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Biodegradation of Used Engine Oil by *Acinetobacter junii* TBC 1.2

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Abstract

The isolates have capability to degrade used engine oil was obtained from soil in the beach contaminated with used engine oil. One of the selected isolates TBC 1.2 was identified by its 16s rDNA as Acinetobacter junii. The microorganism can use hydrocarbons in used engine oil as the sole carbon source and energy, also it significantly degraded almost all hydrocarbon compounds in used engine oil. With its ability Acinetobacter junii TBC 1.2 has a potency to be utilized for bioremediation of soil polluted with used engine oil.

Keywords : biodegradation, used engine oil, *Acenitobacter junii* TBC 1.2

Introduction

Lubricating oils are manufactured in various formulations for different applications. Most formulas generally consist of two fractions: chemicals additives and base fluid. The chemical additives, about 5 -20% (w/v), are selected compounds added for specific functions (Vazouez-Duhalt, 1989). The main component of the base oil is cyclic alkanes (c-alkanes). Long-chain hydrocarbon and c-alkanes are known to be recalcitrant to microbial degradation. The base oil contains C_{16}-C_{36} hydrocarbon, and more than 75% c-alkanes. The ring number of c-alkanes in the base oil are from 1 to 3 and any ring contains 5 or 6 members. Most of the c-alkanes in the base oil have long alkyl side chains (Koma *et al*., 2003).

After a period of usage of lubricating oil ends, used engine oil contain more metals and heavy polycyclic aromatic hydrocarbons (PAHs) that would contribute to chronic hazard including mutagenicity and carcinogenicity (Keith and Telliard, 1979). Prolong exposure and high oil concentration may cause the development of liver or kidney disease, possible damage to the bone marrow and increased risk of cancer (Mishra *et al*., 2001). In addition, PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment (Van Hamme *et al*., 2003).

Mechanical method to reduce hydrocarbon pollution is expensive and time consuming. The cheap, effective and safe method for reducing hydrocarbon pollution could possibly be done through microbial degradation. Microbes are the main degraders of petroleum hydrocarbons contaminated ecosystems (Leahy and Cowell, 1990). Bioremediation has become an alternative way of remediation of oil polluted sites, where the addition of specific microorganisms (bacteria, cyanobacteria, algae, fungi, protozoa) or enhancement of microorganisms already present can improve

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biodegradation efficiency in both in-situ or ex-situ procedures (Cookson, 1995).

Mechanism of bioremediation in principle is a decomposition process of organic material in biosphere by group of degrading microbes (heterotrophic). Heterotrophic microbe has ability to utilized organic compound, in this case petroleum as substrate. Decomposition of petroleum will yield CO_2, CH_4, water, microbe biomass, and by-product in the form of simpler compound (Leisinger, 1981). The petrophile microbe in biosphere can be isolated and purified in the form of isolate. From isolate collection can be selected strongest or specific strain for degrading hydrocarbon in used engine oil.

In general, isolation of petrophile bacteria is using enriched medium with pure hydrocarbon compound or hydrocarbon mixture such as crude oil. Location of isolation can be choose which has been long time contaminated with used engine oil. The existence of hydrocarbon in such area wills naturally resulting various type of bacteria.

This research is aimed at examining microorganism that has capability to degrade used engine oil which illegally thrown in the locations that is not addressed for used engine oil disposal.

Materials and methods

Materials

Soil sample contaminated with used engine oil was obtained from the beach in Jakarta Bay. Soil sample aseptically collected by soil sampler with 20 cm depth, wrapped with aluminum foil and brought to laboratory within 48 h, for the bacterial isolation.

Used engine oil sample for hydrocarbon sources is used engine oil of Synthetic Top One Motor Oil SAE 20W-50, FIRE SL, SJ FOR GASOLINE ENGINES from TOP 1 Oil Products Co. (California, USA) which obtained from car workshop in Tangerang region.

Isolation of Used Engine Oil Degrading Bacteria.

Engine oil degrading bacteria is growing in Bushnell-Hass (1941) media, enriched with used engine oil 10% (v/v) as a single carbon source (Mandri and Lin, 2007). Bushnell-Hass media consisted of: K_2HPO_4 1.0 g/L, KH_2PO_4 1.0 g/L, NH_4NO_3 1.0 g/L, $MgSO_4$ 0.2 g/L, $CaCl_2$ 0.02 g/L and $FeCl_3$ 0.005 g/L with pH value 7.0. One gram soil sample was added, and media incubated in shaker with speed of 170 rpm, at room temperature for 1 week.

