ASM Sc. J., 16, Special Issue 1, 2021 for SCIEMATHIC2019, 93-99

Bioremediation of Oil Spill in Sea Water Medium by Mixed cultures of *Aspergillus* sp. and *Pseudomonas aeruginosa*

Hamdan Dwi Rizqi and Adi Setyo Purnomo*

Department of Chemistry, Faculty of Science and Data Analytics, Institut Teknologi Sepuluh Nopember (ITS), Kampus ITS Sukolilo, Surabaya 60111, Indonesia

One of the serious marine environment problems is Oil Spillage due to its high risk for the marine ecosystem. In this study, the application of mixed cultures of fungus *Aspergillus* sp. and bacterium *Pseudomonas aeruginosa* on spilled oil bioremediation under high salinity medium was investigated. The bioremediation of spilled oil was analysed using Gas chromatography-mass spectrometry (GCMS). The result showed that the application of mixed cultures of *Aspergillus* sp. and *P. aeruginosa* increased the oil spill bioremediation. The highest bioremediation was obtained from the derivation of pentadecane, which degraded up to 65.69%. This study shows that mixed cultures of *Aspergillus* sp. and *P. aeruginosa* have a good potential to use on bioremediation of spilled oil under the seawater medium.

Keywords: *Aspergillus* sp.; bioremediation; oil spill; *Pseudomonas aeruginosa*; seawater medium

I. INTRODUCTION

One of a serious marine environment problem is Oil Spillage due to its high risk for the marine ecosystem (Sun *et al.,* 2019). Recently, the demand for the energy sector was increase. The increase in the demand for the energy sector also had an impact on the level of oil consumption. Currently, the world consumed up to 90 million barrels of oil per year. The high level of oil demand has resulted in increased oil mining activities. In the process of oil mining activities, oil spills are one of the serious environmental problems. The oil spills can be produced from any mining process in the oil distribution process. In the last period, more than 6 million tonnes of oil entered the oceans per year from 1 billion gallons of oil were spilled in worldwide (Abdul-Hamid, 2013). Thus, the oil spill poses a serious threat especially for ecology in the marine environment. The nature of oil which has a lower density than water causes the oil was spread and contaminated the marine environments. The spread of the oil spill can be aided by winds and ocean currents that can widespread damage to marine ecosystems (Li *et al.,* 2016*).* Many oil spills contain low molecular weight compounds and many contain saturated hydrocarbons which are more toxic and difficult to degrade so that they are quite dangerous to the ecosystem, especially in coastal areas. Hydrocarbons derived from the petroleum compound would be toxic and dangerous for marine biota (Wang *et al.,* 2013*).*

Various methods have been developed to recover the marine environment from oil spills pollution such as physicochemical and biological treatment. Physicochemical methods such as incineration, extraction using thermal solvents and desorption can recover quickly but produce a new waste and are quite expensive. Bioremediation is a biological method of environment recovery. Bioremediation was more effective, inexpensive, and environmentally friendly than the other methods. The use of Microorganisms on converting complex compounds to less toxic or non-toxic compounds makes bioremediation more eco-friendly (Prince *et al.,* 2016*).* Some researchers have succeeded in proving that some bacteria and fungi are able to apply on oil spill bioremediation (Mohsenzadeh *et. al.,* 2012; Burghal *et al.,* 2016). The capability of microorganisms on bioremediation the complex compounds are related to their ability to produce some degrading enzymes. Those enzymes are normally used

*Corresponding author's e-mail: adi_setyo@chem.its.ac.id; adi.spurnomo@yahoo.com

as a source of food metabolism to get energy (Purnomo *et. al.,* 2008; Purnomo *et. al.,* 2010; Purnomo *et al.,* 2011).

