This process may slow down the biodegradation process. Unlike the degradation of a single PAH compound, the biodegradation process becomes relatively faster. This result is in accordance with Hennessee and Li (2016) who demonstrated that PAH can be degraded effectively when they are in the form of single compound.

The presence of degraded compounds, such as *Azulene* and *Fluoranthene*, was suspected due to the metabolic activity of *P. chermesinum* in the biodegradation process. These fungal species use the carbon from the PAH compounds as the source of energy to carry out metabolism and reproduction (Nugroho, 2006). Additionally, fungi could reduce the complex-polymer such as polyaromatic compounds (Anastasi *et al*., 2005). This mechanism can be realized by cutting down the long-hydrocarbon chain into shorter hydrocarbon chains by involving enzyme activities.

Azulene has two aromatic rings that may facilitate degradation of this compound. Our result is in coherence with Leonardi *et al*. (2007), and Ali *et al*. (2012) who demonstrated that microorganisms are able to degrade PAH compounds more efficiently when the molecules have lower aromatic rings. On the other hand, Fluoranthene has four aromatic rings and is categorized as a compound with high molecular weight. Degradation of the Fluoranthene may be caused by the presence of other substrates that are easily degraded and is likely the result of interactions between substrates, which may increase the frequency of degradation process in more complex compounds (Juckpech *et al*., 2012).

According to Lau (2003), the ability of microorganism biomass to degrade oil depends on the enzymes produced by hydrocarbon-degrading species. It appears that the presence of high enzymatic capacity allows the community of microorganisms to degrade the complex hydrocarbons. This capacity is their ability to modify or decompose certain pollutants, for instance petroleum. Several enzymes play significant role in biodegradation of PAH, for instance lignin peroxidase, manganese peroxidase, laccase (Peng *et al*., 2008), and oxygenase that incorporates oxygen atoms into hydrogen (Peixoto *et al*., 2011).

The oxygenase enzyme consists of monooxygenase and dioxygenase (Karigar and Rao, 2011). The cytochrome P450 mono-oxygenase catalyzes the oxidation of PAH to arene oxide, which is the initial product of PAH of metabolism (Peng *et* al., 2008). On the other hand, dioxygenase is an enzyme that combines two oxygen atoms into the substrate (Karigar and Rao, 2011). The fungal organisms indeed use the enzyme to cut off the aromatic ring, which will be utilized as their source of energy. This is in accordance with Whiteley and Lee (2005) who found that the presence of dioxygenase could destroy chemical bonds and allow for the opening of the ring. A finding from Hadibarata *et al*. (2008) revealed that *Polyporus* sp. could produce 1,2-dioxygenase (237.5 U/L), and 2,3-dioxygenase (36.8 U/L). The same authors also found that the highest enzyme activity is on day 20. This microorganism can degrade chrysene as much as 65%.

Al-Nasrawi (2012) showed that *Trichoderma* sp. is able to produce 1,2-dioxygenase and 2,3-dioxygenase, and is able to degrade 72% of phenanthrene, and revealed that *Armillaria* sp. is able to degrade Fluoranthene. The ability of this microorganism in degrading Fluoranthene may also be due to the presence of enzymatic activity of laccase and 1,2-dioxygenase. This finding was also supported by the UV-Vis spectrophotometer analysis from the acidic extract, which showed the presence of three intermediate compounds from Fluoranthene. Indeed, Fluoranthene will be broken down into compounds that are similar to intermediate-1,8 naphthalenedicarboxylic acid, benzene-1,2,3-tricarboxylic acid, and phthalic acid. Gadd (2001) revealed that Fluoranthene is metabolized by *Cunninghamella elegans* into trans-2,3-dihydrodiol fluoranthene, 8- and 9-hydroxyfluoranthene trans-2,3-dihydrodiols, 3-fluoranthene b-glucopyranoside, and 3- (8-hydroxyfluoranthene) -β-glucopyranoside.

Decomposed PAH compounds can form new compounds (after decomposition). These compounds are broken down into simpler compounds. Moreover, these simple compounds will combine into more complex compounds. This is in accordance with Młynarz and Ward (1995) that the degradation of PAH by microorganisms is considered to be the main decomposition process. This also confirms the present finding where the formation of Benz[a]azulene and Benz[a]anthracene compounds appeared on the 15^{th} day of experiment. Benz[a]azulene compounds are formed due to the incorporation of Benzene rings and Azulene rings (Yamamura *et al*., 2002). This Benzene ring is the product of breakdown process from the more complex compounds, such as Fluoranthene. Therefore, the formation of the Benz[a]anthracene

compound is probably due to the incorporation of compounds from Benzene rings and Anthracene rings.

