BIODEGRADATION OF PETROLEUM HYDROCARBON BY SINGLE AND CONSORTIUM OF HYDROCARBONOCLASTIC BACTERIA FROM PETROELUM

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Submission date: 16-Jun-2023 08:42PM (UTC+0700)
Submission ID: 2117305579
File name: U_2011_BIODEGRADATION_OF_PETROLEUM_HYDROCARBON_BY_SINGLE_AND.pdf (524.67K)
Word count: 2140
Character count: 11934

ISBN 978-979-98938-5-7

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BIODEGRADATION OF PETROLEUM HYDROCARBON BY SINGLE AND CONSORTIUM OF HYDROCARBONOCLASTIC BACTERIA FROM PETROELUM POLLUTED MANGROVE AREAS

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ABSTRACT

The study on the biodegradation of petroleum hydrocarbons by a single culture and a consortium of hydrocarbonoclastic bacterial isolated from mangrove areas contaminated oil has been done. The study aims to test the ability of single bacteria isolate in degrading petroleum hydrocarbons, to characterize and identify potential bacterial isolate in degrading petroleum hydrocarbon, and to obtain a potential consortium hydrocarbonoclastic bacteria and molds in biodegrading petroleum hydrocarbons. The petroleum biodegradation test was conducted by using a single culture of 29 isolates of bacteria. Out of the 29 isolates, five isolates of bacteria that have a high degradation ability were selected. The five bacterial isolates were further characterized and identified and made into a consortium culture with a combination of 3 (three) bacteria. Petroleum biodegradation tests were conducted by using ten combinations of a consortium culture and 3 (three) replications. Observed variables are the number of bacteria and the percentage of TPH (Total Petroleum Hydrocarbon) degradation. Five of the 29 bacterial isolates tested which have a high ability in degrading petroleum are *Bacillus aminovorans* (53.17%), *Pseudomonas alcaligenes* (53.04%), *Alcaligenes faecalis* (51.54%), *Bacillus cereus* (50.68%), and *Bacillus sphaericus var rotans* (50.30%). The culture of hydrocarbonoclastic bacterial consortium which is capable of degrading oil with the highest percentage of 91.86% consists of *Alcaligenes faecalis*, *Pseudomonas alcaligenes*, and *Bacillus aminovorans*.

Key words : biodegradation, consortium hydrocarbonoclastic bacteria, mangrove areas

INTRODUCTION

Mangrove areas have multi benefits in terms of socio-economic and ecological aspects, including their physical functions as a barrier to coastal erosion, seawater intrusion barrier, windbreaker, reducer of CO_2 gas content in the air and pollutants in coastal swamp waters (Noor *et al*., 2006).

The mangrove forests in South Sumatra and Bangka Belitung cover an area of 1,966,804.94 ha, of which 437,432.10 ha (22.24%) are in damaged condition, 1,251,769.31 ha (63.64%) are severely damaged, and 277,612.53 ha (14.11%) are not damaged (Santoso, 2006). The mangrove forests quantitatively decline from year to year because of the land conversion for industrial, residential, and transportation needs. Mangrove forest area has decreased quantitatively, and qualitatively mangrove forests have also suffered environmental degradation due to pollution. This has happened, for example, in the mangrove areas in South Sumatra, especially the area of Tanjung Api-api, Sungsang, and Upang. The three areas are the areas of Watershed (DAS) of Musi and the Watershed (DAS) of Dawas which serve as the main shipping lines for transporting crude oil from oil fields of Sekayu (MUBA Regency) and Palembang oil fields. Oil pollution in the region is caused by spills and oil spills during the activities of drilling, producing, refining and transporting, permeation of the reservoir; activities of loading and unloading at the port; and the waste of tanker/vessel (Ridho *et al*., 2005). Sungsang area is an area of mangrove ecosystem with the most severe level of damage. This is

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Treatment of waste oil in the waters can be carried out by means of physical, chemical, and biological efforts. Physical and chemical methods are usually more expensive, not environmentally friendly, and the application can create new problems. Therefore, an alternative effort of treating waste oil which requires relatively cheaper cost and does not cause any negative impact on aquatic ecosystems is sought after. The treatment is handling of petroleum waste biologically. One method of biological treatment of waste oil is using bioremediation techniques. Bioremediation, the management which relies on the degradation by means of degrading microorganisms is an environmentally friendly way, which is effective, efficient, economical, and environmentally acceptable (Mangkoedihardjo, 2005 and Syakti, 2004).

