Degradation of Petroleum Refinery Waste by A Consortium of Hydrocarbonoclastic Bacteria Cultured on Combinations Of Medium C:N:P Ratio

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ABSTRACT

Bioremediation is an alternative method to treat petroleum waste using microorganism into nontoxic end product. The method is relatively cheap, effective, and environmental friendly. A key factor influencing bioremediation process for petroleum refinery waste treatment is C:N:P ratio of bacterial growth medium. The objective of this research was to obtain C:N:P ratio of Stone Mineral Salt Solution (SMSS) medium that allow optimal degradation of petroleum refinery waste by consortium of hydrocarbonoclastic bacteria. C:N:P ratio of SMSS medium was adjusted to ratio of 100:10:1, 100:10:0.5, 100:5:1, and 100:5:0.5. We demonstrate that optimal degradation of petroleum refinery waste by a consortium of hydrocarbonoclastic bacteria was achieved in SMSS medium with C:N:P ratio of 100:5:1. It allowed 66.55% degradation of total petroleum hydrocarbon (TPH) and 85.18 % decrease of chemical oxygen demand (COD) value.

Keywords: bioremediation, C:N:P ratio, hydrocarbonoclastic bacteria

INTRODUCTION

Bacteria that have ability to degrade hydrocarbon compound are termed as hydrocarbonoclastic bacteria [1]. Naturally, they can bind, emulsify, transport, and degrade hydrocarbon compound.The hydrocarbonoclastic bacteria degrade hydrocarbon compound by excising hydrocarbon chain to make it shorter, involving some specific enzymes. The synthesis of those enzymes is coded by bacterial chromosome or plasmid depending on the type of the bacteria [2].

The biodegradation of hydrocarbon compound originated from petroleum refinery waste is affected by biological and physicochemical factors. The biological factors cover the nature and quantity of the bacteria and availability of the bacterial enzymes. Physicochemical factors which affect bacterial degradation of hydrocarbon compound are the composition, chemical structure and concentration of hydrocarbon, temperature, oxygen availability, salinity, pH, nutrition, light, and osmotic pressure [3, 4].

Petroleum is a great source of carbon while it is deficient on nitrogen and phosphor. Such condition leads to the availability of both elements becomes the limiting factor on hydrocarbon degradation by hydrocarbon-clastic bacteria. The ratio adjustment of C:N:P by adding urea, NPK fertilizer, ammonium salt, and phosphate salt could optimize bacterial capacity on hydrocarbon degradation. Therefore, this research aimed to obtain the C:N:P ratio that support optimal degradation of petroleum refinery waste by hydrocarbonoclastic bacteria [4].

MATERIALS AND METHODS

Bacterial consortium and combination of C:N:P ratio

Petroleum refinery waste was obtained from Bengkalis, Riau Province in Indonesia. It was a black colored and flammable liquid. Ten species of hydrocarbonoclastic bacteria which were isolated previously from the same petroleum refinery waste were applied on this work as a consortium. They were *Acinetobacter baumannii*, *Alcaligenes eutrophus*, *Bacillus cereus*,*Bacillus* sp1., *Bacillus* sp2., *Flavobacterium ranchiophiia*,*Methylococcus capsulatus*, *Morococcus* sp., *Pseudomonas diminuta*, and *Xanthomonas albilineans*. To support bacterial growth, the Stone Mineral Salt Solution (SMSS) was used as a minimum medium which was consists 5.0 grams of $CaCO_3$; 2.5 grams of NH_4NO_3; 1.0 gram of $Na_2HPO_4.7H_2O$; 0.5 gram of KH_2PO_4; 0.5 gram of $MgSO_4.7H_2O$; 0.2 gram $MnCl_2.7H_2O$; and 1% yeast extract per liter. The final pH was

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adjusted to 6.5 [5]. The medium was supplemented with 50% (v/v) petroleum refinery waste as C source and combinations of NH_4NO_3 and superphosphate fertilizer (SP36)as N and P source that fall into four C:N:P ratios. The ratio combinations are 100:10:1 (100 mL petroleum refinery waste, 3.52 g NH_4NO_3, 0.34 g SP36), 100:10:0.5 (100 mL petroleum refinery waste, 3.52 g NH_4NO_3, 0.17 g SP36), 100:5:1 (100 mL petroleum refinery waste, 1.76 g NH_4NO_3, 0.34 g SP36), and 100:5:0.5 (100 mL petroleum refinery waste, 1.76 g NH_4NO_3, 0.17 g SP36) [6].

Culturing bacterial consortium in media with various C:N:P ratio

Each bacterial species was cultured separately in SMSS medium supplemented with 10% (v/v) petroleum refinery waste and incubated at room temperature with agitation 120 rpm until exponential growth reached. Afterward, they were mixed together on equal cell density and transferred, as much as 10% (v/v), to SMSS medium with four combinations of C:N:P ratio in 500 mL conical flask. The consortium were incubated at room temperature with agitation 120 rpm for seven days. Every twenty-four hours, a total plate count was performed to provide the bacterial growth curve. On the initial and last day of incubation period the TPH and COD level were evaluated.

