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Recommended Citation
Basuki, Witono (2017) "Biodegradation of Used Synthetic Lubricating Oil by Brevundimonas diminuta AKL 1.6," Makara Journal of Science: Vol. 21 : Iss. 3 , Article 6.
DOI: 10.7454/mss.v21i3.7382
Available at: https://scholarhub.ui.ac.id/science/vol21/iss3/6

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Cover Page Footnote
I would like to thanks to Khairul Syahputra, Ayu Tri Suryani, and Ilham Pradipta from Faculty of Sciences, University of Al Azhar Indonesia who have assisted me in this work.

This article is available in Makara Journal of Science: https://scholarhub.ui.ac.id/science/vol21/iss3/6
Biodegradation of Used Synthetic Lubricating Oil by *Brevundimonas diminuta* AKL 1.6

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Received February 9, 2016 | Accepted May 26, 2017

Abstract

Illegal disposal of used lubricating oil is a serious hazard to the environment and public health. This study is aimed at examining the biodegradation process of used synthetic lubricant using local microorganism. Oil-degrading microorganism were obtained from sea water contaminated with used lubricating oil. Those that showed some growth activity in Bushnell-Haas media containing used synthetic lubricating oil as a sole carbon source were selected. Its single colony was then generated by performing the dilution and scratch technique in the isolation and purification process. By using 16s rDNA, a potential strain namely AKL 1.6, was identified as *Brevundimonas diminuta*. Gas chromatography analysis was performed before and after the biodegradation process of the used oil. As a result, *B. diminuta* AKL 1.6 was found to utilize used synthetic lubricating oil as the sole source of carbon and to degrade most of the hydrocarbon within the oil for 14 days. Thus, this particular microorganism has the potential to be the single microbe for bioremediation of sea water contaminated by lubricating oil.

Keywords: biodegradation, *Brevundomonas diminuta*, used synthetic lubricating oil

Introduction

A huge amount of lubricating oil is produced worldwide. The illegal dumping of used engine oil is dangerous to the environment and constitutes a serious threat to humans, animals, and vegetation [1].

Among the many kinds of lubricating oil, synthetic base oils are among the most widely used. Most synthetic motor oils are fabricated by polymerizing short chain hydrocarbon molecules called alpha-olefins into longer chain hydrocarbon polymers called polyalpha-olefins (PAOs). The degree of variation in molecular size, length, and branch chain in synthetically produced fluids occurs more or less in base stocks extracted from crude oil [2]. While they appear chemically similar to the mineral oils refined from crude oil, PAOs do not contain the impurities or waxes inherent in conventional mineral oils. PAOs constitute the most widely used synthetic motor oil in the U.S. and Europe [3].
Synthetic lubricant technology allows products to be designed for particular applications, and in combination with additives, provides targeted performance. Synthetic lubricants are frequently blended with mineral oil in order to provide desired properties [4]. Among the advantages of synthetic lubricants over mineral base oils are low temperature fluidity (and thus better cold weather performance), low volatility, high-temperature thermal stability, oxidation resistance [5], and high natural detergent characteristics [6].

After a period of usage, the lubricating oil contain more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that could pose chronic hazard including mutagenicity and carcinogenicity [7]. These oils become “enriched” with PAHs during engine operation. These contaminants are fuel combustion products that are transported into the crankcase and concentrate in the oil. In an early study using a 1981 gasoline-powered vehicle, PAHs were not detected in new lubricating oil; however, concentrations increased rapidly with increased miles driven [8]. Prolong exposure and high oil concentration may lead to liver or kidney disease, possible damage to the bone marrow, and increased risk of cancer [9].

Mechanical methods to reduce hydrocarbon pollution are expensive and time-consuming. Bioremediation of soils has become an important issue, because it implies a process in which organic contaminants in the subsoil are biodegraded and become mineralized so that eventually become non-toxic compounds. The contaminant does not enter another physical state because it is degraded [10].

