ROLE OF NUTRIENT AND BACTERIA IN REDUCTION OF OIL IN BIOREMEDIATION OF WASTEWATER FROM OIL REFINERY INDUSTRY

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Abstract

Research concerned with role of nutrient and bacteria as biological agent in reducing oil concentration in waste water of oil refinery industry has been done. The aim of research was to investigate the effect of nutrient addition such as KNO\textsubscript{3} and K\textsubscript{2}HPO\textsubscript{4} as well as, bacteria as biological agent in reducing oil concentration in the waste water of oil refinery industry. The research was conducted by adding KNO\textsubscript{3} and K\textsubscript{2}HPO\textsubscript{4} nutrient with various concentration in the waste water. Bacteria were inoculated into the waste water and it was incubated for 15 days. Bacterial population and concentration of oil after treatment were analyzed. Data analysis was treated using Analysis of Variance at significant level of 5\%. Result of the research showed that addition of KNO\textsubscript{3}, K\textsubscript{2}HPO\textsubscript{4}, and bacteria inoculum’s influenced the bacteria population and concentration of oil at the end of treatment of bioremediation process.

Key words : Nutrient, Bacteria, Bioremediation, Wastewater, Refinery Industry

INTRODUCTION

Oil industry up to now still becomes important industry at energy field. The industry is divided into two parts, that is upstream and downstream industry. The upstream oil industry conducts exploration to produce crude oil. It is transferred to downstream oil industry as known as refinery plant to treat the crude oil into various fuels.

In spite of oil refinery industry produces petroleum products, it also produces waste such as wastewater. The wastewater discharged from the oil refinery still contains residual oil. The residual oil still contains hydrocarbon that could achieve 15\%. The residual oil in the wastewater can cover the surface water and it can prevent oxygen diffusion into the surface water so that it can disturb aqua biotic in the water body.

One of efforts to reduce impact resulted by the residual oil is through bioremediation before the wastewater discharged to the environment. Nutrient is required to reduce the residual oil
in the wastewater. One of nutrient added in the waste water is source of N and P. The compounds added as source of N and P are KNO$_3$ and K$_2$HPO$_4$ (Munawar, 2000).

Besides addition of nutrient, biological agent with hydrocarbonoclastic property is also added in the wastewater. Addition of isolate of indigenous bacteria in bioremediation process of oily contaminated soil can reduce concentration of hydrocarbon in terms of Total Petroleum Hydrocarbon (TPH) along the bioremediation process (Munawar, 2001)

The purpose of the research is to acknowledge the effect of nutrient of KNO$_3$ and K$_2$HPO$_4$ as source of N and P in bioremediation process to oil concentration in the wastewater and to acknowledge the effect of addition of hydrocarbonoclastic bacteria inoculum to the oil concentration and bacterial population after bioremediation process.

**MATERIALS AND METHODS**

*Experimental setup and laboratory conditions*

The experimental design consisted of twenty seven treatments with three factors: (1) concentration of KNO$_3$ as nitrogenous sources (0%, 5%, and 10%), (2) concentration of K$_2$HPO$_4$ as phosphorus sources (0%, 0.5%, and 1%), and (3) concentration of inoculants (0%, 5%, and 10%). Each of these treatments was replicated two times. A total of 54 experimental units were placed in a completely randomized design in the laboratory condition (Modified Steel, and Torrie. 1981). For each experimental unit, a total of 100 ml of wastewater contained 15% oil was added to flash and incubated at room temperature with shaker 100 rpm during 15 days.

*Measurement of Oil Concentration and Population of Bacteria*

Measurement of oil concentration was conducted using gravimetric method, so that the oil content can be determined in percent unit (Modified Neumann, et. all. 1981), while bacterial population was measured with method of Total Plate Count (TPC), that is method to calculate life bacterial so that the amount of bacterial population can be known in the wastewater in unit of cfu/ml (Colome, et. all., 1986).

