Science, Engineering and Social Science Series
ISSN/e-ISSN: 2541 – 0369/2613 – 988X
Vol. 3, No. 3, 2019, Printed in the Indonesia

Isolation and Characteristization of Indigenic Diesel Fuel and Gasoline Bacteria from Water Quayside Teluk Bandar Lampung

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Isolation and Characterization of Solar and Gasoline Indigent Bacteria from the Waters of Teluk Bandar Lampung Pier. Lampung Province is a province surrounded by sea waters. Sea water plays an important role in people's lives, where the sea becomes an important commodity for the production of marine biota that can be consumed by the community, because of the high demand for sea transportation. The expenditure is to remove oil which is dumped into the marine environment, especially in the place where the ship's anchorage is anchored. The methodology in this research is used *Poured plate* and SPC (*Standard Plate Count*). Conclusions obtained from the Bacteria coding S2 study have potential bioremediation with the highest S2 cell count of 120 x 105 cells / ml at solar concentrations 3. Bacteria coding S4 has the potential for remediation with a cell number of 130 x 105 cells / ml at 2% gasoline concentration. Petroleum breakers bacteria come from the types of *Nitrococcus, Enterobacter*, and *Achromobacter*.

Keywords: *Bacteria, Isolation, Poured Plate,* SPC *(Standard Plate Count),* Lampung.

1. INTRODUCTION

Oil and gas are the main energy sources for several large and small industries, transportation and households. Industrial activities which include drilling, refining, production and transportation processes generally can produce oil waste and oil spills both on land and in waters. Industrial activities, both near the coast and in the offshore area, are in the spotlight of the community because of the increasing pollution caused. This can increase pollution of the marine environment. Oil waste is B3 (Toxic Hazardous Material), where if it is disposed of in the environment it can damage existing biota.

The most widely used petroleum products are diesel fuel, diesel and gasoline. Solar is composed of benzene, toluene, xylene, and various alkyls in poliaromatic hydrocarbons while gasoline or mogas (motor g asoline) is composed of a mixture of heptane and octane monomers. These compounds will cause chronic effects on mammals such as immunological, reproductive, and fetotoxic and genotoxic effects [1]. Water pollution is the entry or inclusion of living things, substances, energy and / or other components into water and / or changes in the water structure by human activities or natural processes so that the quality of water drops to a certain level which causes water to be less or unable to function according to its designation [1]. Most of the water oil waste comes from the results of several stages of the use of ships, such as the maintenance of production facilities, storage facilities, processing, and oil storage tanks on ships.

Gasoline that can be used in overcoming oil pollution is bioremediation. Bioremediation can be a biological recovery process for polluted environmental components. One method of bioremediation is biodegradation, where the decomposition process by microbial activity results in the transformation of the structure of a compound resulting in changes in molecular integrity and the toxicity of the compound decreases or

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becomes not toxic at all. There are several bacteria that are able to oxidize aliphatic hydrocarbons with the help of the enzyme monooxygenase and produce the final product in the form of acetyl Ko-A which will be catabolized through the citric acid cycle. While aromatic hydrocarbons, will be catalyzed using several enzymes including monookigenase, dioxygenase, sequential dioxygenase to form several simpler compounds including catechol or cis-cismuconate, respective. In the next stage these two compounds will be degraded to succinate, pyruvate, or acetyl Co-A so that it can be catabolized.

2. LITERATURE REVIEW

Bandar Lampung as a province surrounded by the sea and as the largest port of goods and equipment on the island of Sumatra. Marine catches in Bandar Lampung will be supplied to all regions both within the province and outside. Marine catch biota is the main consumption commodity for the people of Lampung. The cconsumption of Bandar Lampung Province's seafood is getting higher, resulting in more transportation of fishing boats.

The Gulf Pier is one of the piers that have high activity, where the pier is a place where ships dock to transport fishermen's catches. The fishermen who were transported were marine biota consumed by the people of Bandar Lampung and as the main gate for the arrival of the most marine biota, so that there were some of them being the place of auction. The high interest in public consumption towards Marine Biota, sea transportation is increasing where fishing vessels can experience oil spills that are used as fuel. Pier is one of the gates that serves the flow of passengers and goods [2].

