

Biodegradation of Phenolic Hydrocarbon Waste Using Bacterial Consortium from Cattle Rumen

Ibnu Maulana, Heri Hermansyah, Arina Shallyta, and Hanif Yuliani

Abstract—Waste water in Indonesia especially from industries contains phenol around 16 ppm which is higher than regulated standard quality of phenol in waste water (2 ppm). Phenol is a toxic aromatic hydrocarbon which is dangerous and difficult to degrade. Therefore, we conduct a study about biodegradation process of hydrocarbon (phenol) liquid waste using bacterial consortium from cattle rumen. In this paper, the researchers discussed how to produce, effect of incubation medium, and identification of bacterial consortium which is isolated from the cattle rumen and petroleum sludge, both solid and liquid form. Based on gram-staining analysis, cattle rumen bacteria are gram-positive and gram-negative bacteria. Phenol biodegradation using cattle rumen bacterial consortium, the obtained k value sure 0.174; 1.125; 1,527; 0.007 and 0.116, which are based on the concentration of phenol of 12, 24, 48, 72 and 120. Similarly, for petroleum sludge, the obtained k values respectively are 0.212; 1.029; 1.26; 1.74 and 2.196.

Index Terms—Bacterial consortium, cattle rumen, phenol biodegradation.

I. INTRODUCTION

Indonesia, is one of petroleum producer country, has problem in treating distillation process waste water. Petroleum industry has an important role in Indonesia because of abundant petroleum resources make Indonesia become an attractive investment destination in the petroleum sector. However, processing of waste coming from the petroleum industry has not been much so that waste from the petroleum industry to pollute the waters especially phenol exceeds a specified limit. Lack of waste treatment leads to accumulation of waste petroleum industry in particular phenol were quite high in the polluted waters [1]. The phenol content in wastewater is around 10-50 ppm [2]. This concentration is much higher than the maximum allowable limit (2 ppm) as specified based on Regulation of the Environment Ministry by the standard quality of waste water exploration and production activities of oil and gas from onshore facilities type of waste produced water

Phenol is a toxic aromatic hydrocarbon which can reduce enzymatic activity, dangerous for health, cause death of animals in the water (phenol content ranging from 5-15 mg/l can kill fish) and increase the growth of harmful aquatic plants [3]. In another research, once wastewater containing phenolic compounds is discharged into a receiving body of water, it endangers for fish live even at a relatively low

concentration (5-25 mg/l) [4]. Existing phenol degradation is often inefficient, due to self-inhibition on high concentration, accumulation of intermediates in the metabolism of phenol, and the release of soluble microbial products [5], [6]. There are some methods to treat the phenolic hydrocarbon waste and one of treatment method is biodegradation. Biodegradation is treatment method using microorganism to decrease organic and toxic substance. Principally, microbial biodegradation release into the environment is contaminated, increasing the ability of indigenous microbes and the use of microbes in special reactors [7]. Many microorganisms that can be used as bioremediation agents are bacteria, fungi, and algae with certain species as bioremediation agents. However, single microorganisms are not able to lose all of the compounds from the waste mixture so that the required form of bacterial consortium that not only has a broad substrate specificity, but also the degradation in achieved in cooxidation and commensalism systems. In recent years, study about capability of bacterial consortium as microorganism from petroleum sludge and lubricant for phenolic hydrocarbon waste biodegradation are conducted [8], [9] and studies on the ability of the bacterial consortium to degrade hydrocarbons from waste and petroleum products. like petroleum sludge, lubricating oil, and turbine oil. However, no studies related to the use of cattle rumen bacterial consortium as phenol waste bioremediation agent. Bacterial consortium live in anaerobic conditions make bioremediation process is advantageous economically because aeration process is not required to meet the needs of oxygen in the process of biodegradation. In addition, bacterial consortium can be compared with the bacterial consortium isolation results that have been there for the degradation of petroleum sludge so that the results can be seen whether the bacteria consortium derived from cattle rumen can compete with the bacterial consortium which has no isolation results for the degradation of petroleum sludge to reduce phenol in laboratory scale. Now, in this research, we conduct a study about biodegradation of phenolic hydrocarbon waste using bacterial consortium from cattle rumen because bacterial in cattle rumen usually work at anaerob condition and appropriate to be implemented for anaerobic biodegradation for phenolic hydrocarbon waste. Objectives of this research are to produce bacterial consortium by isolating and culturing microbial consortium from cattle rumen. Then, characteristics bacterial consortium from cattle rumen will be compared with bacterial consortium from petroleum sludge and will be used for phenol biodegradation.