Widjajanti *et al.* (2008) had found 29 hydrocarbonoclastic bacteria from petroleum contaminated mangrove area. All of the isolates can grow in oil contaminated environment and can used oil as sole carbon source, but quantitatively their ability to degrade petroleum hydrocarbon still unknown. For used as a bioremediation agent so must known their potency in petroleum hydrocarbon biodegradation.

Degrading bacteria used in treating waste oil usually have higher ability if used as a consortium culture or a mixed culture (Alpentri *et al.*, 2001 and Mukred *et al.*, 2008). Oil-degrading microbes do not work as individual species but in the form of multi-species consortium (Mangkoedihardjo, 2005). According to Sanchez (2006), the consortium is a group of microbes which are mutually beneficial to one another and carry out the process by which individual organisms can not do separately. According Alpentri *et al.* (2001) the process of biodegradation of hydrocarbons to perfection cannot possibly be done by only one type of microbe, but it is always done by a set of microbes. The rate of biodegradation of hydrocarbon compounds is more rapidly in mixed culture compared with single cultures.

The study aims to 1) test the ability of single bacteria isolate in degrading petroleum hydrocarbons, 2) characterize and identify potential bacterial isolate in degrading petroleum hydrocarbon, 3) obtain a potential consortium hydrocarbonoclastic bacteria and molds in biodegrading petroleum hydrocarbons.

MATERIALS AND METHODS

Petroleum hydrocarbon biodegradation by bacterial single culture

The bioreactor for biodegradation by bacteria is made by filling the bioreactor with a total volume of 100 ml which consists of 5% of petroleum (w/v), the source of N (KNO_3) and the source of P (K_2HPO_4) with a 10:1 ratio, 10% of bacterial inoculum with a density of 10^6 cells/mL, and Soeminarti liquid medium. The mixture was adjusted to reach pH 7 (Modification of Okoh & Trejo, 2006). Each treatment unit is agitated on a shaker with a speed of 120 rpm at room temperature for 7 days (Mukred et al., 2008).

Of the 29 bacterial isolates whose potential in degrading petroleum hydrocarbons are tested, 5 (five) isolates with the highest capability are selected. The five isolates with the highest ability will then be made into 10 combinations of bacterial consortium cultures with each consortium culture consists of 3 (three) different bacterial species and repetitions are conducted 3 (three) times.

Characterization and identification of hydrocarbonoclastic bacteria

The characterization and identification are performed on five isolates of hydrocarbonoclastic bacteria with high ability. Morphological characters of the bacteria being observed includes the growth in the plate Nutrient Agar (NA), tilt NA medium, and straight NA medium. Cell morphology being observed includes size, shape, and cell chains, the nature of Gram, and the presence/ the absence of endospores (Pikoli *et al.*, 2000). The characterization of physiological and biochemical properties covers : the hydrolysis of starch, fat, casein, and gelatin, the fermentation of glucose, sucrose, and lactose, the production of H_2S, indole, urea, and catalase, the tests of Methyl Red, Voges-Proskauer, TSI, and Simmon's citrate, and the reduction of the nitrate (Cappuccino & Sherman, 1992). Identification of the bacteria is based on the characters of each of the bacterial isolates obtained from the characterization process which is then further identified based on Holt *et al.*, (1994).

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Petroleum hydrocarbons biodegradation by 10 combinations of culture consortium

This stage begins with making a standard curve of bacteria which is prepared by following the Hadioetomo's modification method (1990), which is then followed by making of bacterial growth curves, consortium of bacteria, and bacterial culture consortium standard curves.

