**Biodegradation Capability of Some Bacteria Isolates to Used Lubricant Oil *in Vitro***

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**Abstract.** Our previous study identified three species of bacteria i.e. *Alcaligenes sp*., *Bacillus sp*1, and *Bacillus sp*2 isolated from used lubricant oil-contaminated soil in a Padang’s workshop. However, its ability to degrade hydrocarbon were not known yet. In this extension study we explore wider area to find more hydrocarbonoclastic bacteria and examined its capability to degrade hydrocarbon *in vitro*. Seventeen isolates were characterized its capability using NA + used lubricant oil + tween + neutral red medium. Isolates A1, B2, D1 and D4 shows high degradative index, whereas isolates A2, A3, A5, D2, B1, B3 and isolates A4, B4, D3 have medium and low degradative index, respectively. These potential hydrocarbonoclastic bacteria need *in situ* characterization to know their actual activities for bioremediation.

Keywords: biodegradation, hydrocarbonoclastic bacteria, bioremediation, *Alcaligenes* sp, *Bacillus*

 sp.

1. **Introduction**

Bacteria are ubiquitous and incredible microorganisms on earth. Bacteria can live in water, soil, air, and living thing organisms. Moreover, bacteria have the incredible ability to degrade the natural or synthetic materials. This degradation process is known as biodegradation. Nowadays, bacteria or other microorganisms is utilized for clean up the polluted environment. This activity is known as bioremediation[1].

Waste lubricant oil is one of the environment pollutant[2,3,4]. It could damaged our health when it is oxidated, nitration, cracking of polymers and decomposition of organometallic compounds in diesel engine for long period and high temperature [4]. Lubricant oil is one of the petroleum derivative. Waste lubricating oil also contain other dangerous compound like polyaromatic hydrocarbons (PAHs), toxic heavy metal dan chlorinated hydrocarbons (PCBs)[2]. These compunds are carcinogenic dan neurotoxic on human life system[5,6]. Uncontrolled disposal of waste lubricant oil to environment cause human and other living organisms to be contaminated.

In plant, waste lubricant oil caused the decrease of chlorophyl and protein[7,8], while lower photosinthesis observed on phytoplankton[9]. Human as the key actor of pollution also get the negative impact of carelessly throw waste lubricant away. In human, PAHs and PCBs could induce liver or kidney disease, possible damage to the bone marrow and increased risk of cancer[10,11].

Regarding of high risk of waste lubricant oil to living organisms especially human being, it is an urgent way to do bioremediation. Many studies found some potential genus of bacterial for waste lubricant oil biodegradation like *Acinetobacter, Achromobacter, Arthrobacter, Flavobacterium, Pseudomas* and many others[12,13,14,15,16,17]. These bacteria known as hydrocarbonoclastic bacteria[18]. Our previous study in a Padang’s workshop also isolated and identified three species of bacteria, i.e. *Bacillus* sp1, *Bacillus* sp2 and Alcaligenes from waste lubricant oil contaminated soil[19].

In this extended study we explore wider area to find out more indigenous bacteria in waste lubricant oil contaminated soil of workshops and examined its capacity *in vitro*. The aim of this tudy is to identify the potential clones that can degrade waste lubricant oil effectively *in vitro.*