The mechanism of bioremediation is, in principle, a decomposition process of organic material in the biosphere by a group of degrading heterotrophic microbes. A heterotrophic microbe has the ability to utilize organic compounds, in this case hydrocarbons, as a substrate. Decomposition of the hydrocarbon result in CO₂, CH₄, water, microbe biomass, and by-product in the form of simpler compounds [11].

Microbial bioremediation is one way to preserve our natural environment. Many species of bacteria, yeast, and fungi obtain both energy and tissue-building material from petroleum. Several species of fuel-eating bacteria have evolved a taste for hydrocarbons. These include *Pseudomonas* [12], *Methylobacterium* [13], *Bacillus megaterium* [14], *Ochrobactrum* [15], *Acinetobacter* [16], *Streptococcus* [17] and *Candida* [18]. Individual microbial population usually metabolize only a limited range of substrates, therefore, the performance of mixed population with different degradation capabilities was studied.

In general, isolation of petrophilic bacteria uses an enriched medium with a pure hydrocarbon compound or hydrocarbon mixture such as used lubricating oil. The location is an area that has been contaminated for a long time with oil. The existence of hydrocarbon in such an area will naturally yield various types of bacteria. The petrophilic microbes in this biosphere can be isolated and purified in the form of isolates. From the isolate collection can be selected the strongest or most specific strain for degrading hydrocarbon in used oil.

However, to the author’s knowledge, there is very little published literature available that describe the biodegradation of used synthetic lubricant oil. This experiment is aimed at examining microorganisms that have the capability to degrade used synthetic lubricating oil which has been illegally dumped in locations that are not designated for such disposal.

**Materials and Methods**

**Sampling:** Sea water contaminated with used lubricating oil was obtained from a beach in Jakarta Bay. This location was chosen because many ships dock nearby and the adjacent sea water contaminated with used lubricating oil. Forty milliliters of contaminated sea water were aseptically collected in a 50 mL bottle, wrapped with aluminum foil, and brought to the laboratory within 48 hours for bacterial isolation. A sample of used synthetic oil from Synthetic Top One Motor Oil SAE 20W-50, TOP 1 Oil Products Co. (California, USA) was obtained from a car workshop in the Tangerang region.

**Isolation of used synthetic lubricating oil degrading bacteria.** Synthetic lubricating oil-degrading bacteria was grown in Bushnell-Hass media [19] that has been enriched with used synthetic lubricant oil 10% (v/v) as a single carbon source [20]. Bushnell-Hass media composed of K₂HPO₄ 1.0 g/L, KH₂PO₄ 1.0 g/L, NH₄NO₃ 1.0 g/L, MgSO₄ 0.2 g/L, CaCl₂ 0.02 g/L, and FeCl₃ 0.005 g/L with a pH value of 7.0. These compounds were placed in 10 mL of contaminated sea water sample, and then incubated in a shaker at a speed of 170 rpm, at room temperature for 1 week. At the end of the week, 1 mL fermentation broth (5.2 x 10⁸ CFU/mL) was transferred into 9 ml enriched Bushnell-Hass media and incubated again under the same conditions mentioned above. Six series of dilution were required to obtain the separated colonies. After incubation for 1 week, one mL fermentation broth was poured into an agar plate containing the Bushnell-Hass media. The surface of the agar plate was covered with 100 μL of used synthetic lubricating oil and incubated at 30 °C for 3-4 days. Isolates that grew in the Bushnell-Hass media and then the separated colonies were purified by scratch technique until a single colony was obtained. Pure isolate were then inoculated with agar slant and incubated at 30 °C for 72 hours and kept at 4 °C as stock culture. Media for stock culture was yeast extract media which consist of peptone 5.0 g, yeast extract 3.0 g, and agar 1.5% in 1000 mL distilled water [21].
Bacterial gram staining: Bacterial Gram staining was done by the method of Balow et al. [22].

