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

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Abstract. Our previous study identified three species of bacteria, i.e. Alcaligenes sp., Bacillus sp1, and Bacillus sp2 isolated from using 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 a 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 the high degradation index, whereas isolates A2, A3, A5, D2, B1, B3 and isolates A4, B4, D3 have medium and low degradation index, respectively. These potential hydrocarbonoclastic bacteria need in situ characterization to know their actual activities for bioremediation.

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.*

2. Materials and Methods

Isolation of microorganisms

Waste lubricant contaminated soil was taken from four automobile or motorcycle workshops in West Sumatera i.e. *Padang*, *Pasaman Barat*, *Pesisir Selatan* 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}. The suspension was grown on 2% NA medium by a streak quadrant method for 48 hours at room temperature. Mineral salt medium (MSM) with the following composition (g/L): 1.2 g NH_4Cl; 1.6 g K_2HPO_4; 0,4 g KH_2PO_4; 0.1 g NaCl; 1 g KNO_3; 20 g $MgSO_4.7H_2O$; 10 g $CaCl_2.2H_2O$; 0.05 g $FeCl_3$, 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 was isolated as single colony and spread out on Nutrient Agar (NA) media by streak quadrant method. Single colony of each NA-containing petri dishes then were maintained 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. The degradation - potential of bacterial *in vitro* is revealed through degradation index.

$$Degradation\ index = \frac{HZ - CZ}{CZ} \times 100 \quad (1)$$

3. Results and Discussion

a. Results

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 surrounding the colony. It is known as halo area (Figure 1).

Table 1. Bacterial 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

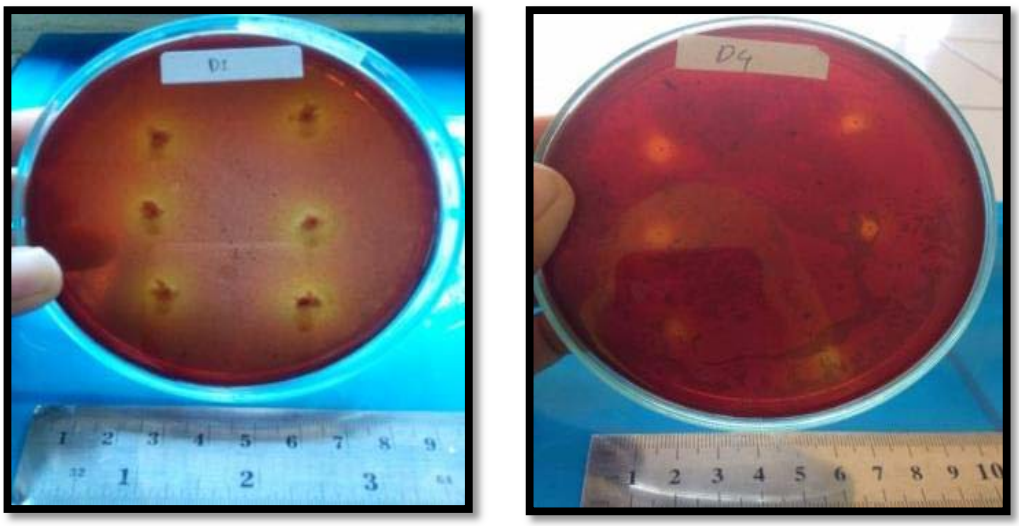

Figure 1. The representative (D1 and D4) colonies showing halo area indicated by clear circles.

Isolates that have potential to degrade 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 degradation 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 degradation index, respectively.

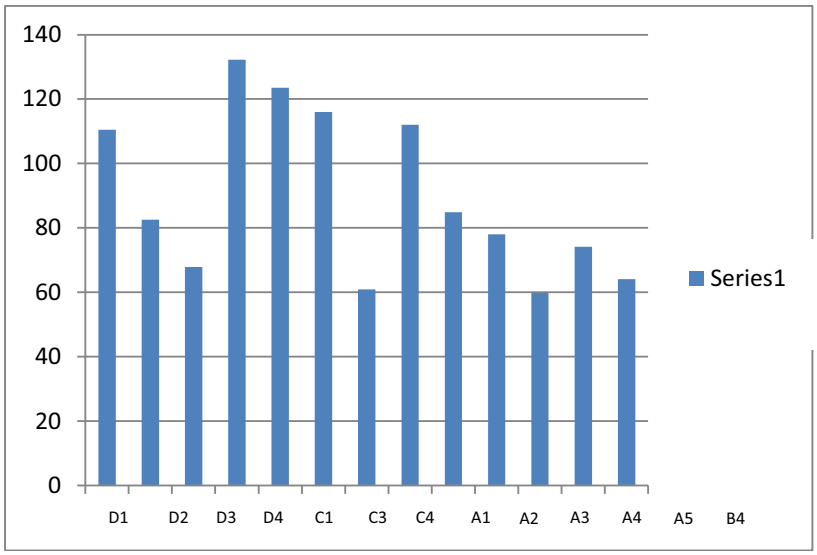

Figure 2. Mean of Degradation Index of Isolates from *Padang*, *Pasaman*, *Pesisir Selatan* and *Bukit Tinggi* workshops. D1 – D4 are isolates from *Padang*, C1 – C4 are isolates from *Pasaman*, A1 – A5 are isolates from *Bukit Tinggi*, and B4 is isolate from *Pesisir Selatan*.

b. Discussion

Bioremediation including bio-degradation in its process. Bio-degradation 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 bio-degradation 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 using 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 the 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 with its potential to degrade heavy metals.

4. 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.

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