**Materials and Methods**

Isolation of microorganisms

Waste lubricant contaminated soil were taken from four automobile or motorcycle workshops in West Sumatera i.e. Padang, Pasaman Barat, Tarusan and Bukit Tinggi, and brought asepticaly to our laboratory for bacterial isolation. One gram soil was added to 99 mL sterilized water and diluted until 10-3. Suspension was grown on 2% NA medium by streak quadrant method for 48 hours in room temperature. Mineral salt medium (MSM) with the following composition (g/L): 1.2 g NH4Cl; 1.6 g K2HPO4; 0,4 g KH2PO4; 0.1 g NaCl; 1 g KNO3; 20 g MgSO4.7H2O; 10 g CaCl2.2H2O; 0.05 g FeCl3, 1 mL of trace element solution[20,21] per liter containing 50 mg MnCl2.H2O, 300 mg H3BO3, 1.1 mg FeSO4.7H2O, 190 mg CoCl2.6H2O, 2 mg CuCl2.2H2O, 24 mg NiCl2.6H2O, 18 mg NaMoO4.2H2O, 42 mg ZnCl2.7H2O, 1 mL vitamin solution[21] were used as selective medium for hidrocarbon-biodegradation bacterial isolation. Single colony was inoculated to 10 mL MSM medium, incubated in a shaker incubator at 37°C at 200 rpm for 12 hours. After 12 hours of incubation, the culture were isolated as single colony and spread out on Nutrient Agar (NA) media by streak quadrant method. Single colony of each NA-containing petridishes then were maintened in slant cultures by preserving at 4°C and subculturing at 2 weeks interval[2].

Determination of biodegradation capabilities *in vitro*

Single colony was inoculated to 2% NA medium enriched 10 mL waste lubricating oil, 10 mL Tween 80 and 0.5 g neutral red[22] by toothpick method. Isolates then incubated at 37°C till found colonies with clear zones. Degradation-potential of bacterial *in vitro* is revealed through degradation index. Degradation index[22] = 

**Result and Discussion**

1. **Result**

Table 1. Bacteria isolates from several workshops in West Sumatera

|  |  |
| --- | --- |
| Location | Isolates |
| Bukit Tinggi | A1, A2, A3, A4, A5 |
| Pesisir Selatan | B1, B2, B3, B4 |
| Pasaman | C1, C2, C3, C4 |
| Padang | D1, D2, D3, D4 |

Exploration in four automobile or motorcycle workshops in West Sumatera found 17 isolates (Tabel 1). These isolates were characterized its potential to degradate waste lubricant oil *in vitro*. Thirteen out of seventeen isolates shows its potential to degradate waste lubricating oil while the other four isolates were not. The potential of isolate to degradate the waste lubricant oil is showed by the clear area surronding the colony. It is known as halo area (Figure 1).

|  |  |
| --- | --- |
| E:\ayang\KAMPUS\penelitian\foto penelitian\zona bening\D1.jpg | E:\ayang\KAMPUS\penelitian\foto penelitian\zona bening\D4.jpg |

Figure 1. The representatif (D1 and D4) colonies showing halo area indicated by clear circles.

D1

D2

D3

D4

C1

C3

C4

A1

A2

A3

A4

A5

B4

Figure 2. Mean of Degradatif Index of Isolates from Padang, Pasaman, Pesisir Selatan and Bukit Tinggi workshops. D1 – D4 are isolates from Padang, C1 – C4 are isolates from Pasaman, A1 – A5 are isolates from Bukit Tinggi, and B4 is isolate from Pesisir Selatan.

Isolates that have potential to degradate waste lubricant oil are D1, D2, D3, D4 (Padang workshop), C1, C3, C4 (Pasaman workshop), A1, A2, A3, A4, A5 (Bukit Tinggi workshop) and B4 (Pesisir Selatan workshop) (Figure 2). Isolates A1, B2, D1 and D4 shows high degradative index. However, the highest potential is shown by D4. In contrast, A2, A3, A5, D2, B1, B3 and isolates A4, B4, D3 have medium and low degradative index, respectively.

1. **Discussion**

Bioremediation including biodegradation in its process. Biodegradation is a process involving microorganisms to separate the toxic macromolecules to be simpler components that are environmentally friendly. One of the microorganisms that play a major role in the biodegradation process is bacteria[23,24,25].

 There are many research that proved the ability of various species of bacteria to degrade hydrocarbons. Bacteria can degrade marine oil spills[26] and petroleum hydrocarbon in a polluted tropical stream[27]. Bacteria also have capability to degrade petroleum in soil[28,29]. Other studies revealed that bacteria can degrade hydrocarbon in used engine oil[3,4, 30,31]. Moreover, Bhattacharya[2] found a species of bacteria that have the capability of degrading hydrocarbons in different waste lubricant oil sources. In this study we also identified some bacteria isolates that could degrade waste engine oil. Our bacteria could be same with other published studies, but did not rule out the discovery of new species. It is therefore necessary to identify the isolates.