Bacterial identification. Genomic DNA from a pure culture of isolate AKL 1.6 was extracted and purified. The DNA was then amplified by PCR with 27F primer (5’-GAG TTT GAT CCT GGC TCA G-3’) and 1525 R primer (5’-AGA AAG GAG GTG ATC CAG CC-3’). The PCR program consisted of pre-denaturation at 96 °C for 3 minutes, then 30 cycles of denaturation at 96 °C for 45 seconds, annealing at 56 °C for 30 seconds, and elongation at 72 °C for 2 minutes. Post elongation was done at 72 °C for 7 minutes and finally held at 4 °C until the process was completed. The 16S rDNA sequences (500 nt) of AKL 1.6 were then analyzed for similarity using BLAST Search [23].

Bacterial capability for consuming used synthetic lubricating oil. In order to test the ability of bacteria to consuming used synthetic lubricating oil, the following experiment was carried out. Strain AKL 1.6 was precultured in 20 mL yeast extracts media and shaken at 120 rpm, 30 °C for 24 hours [24]. Then, 0.2 mL preculture broth (5.2 x 10⁶ CFU/ml) was transferred to 20 mL Bushnell-Hass media containing 400 mg used synthetic lubricant oil, and then shaken at 120 rpm and 30 °C. The used oil had previously been centrifuged at 6000 x g for 10 minutes in order to separate the metals and other precipitated impurities. Later, 1 μL of this used lubricating oil was used as a sample in GC-MS analyses as a control. After 7 and 14 days, fermentation was terminated. Eighteen ml of chloroform-methanol (3:1 v/v) was added to extract the remaining oil. Mixture solvent and culture broth were mixed and settled for 2 hours at room temperature, which allows the separation of the solvent containing the used synthetic lubricant oil from the water. The separated water was then discarded, and the solvent containing the oil was transferred to a centrifuge tube and centrifuged at 6000 x g for 10 minutes at room temperature. Precipitate was then separated from the solvent, and solvent was evaporated for 3 days. The remaining oil was weighted. One μL of this remaining used oil had its composition analyzed using GC-MS. The percentage of used lubricating oil by microbe was calculated gravimetrically as follows:

\[
\text{Consumption} (%) = \frac{\text{original weight} - \text{final weight}}{\text{original weight}} \times 100\%
\]

Gas chromatography analysis. Gas chromatography analysis was done using a Shimadzu GC-MS QP 2010 equipped with a flame ionization detector (FID) with capillary column Rtx-1MS 100% dimethyl polysiloxane (30 m x 0.25 m), J & W Scientific SA, USA. Helium was used for the gas carrier and speed was maintained at 154 mL/min. As mentioned above, 1 μL of the used lubricating oil as a control was injected with temperature injection port at 280 °C, oven column temperature at 50 °C, and pressure at 90.7 mmHg. Column temperature was maintained at 50 °C for 3 minutes, and then increased to 260 °C for 10 minutes. One microliter of the remaining used synthetic lubricating oil as a sample was also injected with the same conditions as described above.

Results and Discussion

Isolation of used synthetic lubricating oil-degrading bacteria and capability of bacteria to consume used synthetic lubricating oil. According to Bushnell and Haas [19], the most successful method of isolating organisms capable of consuming hydrocarbons is to use of a mineral-salts-hydrocarbon enrichment medium in which the hydrocarbon is the only source of carbon and energy for bacterial growth. Bacteria were isolated based on their ability to grow in Bushnell-Haas media containing used lubricating oil as the single carbon source. Out of 35 isolates grown in the Bushnell-Haas media, 10 isolates showed a capability to consume used synthetic lubricating oil as the single carbon source [25]. After observing the GC-MS chromatographic data, AKL 1.6 was selected as one of the isolate which has a strong capability for degrading used synthetic lubricating oil.

Characterization and identification of AKL 1.6. Microscopic observation and Gram staining tests as shown in Figures 1 and 2 indicated that strain AKL 1.6 is a bacterium with a rod form and is classified as Gram negative. In addition, 16S rDNA sequence showed that strain AKL 1.6 has a 99% similarity with Brevundimonas diminuta. Therefore, strain AKL1.6 was named Brevundimonas diminuta AKL 1.6.