4. CONCLUSIONS

The present study revealed that the biomass growth profile of *P. chermesinum* experienced a lag phase for 6 days. The exponential phase occurred until day 12, and followed by the death phase on day 15, with the total TPH degradation as much as 29.16%. We also observed that there was a change in PAH compounds such as Azulene and Fluoranthene as well as the presence of new compounds such as Benz[a]azulene and Benz[a]anthracene.

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References

[1] Ali, M.I.A., N.M. Khalil, and M.N.A. El-Ghany. Biodegradation of some polycyclic aromatic hydrocarbons by *Aspergillus terreus*. *African Journal of Microbiology Research* 6 (16) (2012) 3783-3790.

[2] Al-Nasrawi, H. Biodegradation of crude oil by fungi isolated from Gulf of Mexico. *Journal of Bioremediation and Biodegradation* 3 (4) (2012) 1-6.

[3] Anastasi, A., G.C. Varese, and V.F. Marchisio. Isolation and identification of fungal in compost and vermicompost. *Mycologia* 97 (1) (2005) 33-44.

[4] Atlas, R.M. Stimulated Petroleum Biodegradation. *CRC Critical Reviews in Microbiology* 5 (1977) 371-386.

[5] Atlas, R.M. and R. Bartha. Hydrocarbon Biodegradation and Oil Spill Bioremediation. Advances in Microbial Ecology Vol. 12, 1992. Edited by K.C. Marshall. Plenum Press. New York.

[6] Balaji, V., P. Arulazhagan, and P. Ebenezer. Enzymatic bioremediation of polyaromatic hydrocarbons by fungal consortia enriched from petroleum contaminated soil and oil seeds. *Journal of Environmental Biology* 35 (2013) 1-9.

[7] Bojes, H.K. and P.G. Pope. Characterization of EPA's 16 Priority Pollutant Polycyclic Aromatic Hydrocarbons (PAHs) in Tank Bottom Solids and Associated Contaminated Soils at Oil Exploration and Production Sites in Texas. *Regulatory Toxicology and Pharmacology* 47 (2007) 288-295.

[8] Cappuccino, J.G. and N. Sherman. Microbiology: A Laboratory Manual. 5th Ed. Benjamin/Cummings Science Publishing. California, 1987.

[9] Chikere, C.B. and C.C. Azubuike. Characterization of hydrocarbon utilizing fungi from hydrocarbon polluted sediments and water. *Nigerian Journal of Biotechnology* 27 (2014) 49-54.

[10] Das, N. and P. Chandran. Microbial degradation of petroleum contaminants: an overview. *Biotechnology Research International* 2011 (2010) 1-13. https://doi.org/10.4061/2011/941810

[11] Enabulele, O.I. and O.N. Obayagbona. Biodegradation potentials of mycoflora isolated from auto mobile workshop soils on flow station crude oil sludge. *International Research Journal of Biological Sciences* 25 (5) (2013) 9-18.

[12] EPA Method 1664. Method for N-Hexane Extractable Material (HEM; Oil and Grease) and Silica Gel Treated N-Hexane Extractable Material (SGT-HEM; Non-polar Material) by Extraction and Gravimetry. 3M Bioanalitical Technological Project, 2004.

[13] Fan, Chi-Yuan and S. Krishnamurthy. Enzymes for enhancing bioremediation of petroleum-contaminated soils. *Journal of the Air and Waste Management Association* 45 (1995) 453-460.

[14] Gadd, G. M. Fungi in Bioremediation. Cambridge University Press. Cambridge, 2001.

[15] George-Okafor, U., F. Tasie, and F. Muotoe-Okafor. Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. *Journal of Physical and Natural Sciences* 3(1) (2009) 1-6.

[16] Ghazali, F.M., R.N.ZA. Rahman, A.B. Salleh, and M. Basri. Biodegradation of Hydrocarbons in Soil by Microbial Consortium. *International Biodeterioration and Biodegradation* 54 (2004) 61-67.

[17] Gilman, J.C. *A Manual of Soil Fungi*. Oxford and IBH Publishing. New Delhi, 1975.

[18] Gofar, N. Characterization of petroleum hydrocarbon decomposing fungi isolated from mangrove rhizosphere. *J. Trop. Soils* 16(1) (2011) 39-45.

[19] Hadibarata, T., S. Tachibana, and K. Itoh. Biodegradation of Phenanthrene by Fungi Screened from Nature. *Pakistan Journal of Biological Sciences* 10(15) (2007) 2535-2543.

[20] Hadibarata, T., S. Tachibana, and K. Itoh. Biodegradation of chrysene, an aromatic hydrocarbon by *polyporus* sp. S133 in Liquid Medium. *Journal of Hazardous Materials* 164 (2009) 911-917.

