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Optimization of Nutrient Supplement In Hydrocarbon Bioremediation Process with Biostimulation Techniques in Coastal Regions

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***Abstract*— Bioremediation is the application of the principles of biological processes to cultivate the land, and clay which are contaminated by dangerous chemicals. By adding nutrient, it is known as biostimulation. Biostimulation needs nutrient addition to stimulate microbial growth. Bioremediation's success often measured by contaminants concentration reduction percentages in soil or ground water. From this results, the additional nutrient's optimum value obtained with a ratio of N: P at 107.63: 1, with the amount of nutrients (% P) of 8.471 and a decrease in oil concentration coefficient (k) 0,033 / day. Meanwhile, in the experiment with additional nutrients as much as 8.47% P at a ratio of N: P = 105.5: 1, while the percentage of bioremediation obtained approximately 88.47%. The output value of the regression analysis of R-Sq (adj) = 82.2%, this indicates that the expected response of high significance. The research was conducted in the pond area of District Kalang Anyar Sedati Sidoarjo, implemented from May to August 2014 [11].**

***Keywords*— optimization, nutrient, bioremediation, hydrocarbon, biostimulation.**

I. INTRODUCTION

Nutrient's main functions are as an energy source, cell synthesis ingredients, and electron acceptor in the energy producing reaction. Nutrient ant substrate (pollutant) must be able to be transported into microorganism's cell. The entry of substrates into the cell requires that the substrate is available in a form that allows the substrate to enter the cell through the membrane without damaging the integrity of the membrane sets. Therefore, molecules that will be taken to be small enough to pass through cell membranes and should be in the dissolved phase. The solubility of pollutants in the water is the most important factor for bioavailability. High solubility produces high availability compounds [5]

Bioremediation is the application of the principles of biological processes for treating groundwater, soil, and sludge contaminated with hazardous chemical substances [10]. The ultimate goal of bioremediation is contaminants mineralization, which is changing harmful chemical compounds into less harmful as carbon dioxide or some other contaminants, inorganic compounds, water, and materials needed by microorganisms to degrade [9]. In a bioremediation system, condotion which does not support the modifying of biodegradation reactions is enhanced by improving the environmental factors that limit the biological activity [9].

Bioremediation process makes the familiar terms of bioavailability. According to the Commission on Soil and Water Chemistry, bioavailability is part of a pollutant that can be taken up by an organism from its environment and then transported, distributed, and metabolized by the organism. The definition indicates that only a portion of the substances present in the soil that can be utilized by the organism. In the soil environment, bioavailability becomes more complex due to the transfer of the solution into the cell can be hindered and slowed by several factors such as confinement and sorption-desorption processes. This occurred because the dissolved pollutants molecules are much smaller than bacteria so that these pollutants can get into the soil micropores where bacteria can not enter into it. It is more common in clay than in sandy soil, because it contains more micropores. With the degradation of pollutants by bacteria in the larger pore spaces, the pollutant molecules in micropores can go back out because of the equilibrium concentration. The process of discharge of pollutant molecules from micropores may take longer depending on the diffusion distance and velocity of molecular that is limited by the pore space [5].

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The success of bioremediation is often measured by the reduction percentage of the concentration of contaminants in soil or groundwater. It is not appropriate because although bioremediation achieve high percent allowance, not necessarily to achieve the desired level of allowance. It's possible that the contaminants can come out of the ground or water, or transformed abiotically through volatilization, migration, or photo-oxidation. If this is the case, the purpose of bioremediation to detoxify and immobilization of contaminants can not be reached. Prospects for successful bioremediation should exercise control over the transport of contaminants, such as by making a cover for collecting volatile material or make the monitor wells to detect the migration of contaminants. At the same time, it is required evidence that biodegradation has occurred. Such evidence may include the increase in the activity of microorganisms, the increase in the release of carbon dioxide, the increase in oxygen uptake, or the presence of metabolite products. In-situ is a bioremediation process carried out at the site of contamination. In-situ Bioremediation requires the provision of oxygen and nutrients needed in contaminated soil. In some cases, populations of indigen microorganisms are less suitable for bioremediation of soil so that appropriate microorganisms culture needs to be added [9].

Bioremediation continues to evolve as an effective and environmentally friendly treatment to the oil spill contaminated beaches [2]. Although bioremediation is known for a long time, but there are factors that control bioremediation, which are not well understood. The addition of nutrients is bioremediation practices that have been received, but there is still little understanding of the systematic effect of the addition of nutrients to the microbes population or the course of bioremediation. Mineral nutrients have been used to enhance the biodegradation of crude oil in the oil spill events. However, there are still many unresolved issues relating to the bioremediation of crude oil.

