

Characteristics and Growth of Microbial Consortia in Media Containing Earth Oil

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Abstract

Laboratory microcosm observation were conducted to study the effect of media culture containing oil spill in microbial growth ability . Laboratory microcosms was inoculated with oil microbial consortia isolated from oil spill sample in Cepu oil field. Based on the colony characteristic differentiation, 5 microbial types identified from the sample. At the end of enrichment period and acclimatization on the BH media containing 5% Cepu crude oil, all isolates as microbial consortia put into growth experiment media. The experiment proved that microbes could be able utilizing oil as carbon source for their cell multiplication. The results showed the more the turbidity concentration increase, the more the microbial population rise. These studies indicates that reducing oil spill utilizing in situ bioremediation technologies can be realized.

Keywords : Spill Oil, Oil Pollution, Bioremediation



A. INTRODUCTION

Petroleum is one of the main energy sources that can never be separated from human life. Apart from all the positive things that are obtained from the use of petroleum as an energy source, there are some negative things that should be taken into consideration when using petroleum in exploitation, processing, and in distribution. Therefore, the use of petroleum that does not pay attention to environmental sustainability will certainly harm humans themselves and ultimately environmental pollution will have a negative impact, especially on public health.

This phenomenon spurred efforts to overcome through various chemical, physical and biological processes to suppress the accumulation of toxic organic compounds in the environment without causing damage to the ecosystem in the future. Efforts to restore the environment polluted by hazardous toxic waste (B3) require a systematic procedure, carried out in a gradual manner and implemented with a multidisciplinary approach that is integrated from a variety of different scientific disciplines. Actually, there are already many technologies available for use in the context of efforts to restore polluted land (remediation), but the most important thing is how to choose the technology that is suitable with the type of pollutant, according to the characteristics of the polluted land, the cost required and the time factor as a limitation. One remediation technique that utilizes microbes is known as bioremediation. This is very interesting, because the process is environmentally friendly at a cost more competitive than physicochemical techniques.1)

The definition of bioremediation itself is the process of decomposing waste (pollutants) using biological agents (microbes) which are carried out under controlled conditions. The bioremediation process can occur naturally by microbes present in a polluted environment (intrinsic bioremediation). However, several things are often done to speed up the process. For example by adding microbes (exogenous microbe), nutrients, donors and or electron acceptors Bacteria known to have the ability to degrade oil include *Pseudomonas aeruginosa*, *Serratia marcescens*, *Acinetobacter baumannii*, *Baccillus megaterium*, *Baccillus cereus*, *Fussarium verticillioide*, and *Candida tropicalis*.

Bacteria found in nature are not only in the singular but mixed form. Likewise, bacteria found in areas contaminated with crude oil. Petrophilic microbes found in polluted areas of crude oil are not only in the singular but mixed form. One type of microbe that works alone will not be able to degrade various compounds found in crude oil. The use of microbial consortium tends to give better results than the use of a single isolate, because it is expected that the work of enzymes of each type of microbe can complement each other to be able to survive using the nutrient sources available in crude oil.

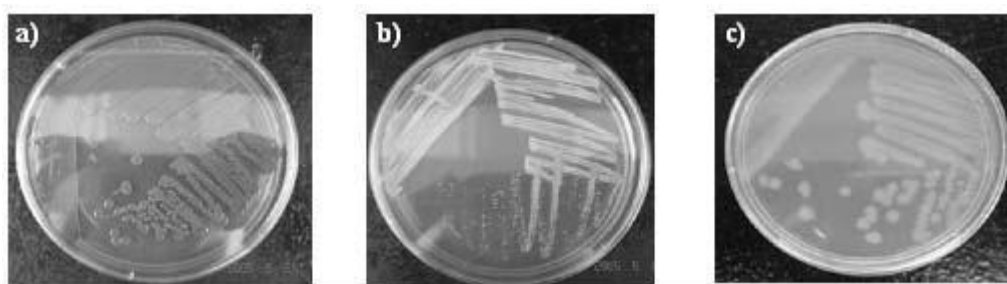


Figure 1. Hydrocarbon degrading bacteria profile.

- a) *Serratia marcescens*;
- b) *Acinetobacter baumannii*;
- c) *Pseudomonas sp.* (Malatova 2005).

This technique is based on the optimization of biological processes in reducing or even recovering from pollutants. The essence of bioremediation of land polluted by organic matter is efforts to eliminate the effects of poisons (detox) or by hydrolyzing pollutants to carbon dioxide (CO₂) and water (H₂O). Therefore, it is important to know information about the characteristics and growth of a local (indigenous) microbial consortium that has the potential to degrade various compounds found in crude oil.