Bacterial capability for consuming used synthetic lubricating oil. Table 1 shows the percentages of used synthetic lubricating oil consumed by B. diminuta AKL 1.6. The table indicates that, during 7 days of incubation 1.89% of the oil was consumed. After 14 days, 5.6% was consumed. Analysis of used oil components via gas chromatography (GC) was aimed at determining the change in hydrocarbon composition resulting from biodegradation activity of B. diminuta AKL 1.6.

As shown in Figure 3, GC analyses of the control oil before biodegradation indicated that used synthetic lubricating oil is composed of 47 compounds. The compounds are shown by peak no.1 to 47 which emerged from a retention time of the third minute until the twenty-ninth minute. The compounds were composed of short chain hydrocarbons (≤C9), medium chain hydrocarbons (C10-C24), and long chain hydrocarbons (≥C25) in linear, cyclic, and polycyclic forms. Identification of the component (as shown in Table 2) was carried out using Wiley 7 and NIST 147 library software which was available in the CG-MS instrument. GC analysis of all
hydrocarbon components in the oil sample after 14 days of biodegradation by *B. diminuta* AKL 1.6 is shown in Figure 4. Table 3 shows the remaining components of used synthetic lubricating oil after biodegradation. It shows that the number of peak hydrocarbon components decreased significantly.

Table 1. Percentage of Used Lubricant Oil Consumed by *B. diminuta* AKL 1.6

| Incubation Time | Weight (gram) used synthetic lubricant oil | % Consumed |
|-----------------|-------------------------------------------|------------|
| 7 days          | Original 0.3962 | Final 0.3887 | Consumed 0.0075 | 1.89 |
| 14 days         | Original 0.3962 | Final 0.3740 | Consumed 0.0222 | 5.60 |

Short (≤C9) and long (≥C25) chain hydrocarbons, which were detected in the control (Figure 3), were lost. Most of the hydrocarbon components in used synthetic lubricating oil in the form of aliphatic, aromatic, and polycyclic were degraded by this microorganism. The data indicate that

Figure 1. *B. diminuta* AKL 1.6

Figure 2. Gram staining of *B. diminuta* AKL 1.6

Figure 3. Gas Chromatogram of Used Synthetic Lubricant Oil at 0 Day Biodegradation

Figure 4. Gas Chromatogram of Used Synthetic Lubricant Oil after 14 Days Biodegradation
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dility of PAO can range from 20 to 100 percent. Rates and extents of biodegradation vary considerably between laboratories and field situations, largely due to the influence of factors such as temperature, the type and number of microbes, and the availability of oxygen and water.

Tamada et al. [27] found that synthetic lubricating oil is the most biodegradable due to the fact that it is composed of molecules which are predominantly formed by only one type of chemical structure. As the microorganisms adapted to this substrate, biodeg-