There are two techniques of using of bacteria to degrade the contaminants contained in soil, sludge, sediment, and wastewater. The first technique is to use bacteria that have been available in the soil. These bacteria are stimulated to grow by optimizing the factors that affect the growth of bacteria accelerate the degradation process, the process is called biostimulation. Another technique is by adding a bacterial culture on contaminated sites, further enhances the biodegradation by microorganisms.

Hydrocarbons are petroleum compounds derived from fossil experiencing a very long process and even thousands of years, so that many of the chemical compounds in it.

One type of hydrocarbon compounds is an aromatic hydrocarbon containing one or more benzene ring structure, is characterized by its very typical smell. There are Aromatic compounds that are easily degraded, some are resistant, and also in the transformation can produce unwanted intermediates. The process of decomposition of aromatic compounds and polycyclic aromatic basically follow the same path, but monoaromatic or diaromatic will be more easily degraded than polycyclic aromatic hydrocarbons due to the less amount of the aromatic ring. The simplest Monoaromatic is benzene. Benzene has the properties of nonpolar, insoluble in water but soluble in organic solvents such as diethyl ether, carbon tetrachloride, or hexane. Benzene, toluene, ethyl benzene, and xylene are known as BTEX group. Volatile organic compounds is the most potentially dangerous, especially benzene, because it is toxic and carcinogenic. So that BTEX is often used as an indicator of contamination of soil and groundwater [9].

Coast is an area that stretches from the coastline to relatively wide inland, stretching for hundreds of kilometers along the coastline and often a few kilometers inland from the coast. Coastal line is a line that forms the boundary between the coast and the beach. Coastline limit coasts and beaches with relatively fixed positions, will coincide with the shoreline during a highest tide or relatively large wave. In biology, coastal characteristics can be determined from the distribution of biota towards the land, both vegetation and animal distribution. In climatology, coastal characteristics are determined by the influence of the sea breeze. In hydrology, coastal characteristics determined by how far the influence of tides coming ashore. Seaward limit of the coastal area is the location of the beginning of the first wave breaking occurs when the lowest tide [1].

This paper aims to present the results of the study with additional nutrients optimization of oil bioremediation with biostimulation techniques in coastal environments, and provide additional information regarding the conduct of bioremediation.

II. Material And Methods

A. Location

The location of this study is in the coastal areas of East Surabaya precisely Keputih Village in the Sukolilo District. The study held from April to August 2014. The location is situated at latitude $07^o\ 12'\ 20''$ LS ; Longitude $112^o\ 44'\ 08''$ BT; Altitude 3 meters above sea level; The average temperature is 30.3^oC, maximum of 33.1^oC and minimum of 26.1^oC [11].

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B. Materials

This study uses organic nutrients derived from the composting process commonly found in the market, the results of laboratory analysis of nutrients containing N = 1.46%; P = 1.03%. Oil is used in the form of crude oil taken from oil wells owned by Pertamina Cepu, Central Java, oil spill events that often occur in the form of crude oil.

C. How it works

In this research, a trial plot measuring 1 x 1 m^2 is created, where one plot apart from another plot at a distance of 0.5 meters. This research method refers to research conducted by Delille et al. [8] Picture sample plots as shown in Figure 1.

Figure 1. Experiment Plot in the Field

Optimization is done to observe the ratio of N: P, and the optimum number of additional nutrients in the bioremediation of oil. The experimental data to determine the predictor X1 and X2 values obtained from the results of experiments using the central composite design (CCD) experimental plan. Predictors used a number of two variables with X1 is an additional nutrient ratio of each plot and X2 is the number of additional nutrients (% P). Because the number of variables is independent or predictor by 2, the value of *k* (number of factors) = 2. This design consists of a draft 2*k* = 4. Axial point obtained is α = 4, 2*k* = 1.414. Taking a sample of 13, consisting of observations axial α 4 units were obtained from as many as 2*k* = 2x2 = 4 and the observations at the central point of 5 pieces [6]. The experimental plot design results obtained as shown in Table I.