[21] Hadibarata, T. and R.A. Kristanti. Biotransformation Studies on Fluoranthene, a Four-ring Polycyclic Aromatic Hydrocarbon, by White-Rot Fungus Armillaria sp. F022. *Agriculture and Agricultural Science Procedia* 3 (2015) 45-50.

[22] Haritash, A.K. and C.P. Kaushik. Biodegradation aspects of polycyclic aromatic hydrocarbons (pahs): a review. *Journal of Hazardous Materials* 169 (2009) 1-15.

[23] Helmy, Q., E. Kardena, Z. Nurachman, and Wisjnuprapto. Application of Biosurfactant Produced by *Azotobacter vinelandii* AV01 for Enhanced Oil Recovery and

Biodegradation of Oil SLudge. *International Journal of Civil and Environmental Engineering* 10(1) (2010) 6-12.

[24] Hennessee, C.T. and Q.X. Li. 2016. Effect of PAH Mixtures on Degradation, Gene Expression, and Metabolite Production in Four Mycobacterium Species. *Applied and Environmental Microbiology* 82(11) (2016) 3357-3369.

[25] Ikechukwu, A.A., S.M. Tanko, D.B. Emmanuel, S.O. Suleiman, and F. Aberuagba. Effect of extraction methods on the polycyclic aromatic hydrocarbons content Smoked Catfish Species in Niger State of Nigeria. *Jordan Journal of Biological Sciences* 5(1) (2012) 71-78.

[26] Juckpech, K., O. Pinyakong, and P. Rerngsamran. Degradation of Polycyclic Aromatic Hydrocarbons by Newly Isolated *Curvularia* sp. F18, *Lentinus* sp. S5, and *Phanerochaete* sp. T20. *Science Asia* 38 (2012) 147-156.

[27] Karigar, C. S. and S.S. Rao. Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. *Enzyme Research* Vol. 2011, Article ID 805187, 1-11, doi:10.4061/2011/805187 1-11

[28] Kota, M.F., A.A.S.A. Hussaini, A. Zulkharnain, and H.A. Roslan. Bioremediation of crude oil by different fungal genera. *Asian Journal of Plant Biology* 2(1) (2014) 11-18.

[29] Kumari, B., S.N. Singh, F. Deeba, M. Sharma, V. Pandey, and D.P. Singh. Elucidation of Pyrene Degradation Pathway in Bacteria. *Advances in Bioresearch* 4(2) (2013) 151-160.

[30] Leahy, J.G. and R.R. Colwell. Microbial degradation of hydrocarbons in the environment. *Microbiological Reviews* 54(3) (1990) 305-315.

[31] Lee, S.H., S. Lee, D.Y. Kim, and J.G. Kim. Degradation Characteristics of Waste Lubricants Under Different Nutrient Conditions. *Journal of Hazardous Materials* 143(1-2) (2007) 65-72.

[32] Leitao, A.L. Potential of Penicillium Species in the Bioremediation Field. *International Journal of Environmental Research and Public Health* 6 (2009) 1393-1417.

[33] Leonardi, V., V. Sasek, M. Petruccioli, A. D'Annibale, P. Erbanova, and T. Cajthaml. Bioavailability Modification and Fungal Biodegradation of PAHs in Aged Industrial Soil. *International Biodeterioration and Biodegradation* 60 (2007) 165-170.

[34] Liu, W., Y. Luo, Y. Teng, Z. Li, and Q. Ma. Bioremediation of oily slude-contaminated soil by stimulating indigenous microbes. *Environ. Geochem. Health* 32(1) (2010): 23-29.

[35] Lotfinasabasl, S., V.R. Gunale, and N.S. Rajurkar. Assessment of petroleum hydrocarbon degradation from soil and tarball by fungi. *Bioscience Discovery* 3(2) (2012) 186-192.

[36] Mandal, A.K., P.M. Sarma, B. Singh, C.P. Jeyaseelan, V.A. Channashettar, B. Lal, and J. Datta. Bioremediation: an environment friendly sustainable biotechnological solution for remediation of petroleum hydrocarbon contaminated waste. *ARPN Journal of Science and Technology* 2 (2012) 1-12.

[37] Margesin, R. and F. Schinner. Bioremediation (Natural Attenuation and Biostimulation) of Diesel-Oil-Contaminated Soil in an Alpine Glacier Skiing Area. *Applied and Environmental Microbiology* 67(7) (2001) 3127-3133.

[38] Milic, J.S., V.P. Beskoski, M.V. Ilic, S.A.M. Ali, G.D. Gojgic-Cvijovic, and M.V.M. Vrvic. Bioremediation of soil heavily contaminated with crude oil and its products: composition of the microbial consortium. *Journal of The Serbian Chemical Society* 74(4) (2009) 455-460.