In some previous studies, fungi from class *Aspergillus* have been reported to have the capability to spill oil waste bioremediation. Ojewumi *et al.* (2018) reported that *Aspergillus niger* was significantly remediation the oil pollution on the soil in 45 days of incubation. In another study, the capability of *Aspergillus* sp. to reduce oil on Bushnell Hass Broth media in 16 days incubation period has been reported by Vanishree *et al.* (2014). Thus, *Aspergillus* sp. was indicated capable and has the potential to be a bioremediation agent. However, from several studies that have been conducted, bioremediation using the fungus *Aspergillus* sp. still takes a long time and has not been tested on seawater media, so this research gap must be resolved immediately (Ojewumi *et al.,* 2018).

The length of the oil spill bioremediation process by *Aspergillus* sp. indicates that the use of *Aspergillus* sp. on oil spill bioremediation still needs to develop, in order to get a shorter bioremediation duration. Several studies have been conducted to develop bioremediation, one of which is the addition of biosurfactant-producing bacteria (BPS). Biosurfactants produced by BPS can increase the oil spill solubility in the medium so that it is easier to be accessed by fungi to be degraded. In the previous study, Li *et al.* (2008) reported that the bacteria addition was able to enhance the diesel oil waste degradation ratio. In another study, Wang *et al.* (2012) also investigated the method of oil spills remediation on the soil by mixed cultures using 7 species of fungi and 4 species of bacteria. The study indicates that bacteria have an important role in increasing the oil spill bioremediation.

In this study, the application of mixed cultures of fungus *Aspergillus* sp. and bacterium *Pseudomonas aeruginosa* on spilled oil bioremediation was tested in seawater medium on a laboratory scale. *P. aeruginosa* was chosen because it often found in the environment and proved capable to increase the organic pollutant bioremediation capability by fungi (Purnomo *et. al.,* 2017; Purnomo *et al.,* 2018). This research was conducted on an artificial seawater medium to adjust the culture conditions with sea conditions. This study is expected to help solve the problem of oil spills in the marine environment utilising bioremediation methods that are cheap, effective, and eco-friendly.

II. MATERIALS AND METHOD

A. Material

In this study, the oil spill sample was collected from Oil Site in Cepu, Central Java, Indonesia. *Aspergillus* sp. and *Pseudomonas aeruginosa* as bioremediation agents were obtained from Microbial Chemistry Laboratory, ITS. Instant medium Nutrient Broth (NB) and Potatoes Dextrose Broth (PDB)) from Merck, Darmstadt, German were used as a pre-incubated medium. Some chemicals such as KCl (Potassium Chloride), NaCl (Sodium Chloride), $CaCl_2$ (Calcium Chloride), $MgCl_2$ (Magnesium Chloride), $SrCl_2$ (Strontium Chloride), NaF (Sodium Fluoride), $NaHCO_3$ (Sodium Bicarbonate), Na_2SO_4 (Sodium Sulfate), KBr (Potassium Bromide), and H_3BO_3 (Boric Acid) from SAP chemicals, Indonesia were used to make artificial seawater medium. Some solvents such as n-hexane, acetone, and methanol from Anhui Fulltime Specialised Solvent & Reagent Co., Ltd (Anhui, China) were used for oil spill extraction (oil spill recoveries). DDT (Dichloro diphenyl trichloroethane) from Tokyo Chemical Industry Co. was used as the internal standard.

B. Artificial Seawater Preparation

The seawater medium was made on Standard Practice for the Preparation of Substitute Ocean Water (ASTM D1141-98). 24.5 g NaCl and 4 g Na_2SO_4 was dissolved into 800 mL water. Then, the mixture of 57.9 g L^{-1} $CaCl_2$; 55.6 g L^{-1} $MgCl_2$; and 2 g L^{-1} $SrCl_2$ in 20 mL solution as Solution A was added slowly into the previous solution. Then 10 mL of Solution B (69.5 g L^{-1} KCl; 10 g L^{-1}KBr; 20 g L^{-1} $NaHCO_3$; 0.3 g L^{-1} NaF; and 2.7 g L^{-1} H_3BO_3) was added into the solution. All solution mixture was diluted to 1 L solution and used as a seawater medium.