TABLE I
THE EXPERIMENTAL PLOT DESIGN RESULTS OCCURRED

Partition	Code of			
	X1	X2	X1	X2
1	-1	1	31	1370
2	1	1	180	1370
3	-1,414	0	1	822
4	0	0	105,5	822
5	0	1,414	105,5	1597
6	0	-1,414	105,5	47
7	-1	-1	31	274
8	1	-1	180	274
9	0	0	105,5	822
10	0	0	105,5	822
11	0	0	105,5	274
12	1,414	0	211	822
13	0	0	105,5	822

Response data that were also obtained are increase percentage in the number of microbes and the decrease percentage in the amount of oil content represented BTX compounds, and then performed the statistical analysis with the response surface method. Variables used in this study are, the first one is predictor variable X1 = nutrients to the ratio of N: P (31: 1 to 180: 1), X2 = the amount of nutrients added (274g to 1370g), the second response variable Y1 = percentage the total number of microbes, Y2 = percentage reduction in oil concentration. To measure the total number of microbes (TPC) using the pour-plate method, the number of microbes was measured as the change in cell number or cell mass. This method receipts *agar* medium containing nutrients needed for microbial growth. A little mixed culture rubbed on the diluted *agar* surface then put on a petri dish. Then the petri dishes are incubated and the colonies formed on the surface are assumed to be a living cell in the real culture, the cells measured as colony forming units (CFU). To measure the oil concentration is by looking at BTX compounds using a Gas Chromatography/ Mass Spectrometer (GC / MS). Sampling was carried out periodically, as is shown in Table II.

TABLE II
SAMPLING SCHEDULE

No.	Day	Sampling date
1	0	April 25th, 2014
2	15	May 10th, 2014
3	30	May 25th, 2014
4	45	June 10th, 2014
5	60	June 25th, 2014
6	75	July 10th, 2014
7	90	July 25th, 2014
8	105	August 10th, 2014
9	120	August 25th, 2014

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III. RESULT AND DISCUSSION

The success of bioremediation is often measured by the reduction percentage of the concentration of contaminants in soil or groundwater [9]. Bioremediation process can be determined by measuring the decrease in the concentration of oil in the land affected by the oil spill. To determine the rate of decrease in oil concentration is find the difference between the initial oil concentration and final oil concentration of each period of observation.

dL is the change in the value of oil concentration, *L* is the concentration of oil and *dt* is the change in time. *k* values obtained from the regression equation constants between *dL/dt* as the predictor variable and *L* as the response variable. For example the first plot, oil concentration data taken at each time from day 1 to day 60. The next step, performed a simple regression between time and concentration of oil by using the following formula:

$$-\ln(C/Co) = k\, t \qquad (1)$$

The value of k is the coefficient of the rate of decomposition of oil. From these equations, the value of k obtained with units of g/day for each experimental plot, as shown in Table III. The values listed in Table III are inputs to determine the desired optimum value, then this is done by using response surface analysis, the results shown in Table IV.

From Table IV, the output of the regression analysis shows that the value of R-Sq (adj) = 82.2%, this indicates that the expected response of high significance, means that the ratio of N: P and nutrients were added (% P) is the optimum nutrient composition in oil bioremediation process, meaning that the composition of these nutrients could stimulate soil microorganisms to achieve significant growth. In this condition, the microbes work quickly so that the decrease of the concentration of oil happened very quickly as well, because the oil in the ground serves as a substrate, the substrate in the presence of the microbes will get a carbon source for growth further define the optimum point. In determining the optimum point using Canonical analysis obtained the following results as shown in Table III.

TABLE III

COEFFICIENT OF THE RATE OF OIL CONCENTRATION DECREASE (K)

No. of partition.	Additional Nutrient (g)	N:P Ratio	P (%)	Value of *k* (g/day)
1	1370	31	14,11	-0,012
2	1370	180	14,11	-0,011
3	822	10	8,47	-0,012
4	822	105,5	8,47	-0,033
5	1597	105,5	16,45	-0,011
6	47	105.5	0,48	-0,011
7	274	31	2,82	-0,011
8	274	180	2,82	-0,011
9	822	105,5	8,47	-0,033
10	822	105,5	8,47	-0,034
11	274	105,5	2,82	-0,021
12	822	211	8,47	-0,011
13	822	105,5	8,47	-0,021

In Table III, using SAS software obtained optimum value. It can be explained that the optimum value of the ratio of N: P of 107.63 N: 1 P, the number of nutrients (% P) of 8.4711 and oil concentration reduction coefficient (k) of 0.033237. This is the achievement of this study that the study site with the condition and character of the soil and the type of oil that pollutes the mainland coast. Value of bioremediation coefficient (k) 0.033237 means the rate of decomposition of the oil occurs more quickly, because if inserted into equation (1), it is obtained a steep line position. To predict the required time, bioremediation process can be calculated using equation (1) by inserting *k* value. Likewise, to predict the final concentration of the bioremediation process.