After 1 week, 1 mL fermentation broth transferred into 9 mL enriched Bushnell-Hass media and incubated again with the same condition as mention above. After incubation for 1 week, the enriched Bushnell-Hass media was diluted with 6 dilution series each 10^{-1} fold. One mL of each fifth and sixth dilution series was taken and poured in agar plate containing Bushnell-Hass media. Surface of agar plate was poured with 100 µl used engine oil and incubated at 30°C for 3-4 days.

Isolates that growth at Bushnell-Hass media then purified by scratch technique until single colony was obtained. Pure isolate then inoculated at agar slant and incubated at 30°C for 72 h and kept at 4°C as stock culture. Media for stock culture is yeast extract media which consist of peptone 5.0 gr; yeast extract 3.0 gr; agar 1.5 % in 1000 mL distilled water.

Bacterial Gram staining.

Bacterial Gram staining is done by the method of Balow *et al*. (1992).

Identification of Used Engine Oil Degrading Bacteria.

Genomic DNA from pure culture of isolate TBC 1.2 was extracted and purified. The DNA, 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 16S rDNA sequences (500 nt) of TBC 1.2 was then analyzed its similarity using BLAST Search (http://www. ncbi.nlm. nih. gov/ blast/).

Bacterial Capability in Consuming Used Engine Oil.

In order to test the ability of bacteria in consuming used engine oil, an experiment as follows was done. Strain TBC 1.2 was precultured in 20 mL yeast extracts media and shakes at 120 rpm, at 30°C for 24 h (Aoshima, 2006). Then, 0.2 mL preculture broth transferred to 20 mL Bushnell-Hass media containing 400 mg used engine oil, and then shake at 120 rpm, 30°C. After 7 days and 14 days fermentation is terminated and added with 18 mL chloroform-methanol (3:1 v/v) for extracting the remaining used engine oil.

Mixture solvent and culture broth was mixed, and settled for 2 h at room temperature, allowing separation of solvent containing used engine oil from water. Separated water then was thrown, and solvent containing used engine oil was transferred to centrifuge tube and centrifuged at 6000 x g, for 10 min at room temperature. Precipitate was separated from solvent, and solvent was evaporated for 3 days. The remaining used engines oil was then weighted. Percentage used engine oil by microbe is calculated gravimetrically as follows:

$$\text{Consumption} = \frac{\text{original weight–final weight}}{\text{original weight}} \times 100\%$$

Gas Chromatography Analyses of Used Engine Oil Biodegradation,

Gas chromatography analyses were done by Shimadzu GC-MS QP 2010 equipped with flame ionization detector (FID) with capillary column Rtx-1MS 100% dimethyl polysiloxane (30 m x 025 m), J & W Scientific, SA, USA. Helium was used as gas carrier and speed was maintained at 154 mL/min. One µL of sample injected with temperature injection port at 280°C; oven column temperature at 50°C, pressure at 90.7 mmHg, column temperature maintained at 50°C for 3 min, then increased to 260°C for 10 min.

Results and Discussion

Isolation of Used Engine Oil Degrading Bacteria and Capability of Bacteria to Consume Used Engine Oil.

Bacteria were isolated based on its ability to grow in Bushnell-Haas media containing used engine oil as single carbon source. Out of 35 isolates growth at Bushnell-Haas media, 10 isolates showed its capability to consume used engine oil as single carbon source (Syahputra and Witono, 2009). TBC 1.2 was selected as one of the excellent isolate.

Characterization and Identification of TBC 1.2.

Microscopic observation and gram staining test indicated that strain TBC 1.2 was bacteria with rod form and classified as gram negative bacteria. Whereas result of I6S rDNA sequence showed that strain TBC 1.2 has similarity (99%) with *Acinetobacter junii*. Therefore, strain TBC 1.2 then is named as *Acinetobacter junii* TBC 1.2.

Capability of Bacteria in Consuming Used Engine Oil.

Table 1. Percentage of Used Engine Oil Consumed by A. junii TBC 1.2

Incubation Time	Weight (Gram) Used Engine Oil			% Used Engine Oil Consumption
	Original	Final	Consumption	
1 day	0.4063	0.4063	0.0000	0.00
7 days	0.4062	0.4034	0.0028	0.68
14 days	0.4060	0.3896	0.0164	4.04

Table 1, showed percentage of used engine oil consumed by isolate TBC 1.2. The tables indicated that during 7 days incubation 0.68% used engine oil was consumed; after 14 days, 4.04% used engine oil was consumed.