TPH and COD analysis

Total petroleum hydrocarbon analysis of the petroleum refinery waste from each culture was performed using Gravimetric method before and after incubation period. Five milliliter petroleum sample was extracted with5 mL benzene, pentane, and diethylether (3:1:1). The petroleum was then weighed to get the amount of petroleum contained in the sample. Degradation level was detected by calculating the ratio of initial TPH subtracted by final TPH and the initial TPH [7]. Chemical oxygen demand level was detected to examine the total chemical substance concentration in the waste before and after incubation period. It was performed regarding to *Dichromate Reflux Technique Standard Method.* Twenty five milliliters sample was transferred to 500 mL conical flask. Add 1.0 gram $HgSO_4$ and 5.0 mL concentrated H_2SO_4 into the conical flask and stir-dissolved. Add 12.5 mL of 0.25 N $K_2Cr_2O_7$ slowly to get homogenous solution. During stirring process,

35 mL of concentrated H_2SO_4 was added. The solution was refluxed for 2 hours and cooled down. Aquadest was added to get the total volume of 175 mL. Then 2-3 drops of Ferroin indicator was added and titrated with FAS [Fe $(NH_4)_2(SO_4)_2$] until the color changed to brownish red. The value of COD was calculated using the formula according to Horwitz (2000) [8].

RESULTS AND DISSCUSION

The optimal C:N:P ratio for petroleum biodegradation process was around 100:10:1 [6]. Furthermore, we examined four combination of C:N:P ratio which were 100:10:1, 100:10:0.5, 100:5:1, and 100:5:0.5 in SMSS medium. Considered parameters on deciding the optimal degradation of petroleum refinery waste were bacterial growth rate, level of TPH degradation and COD reduction. Based on our result, combination of C:N:P ratio at 100:5:1 provided the highest value of TPH degradation and COD reduction which were 66.55% and 85.18%, respectively (Figure 1). Figure 2 shows bacterial growth curves between four combination of C:N:P ratio and the 100:5:1 ratio provide better exponential growth than others with the growth rate was 0.0457 cell per hour (data not shown). The result suggest that such condition is an optimal ratio of C:N:P elements for hydrocarbonoclastic bacteria to grow and degrade petroleum optimally. This correspond to Leahy and Colwell (1990)[4] which stated that appropriate comparison of nutrients, especially nitrogen and phosphor are main critical factors to improve petroleum degradation level. Addition of nitrogen and phosphor can increase the respiration rate of bacterial cell. It amplified the amount of energy production which lead to improvement of hydrocarbon degradation. Besides, C:N:P ratio is one of limiting factors in bioremediation process [9]. The original comparison of the ratio was very dependent on the waste characteristics, so an appropriate comparison of nutrients will give an optimal bioremediation result.

The study showed that C:N ratio at 100:5 has better bacterial growth and biodegradation result compared to that at 100:10. Metabolism of high nitrogen concentration resulted in high nitrite formation. It was a toxic compound that disturbed the bacterial growth and the biodegradation process. Hydrocarbon degradation and bacterial growth were stimulated by nitrogen addition [10]. Nitrogen compound in a significant amount can suppress

bacterial growth. A suitable nitrogen level was necessary to avoid the present of toxic compounds [11].
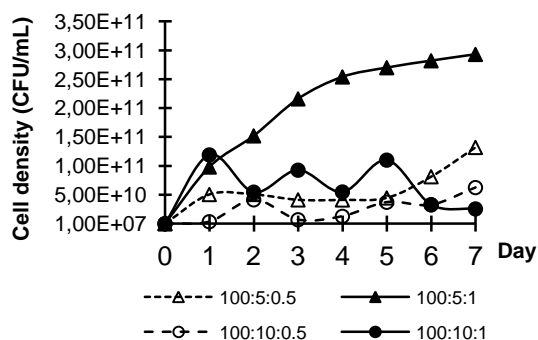

Figure 1. A growth curve of bacterial consortium in SMSS media supplemented with 50% petroleum refinery waste at combinations of C:N:P ratio.
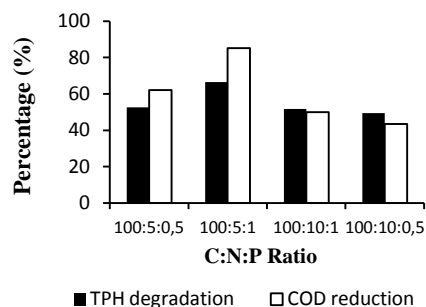

Figure 2. TPH degradation and COD reduction level by bacterial consortium in SMSS media supplemented with 50% petroleum waste at combinations of C:N:P ratio.

In Figure 2, it indicates that C:N:P ratio at 100:5:1 supported degradation of petroleum refinery waste and reduction of COD level significantly compared to other ratios. A balanced C:N:P ratio will allow optimal bacterial growth and degradation of hydrocarbon and non-hydrocarbon compound resulting in reduction of COD level. Nitrogen is necessary for protein synthesis, while phosphor source is necessary for DNA, RNA, and ATP synthesis [12, 13]. Protein, DNA, RNA, and ATP are important molecules in cells. If those molecules are present in optimal amount, bacterial growth and ability to degrade petroleum waste will improve. An adequate C:N:P ratio could increase the biosurfactant production. The presence of biosurfactant could increase petroleum waste solubility in water and widen the contact between bacteria and petroleum waste. It resulted in improving the biodegradation process. According to Kinbal (1994)[14], surfactant serve to widen the contact

surface between hydrocarbon and bacteria through micelle formation thus improve hydrocarbon solubilization and emulsification.

CONCLUSIONS

Based on the study, we conclude that the ratio of C:N:P at 100:5:1 was optimal condition for bacterial consortium to degrade Bengkalis petroleum refinery waste. The condition resulted in 66.55% decrease of TPH level, 85.18% reduction of COD level with growth rate of hydrocarbonoclastic bacterial consortium was 0.0457 cell per hour.

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