[39] Młynarz, D.T. and O.P. Ward. Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) by a Mixed Culture and its Component Pure Cultures, Obtained from PAH-Contaminated Soil. *Can. J. Microbiology* 41 (1995) 470-476.

[40] Mohan, S.R.V., M.P. Devi, M.V. Reddy, K. Chandrasekhar, A. Juwarkar, and P.N. Sarma. Bioremediation of petroleum sludge under anaerobic microenvironment: influence of biostimulation and bioaugmentation. *Environmental Engineering and Management Journal* 10(11) (2011) 1609-1616.

[41] Obire, O. and R.R. Putheti. Fungi in bioremediation of oil polluted environments. *J. Appl. Sci. Environ. Manag.* 7 (2008) 61-67.

[42] Okoro, C.C. Biodegradation of hydrocarbons in untreated produce water using pure fungal cultures. *African Journal of Microbiology Research* 2 (2008) 217-223.

[43] Olajire, A.A. and Essien J.P. Aerobic degradation of petroleum components by microbial consortia. *Journal Petroleum and Environmental Biotechnology* 5(5) (2014) 1-22.

[44] Peixoto, R.S., A.B. Vermelho, and A.S. Rosado. Petroleum-Degrading Enzymes: Bioremediation and New Prospects. *Enzyme Research* 3 (2011) 1-7. https://doi.org/10.4061/2011/475193

[45] Peng, R.H., A.S. Xiong, Y. Xue, X.F. Fu, F. Gao, W. Zhao, Y.S. Tian, and Q.H. Yao. Microbial Biodegradation of Polyaromatic Hydrocarbons. *FEMS Microbiol. Rev.* 32 (2008) 927-955.

[46] Refai, M., H.A. El-Yazid, and W. Tawakkol (2015). Monograph on the Genus Penicillium. https://www.academia.edu/11498579/Monograph_on_the_genus_Penicillium

[47] Rossiana, N., A.P. Wulandari, F. Fiandisty, A.S. Rescho, and F.Z. Ghassani. Biosurfactants activity from exogenous fungi to decreasing total petroleum hydrocarbon (TPH) of Balongan oil sludge. *Asian Journal of Microbiology Biotechnology Environmental Science* 17(4) (2015) 1-4.

[48] Salleh, A.B., F.M. Ghazali, R.N.Z. Abd Rahman, and M. Basri. Bioremediation of petroleum hydrocarbon pollution. *Indian Journal of Biotechnology* 2 (2003) 411-425.

[49] Santosa, D.A., Listiyawati, T. Irawathi, D. Herdiyantoro, R.W.U. Ananda, and S. Adiwibowo. Biotechnology for Remediation of Oil Sludge and Petroleum Contaminated Ecosystem Using Bacteria Isolated from Indonesia's Region. Environmental Research Center-Institute Pertanian Bogor. Bogor, 2004.

[50] Saraswathy, A. and R. Hallberg. Degradation of pyrene by indigenous fungi from a former gasworks site. *FEMS Microbiology Letters* 210 (2002) 227-232.

[51] Sharpley, J.M. Elementary Petroleum Microbiology. Gulf Publishing Company. Texas, 1996.

[52] Singh, H. *Mycoremediation: Fungal Bioremediation*. John Wiley and Sons, Inc. Hoboken, 2006.

[53] Singh, P.D., J.H. Johnson, C.A. Aklonis, K. Bush, S.M. Fisher, and J. O'Sullivan. Two New Inhibitors of Phospholipase A_2 Produced by *Penicillium chermesinum*. *The Journal of Antibiotics* 38(6) (1985) 706-712.

[54] Sorkhoh, N.A., R.H. Al-Hasan, M. Khanafer, and S.S. Radwan. Establishment of Oil-Degradation Bacteria Associated with Cyanobacteria in Oil-Polluted Soil. *Journal of Applied Bacteriology* 78 (1995) 194-199.

[55] Stapleton, R.D., D.C. Savage, G.S. Sayler, and G. Stacey. Biodegradation of Aromatic Hydrocarbons in an Extremely Acidic Environment. *Applied and Environmental Microbiology* 64(11) (1998) 4180-4184..

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

[57] Whiteley, C.G and D.J. Lee. Enzyme technology and biological remediation. *Enzyme and Microbial Technology* 38(3-4) (2006) 291-316.

[58] Yamamura, K., Y. Kitagawa, and M. Hashimoto. Crystal Structure of 1,1,1-Triphenyl-4-(10-benz[a]azulenyl)-3,4-buten-2-one. *Analytical Sciences* 18 (2002) 373-374.

[59] Ze Bilo'o, P. and M.B. Ngassoum. Characterization of polycyclic aromatic hydrocarbons (PAHs) in oily sludge from Cameroon petroleum refinery. *International Journal of Environmental Science* 3(1) (2012) 509-517.