C. Bioremediation of Oil Spill by Aspergillus sp.

Aspergillus sp. has inoculated on the PDA medium for 7 days incubation, then pre-incubated in 10 mL of PDB medium for 7 days at 30°C. The pre-incubated culture was centrifugated at 3000 rpm for 10 min. The supernatant was decanted, and the biomass of *Aspergillus* sp. was washed with sterile water and then inoculated into 10 mL seawater medium. There are two variations done in this study, treatment and control, with

2 repetitions for each variation. Treatment was done by adding 50 µL oil spill, while control was done the same as treatment, except the culture was non activated by autoclaving in 121°C for 15 minutes before being added oil spill. Both treatment and control were flowed with oxygen for 30 seconds and then incubated for 7 days.

D. Bioremediation of Oil Spill by P. aeruginosa

P. aeruginosa has inoculated on the NA medium for 21 hours incubation, then pre-incubated in 10 mL of NB medium for 21 hours at 30°C. The pre-incubated culture was centrifugated at 3000 rpm for 10 min. The supernatant was decanted, and the biomass of *P. aeruginosa* was washed with sterile water and then inoculated into 10 mL seawater medium. There are two variations done in this study, treatment and control, with 2 repetitions for each variation. Treatment was done by adding 50 µL oil spill, while control was done the same as treatment, except the culture was non activated by autoclaving in 121°C for 15 minutes before being added oil spill. Both treatment and control were flowed with oxygen for 30 seconds and then incubated for 7 days.

E. Bioremediation of Oil Spill by Mixed Culture Aspergillus sp. And P. aeruginosa

After pre-incubation, both fungus dan bacterium cultures were centrifugated at 3000 rpm for 10 min. The initial medium was decanted, washed and then the biomass was mixed and inoculated to 10 mL seawater medium. Treatment was done by adding 50 µL oil spill to the culture, while control was done the same as treatment, except the culture, was non activated by autoclaving in 121°C for 15 minutes before being added oil spill. Both treatment and control were flowed with oxygen for 30 seconds and then incubated for 7 days.

F. Oil Spill Recovery

After the incubation process, 10 mL of methanol was added to the culture to deactivate the metabolism process, then 50 µL of DDT 5 mM in DMSO (dimethyl sulfoxide) (final concentration 0.25 µmol) was added as the internal standard. The culture was homogenised and centrifuged at 3000 rpm for 10 min. The supernatant was filtered with Whatman filter paper No. 41. and evaporated at 64°C to remove the methanol. Then the supernatant was extracted with 100 mL n-hexane and the organic fractions were collected and dried over anhydrous sodium sulfate. The extracts were concentrated by evaporated at 68°C and analysed using GCMS. GCMS Agilent Technologies 7890 GC System linked to an Agilent Technologies 5975C VL MSD Detector with a 30 m × 50 µm × 0.25 µm Agilent 19091S-433 column was used in analysing the sample with the oven temperature was programmed start from 80°C and hold for 2 min, and then, the temperature was increased to 280°C at 5°C min^{-1} and held for 15 min.

III. RESULT AND DISCUSSION

In this study, the application of mixed cultures of fungus *Aspergillus* sp. and bacterium *P. aeruginosa* on spilled oil bioremediation was tested in seawater medium on a laboratory scale. The used of artificial seawater medium was to simulate the capability of the cultures on oil spills remediation in the marine environment which gives high salinity conditions. The artificial seawater medium was made based on Standard Practice for the Preparation of Substitute Ocean Water (ASTM D1141-98). The result shows that the pH of artificial seawater is 8.3, which is in the seawater pH range (7.5-8.4) (Chen *et al.,* 2017). In this study, both *Aspergillus* sp. and *P. aeruginosa* could alive under high salinity conditions and able to degrade the oil spill substrate. It indicated that *Aspergillus* sp. and *P. aeruginosa* are had the potential to be bioremediation agents in a marine environment. This result was similar to the previous research by Haenseler (1921). Haenseler (1921) reported that some *Aspergillus* species were able to grow in high salt proportions. In another research, Nakbanpote *et al.* (2014) also report that some *Pseudomonas* species are salt-tolerant and can grow under saline condition.