The Experiments are prepared by using Completely Randomized Design with a combination of treatment of the combination of 3 (three) species of bacteria of the 5 (five) species of bacteria with the highest capability, so there are 10 (ten) treatments, each of which is repeated 3 (three) times.

The bioreactor for biodegradation by bacteria is made by the same procedure in biodegradation by single bacterial but bioreactor incubated for 14 days. The variables of observations measured are the bacterial cell number and the percentage of degradation of petroleum hydrocarbons. The counting of the number of the bacteria is measured by using a spectrophotometer (at wavelength $\lambda = 620$ nm). The absorbance value is entered into the equation of mixed culture (Hadioetomo's modification, 1990).The measurement of the percentage of the degradation of petroleum hydrocarbons is done by means of gravimetric method. According Nugroho (2006), the percentage of weight loss of petroleum sludge during the testing treatment showed the percentage of degradation that occurred. Analysis of variance (ANOVA) at α level of 0.05 is performed on data obtained from the observations. If there is a significan difference, then it will proceed with a further test of Duncan's New Multiple Range Test (DNMRT) using the Statistics Program-6.

RESULT AND DISCUSSION

Petroleum hydrocarbon biodegradation by single culture bacteria

The results of the ability tests of the 29 bacterial in degrading petroleum hydrocarbons and the selection of 5 (five) species of bacterias and molds with the best capabilities are presented in Table 1.

Table 1. Percentage of petroleum hydrocarbon degradation by single culture bacteria

No.	Isolate Code	Petroleum degradation (%)	No.	Isolate Code	Petroleum degradation (%)
1.	B_{25}	53.17	16.	B_{27}	47.18
2.	B_{21}	53.04	17.	B_{18}	46.32
3.	B_{20}	51.54	18.	B_5	45.71
4.	B_{24}	51.68	19.	B_1	45.46
5.	B_{29}	51.30	20.	B_9	43.57
6.	B_{15}	50.28	21.	B_{14}	42.56
7.	B_{11}	50.25	22.	B_3	40.67
8.	B_4	50.23	23.	B_{12}	40.21
9.	B_8	50.23	24.	B_{10}	38.87
10.	B_{22}	50.21	25.	B_2	38.76
11.	B_{17}	50.16	26.	B_{13}	38.45
12.	B_{23}	50.02	27.	B_7	37.81
13.	B_{19}	49.98	28.	B_{16}	37.54
14.	B_{28}	49.78	29.	B_6	35.25
15.	B_{26}	47.73			

Characteristerization and identification 5 (five) species of bacteria

Of the 29 species of bacteria, 5 (five) species of bacteria with the highest degradation capability are used to create a mixed culture formula, namely : isolates B_{25} (*Bacillus aminovorans*), B_{21} (*Pseudomonas alcaligenes*), B_{20} (*Alcaligenes faecalis*), B_{24} (*Bacillus cereus*), B_{29} (*Bacillus sphaericus var rotans*) (Table 2). This result is in accordance with Leahy and Colwell (1990) there are 200 bacteria species that can degrade petroleum on the sea. The important genera bacteria are*Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Brevibacterium, Cornybacterium, Flavobacterium, Nocardia, Pseudomonas,* and *Vibrio.*

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Petroleum hydrocarbons biodegradation by consortium culture bacteria

The combination of a consortium of bacterial culture significantly influences both the number of bacteria and the percentage of oil degradation. The bacterial population and the percentage of oil degradation of 10 (ten) a combination of bacterial culture consortium is presented in Table 3.