| No. Peak | Retention time (min) | Percentage Area (%) | Component |
|----------|----------------------|---------------------|-----------|
| 1        | 3.641                | 1.79                | Toluene   |
| 2        | 3.809                | 0.27                | 2.5-Dimethyl hexane |
| 3        | 3.939                | 0.30                | 2.4-Dimethyl hexane |
| 4        | 4.410                | 0.38                | n-Octane  |
| 5        | 5.350                | 0.86                | Ethylbenzene |
| 6        | 6.519                | 3.85                | ω-Dimethylbenzene |
| 7        | 7.680                | 0.45                | 5-Methylundecane |
| 8        | 8.815                | 0.28                | 3-Methyloctane |
| 9        | 9.960                | 1.78                | p-Dimethylbenzene |
| 10       | 6.364                | 0.37                | n-Nonane  |
| 11       | 7.167                | 0.60                | n-Propylbenzene |
| 12       | 7.314                | 2.25                | n-Ethylmethylbenzene |
| 13       | 7.351                | 0.97                | ω-Ethylmethylbenzene |
| 14       | 7.745                | 0.24                | 3-Methylnonane |
| 15       | 7.924                | 5.49                | 1,3,5-Trimethylbenzene |
| 16        | 8.271               | 0.45                | n-Decane |
| 17        | 8.422               | 1.73                | 1,2,3-Trimethylbenzene |
| 18        | 8.622               | 0.76                | Benzo cyclopentane |
| 19        | 8.925               | 0.50                | 1,4-Diethylbenzene |
| 20        | 8.973               | 0.86                | 1-Methyl-3-propylbenzene |
| 21        | 9.040               | 0.64                | 2-Tolylxylene |
| 22         | 94.093              | 1.05                | 1,4-Dimethyl-2-ethylbenzene |
| 23        | 9.238               | 0.40                | 1-Methyl-2-propylbenzene |
| 24         | 9.301               | 0.21                | 5,6-Dimethylundecane |
| 25         | 9.356               | 0.18                | 4-Methyldecan |
| 26         | 9.546               | 2.11                | 1,2-Dimethyl-4-ethyl benzene |
| 27        | 10.009              | 0.65                | n-U ndecane |
| 28        | 10.145              | 1.75                | 1,2,3,5-Tetramethylbenzene |
| 29        | 10.418              | 1.33                | 2-Alytoluene |
| 30         | 10.910              | 0.80                | Isopropylbenzaldehyde |
| 31         | 11.048              | 2.27                | Naphthalene |
| 32         | 11.591              | 0.77                | n-Dodecan |
| 33         | 12.618              | 1.07                | Pentamethylbenzene |
| 34         | 12.732              | 4.77                | 2-Methyl phenalan |
| 35         | 12.956              | 2.72                | 3-Methylphenalan |
| 36         | 13.047              | 1.13                | n-Tridecan |
| 37         | 19.071              | 1.15                | n-Octadecane |
| 38         | 19.884              | 0.39                | 2-Methyltriacene |
| 39         | 19.936              | 0.58                | ω-Methylpentan tren |
| 40         | 20.042              | 0.57                | 1-Methy lantracene |
| 41         | 20.093              | 1.61                | n-Dodecan |
| 42         | 22.483              | 1.57                | n-Heneicosan |
| 43         | 23.602              | 3.82                | n-Eicosan |
| 44         | 23.985              | 7.36                | n-Tetracosan |
| 45         | 24.541              | 9.41                | 8-Hexylpentadecane |
| 46         | 25.776              | 12.91               | Tetracontane |
| 47         | 28.025              | 14.58               | n-Tetra tricontane |

| No. | Retention time (min) | Percentage area (%) | Component |
|-----|----------------------|---------------------|-----------|
| 1   | 5.413                | 0.08                | 2,3-Dimethyl-1,3-cyclohexadiene |
| 2   | 5.801                | 0.08                | 1,4-Dimethyl-cyclohexa-1,3-diene |
| 3   | 7.868                | 0.12                | 1,2,5,5-Tetramethyl-1,3-cyclopentadiene |
| 4   | 9.513                | 0.19                | Benztiole, 2-methyl |
| 5   | 10.972               | 0.32                | 3,5-Diisoprophyl-1,2,4-tritiolene |

**B. dimuita** AKL 1.6 can significantly degrade short and long chain aliphatic, aromatic, and polycyclic hydrocarbons.

According to the GC results, after 14 days of biodegradation of used oil (Figure 3), there emerged small peaks of new compounds in the retention times of 5 to 15 minutes, which was previously not detected before biodegradation. The new compounds are assumed to be a result of biodegradation of high molecule weight compounds which are not detected by GC or gathering of fractions resulting from degradation of compounds which experienced declining peak areas [26]. Synthetic oil presents known structures. It is composed of molecules produced in the refinery, and in many cases it becomes more biodegradable than used lubricating oil. Its molecules are biodegraded most easily, followed by semi-synthetic oil, and finally mineral oil [27]. PAOs show higher biodegradability than mineral oils of equivalent viscosity because of their higher degree of hydrocarbon chain linearity [4]. Within a class of synthetic lubricants, the percentage of materials is biodegraded for the same lubricant type. For example, biodegradability of PAO can range from 20 to 80 percent after 21 days using a primary biodegradability test, which measures the initial transformation from the parent material [4].