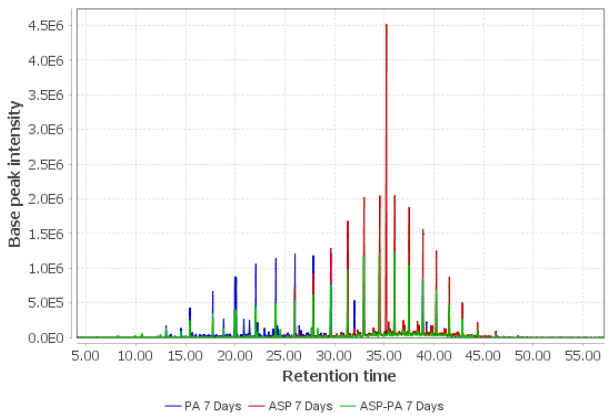

Figure 1. The Chromatogram of Oil Spill Recovery after 7 days of incubation treatment. Red = by *Aspergillus* sp. only, Blue = by *P. aeruginosa* only, and Green = by Mixed Cultures *Aspergillus* sp. and *P. aeruginosa*.

In this study, the ability of *Aspergillus* sp. and *P. aeruginosa*, as well as mixed cultures of them were investigated and compared. The result of analysing using GCMS was shown in Figure 1. The chromatogram of mixed cultures was lower than the chromatogram of particular *Aspergillus* sp. and *P. aeruginosa*. It indicated that the mixed culture treatment increased the oil spill bioremediation capability of *Aspergillus* sp. and *P. aeruginosa*. The capability of *Aspergillus* sp. and *P. aeruginosa* on oil spill bioremediation was due to their ability to produce some degradation enzyme. *Aspergillus* sp. can produce ligninolytic enzymes such as dehydrogenase, peroxidase, and laccase enzymes, which very useful to get energy from their food resources (Wemedo *et. al.,* 2018; Gulzar *et al.,* 2017). Besides, *P. aeruginosa* produces some degradation enzymes such as amylase, protease, lipase, and cellulose (Gill *et al.,* 2018).

The ability of mixed cultures treatment to increase oil spill bioremediation might be associated with their synergetic effect. It can be correlated to biosurfactant produced by *P. aeruginosa*. *P. aeruginosa* is one of the bacteria which can produce rhamnolipid biosurfactant (Joice & Parthasarathi, 2014). The biosurfactant that produces by *P. aeruginosa* can involve the increasing oil spill bioremediation capability. The presence of biosurfactants can enhance the solubility of the oil spill and reduce the surface tension and interface of oil spills under high salinity water conditions. The increase of oil spill solubility makes the oil spill easier to be accessed by microorganisms and easier to be degraded (Plaza *et al.,* 2008). This result similar to research reported by Sariwati (2018). Sariwati (2018) report that the addition of *P. aeruginosa* can enhance DDT remediation capability by *Fomitopsis pinicola*. It suggested that the application of mixed cultures can increase the bioremediation capability of organic pollutants.

The oil spill recovery compounds were shown in Figure 2 and Table 1. There are 10 compounds that recovered in all cultures from oil spill remediation for 7 days incubation bioremediation prosses (Figure 2) such as 2-methyl-dodecane; 2,6,10-trimethyl-dodecane; pentadecane; 2,6,10-trimethyl-pentadecane; hexadecane; 2,6,10,14-tetramethyl-hexadecane; heptadecane; octadecane; nonadecane; and eicosane. This result was supported by previous research that reported by Atlas *et al.* (1991) that reported oil spill converted to some compound including aliphatic hydrocarbon and polycyclic aromatic hydrocarbon. However, even these recovered compounds were found in all cultures, but the recovery in mixed cultures was the lowest. It indicated that the degradation of these compounds larger in the mixed cultures rather than particular *Aspergillus* sp. and *P. aeruginosa* itself.