Table 2. Characteristic and identity 5 (five) spesies of bacteria

Isolate character	Isolate B_{20}	Isolate B_{21}	Isolate B_{24}	IsolateB_{25}	Isolate B_{29}
Macroscopic colony morphology	*Circular, entire, umbonate, fluorescent/bi olumines cent*	*Circular, entire, convex, translucent (more fluorescent)*	*Circular, serrate, raised, rough surface, opaque, waxy*	*Circular, lobate, raised, opaque, big colony like flower*	*Circular, undulate, flat, translucent, unpigmented*
Microscopic cell morphology	Coccus, Gram negative, did not produce spore	Bacillus, gram negative, did not produce spore	Bacillus, Gram positive, produce spore	Bacillus, Gram positive, produce spore	Bacillus, Gram positive, produce spore
Motility	Motile	Non motile	Motile	Motile	Non motile
Biochemical tests					
-Starch Hydrolysis	Negative	Positive	Positive	Positive	Negative
-Lipid Hydrolysis	Positive	Negative	Negative	Negative	Negative
-Casein Hydrolysis	Positive	Negative	Positive	Positive	Negative
-Gelatine Hydrolysis	Negative	Negative	Positive	Positive	Negative
-Glucose Fermentation	Negative	Negative	Positive	Negative	Positive
-Sucrose Fermentation	Negative	Negative	Negative	Negative	Positive
-Lactose Fermentation	Negative	Negative	Negative	Negative	Negative
-H_2S production	Negative	Negative	Negative	Negative	Negative
-Indole Production	Negative	Negative	Negative	Negative	Negative
-Urease Produktion	Positive	Negative	Negative	Negative	Negative
-Catalase Production	Positive	Positive	Positive	Positive	Negative
-Metyl red test	Positive	Negative	Negative	Negative	Negative
-Voges Proskauer test	Negative	Negative	Negative	Positive	Negative
-TSI test	Negative	Positive	Negative	Positive	Positive
-Simmon's citrate test	Positive	Positive	Positive	Positive	Negative
-Nitrat reduction	Negative	Positive	Positive	Negative	Positive
CONCLUSION	*Alcaligenes faecalis*	*Pseudomonas alcaligenes*	*Bacillus cereus*	*Bacillus aminovorans*	*Bacillus sphaericus var rotans*

The combination treatment of mixed cultures of bacteria influences the number of bacteria, due to differences in bacterial activities which are mixed and allegedly due to a synergistic interaction. According Suardana *et al*., (2002), if two or more different bacteria are grown together in a single treatment medium, the metabolic activity will be quantitatively and qualitatively different from the amount of metabolic activity of each species of bacteria that are grown in the same treatment medium separately. The beneficial effect of bacteria which are mixed in a consortium is that their activities become more optimal, and this condition can help speed up the process of biodegradation of petroleum hydrocarbons. Consortium culture supports the growth of indigenous bacteria that work synergistically.

The highest average of bacterial populations is obtained from combination treatment of consortium cultures of *Alcaligenes faecalis*, *Pseudomonas alcaligenes*, and *Bacillus aminovorans*, and is significantly different from 8 other combinations, but non significantly different with combination of consortium cultures of *Pseudomonas alcaligenes*, *Bacillus aminovorans*, and *Bacillus sphaericus* var *rotans*. This is due to a synergistic association among bacteria. According to Atlas & Bartha (1998), cell growth is at its best when a combination of consortium cultures uses *Pseudomonas*. *Pseudomonas* causes an association which is synergistic in nature. The synergistic association gives the ability to the combination of the bacterial population to perform the synthesis of a product that cannot be done alone. The hydrocarbon fraction which is used by the bacteria as carbon and energy source can be derived from a fraction of the breakdown of hydrocarbons by themselves or the fraction of the breakdown of hydrocarbons by the other bacteria. According Nugroho (2006) each species of

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bacteria requires a specific substrate. Some bacteria interact beneficially with each other in the form of consortium.

The percentage of biodegradation of petroleum hydrocarbons is greater by using consortia of bacterial culture compared to using a single culture. This is because the bacteria used in degrading waste oil typically have higher ability if used as a consortium culture. Consortium cultures have a more complete profile of enzymes than a single culture. According to Mukred *et al.* (2008) hydrocarbon compounds which are lighter and simpler can be degraded earlier at an early stage by a

Table 3. The average bacterial population and the percentage of petroleum degradation of 10 (ten) combinations of consortium cultures.