Gasoline is generally a mixture of refineries containing paraffin, naphthenic and aromatic with varying comparisons. Today there are three types of gasoline, namely premium, pertamax, and pertamax plus. All three have different qualities or behaviors. Gasoline quality is used with the term octane number (Octane Number). Diesel oil is a clear yellow brown oil fraction that boils around 175-370 ° C and is used as diesel engine fuel. Generally, solar contains sulfur with a high enough level. The use of diesel fuel in general is for fuel on all types of diesel engines with high speed (above 1000 rpm), which can also be used as fuel in direct combustion in small kitchens which are especially desirable for clean combustion. This diesel oil is also called Gas Oil, Automotive Diesel Oil, and High Speed. The perfection of the combustion process in the engine will affect fuel consumption and pollutant content in the exhaust gas. Fuel as a basic element in the combustion process has an important role in the perfect combustion process in the combustion chamber. In this study, an experiment was carried out which was to provide a treatment of premium fuel by heating the fuel tank through a pipe mounted on upper tank radiator, so that it is expected to obtain a condition where the mixture of fuel and air is expected to be better [3].

The isolation of the Solar and Oil bacteria in this study was carried out by using a Sleek Medium with the addition of oil. This is done so that the bacterial enrichment medium is similar to field conditions so that the possibility of acquired isolates is more adaptable for people. The culture method in the laboratory will only show physiological and nutritional types that can grow in the environment provided by the laboratory, so that a particular breeding procedure will only allow a small part of the total microbial population to grow [4]. If after the incubation period the colonies are separated far enough so that they do not touch, pure colonies are obtained. The concept of pure culture is needed to be able to identify various species in a particular habitat. After obtaining pure culture, the characteristics and abilities of the organism are carried out by conducting various laboratory tests. Researchers usually require a combination of microscopic observations, growth in culture media, environmental responses, nutritional needs and physiological reactions in identifying unknown isolates [5, 6].

3. METODOLOGY

3.1 Sampling Technique

In this study, we taken the samples at Gulf Island Pier, Teluk Betung Barat market and TPI Pier (Fish Auction Place) using the random sampling method. Sampling is located in areas contaminated with diesel fuel. The representative sampling point is determined and 1 L is taken from the sampling point. Then the water sample is put into a sterile container. To keep the condition of the sample unchanged, the sample wrapped in the container is inserted into the cool box plus ice [7].

3.2 Sterilization tool

Tools made of glass are sterilized using an oven at a temperature of 180ºC for 2 hours. Previously the tool was wrapped in paper. Tools that cannot stand the heat / temperature that are too high are sterilized by using an autoclave at 121ºC, at a pressure of 2 atm for 15 minutes, while a tool made of aluminum is sterilized using a flame incandescent [8].

3.3 Salt Solution Stone Mineral medium (SMSSe)

All ingredients of Stone Mineral Salt Solution (SMSSe) are dissolved in 1000 ml of distilled water. Into the medium added 1% diesel oil (1.5 ml), 2% (3 ml) and 3% (4.5 ml) as a carbon source, set the pH to 6.5 by using 1% NaOH or HCl 1 %. Then the intermedium is inserted into the Erlenmeyer flask and sterilized by using an autoclave at a temperature of 121ºC at a pressure of 2 atm for 15 minutes.

3.4 Isolation

Each sample in the liquid Broth Nutrient medium which turned cloudy was diluted from 10-1 to 10-6 dilution by homogenizing 1 mL of the sample in the liquid Broth medium with 9 mL of physiological salt. Then from each dilution it is grown in NA medium with poured plate method and incubated 2 x 24 hours at 370C until it shows growth. The each bacterial colony observed that have grown factors with different characteristic.

3.5 Observation of bacterial colonies

Morphological observation was carried out in two ways, namely observation of Gram colonies and staining. Colony observation was carried out directly on isolates to see the appearance of colonies in the form, texture, color and elevation while Gram staining is done to look at bacterial cell forms and to find out the bacterial group of isolates produced. Observations were then continued with biochemical tests to determine the type of bacterial isolate obtained.

3.6 The Ability of Bacterial Isolate in Degradating Solar Gasoline Oil

Each of the 10-5 cell / ml purified bacterial isolates was taken 1 ml then added to the liquid SMSSe medium (150 ml) containing 1% diesel oil (1.5 ml), 2% (3 ml) and 3% (4.5 ml) separately. The culture was incubated at room temperature and shaken on a shaker at a speed of 120 rpm, then estimated bacterial density on days 1, 3, 5 and 7. The *Standard Plate Count* (SPC) method is used to count the number of bacterial colonies. The same treatment was also carried out on the control but this treatment did not use bacterial isolates.