 Our identification revealed that thirteen of seventeen isolates have the potential to degradate waste lubricant oil. This is also indicated by Ibrahim’s study[3]. Two of ten isolates have the degradation capacities more than 45%. Our study have not either identify the characteristic of carbon source for optimal bacteria activies yet. However, Bhattacharya’s study[2] show that Ochrobactrum sp.C1 could degrade several kind of hydrocarbon like naphtalene, xylene, diesel, and kerosene. The best result is found in naphthalene biodegradation. It is one of our focus in the next study.

Moreover, bacteria have the ability to degrade heavy metal in waste lubricant oil. *Pseudomonas* *aeruginosa* strain RM1 and strain SK1 could digest zinc, iron and nickel[4]. Those three metals were found in large quantities in lubricant oil. Our study identify two best isolates namely D4 and C1. These two isolates have chance to be identified its potential to degrade heavy metals.

**Conclusion**

This study has established 13 bacteria isolates that have ability to degrade waste lubricant oil. Highest activity is shown by isolate D4. For the future, our study will be focussed on several data like the species of isolates, the optimal activity of isolates to degrade several hydrocarbon sources and the ability of isolates to degrade heavy metal contained in waste lubricant oil.

**REFERENCES**

[1] Habe H, Chung JS, Lee JH, Kasuga K, Yoshida T, Nojiri H and Omori T 2001 Degradation of chlorinated dibenzofurans and dibenzo-p-dioxins by two types of bacteria having angular dioxygenases with different features. Appl Environ Microbiol 67:3610-17

[2] Bhattacharya M, Biswas D, Sana S and Datta S 2015 Biodegradation of waste lubricants by a newly isolated Ochrobactrum sp. C1. 3 Biotech 5:807-17 doi:10.1007/s13205-015-0282-9

 [3] Ibrahim HMM 2016 Biodegradation of used engine oil by novel strains of Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 isolated from oil-contaminated soil. 3 Biotech 6:226 doi:10.1007/s13205-016-0540-5

[4] Salam LB 2016 Metabolism of waste engine oil by *Pseudomonas* species. 3 Biotech 6:98 doi:10.1007/s13205-016-0419-5

[5] Ray S, Khillare PS, Agarwal T and Shridhar V 2008 Assessment of PAHs in soil around the International Airport in Delhi, India. J Hazard Mater 156:9-16

[6] Obayori OS, Ilori MO, Adebusoye SA, Oyetibo GO and Amund OO 2008 Pyrene degradation potentials of *Pseudomonas* species isolated from polluted tropical soils. World J Microbiol Biotechnol 24:2639-46

[7] Oluwole OS, Makinde SCO and Philips DA 2005 The impact of spent engine oil pollution on the growth of Celosia argentea. Proc. of the Fac. of Sci. Conf. Lagos States Univ. (LASU) (Nigeria)

[8] Umechuruba CI 2005 Health impact assessment of mangrove vegetation in an oil spilled site at the Bodo West field in Rivers State, Nigeria. J Appl Sci Environ Manag 9:69-73

[9] Vazquez-Duhalt R 1989 Environmental impact of used motor oil. Sci Total Environ 79:1-23

[10] Llyod CA and Cackette TA 2001 Diesel engines: environmental impact and control. Air Waste Manag Assoc 51:805-47

[11] Mishra S, Jyot J, Kuhad R and Lal B 2001 Evaluation of inoculum addition to stimulate in situ bioremediation of oily sludge-contaminated soil. Appl Environ Microbiol 67(4):1675-81

[12] Adelowo OO, Alagbe SO and Ayandele AA 2006 Time-dependent stability of used engine oil degradation by cultures of *Pseudomonas fragi* and *Achromobacter aerogenes*. Afr J Biotechnol 5:2476-9

[13] Mandri T and Lin J 2007 Isolation and characterization of engine oil degrading indigenous microorganism in Kwazulu-Natal, South Africa. Afr J Biotechnol 62:23-6

[14] Bagherzadeh-Namazi A, Shojaosadati SA and Hashemi-Najafabadi S 2008 Biodegradation of used engine oil using mixed and isolated cultures. Int J Environ Res 2:431-40.