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II. MATERIALS AND METHODS

A. Materials

The materials used in this study are cattle rumen, petroleum sludge, Na₂HPO₄, KH₂PO₄, NH₄Cl, NaCl, phenol, nutrient broth, nutrient gelatin, yeast extract, blood, violet crystal, iodine solution, 95% alcohol, acetone (3:1), concentrated NH₄OH, distilled water, 4 amino antipirin solution, K₃Fe(CN)₆ solution, and carrageenan.

B. Methods

1) Bacteria isolation

Bacteria from petroleum sludge and cattle rumen mucus which fermented for 3 days has been inoculated in 3 kinds of medium: yeast extract, nutrient agar, and blood. Then, the bacteria of each inoculation were isolated in agar medium which added with synthetic phenol to obtained pure culture of bacterial consortium.

2) Bacteria identification

Identification of bacteria are colony form, gram staining, and bacterial growth. The colony form and gram staining identification refer to *Bergey's Manual of Determinative Bacteriology* [10]. Bacterial growth was determined by optical density of medium using UV-VIS spectrophotometer 600 nm.

3) Phenol biodegradation

Bacteria from cattle rumen were dissolved in 100 ml NPK medium (6% wt Na₂HPO₄, 3% wt KH₂PO₄, 1% wt NH₄Cl, 0.5% wt NaCl dissolved in distilled water). Then, various concentration of phenol was added to medium and shaken at 25-30°C (room temperature), 90 rpm, for 16 h. Sampling for activity test every 2 h during 16 h.

4) Activity test

The activity of bacteria for phenol biodegradation was determined by calculate difference of phenol concentration in initial medium and sample medium. The phenol concentration in medium was determined according 4-aminoantipirin method using UV-VIS spectrophotometer

510 nm (SNI 06-6989.21-2004). 5 ml sample was incubated with NH₄OH, buffer phosphate, 4 aminoantipirin 2% solution, and K₃Fe(CN)₆ 8% before measured by spectrophotometer.

5) Bacterial immobilization

6 g carrageenan was dissolved in 200 ml distilled water and sterilized by autoclave. Then, 2 ml of bacteria-nutrient both solution was added to 100 ml carrageenan solution and shaking for 5 minutes. KCl 3 M was slowly dropped to the solution and incubated for 24 h, then separated by filtration.

III. RESULT AND DISCUSSIONS

A. Cattle Rumen Bacteria Identification

After the test morphology and physiology, isolates - isolated bacteria species were identified through the identification table of *Bergey's manual* [10], it is obtained that in cattle rumen, the bacteria are gram-positive, regular rod-shaped, irregular rod-shaped and irregular spherical-shaped, while the gram negative bacteria have regular rod-shaped and spherical-shaped. These results are similar to the results obtained from the bacteria isolated from petroleum sludge.

The results of the bacterial isolation from a variety of media, that is from on isolate from cattle rumen and petroleum sludge could be gram-positive or gram-negative, and have rod, irregular rod, and spherical shaped as shown at table 1 below. The results of the bacterial isolation from petroleum sludge are almost the same as the results of the isolation from cattle rumen, yet for blood medium, there are a lot of bacteria that are suspected to belong to the genus of *Bacillus sp.* as much as 55% and as much as 45% of the bacteria are gram-negative spherical-shaped bacteria. *Bacillus sp.* have ability to live in many medium at extreme condition. Furthermore, yeast extract medium contain more nutrition that bacterial needed than nutrient broth and blood medium.