3.7 Analysis and Presentation

Isolation Data, Capability Test, Characterization of bacterial colonies obtained were analyzed descriptively, displayed in the form of tables, graphs and images / photographs. The sample is analyzed by calculating the rate of growth of bacteria that grows onthe medium being tested. The formula to calculate determining of bacterial colonies number using the *Standard Plate Count* (SPC) method expressed in equation one and two [9]:

$$Fa = D \times NS \quad (1)$$

$$Nc = Ncc \times 1 / Fa \quad (2)$$

where, *Fa* is diluent factor, *D* is level of dilution performed, *Nc* is Number of samples tested, *Ncc* is Number of colonies in each 1 ml sample (one cup).

4. RESULT AND DISCUSSION

In order to obtain Isolation and Purification of Indigenic Bacteria in Solar and Gasoline, we analyze the calculation of gasoline bacteria. Here, we obtain three type sample water with different Isolat code (see Table 1).

Table I. Results of Isolation and Purification of Indigenic Solar and Gasoline Bacteria

Sampel Type	Total Sample	Isolat Code
Seawater Cotamined	3	S1, S2, and S4

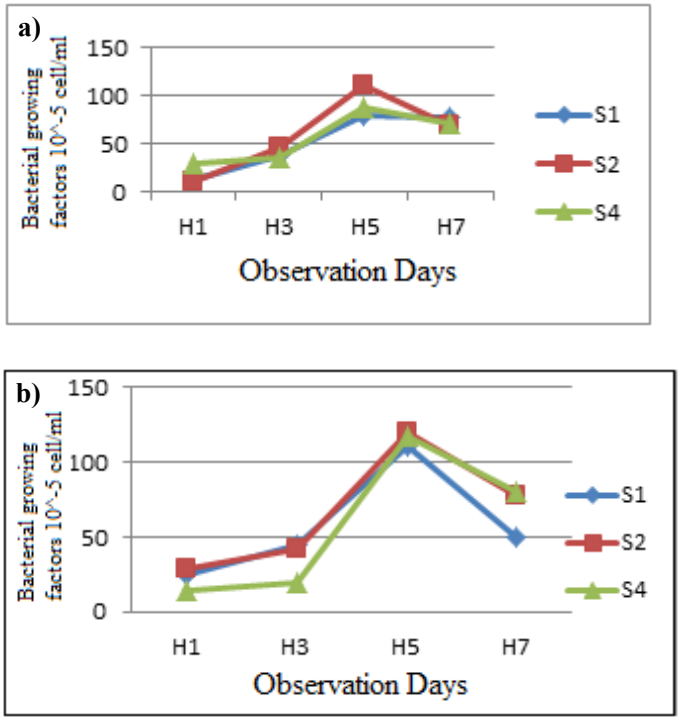

Figure 1. Growth rate of bacterial isolates at (a) 1% and (b) 2% over solar concentration 10^{-5} dilutions

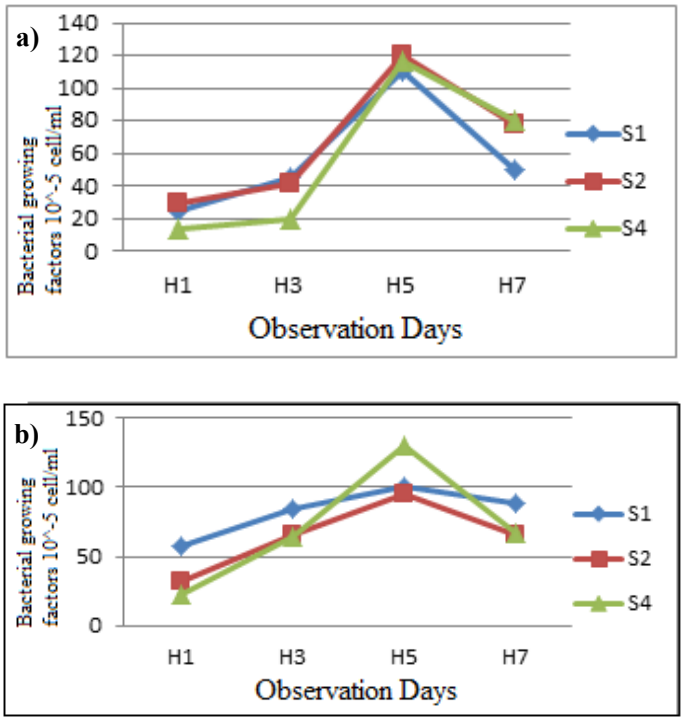

Figure 2. Growth rate of bacterial isolates at (a) 3% and (b) 1% over solar concentration 10^{-5} dilutions