*Statistical Analysis*

Concentration of oil and bacterial population was analyzed using analysis of variety, If the analysis showed the significant results, it was continued with Duncan test. All tests were conducted at level of significance of 0.05. (Steel and Torrie, 1981)
RESULTS AND DISCUSSION

The effect of each KNO₃ on oil residual and population of bacteria

The effect of addition of nutrient of KNO₃ to oil concentration and bacterial population in bioremediation process held for 15 days was shown at Figure 1. Addition of KNO₃ reduced the oil content, while it had effect in increasing the bacterial population. Reduction of the oil content due to addition of KNO₃ was 21% to 23%, while the increase of population of bacteria was 47% to 54%.

As shown in Figure 1, higher concentration of KNO₃ had tendency to reduce more oil concentration and to increase the bacterial population. The phenomena can be explained that addition of KNO₃ in bioremediation process is optimization process by adding N source as nutrient. With addition of N, the required N for metabolism process and bacterial growth increase, therefore the population of bacteria in bioremediation process increase. The condition of high bacterial population will increase the bioremediation performance so that oil content at the end of bioremediation process reduces. It was suitable with opinion of Wrenn et. al. (1994) saying that N was one of necessary nutrient for bacteria to degrade hydrocarbon.

Figure 1. Graph show effect of nutrient addition (KNO₃) on oil residual and Population of bacteria in medium contaminated with crude oil
Bacteria requires N for metabolism of protein. It is divided into two groups, that is structural protein that has function to arrange the cell component, and functional protein that has role in degrading substrate such as hydrocarbon in the oil as source of carbon and energy. If the requirement of N for bacteria is fulfilled, enzyme synthesis for degradation of hydrocarbon also increases. The degradation activity increases so that the hydrocarbon concentration will reduce along the bioremediation process.

*The effect of each K$_2$HPO$_4$ on oil residual and population of bacteria*

The effect of nutrient addition in terms of K$_2$HPO$_4$ to oil concentration and population of bacteria along 15 day bioremediation process was shown in Figure 2. The effect was similar to that of addition of KNO$_3$ to the oil content, that is to reduce the oil content and to increase the population of bacteria. The reduction of oil content due to addition of K$_2$HPO$_4$ was from 17% to 24%, and the increase of population bacteria was 38% to 48%.

Figure 2 also showed similar tendency with the effect of KNO$_3$, that is higher concentration of K$_2$HPO$_4$, the oil content was less, and population of bacteria increased. It can be explained that K$_2$HPO$_4$ is nutrient required by bacteria for metabolism and growth. The compound provides source of P for the bacteria. With addition of P, requirement of P for metabolism and bacteria growth increase. The condition will increase population of bacteria in bioremediation process. High population of bacteria will increase the performance of bioremediation so that the oil content at the end of bioremediation process decreases.

![Figure 2: Graph show effect of nutrient addition (K$_2$HPO$_4$) on oil residual and Population of bacteria in medium contaminated with crude oil](image-url)
Utilization of P for bacteria is in the form of phosphate group for arranging DNA or RNA. One of arranging DNA was phosphate group, DNA will be replicated when cell growth, while RNA will be transcription at process of expressing gen including gen being responsible for hydrocarbon breakup along metabolism of hydrocarbon. Therefore P is required by bacteria along its growth and enzyme production for degradation process of hydrocarbon. According to Margesin and Schinner, (2001), the fulfillment of nutrient as source of P can be conducted by biostimulation, that is by adding nutrient along the bioremediation process.

**The effect of each Bacteria inoculum on oil residual and population of bacteria**

The effect of addition of bacteria inoculum to oil concentration and bacterial population is shown in Figure 3. Reduction of oil content due to addition of bacteria ranges from 25% to 37%. The reduction of oil content was explained by Whittenbury, (1971). Oil contained hydrocarbon compounds utilized by bacteria as substrate for carbon and energy sources.

High concentration of bacteria inoculum provided will increase bacterial population. Innoculum provided in the bioremediation process is initial population of bacteria. If it provided in great number, its growth rate will be faster so that bacterial population in the bioremediation is high.