Using this same test, biodegradation of mineral-based oil can range from 10 to 45 percent. Rates and extents of biodegradation vary considerably between laboratory and field situations, largely due to the influence of factors such as temperature, the type and number of microbes, and the availability of oxygen and water.
radiation was accelerated and in 60 days the toxicity was reduced in all samples.

From the description above it can be concluded that \textit{B. diminuta} AKL 1.6 has a proven potency to be the single microbe for bioremediation of sea water contaminated by used lubricating oil.

Conclusions

From the description above it can be concluded that \textit{B. diminuta} AKL 1.6 was found to utilize used synthetic lubricating oil as the sole source of carbon and to degrade most of the hydrocarbon within the used synthetic lubricating oil. This microorganism has the potential to be used as the single microbe for bioremediation of sea water contaminated by lubricating oil.

Acknowledgments

I would like to thanks to Khairul Syahputra, Ayu Tri Suryani, and Ilham Pradipita from Faculty of Sciences, University of Al Azhar Indonesia who have assisted me in this work.

References

[1] Bhattacharya, M., Biswas, D. 2014. Enhancement of waste engine oil biodegradation by optimization of media using factorial design study. \textit{Indian J. Biotech.} 13: 293-300.

[2] OECD, 2004. Emission scenario document on lubricants and lubricant additives. Organization for Economic Cooperation and Development. ENV/JM/MONO.21.

[3] Denton, J.E. 2007. A review of the potential human and environmental health impacts of synthetic motor oils, Office of Environmental Health Hazard Assessment California Environmental Protection Agency.

[4] Boyde, S. 2002. Green lubricants: environmental benefits and impacts of lubrication. \textit{Green Chem.} 4: 293-307.

[5] Tripathi, A.K., Vinu, R. 2015. Characterization of thermal stability of synthetic and semi-synthetic engine oils. \textit{Lubricants} 3: 54-79. doi:10.3390/lubricants3010054.

[6] Kroschwitz, J.I. 2004. Kirk-Othmer Encyclopedia of Chemical Technology, 5th ed. Wiley Interscience, N.J.

[7] Wong, P.K., Wang, J. 2001. The accumulation of poly cyclic aromatic hydrocarbons in lubricating oil over time – a comparison of supercritical fluid and liquid-liquid extraction methods. \textit{Environ. Pollut.} 112: 407-415.

[8] Mishra, S., Jyot, J., Kuhad, R.C., Lai, B. 2001. Evaluation of inoculum addition to stimulate \textit{in situ} bioremediation of oily-sludge-contaminated soil. \textit{Appl. Environ. Microbiol.} 67: 1675-1681.

[9] Khan, J.A., Asthana, A. 2011. A Study on oil degradation potential of \textit{Bacillus megaterium} isolated from oil contaminated sites in Lucknow. \textit{Arch. Appl. Sci. Res.} 3: 513-517.

[10] Iturbe, R., López, J., Pet, J. 2015, Bioremediation for a soil contaminated with hydrocarbons. \textit{Environ. Biotecnol}. 6:208-214. doi:10.4172/2157-7463.1000208.

[11] Mbachu, A.E., Onochie, C.C., Agu, K.C., Okafor, O.I., Awah, N.S. 2014. Hydrocarbon degrading potentials of indigenous bacteria isolated from automechanic workshop at Mgbuka-Nkpor, Nigeria. \textit{J. Global Biosci.} 3: 321-326.