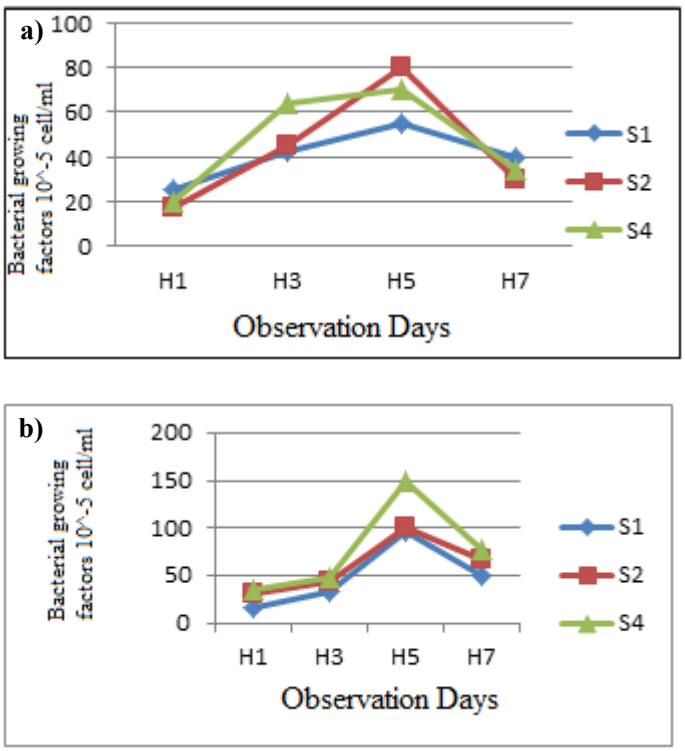

Figure 3. Growth rate of bacterial isolates at (a) 2% and (b) 3% over solar concentration 10^{-5} dilutions

The results of isolation from the two sample points tested in all dilutions have 3 different bacterial colonies for each dilution. There is diversity in homogenized samples. The bacterial isolates are able to live in several areas around Solar and Gasoline and it can be indicated that the isolates have a wide tolerance range for the presence of oil contamination that has been mixed with clay. The effect of oil contamination on microbial populations on the ground depends on the amount of concentration, the duration of contamination taking place, and also the environmental conditions presented in Table 1. The bacterial cell morphology for S1, S2 and S4 has the same cell form, namely Coccus. The gram properties of S1, S2 and S4 are gram negative with bacterial cells in red at this stage of coloring, we need coloring agents such as violet crystals, lugol, and safranin and the addition of alcohol. This gram staining is to see the difference between gram-positive and gram-negative bacteria. When bacteria is given crystal violet as the primary dye, hen both gram positive and negative bacteria will be both purple. The next stage is giving lugol solution as mordan to increase the affinity of dyes by bacteria. Violet and lugol crystals will give a strong purple so that the dye is clearer. When bacteria are given a solution of pale (alcohol), the gram negative bacteria become colorless because lipids dissolve in the bleaching solution so that the cell wall pores become large and the violet crital complex is released, while the gram positive bacteria remain purple because violet crystals are maintained and firmly attached on the cell wall. Then the addition of safranin as a secondary dye causes gram negative to be red because violet-lugol crystals have dissolved and the cell wall binds securely to safranin, while gram-positive remains purple because the cell wall binds to crystal violet so that it cannot bind safranin anymore. The isolates of enter the genus *Nitrococcus, Achromobacter, and Enterobacter* are S4 isolates. The morphological characteristics of cells are found in crocus-shaped bacterial cells, gram negative properties and do not form endospores, for the biochemical test results, the bacteria are motile (S2 and S4) with a positive catalase test (S1). Bacterial identification was carried out after data obtained from morphological characterization and physiological characterization showed that isolates obtained as many as 3 isolates were grouped into 3 different genera, namely the *Nitrococcus* genus with code S1 isolate, *Enterobacter* with code S4 and Achromobacter isolates with code isolate S2. Bacteria that are able to utilize petroleum hydrocarbons as a source of carbon and much of their energy are found in Sumatra derived from the types of *Nitrococcus, Enterobacter, and Achromobacter*[4].

5. CONCLUSION

This study is obtained from the bacterial isolation process, 3 bacteria with different characteristics are obtained. S2 bacteria have bioremediation potential with the highest S2 cell count of 120 x 10^5 cells / ml at 3% solar concentration. S4 bacteria have remediation potential with the highest cell count of 130 x 10^5 cells / ml at 2% gasoline concentration. Petroleum remodel bacteria in Sumatra come from the types of Nitrococcus, Enterobacter, and Achromobacter.

Acknowledgement

The authors wish acknowledge the financial support by the Institut Teknologi Sumatera for ones who successfully received the selection.

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Received: 30 July 2019, Accepted: 29 September 2019