Gas Chromatography Analyses of Used Engine Oil Biodegradation.

Analyses of used engine oil component with gas chromatography (GC) is aimed

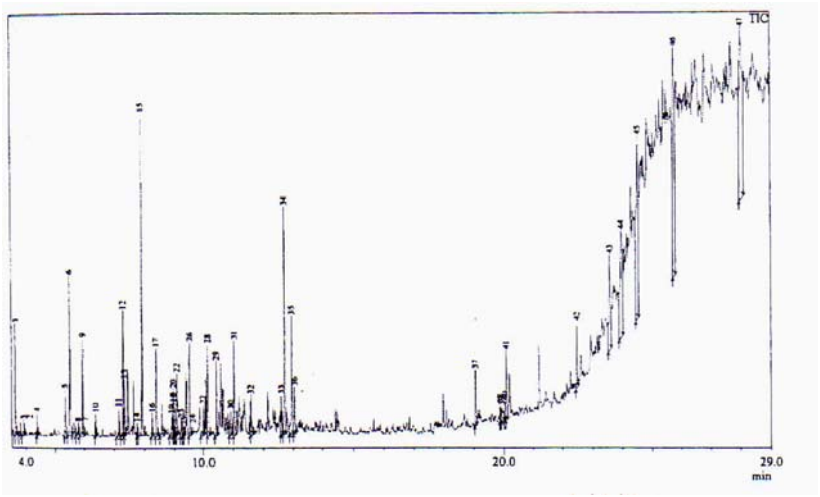

Figure 1. Gas Chromatogram of Used Engine Oil Before Biodegradation.

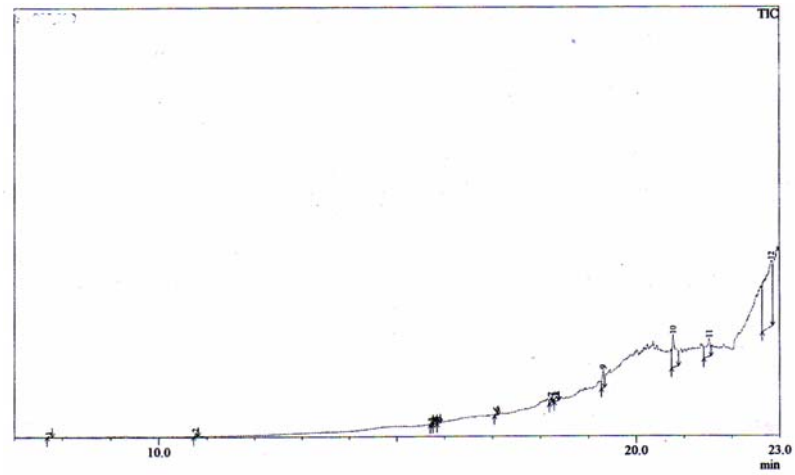

Figure 2. Gas Chromatogram of Used Engine Oil After Biodegradation.

at knowing the changing of hydrocarbon composition in used engine oil resulted by biodegradation activity of *Acinetobacter junii* TBC 1.2.

As shown in Figure 1, GC analyses of used engine oil before biodegradation indicated that used engine oil component composed 47 compounds. The compounds shown by peak number 1 to number 47 which emerged from retention time of third min until twenty-ninth min. The compounds composes short chain hydrocarbon (≤ C9), medium chain hydrocarbon (C10 - C24), and long chain hydrocarbon (≥ C25) in linear and cyclic form. The GC analysis of the component of hydrocarbon of used engine oil before biodegradation showed in Table 2. Figure 2 shows result of GC analysis of hydrocarbon component of used engine oil after 14 days biodegradation by *Acinetobacter junii* TBC 1.2.