[12] Obayori, O.S., Salam, L.B., Ogunwumi, O.S. 2014. Biodegradation of fresh and used engine oils by \textit{Pseudomonas aeruginosa} LP5. \textit{J. Bioremed. Biodeg}. 5: 213-220. doi:10.4172/2155-6199.1000213.

[13] Salam, L.B., Obayori, O.S., Raji, S.A. 2015. Biodegradation of used engine oil by a methylo trophic bacterium, \textit{Methyllobacterium mesophilicum} isolated from tropical hydrocarbon-contaminated soil. \textit{P Petro. Sci. Tech}. 33:186-195.

[14] Gopinath, S.M., Shareef, M.I., Ashalatha, Ganessin, A. 2015. Bioremediation of lubricant oil pollution in water by \textit{Bacillus megaterium}. \textit{Int. J. Innov. Res. in. Sci. Eng. Tech.} 4: 6773-6780. doi: 10.15680/ IJIRSET.2015.0408010.

[15] Bhattacharya, M., Biswas, D., Sana, S., Datta, S. 2015. Biodegradation of waste lubricants by a newly isolated Ochrobactrum sp. C1. \textit{3 Biotech}. 5: 807-817. doi: 10.1007/s13205-015-0282-9.

[16] Sihag, S., Sharma, S., Pathak, H., Dave, S., Jaroli, D.P. 2013. Biodegradation of engine oil by \textit{Acinetobacter calcoaceticus} BD4, isolated from coastal area Mumbai. \textit{Int. J. Biotech. Bioeng. Res}. 4: 235-242.

[17] Eniola, K.I.T., Adegbola, G.M., Opasola, O.A. 2014. Biodegradation of used engine oil by bacteria isolated from soil contaminated with used engine oil in Ogbomoso, Nigeria. \textit{IOSR J. Environ. Sci. Toxic. Food Tech. (IOSR-JESTFT)}. 8: 66-70.

[18] Ramadan, K.M.A., Aizez, A.Z.A., Hassanien, S.E., Eissa, H.F. 2012. Biodegradation of used lubricating and diesel oils by a new yeast strain \textit{Candida viswanathii} KA-2011. \textit{African J. Biotech}. 11: 14166-14174. doi: 10.5897/AJ B 12.1339.

[19] Bushnell, L.D., Haas, H.F. 1941. The utilization of certain hydrocarbons by microorganisms. \textit{J. Bacteriol}. 41: 653-673.

[20] Mandri, T., Lin, J. 2007. Isolation and characterization of engine oil degrading indigenous microorganisms in Kwazulu-Natal, South Africa. \textit{African J. Biotech}. 6: 23-27.

[21] Atlas, R.M. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. \textit{Microbiol. Rev}. 45: 180-209.

[22] Balows, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. 1992. The Prokaryotes: A Handbook on the Biology of Bacteria. Springer Verlag, Heidelberg.
[23] U.S. National Library of Medicine. Basic Local Alignment Search Tool. Access from: http://www.ncbi.nlm.nih.gov/blast/.

[24] Aoshima, H., Hirase, T., Tada, T., Ichimura, N., Yamaguchi, H., Taguchi, M., Myoenzono, T. 2006. Improvement of heavy oil degradation by Rhodococcus erythropolis C2. J. Environ. Biotech. 5: 107-109.

[25] Syahputra, K., Basuki, W. 2009. Isolation and Characterization of Used Lubricant Oil Degrading Microorganism. Report of the Competitive Research Grant. Directorate General of Higher Education, Ministry of National Education, Republic of Indonesia.

[26] Gritter, R.J., Bobbin, J.M., Schwating, A.E. 1991. Introduction to Chromatography. Penerbit ITB, Bandung.

[27] Tamada, I.S., Lopes, P.R.M., Montagnolli, R.N., Bidoia, E.D. 2012. Biodegradation and toxicological evaluation of lubricant oils. Brazilian Arch. Biol. Biotech. 55: 951-956.