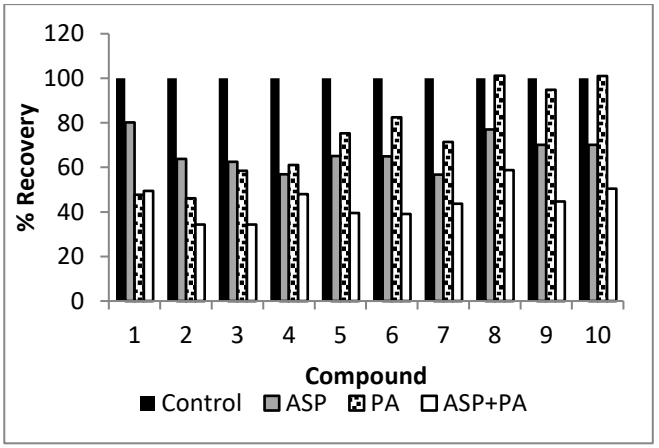

Figure 2. The Recovery of Oil Spill Compounds after 7 days of incubation treatment. Black bar = Control, Grey bar: by *Aspergillus* sp. only, Dot bar = by *P. aeruginosa* only, and White bar = by Mixed Cultures *Aspergillus* sp. and *P. aeruginosa*.

Based on Table 1, the result shows that all culture was able to degrade all oil spill compounds. The mixed culture was given the highest % degradation almost in all compounds after 7 days incubation. The highest degradation compound in mixed culture is pentadecane, which degrades up to

65.69%. In *Aspergillus* sp. culture, the highest degradation compound is 2,6,10,14-tetramethyl-Hexadecane which degrades up to 43.29 % and in *P. aeruginosa* culture, the highest degradation compound is pentadecane, which degrades up to 53.86 %. The highest degradation culture was mixed culture. It's correlated to their synergetic effect between *Aspergillus* sp. and *P. aeruginosa*.

Table 1. % Recovery and % degradation of oil spill compound. ASP = *Aspergillus* sp. only, PA = *P. aeruginosa* only, and Mixed = *Aspergillus* sp. + *P. aeruginosa*.

No.	Compound	% Recovery			% Degradation		
		ASP	PA	Mixed	ASP	PA	Mixed
1	2,6,10-trimethyl- Dodecane	80.25	47.71	49.49	19.75	52.29	50.51
2	Pentadecane	63.84	46.14	34.31	36.16	53.86	65.69
3	Hexadecane	62.49	58.45	34.38	37.51	41.55	65.62
4	2,6,10-trimethyl- Pentadecane	56.86	61.14	47.95	43.14	38.86	52.05
5	Heptadecane	65.06	75.24	39.50	34.94	24.76	60.50
6	Octadecane	64.90	82.48	39.09	35.10	17.52	60.91
7	2,6,10,14-tetramethyl- Hexadecane	56.71	71.41	43.64	43.29	28.59	56.36
8	2-methyl- Dodecane	76.99	101.20	58.75	23.01	0.00	41.25
9	Nonadecane	70.07	94.87	44.76	29.93	5.13	55.24
10	Eicosane	70.17	100.96	50.48	29.83	0.00	49.52

According to Banerjee *et al.* (2016), the degradation of aliphatic hydrocarbon from the oil spills by microorganisms was performed by an oxidation mechanism. The Microorganism enzymes oxidise the aliphatic hydrocarbon by both the terminal and subterminal pathways, in which the aliphatic hydrocarbon is oxidised by the action of monooxygenase and yields secondary alcohol, which is then converted to a ketone and then to a fatty acid (Whyte *et al.,* 1998).