![Figure 3. Graph show effect of bacteria inoculum on oil residual and Population of bacteria in medium contaminated with crude oil](image-url)
According to Munawar (2000), bacteria in bioremediation process is an important component determining the success of bioremediation process. Bacteria as biological agents are equivalent to engines in industry that change substrates into products. In the process of bioremediation, bacteria act as the engines to degrade pollutants to be compounds with less toxicity.

**The effect of interaction $\text{KNO}_3 \times \text{K}_2\text{HPO}_4 \times \text{Bacteria inoculum}$ on oil residual and population of bacteria**

The interaction effect of the three treatment factors to oil concentration and population of bacteria at the end of bioremediation process is shown in Figure 4. It can be seen that interaction of addition of $\text{KNO}_3$ 10% x $\text{K}_2\text{HPO}_4$ 1% x inoculum addition 10% is able to reduce the oil concentration up to 3.7% or a reduction of 68%. The concentration is the lowest concentration of oil when it is compared with interaction of the three treatment factors at all levels. The effective interaction for reduction of oil content in wastewater from refinery industry is by addition of $\text{KNO}_3$ 10%, $\text{K}_2\text{HPO}_4$ 1%, and concentration of inoculum 10%.

![Figure 4. Graph showing effect of interaction $\text{KNO}_3 \times \text{K}_2\text{HPO}_4 \times \text{Bacteria inoculum}$ on oil residual in medium contaminated with crude oil.](image)

The effect of the three treatment factors on bacterial population is presented in Figure 5. The effective interaction for increasing the bacterial population in the bioremediation process is interaction by addition of $\text{KNO}_3$ 5%, $\text{K}_2\text{HPO}_4$ 1%, and inoculum concentration.
10%. The interaction of the three treatment factors at the level is able to increase bacterial population up to $8.5 \times 10^8$ cfu/ml (46%). Therefore, the interaction is used to increase the bacterial population in bioremediation process.

Based on results in Figure 4 and 5, different effective treatment combination exist between effectiveness in reducing the oil concentration and that in increasing the bacterial population. The difference relates to factor of nutrient addition of KNO$_3$. The effective concentration of KNO$_3$ in reducing the oil concentration is 10%, while that in increasing bacterial population is 5%.

![Figure 5. Graph showing effect of interaction KNO$_3$ x K$_2$HPO$_4$ x Bacteria inoculum on Bacteria population in medium contaminated with crude oil](image)

As shown in Figure 6, bacterial population has positive correlation with the oil concentration reduced with coefficient of correlation $r = 0.7536$, and coefficient determination $r^2 = 0.568$. It means that bacterial population in reducing the oil concentration is bioremediation is 56.8%, while 43.2% is effected by other unknown factors in the research.

One of factors effecting reduction of oil concentration is time required for the bioremediation process. The time of bioremediation process used in the research is 15 days and it is not included in the treatment so that the effect of other factors of 43.2% includes the
time for the bioremediation process. If it prolonged, the oil concentration might be lower at the end of bioremediation process.

![Graph showing the effect of bacteria inoculum upon concentration of oil residual in medium contaminated with crude oil.](image)

**Figure 6.** Graph show effect of bacteria inoculum upon concentration of oil residual in medium contaminated with crude oil

**CONCLUSIONS AND SUGGESTION**

Effective treatments are interaction of KNO₃ 10%, K₂HPO₄ 1.0% as nutrient added and Bacterial inoculum of 10% showed reduced oil residual from 12.9% up to 3.7% (decreasing up to 68%) and than interaction of KNO₃ 5%, K₂HPO₄ 1.0% as nutrient added and Bacterial inoculum of 10% showed bacterial population of 8.93 (8.5 x 10⁸ cfu/mL) in bioremediation process during 15 days.

Further research may be performed by adding more independent variables such as time and pH to performance of bioremediation process that utilizes indigenous hidrocarbonoclastic bacteria as biological agent.
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