No.	Consortium cultures	Bacterial population (cfu/mL)	Petroleum degradation (%)
1.	$B_{24} + B_{25} + B_{29}$	$1.9.10^7$ a	91.13 cd
2.	$B_{21} + B_{24} + B_{29}$	$2.9.10^7$ b	89.95 abcd
3.	$B_{20} + B_{21} + B_{24}$	$3.7.10^7$ c	90.04 abcd
4.	$B_{20} + B_{25} + B_{29}$	$3.9.10^7$ c	89.62 abc
5.	$B_{21} + B_{24} + B_{25}$	$5.1.10^7$ d	90.59 bcd
6.	$B_{20} + B_{24} + B_{25}$	$5.2.10^7$ de	91.52 d
7.	$B_{20} + B_{21} + B_{29}$	$5.7.10^7$ de	88.81 a
8.	$B_{20} + B_{24} + B_{29}$	$6.2.10^7$ de	89.79 abc
9.	$B_{21} + B_{25} + B_{29}$	$6.6.10^7$ ef	89.52 ab
10.	$B_{20} + B_{21} + B_{25}$	$8.1.10^7$ f	91.86 d

Notes: the numbers followed by the same small letters on the same column show no significance difference according DNMRT ($\alpha = 5\%$)
B_{20}: *Alcaligenes faecalis* B_{21}: *Pseudomonas alcaligenes*
B_{24}: *Bacillus cereus* B_{25}: *Bacillus aminovorans*
B_{29}: *Bacillus sphaericus* var *rotans*

bacterial culture, then the compounds which have not been degraded in the early stages, are degraded by the second culture and so on. According Kanaly & Harayama (1995) and Nugroho (2006) the composition of complex compounds, such as petroleum hydrocarbons cause a single species of bacteria to be unable to degrade all the components of petroleum, because each species of bacteria require a specific substrate. Some bacteria which interact mutually beneficially with one another in the form of a consortium have a highly beneficial role during the degradation process of petroleum hydrocarbons.

The combination treatment of consortium culture which produces high percentage of degradation of petroleum hydrocarbons is a combination of : *Alcaligenes faecalis, Pseudomonas alcaligenes, and Bacillus aminovorans*, ; *Alcaligenes faecalis, Bacillus cereus, and Bacillus aminovorans*, ; *Pseudomonas alcaligenes*, *Bacillus cereuss*, and *Bacillus aminovorans, Pseudomonas alcaligenes, Bacillus cereus, and Bacillus aminovorans ; Alcaligenes faecalis, Pseudomonas alcaligenes,* and *Bacillus cereus.* Sequentially the average percentages of degradation of petroleum hydrocarbons are as follows: 91.86%, 91.52%, 91.13%, 90.59%, and 90.04% (Table 3). It is allegedly due to the fact that the genus *Bacillus, Pseudomonas* and *Alcaligenes* are able to degrade petroleum hydrocarbons from low molecular weight to high molecular weight resulting in a high percentage of degradation. According to Alexander (1977) genus *Bacillus* and *Pseudomonas* are capable of degrading petroleum hydrocarbons with low molecular weight such as ethane, propane and butane, and the petroleum hydrocarbons with high molecular weight such as aromatic hydrocarbons, phenol, naphthalene and antrasena. According to Atlas & Bartha (1998), the genus of *Pseudomonas* can grow and degrade petroleum hydrocarbons chain C_{18}-C_{36}. According to Desai & Vyas (2006), the genus *Bacillus*, *Pseudomonas* and *Alcaligenes* are able to degrade saturated hydrocarbons, monoaromatic hydrocarbons, and polyaromatic hydrocarbons. According to Zhu *et al.* (2004), the genus *Pseudomonas* and *Alcaligenes* are bacterial consortium cultures which are able to degrade the components of petroleum hydrocarbons alkanes with carbon chain C_{20}-C_{25}. Presumably because of the differences in the ability of each genus in degrading petroleum hydrocarbons so that the hydrocarbon components of the low, the medium and the high molecular weight can be degraded. According to

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