IV. CONCLUSION

Mixed cultures of *Aspergillus* sp. and *P. aeruginosa* enhanced the oil spill bioremediation capability. The highest degradation compound by mixed cultures was pentadecane, which degraded up to 65.69%. In *Aspergillus* sp. culture, the highest degradation compound was 2,6,10,14-tetramethyl-hexadecane which degraded up to 43.29%, while in *P. aeruginosa* culture, the highest degradation compound was pentadecane, which degraded up to 53.86%. This study indicates that mixed cultures of *Aspergillus* sp. and *P. aeruginosa* can be potentially used as a bioremediation agent of the oil spill in a marine environment.

V. ACKNOWLEDGEMENT

This work was financially supported by the research grant from Institut Teknologi Sepuluh Nopember (ITS) (1204/PKS/ITS/2019).

VI. REFERENCES

Abdul-Hamid 2013, The OPEC Annual Statistical Bulletin, Vienna: OPEC.

Atlas, R & Ronald, M 1991, 'Microbial hydrocarbon degradation-bioremediation of oil spills', Journal of Chemical Technical Biotechnology, vol. 52, no. 2, pp. 149-156.

Banerjee, A, Roy, A, Dutta, S & Mondal, S 2016, 'Bioremediation of Hydrocarbon – A Review', International Journal of Advanced Research, vol. 4, no. 6, pp. 1303-1313.

Burghal, AA, Abu-Mejdad, NMJA & Al-Tamimi WH 2016, 'Myc degradation of crude oil by fungal species isolated from petroleum contaminated soil', International Journal of Innovative Research in Science Engineering and Technology, vol. 5, no. 2, pp. 1517-1524.

Chen, Q, Li, J, Liu, M, Sun, H & Bao, M 2017, 'Study on the biodegradation of crude oil by the free and immobilized bacterial consortium in marine environment', PLoS ONE, vol. 12, no. 3, pp. 0174445.

Gill, SS, Shrivastav, A & Jana, AM 2018, 'Evaluation of biodegradability of pseudomonas aeruginosa and bacillus subtilis in bioremediation of kitchen wastes', International Journal of Creative Research Thoughts, vol. 6, no. 1, pp. 18-23.

Gulzar, T, Huma, T, Jalal, F, Iqbal, S, Abrar, S, Kiran, S, Nosheen, S, Hussain, W & Rafique, MA 2017, 'Bioremediation of synthetic and industrial effluents by aspergillus niger isolated from contaminated soil following a sequential strategy', Molecules, vol. 22, pp. 2244.

Haenseler, CM 1921, 'The effect of salt proportions and concentration on the growth of aspergillus niger', American Journal of Botany, vol. 8, no. 3, pp. 147-163.

Joice, PA & Parthasarathi, R 2014, 'Optimization of biosurfactant production from Pseudomonas aeruginosa PBSC1', International Journal of Current Microbiology and Applied Sciences, vol. 3, no. 9, pp. 140-151.

Li, P, Cai, Q, Lin, W, Chen, B & Zhang, B 2016, 'Offshore oil spill response practices and emerging challenges', Marine Pollution Bulletin, vol. 110, pp. 6–27.

Li, YQ, Liu, HF & Tian, ZL 2008, 'Diesel pollution biodegradation: synergetic effect of mycobacterium and filamentous fungi', Biomedical and Environmental Sciences, vol. 21, pp. 181-187.

Mohsenzadeh, F, Rad, AC & Akbari, M 2012, 'Evaluation of oil removal efficiency and enzymatic activity in some fungal strains for bioremediation of petroleum-polluted soils', Iranian Journal of Environmental Health Sciences and Engineering, vol. 9, pp. 26.

Nakbanpote, W, Panitlurtumpai, N, Sangdee, A, Sakulpone, N, Sirisom, P & Pimthong, A 2014, 'Salt-tolerant and plant growth-promoting bacteria isolated from Zn/Cd-contaminated soil: identification and effect on rice under saline conditions', Journal of Plant Interactions, vol. 9, no. 1, pp. 379-387.