[15] Basuki W, Syahputra K, Suryani AT, and Pradipta I 2011 Biodegradation of used engine oil. Indones J Biotechnol 16:132-8

[16] Obayori OS, Salam LB and Ogunwumi OS 2014 Biodegradation of fresh and used engine oils by Pseudomonas aeruginosa LP5. J Bioremediat Biodegrad 5:213. Doi:10.4172/2155-6199.1000213

[17] Salam LB, Obayori OS and Raji SA 2015 Biodegradation of used engine oil by a methylotrophic bacterium, *Methylobacterium mesophilicum* isolated from tropical hydrocarbon-contaminated soil. Petroleum Sci Technol 33:186-195

[18] Nursyirwani dan Amolle KC 2007. Isolasi dan Karakterisasi Bakteri Hidrokarbonoklastik dari Perairan Dumai dengan Sekuens 16S rDNA. Ilmu Kelautan; 12(1):12-17.

[19] Ahda dan Fitri 2016 Karakterisasi bakteri potensial pendegradasi oli bekas pada tanah bengkel di kota Padang Sainstek 8:2:98-103

[20] Brito EMS, Guyonraud R, Goni-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MAC, Wasserman JCA and Duran R 2006 Characterization of hidrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil Res Microbiol 157:752-62

[21] M’rassi AG, Bensalah F, Gury J and Duran R 2015 Isolation and characterization of different bacterial strains for bioremediation of n-alkanes, and polycyclic aromatic hydrocarbon Environ Sci Pollut Res doi:10.1007/s11356-015-4343-8

[22] Yolantika H, Periadnadi and Nurmiati 2015 Isolation of the hydrocarbon degrading bacteria in the contaminated soil of the automotive workshop. J Bio UA 4(3):153-7

[23] Rahman KSM, Thahira-Rahman J, Lakshmanaperumalsamy P, and Banat IM 2002 Toward efficient crude oil degradation by a mixed bacterial consortium. Bio Techno 85(3):257-61

[24] Capello S, Denaro R, Genovese M, Giuliano I and Yakimov MM 2007 Predominant growth of Alcanivorax during experiments on oil spill bioremediation in mesocosms. Microbiol Res 162(2):185-90

[25] Margesin R, Moertelmaiyer C and Mair J 2013 Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene, pyrene) by four actinobacterial strains. Int Biodeterio Biodeg 84:185-91.

[26] Prince RC, Lessard RR and Clark JR 2003 Bioremediation of marine oil spills Oil & Gas Tech Rev 58(4):463-8

[27] Adebusoye SA, Ilori MO, Amund OO, Teniola OD and Olatope SO 2007 Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. World J Microb Biotechnol 23:1149-59

[28] Batista SB, Mounteer AH, Amorim FR and Totola MR 2006 Isolation and characterization of biosurfactant/bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresour Technol 97:868-75

[29] Chandra S, Sharma R, Singh K and Sharma A 2013 Application of bioremediation technology in the environment contaminated with petroleum hidrocarbon. Ann Microbiol 63:417-31

[30] Abioye PO, Agamuthu P and Aziz AR 2012 Biodegradation of used motor oil in soil amended with organic wastes. Biotechnol Res Int. doi:10.1155/2012/587041

[31] Dadrasnia A and Ismail S 2015 Biosurfactant production by Bacillus salmalaya for lubricating oil solubilization and biodegradation. Int J Environ Res Public Health 12:9848-63 doi:10.3390/ijerph120809848