Effect of Agitation to Hydrocarbon Degradation by a Hydrocarbonoclastic Bacterium isolated from Chevron Pacific Indonesia’s Waste Tank in Petapahan, Riau

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Abstract. Oxygen content is a limiting factor in the process of hydrocarbon compounds degradation by hydrocarbonoclastic bacteria. Oxygen may be supplied through agitation (stirring) during fermentation process by hydrocarbonoclastic bacteria. This study aims to to determine the optimal agitation speed for batch fermentation process by hydrocarbonoclastic bacteria isolated from the waste tank of PT Chevron Pacific Indonesia (CPI) Petapahan, Riau. This study was conducted in *Biota* laboratory*,* Universitas Andalas, West Sumatra, Indonesia. Hydrocarbonoclastic bacteria were recovered from waste samples by culturing into nutrient broth. Three different agitation speed viz. 110, 120, and 130 rpm were selected as optimization factors. The results show that the percentage of total petroleum hydrocarbon (TPH) degradation are 79.72, 87.49, and 88.35 for 110, 120, and 130 rpm, respectively. Meanwhile, chemical oxygen demand (COD) monitored during fermentation are 88.48, 90.06, and 90.16%, respectively. The agitation speed of 130 rpm is then designated as optimum factor for hydrocarbon degradation by hydrocarbonoclastic bacteria.

Keyword: *Agitation, Chemical Oxygen Demand (COD), Hydrocarbonoclastic bacteria, Total Petroleum Hydrocarbon (TPH), Optimization*

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1. Introduction

Riau is one of the provinces producing the largest petroleum natural resources in Indonesia. There are several oil and gas blocks found in the Riau region including the Minas, Siak, Duri, and Rokan block. Crude oil production in the Riau region in 2016 reached 98,892,755.91 barrels, regarded as one of national petroleum suppliers in Indonesia [1]. The largest company running petroleum production operations in Riau is PT. Chevron Pacific Indonesia (CPI), in addition to other national and private companies as production partners, e.g PT. Pertamina, Malacca Strait, Petroselat, and PT Sarana Pembangunan Riau. This petroleum processing requires many gathering station (GS) units spread in all oil production lines starting from the production block to the oil refineries in the ports located in Dumai and Sei Pakning. Gathering station functions as the first reservoir for processing crude oil before oil refineries. Petapahan GS is located in Petapahan village, Tapung district, Kampar regency which accommodates the production of petroleum from the Minas and Siak block lines. The GS as a processing unit collects the government oil supply and then process it to yield pure oil.

Oil and natural gas are primary energy source and significant economic forces in Indonesia through their utilization in manufacturing industry and other downstream processing sectors [2]. Common challenge arise from oil and gas refineries is their accidental spillages or discharges into the natural habitat, leading to environmental pollution with hazardous properties [3]. The discharge of petroleum products may cause direct and indirect effects to human and environmental health depending on the amount of spilled product and its localization with serious impacts to marine and continental ecology balance [4].

One way to manage and utilize excessive petroleum waste is through the use of biological agents or bioremediation. Bioremediation is a process of restoring (remediation) land polluted by organic and inorganic waste through microbial utilization and application. The bioremediation technique is an alternative to handling petroleum waste due to its cost-effective and eco-friendly process which results in final, stable and less-toxic compounds. However, physico-chemical characteristics during fermentation by these microbes, or the hydrocarbonoclastic bacteria must be adjusted to achieve optimum degradation and effective transformation of petroleum products [5]. Environmental factors supporting the biodegradation of petroleum include: temperature, oxygen, pH and nutrition [6].

Oxygen supply is an essential factor in enhancing biodegradation of petroleum. Oxygen is utilized for the oxidation and respiration processes of microorganisms. Most of the petroleum-degrading microorganisms belong to aerobic bacteria. Oxygen affects bacterial growth in the hydrocarbon environment through activation of multiple oxygenase enzymes involved in hydrocarbon-compounds conversion [7]. In this study, we evaluated the optimum agitation speed which yield the highest biodegradation capacity to the batch fermentation of indigenous hydrocarbonoclastic bacteria isolated from gas boot in PT. CPI, Riau province.

1. Method

Collection of indigenous hydrocarbonoclastic bacteria were previously isolated from gas boot of PT. Chevron Pacific Indonesia (CPI). Aliquots of serial dilution of petroleum samples (10-6) were spread into isolation agar medium or Nutrient Agar (NA) supplemented with 2% crude oil. Plates were incubated following daily observation until 7 days [8]. Simulation of petroleum degradation in batch fermentation was performed by inoculating bacterial strain namely IMB-2 into modified Stone Mineral Salts Solution (SMSS) medium supplemented with 2% crude oil [9]. Different agitation speed, viz. 110, 120, and 130 rpm was adjusted following incubation period for 14 days. Parameters observed during biodegradation simulation were Total Petroleum Hydrocarbon (%) and Chemical Oxygen Demand (%) from initial and final day of observation.

1. Results and Discussion

Initial isolation efforts have recovered 4 (four) bacterial strains from gas boot or waste tank of PT. CPI, Riau province [8]. The morphological characteristics of a potential isolate namely IMB-2, were identified as *Bacillus* sp. with gram positive and rod cellular shape. In this study, oxygen was supplied through agitation by orbital shaker during fermentation of crude oil. The results showed that biodegradation capacity by isolate IMB-2 increased following the intensity of agitation speed. The highest biodegradation capacity depicted through percentage of total petroleum hydrocarbon (TPH) and chemical oxygen demand (COD) was 88.35 and 90.18%, respectively in 14 days of observation (Figure 1).



Figure 1. Biodegradation capacity (%) of bacterial consortium under different agitation speed

Bioremediation of petroleum-contaminated sites may occur naturally without the enumeration of biological agent. A report from previous study, resulted in 97% of TPH percentage during 122 days of incubation in mixed contaminated soil compared to uninoculated contaminated soil with only 12% of TPH removal [10]. However in this study, the control did not show any biodegradation capacity under static condition which may indicate that *Bacillus* sp. IMB-2 is strictly aerobic to perform the petroleum degradation, also supported by the results of TPH removal following the intensity of agitation speed of 130 rpm.

Meanwhile, the chemical oxygen demand or COD value indicates the amount of organic pollutant in sample yet being used as essential parameter in biodegradation process. The COD removal by *Bacillus* sp. IMB-2 also increased following the intensity of agitation speed or 130 rpm. The biodegradation capacity may be enhanced through the co-culture of different bacterial strains in laboratory condition. *Bacillus cereus* AKG1 and AKG2, the phenol-tolerant strains, were reported to remove 34-83% of COD at their optimum growth (37oC, 120 rpm) from wastewater samples [11]. In comparison, the single isolate, IMB-2 have already shown promising result by reaching >90% of COD removal. Hence, optimization of physico-chemical characteristics for its optimum growth are worth studied since many factors may be involved in the biodegradation process of petroleum or hydrocarbon compounds.

1. Conclusion

Indigenous bacterial strain namely IMB-2 was optimized for its biodegradation capacity to petroleum or hydrocarbon compounds. Modification of agitation speed to 130 rpm in batch fermentation revealed the TPH and COD removal with the percentages of 88.35 and 90.18, respectively.

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