B. METHOD

1. Isolate stock

The equipment used during this research was incubator, shaker, laminar chamber, glass equipment such as petri dishes, erlenmeyer, vortex, goblet, pH meter, JASCO V-530 UV / Vis Spectrophotometers, high blower aerators, analytical balance, water bath, paper, filter, oven, autoclave and magnetic stirrer. Materials used include crude oil (crude oil) from the Cepu block field, distilled water, Bushnell-Haas (BH)

media with a composition of MgSO₄ 0.2 g / L, KH₂PO₄ 1 g / L, K₂HPO₄ 1 g / L, FeCl 3.3H₂O 0.05 g / L, CaCl₂.2H₂O 0.02 g / L, yeast extract 12 g / L, NH₄NO₃ 1 g / L. In addition, agar nutrient media (NA) consisting of peptone 15 g / L, yeast extract 3 g / L, NaCl 6 g / L, glucose 1 g / L, and agar 12 g / L. The isolate used during the study was the result of isolation from crude oil samples from oil wells contained in the cepu block. Samples after being bred and acclimatized in the growth media contain 5% crude oil and visually demonstrate the ability to degrade crude oil, only then tested its ability to use crude oil as a source of nutrients for growth.

2. Analyzed Parameters

Sampling is done once every 3-5 days until the fifth and so on taking samples every once a week. Parameters analyzed include pH, number of colonies (CFU / ml) and bacterial growth. The analyzed samples were from treatment media 1 by giving microbial consortium once, treatment media 2 by giving microbial consortium 2 times, and control media. Number of Colonies and Standard Curves. The number of colonies is calculated using the standard plate count technique. A total of 100 µl of bacteria at dilution factors of 10⁻⁵, 10⁻⁶, and 10⁻⁷ were spread on NA media then incubated at 30°C for 48 hours. The number of growing colonies is then recorded and plotted with absorbance values at a wavelength of 600 nm. The standard curve is obtained from the relationship between the absorbance value and the number of colonies. Bacterial Growth (Ciawi et al.). Bacterial growth was measured by absorbance at a wavelength of 600 nm. The sample was first diluted with physiological NaCl (0.9%) to produce a absorbance value of sample.

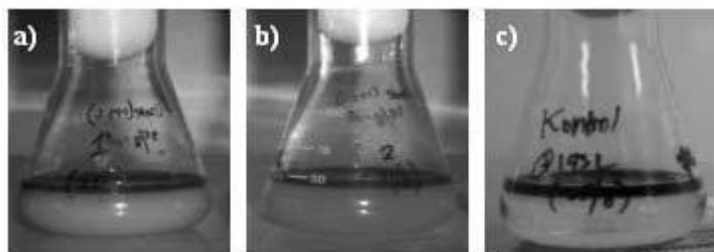


Figure 2. Cepu isolates used in the screening process, a) isolate 1; b) 2; c) control.

C. DISCUSSION

1. Microbial Characteristics

Based on the shape of the colony that appears on the agar plate, an image of several types of microbes with the following characteristics is obtained:

- Colonies are white, shiny, smooth surface, convex, flat sides.
- Colony is yellow, shiny, smooth surface, convex, flat side.
- Colony white, shiny, smooth surface, slightly concave in the middle, flat side.
- Colonies are white, not shiny, flat with the medium, flat sides, on the surface there are streaks.

- e. White, shiny colonies, uneven / jagged sides.

2. Microbial Growth Profile

To make it easier to calculate microbial growth, a standard curve was created which results from a plot of absorbance value on the number of colonies growing on the agar plate. The number of colonies growing from the second to fifth sampling was plotted against the absorbance value at a wavelength of 600 nm, so a standard curve was obtained with the equation $Y = 0,0002X + 0.3455$, where the value $R^2 = 0.713$ as an approach in obtaining the amount of microbial growth density. The bacterial growth profile until the last sampling was calculated based on the absorbance value approach at a wavelength of 600 nm. Although it is known that the absorbance value is generated from the uptake of living and dead biomass, from the standard curve obtained it can be estimated the amount of biomass that lives in the media. Bacterial growth pattern until sampling VIII or after approx