Figure 2 showed that number of peak of hydrocarbon component after 14 days, decrease significantly compared with the first day (Figure 1). Short (≤ C9) and long (≥ C25) chain hydrocarbon which detected at first day (Figure 1) were loose after 14 days. Most all of hydrocarbon components in used engine

Table 2. Component of Used Engine Oil Before Biodegradation

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	5,519	3,85	o-Dimethylbenzene
7	5,680	0,45	5-Methylundecane
8	5,815	0,28	3-Methyloctane
9	5,960	1,78	p-Dimethylbenzene
10	6,364	0,37	n-Nonane
11	7,167	0,60	n-Prophylbenzene
12	7,314	2,25	m-Ethylmethylbenzene
13	7,351	0,97	o-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	Benzocyclopentane
19	8,925	0,50	1,4-Diethylbenzene
20	8,973	0,86	1-Methyl-3-prophylbenzene
21	9,040	0,64	2-Tolyloxyrane
22	9,093	1,05	1,4-Dimethyl-2-ethylbenzene
23	9,238	0,40	1-Methyl-2-prophylbenzene
24	9,301	0,21	5,6-Dimethylundecane
25	9.356	0,18	4-Methyldecane
26	9.546	2,11	1,2-Dimethyl-4-ethylbenzene
27	10,009	0,65	n-Undecane
28	10,145	1,75	1,2,3,5-Tetramethylbenzene
29	10,418	1,33	2-Alyltoluene
30	10,910	0,80	Isoprophylbenzaldehide
31	11,048	2,27	Naftalene
32	11,591	0,77	n-Dodecane
33	12,618	1,07	Pentamethylbenzene
34	12,732	4,77	2-Methylnaftalene
35	12,956	2,72	3-Methylnaftalene
36	13,047	1,13	n-Tridecane
37	19,071	1,15	n-Oktadecane
38	19,884	0,39	2-Methylantracene
39	19,936	0,58	1-Methylpenantrene
40	20,042	0,57	1-Methylantracene
41	20,093	1,61	n-Dodecane
42	22,483	1,57	n-Heneicosane
43	23,602	3,82	n-Eicosane
44	23,985	7,36	n-Tetracosane
45	24,541	9,41	8-Hexylpentadecane
46	25,776	12,91	Tetracontane
47	28,025	14,58	n-Tetratricontane
	Amount	100.00	

Table 3. Component of Used Engine Oil After Biodegradation

No. Peak	Retention Time (menute)	Percentage area (%)	Component
1	7,712	0,21	1,3-Cyclopentadiene, 1,2,5,5- tetrametane
2	10,764	0,49	3,5-Diisopropyl-1,2,4-tritiolane
3	15,728	0,41	2,4-Di-tert-buthylphenol
4	15,815	0,55	4-Metil-2,6-di-tert-buthylphenol
5	15,880	0,41	n-Pentadecane
6	17,073	0,46	n-Nonadecane
7	18,222	126	n-Heptadecane
8	18,329	1,43	2,6,10,14-Tetramethylpentadecane
9	19,310	4,88	n-Octadecane
10	20,770	15,49	Pentadecanoic acid
11	21,528	9,52	n-Eicosane
12	22,827	64,88	n-Nonacosane
		100,00	

oil were degraded except n-octadecane and n-eicosane (Table 3). The data indicates that *Acinetobacter junii* TBC 1.2 can degrade short and long chain hydrocarbon signi-ficantly.

According to the result of gas chromatography after 14 days biodegradation of used engine oil (Figure 2), seems the emerging of small peaks of new compound in the retention time of 7 min to 21 min which previously not detected before biodegradation. The new compounds are assumed as a result of biodegradation of high molecule weight compound which is not detected by gas chromatography or gathering of fractions resulted degradation of compounds which experienced peak area declining (Gritter, 1991).

In general, all medium chain hydrocarbons, starts from C_{10} to C_{17} were declining its abundance. Atlas (1981) explains that only a few of bacteria has ability to degrade branched or ring structure hydrocarbon because such hydrocarbon difficult enter into the cell, while Harayama (1999) explain that high molecular weigh hydrocarbon was difficult to degrade because it has low solubility so that not easy to enter cell membrane.

Besides medium chain hydrocarbon was formed, after 14 days penta decanoic acid also was formed. The forming of this organic acid is assumed caused by the breaking of the short chain hydrocarbon. According to Cookson (1995), short chain hydrocarbon is more difficultly to degrade except methane. To degrade the short chain, methane was used as primary substrate and oxidizes ethane, propane, butane as secary substrate to forms alcohol, aldehyde and carboxylic acid. Other type organic acid is not detected at day 14, this may caused by process evaporation of low organic acids.

From the description above it can be concluded that *Acinetobacter junii* TBC 1.2 was proved has potency for biodegradation of used engine oil.

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