Ojewumi, ME, Anenih, EV, Taiwo OS, Adekeye, BT, Awolu, OO & Ojewumi, EO 2018, 'A bioremediation study of raw and treated crude petroleum oil polluted soil with aspergillus niger and pseudomonas aeruginosa', Journal of Ecological Engineering, vol. 19, no. 2, pp. 226-235.

Plaza, GA, Jangid, K, Lukasik, K, Jawecki, GN, Berry, CJ & Brigmon, RL 2008, 'Reduction of petroleum hydrocarbons and toxicity in refinery wastewater by bioremediation', Bull. Environ. Contam. Toxicol, vol. 81, pp. 329-333.

Prince, RC, Nash, GW & Hill, SJ 2016, 'The biodegradation of crude oil in the deep ocean', Marine Pollution Bulletin, vol. 111, pp. 54–357.

Purnomo, AS, Kamei, I & Kondo, R 2008, 'Degradation of 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT) by brown-rot fungi', Journal of Bioscience and Bioengineering, vol. 105, pp. 614-621.

Purnomo, AS, Mori, T & Kondo, R 2010, 'Involvement of Fenton reaction in DDT degradation by brown-rot fungi', International Biodeterioration and Biodegradation, vol. 64, pp. 560-565.

Purnomo, AS, Mori, T, Takagi, K & Kondo, R 2011, 'Bioremediation of DDT contaminated soil using brown-rot fungi', International Biodeterioration and Biodegradation, vol. 65, no. 5, pp. 691-695.

Purnomo, AS, Ashari, K & Hermansyah, F 2017, 'Evaluation of the synergistic effect of mixed cultures of white-rot fungus Pleurotus ostreatus and biosurfactant-producing bacteria on DDT biodegradation', Journal of Microbiology and Biotechnology, vol. 27, no. 7, pp. 1306-1315.

Purnomo, AS, Rizqi, HD, Fatmawati, S, Putro, HS & Kamei, I 2018, 'Effects of bacterium Ralstonia pickettii addition on DDT biodegradation by Daedalea dickinsii', Research Journal of Chemistry and Environment, vol. 22, no. II, pp. 151-156.

Sariwati, A & Purnomo, AS 2018, 'The effect of Pseudomonas aeruginosa addition on 1,1,1 Trichloro 2,2 bis (4 chlorophenyls) ethane DDT Biodegradation by Brown-Rot fungus Fomitopsis pinicola', Indonesian Journal of Chemistry, vol. 18, no. 1, pp. 75-81.

Sun, H, Liu, H, Wang, S & Liu, Y 2019, 'Remediation of oil spill contaminated sands by chemical-free microbubbles generated in tap and saline water', *Journal of Hazardous Materials,* vol. 366, pp. 124-129.

Vanishree, M, Thatheyus, AJ & Ramya, D 2014, 'Biodegradation of petrol using aspergillus sp', Annual Research and Review in Biology, vol. 4, no. 6, pp. 914-923.

Wang, C, Gao, X, Sun, Z, Qin, Z, Yin, X & He, S 2013, 'Evaluation of the diagnostic ratios for the identification of spilled oils after biodegradation', Environmental Earth Sciences, vol. 68, pp. 917–926.

Wang, S, Nomura, N, Nakajima, T & Uchiyama, H 2012, 'Case study of the relationship between fungi and bacteria associated with high-molecular-weight polycyclic aromatic hydrocarbon degradation', Journal of Bioscience and Bioengineering, vol. 113, no. 5, pp. 624-630.

Wemedo, SA, Nrior, RR & Ike, AA 2018, 'Biodegradation potential of *Aspergillus niger* and *Rhizopus arrhizus* isolated from crude oil spilled site in Rivers State', IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), vol. 12, no. 12, pp. 49-57.

Whyte, LG, Hawari, J, Zhou, E, Bourbonniere, L, Inniss, WE & Greer, CW 1998, 'Biodegradation of variable-chain-length alkanes at low temperatures by a psychotropic Rhodococcus sp', App Environ microbiol, vol. 64, no. 7, pp. 2578-2584.