Figure 3. Cultures resulting from isolation

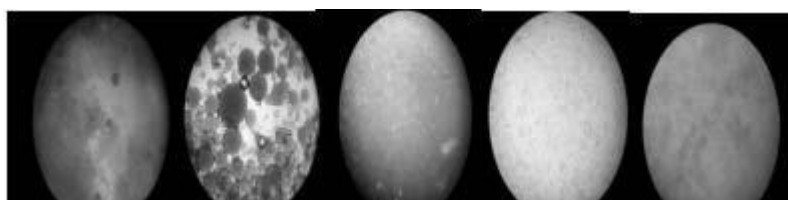


Figure 4. Performance of the five microbial cultures under a microscope

45 days are presented in Figure 5. Up to the fifth sample, parabolic chart forms were observed for all treatments, namely, reaching the highest value in sample III for the same two treatments (1x and 2x) and in sample II for blank (0). The lowest point of the graph observed in sampling V. After sampling V, it was observed that an increase in the absorbance value of each treatment. Addition of the same amount of microbes in the treatment 2x done after sampling IV. oil-media which can be utilized by bacteria as a source of carbon. The data is supported by the results of research which states that the emulsion index (IE24) value is reached maximum after incubation for 5-10 days. Then the second highest point was reached in VIII sampling for 1x and 2x treatment. With the

increasing value of the absorbance is expected as an indication of increasing microbial density increases or in other words the microbial consortium.

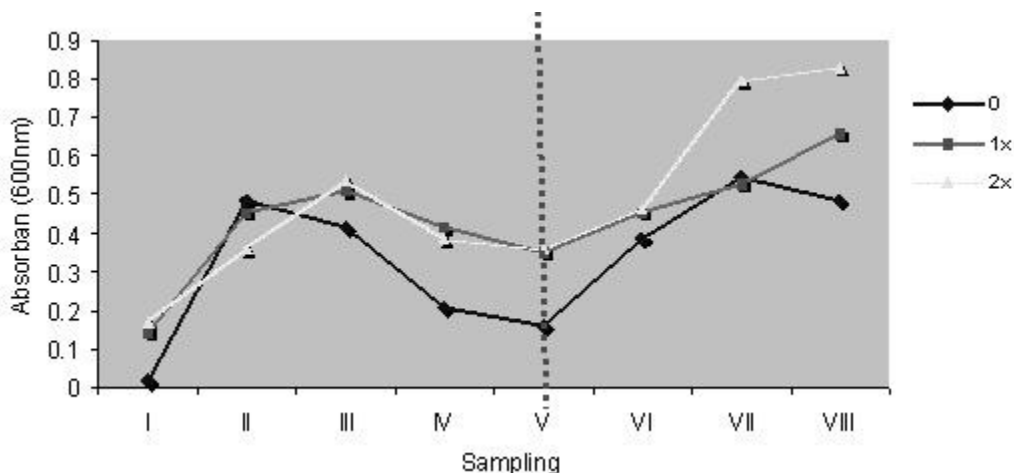


Figure 5 Profile of bacterial growth at each treatment

Bacterial growth is usually influenced by many factors, including the value of pH, temperature, nutrients, oxygen availability, and other factors⁷). From the bacterial growth profile chart (Figure 5), it was observed that bacterial growth between treatments 1x and 2x was different from the blank. The first highest point of microbial growth occurs in sampling III or after an incubation period of 9 days. The difference in growth is thought to occur due to differences in biosurfactant production produced by a bacterial consortium in each treatment. Biosurfactants play a role in the formation of emulsions has been able to use crude oil as a carbon source in doubling the cell density in the test media. Rahman et al. in his research using a consortium of five bacteria stated that the highest bacterial population was reached after 60 days of incubation. These results are obtained from the absorbance value at a wavelength of 595 nm. The incubation time during the study is around 45 days, so it can be ascertained that the number of bacteria that grows in the media is still carrying out its activities in degrading hydrocarbons. Optimal bacterial growth is one indication that the bacteria can survive in a minimal salt media (BH media) supplemented by crude oil. Good microbial growth is supported by changes in pH values during the study in the range of 7.0-8.5 considering that pH is one of the factors that influence bacterial growth. The optimal pH value is closely related to the work of bacterial enzymes, because oil degradation is carried out by a series of enzymes found in bacteria. At a pH value of around 5.5-8.0 the bacteria that degrade hydrocarbons still carry out their activities^{3, 10, 11}). Even so the optimum pH value is in the neutral and slightly alkaline pH range (slight base) 3.7) When linked to the bacterial growth profile graph (Figure 5), the highest absorbance value is reached at pH 7.6, which is VIII sampling.

D. CONCLUSION

From the results of field isolation enrichment, a consortium was obtained to be developed into a group of oil-degrading microbes. In the observation of microbial consortium growth showed a fairly good increase, although it has not been followed by measurements of oil utilization used by microbes as a carbon source.

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