TABLE I : BACTERIAL IDENTIFICATION RESULTS

	Cattle Rumen			Petroleum Sludge		
	Yeast Extract	Nutrient Broth	Blood	Yeast Extract	Nutrient Broth	Blood
Gram-Positive, Rod-Shaped	20%	25%	45%	20%	25%	55%
Gram-Positive, Irregular Rod-Shaped	20%	20%	20%	20%	20%	-
Gram-Negative, Rod-Shaped	20%	15%	-	20%	15%	-
Gram-Positive, Spherical-Shaped	20%	15%	-	20%	15%	-
Gram-Negative, Spherical Shaped	20%	25%	35%	20%	25%	45%

B. Bacteria Consortium Growth

To obtain ideal biodegradation, bacterial growth factors must be optimal, such as substrate concentration, pH, and level of nutrition. In Fig. 1 and 2 below, shown bacteria consortium growth from cattle rumen and petroleum sludge for various phenol concentration. In Fig 1, the bacteria growth is increase along of time, it means that bacteria is grown. Furthermore, the decreasing line in Fig. 1 means that bacteria are in a retardation phase. As known before, there is sixth phase of bacteria growth. First, lag phase when bacteria is adapt with its environment. Second, acceleration phase when bacteria is grown fast. Third, exponential phase when bacteria growth rate is constant. Fourth, retardation phase

when bacteria growth rate is decreasing. Fifth, stationer phase when there is no bacteria growth rate. Last, death phase when bacteria growth rate is negative. In Fig. 2, growth of bacteria from petroleum sludge for phenol concentration 72 and 120 ppm still in acceleration phase.

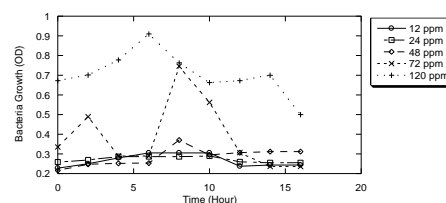


Fig. 1. Growth of bacteria consortium from cattle rumen for various phenol concentration.

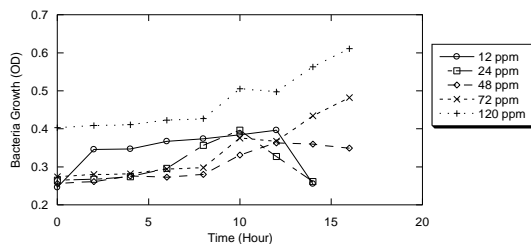


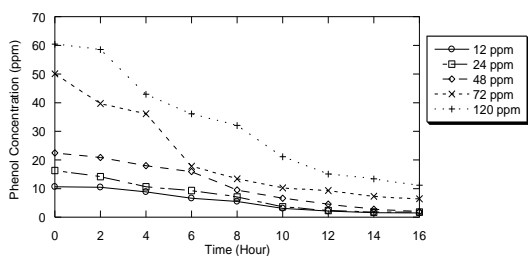
Fig. 2. Growth of bacteria consortium from petroleum sludge for various phenol concentration.

C. Biodegradation Phenol Using Cattle Rumen Bacteria

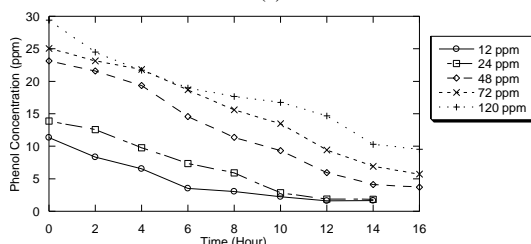
The results of bacteria isolation, from both the cattle rumen and petroleum sludge, are able to degrade phenol up to 120 ppm. The activity of bacterial consortium from cattle rumen and petroleum sludge to phenol degradation with various concentration are shown in Fig. 3. From concentration data every 2 h during 16 h, we can calculate degradation rate (k) with equation in table 2. Phenol biodegradation using cattle rumen bacterial consortium, the obtained k value sure 0.174; 1.125; 1,527; 0.007 and 0.116, which are based on the concentration of phenol of 12, 24, 48, 72 and 120. Similarly, for petroleum sludge at the same concentrations, the obtained k values respectively are 0.212; 1.029; 1.26; 1.74 and 2.196.

TABLE II: KINETICS MODEL FOR BIODEGRADATION [11]

Substrate	Equation
PAHs	Order 0
	$[C]_t = -kt + [C]_0$
	Order 1
	$\ln[C]_t = -kt + \ln[C]_0$
	Order 2
	$\frac{1}{[C]_t} = -kt + \frac{1}{[C]_0}$



(a)



(b)

Fig. 3. Results of biodegradation test using bacterial consortium from cattle rumen (a) and petroleum sludge (b).

D. Biodegradation Phenol Using Cattle Rumen Bacteria

The result of bacterial consortia production in liquid medium is 1.5 liter within one batch; while the immobilization result in one batch is 50 grams. We produce bacteria which have move to liquid medium of about 1.5 liters in one batch in accordance with the procedure. Cell immobilization is done to obtain longer life and keep its catalytic ability.

IV. CONCLUSION

The identification of bacteria from cattle rumen is similar with bacteria from petroleum sludge in yeast extract and nutrient broth medium. It contain gram-positive rod-shaped, gram-positive irregular rod-shaped, gram-negative rod-shaped, gram-positive spherical-shaped, and gram-negative spherical shaped of bacteria. In medium and nutrient broth yeast extract. Bacterial consortium in the cattle rumen and bacterial consortium on petroleum sludge have same bacterial composition. They are gram-positive rod-shaped, gram-positive irregular rod-shaped, gram-negative rod-shaped, gram-positive spherical-shaped, and gram-negative spherical-shaped by 20% for yeast extract. Gram-positive rod shape and gram-negative spherical-shaped by 25%, gram-positive irregular rod-shaped by 20%, gram-negative rod-shaped and gram-positive spherical-shaped by 15% for nutrient broth. While for the blood medium, both in cattle rumen and petroleum sludge. have a varied bacterial composition. Bacterial consortium type of gram-positive rod-shaped and gram-negative spherical-shape in cattle rumen have lower bacterial composition than petroleum sludge. Gram-positive rod-shape in the cattle rumen by 45% and 55% in petroleum sludge. Gram-negative spherical-shaped in the cattle rumen by 35% and 55% in petroleum sludge. But, gram-positive irregular rod-shaped identified in cattle rumen only in blood medium with 20%. Gram-negative rod-shaped and gram-positive spherical-shaped not identified in the two types of bacterial consortium either in cattle rumen and petroleum sludge with blood medium.

From the experiment, bacteria in cattle rumen and petroleum sludge both can live in maximum 120 ppm of phenol concentration. The kinetics result of synthesis of phenol biodegradation is shown with k value for cattle rumen bacterial consortium and petroleum sludge for phenol concentration of 12, 24, 48, 72, and 120 ppm. The k value for phenol biodegradation using cattle rumen bacterial are 0.174; 1.125; 1,527; 0.007 and 0.116, while k value for phenol biodegradation using petroleum sludge are 0.212; 1.029; 1.26; 1.74 and 2.196. From this result, cattle rumen bacterial as good as petroleum sludge to decomposed phenol. Besides that, the cell immobilization result in 50 grams of immobilized cell to improve its catalytic ability. This bacterial consortium is potential to scale up and applied at wastewater treatment in production of petroleum gas, because it can decomposed phenol 48 ppm to less than 2 ppm in 16 hours.

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