To obtain linkages information of k optimum value of nutrients and the ratio of N: P then graphed the response surface obtained Figure 2.

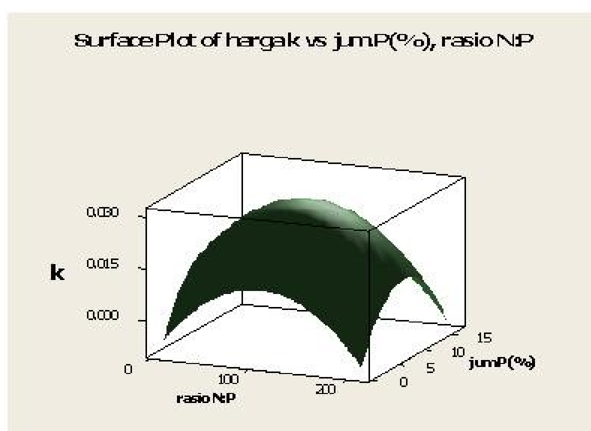

Figure 2. Response to the k value ratio of N: P and nutrients (% P)

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From Figure 2 can be informed that the optimum k value is 0.033 to 0.034. This means that the constant lies between 0.033 to 0.034 where the constants have the peak value in the range of 8% to 9% for the amount of nutrients and 100 to 125 grams for the ratio of N: P This is also seen in the contours of the response to the value of k ratio of N: P and the amount of nutrients (% P), as shown in Figure 3.

In Figure 3, it is shown that the optimum value of k is the value of 0.033 to 0.034, this means that the rate of decline in oil concentration in the soil with additional nutrients as much as 8.74% phosphorus by the ratio of N: P as much as 105.5: 1 oil concentrations decline at most. This is in line with the statement put forward by Venosa, 2003. That amount of nutrients added to the oil spill based on consideration of the amount of N and P are needed to change the amount of certain hydrocarbons into carbon dioxide, water, and biomass of microorganisms. Applied nutrients should be in a certain amount, not exceeding toxic concentrations of ammonia and or nitrate. Nonetheless, types and optimal nutrient concentrations vary greatly depending on properties of oil and environmental conditions [2]. With the results of this study have been obtained the comparison of the optimal amount of nutrients for bioremediation of oil spills in coastal environments.

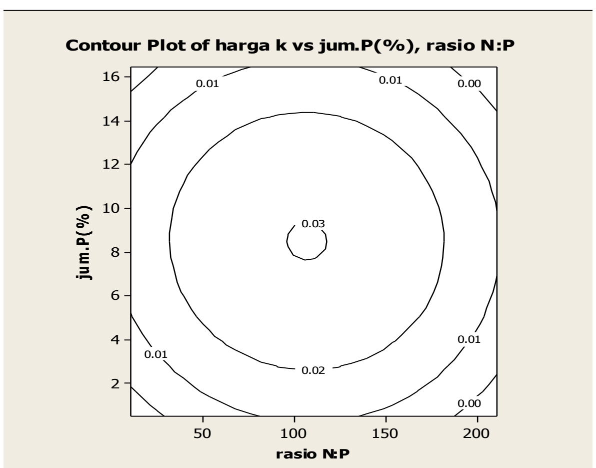

Figure 3. Response Contour of k to the amount of P (%) and the ratio of N: P

To know the nutrient optimization in this study by looking at the percentage of bioremediation for each experimental plot as is shown in Table IV.

TABLE IV
PERCENTAGE DECREASE IN OIL CONCENTRATION

No. of partition	Number of nutrient (g)	N:P Ratio	P (%)	Bioremediation %
1	1370	31	14,11	60,79
2	1370	180	14,11	57,80
3	822	10	8,47	60,79
4	822	105,5	8,47	87,51
5	1597	105,5	16,45	58,47
6	47	105,5	0,48	57,63
7	274	31	2,82	60,34
8	274	180	2,82	60,34
9	822	105,5	8,47	88,26
10	822	105,5	8,47	88,87
11	274	105,5	2,82	72,96
12	822	211	8,47	59,80
13	822	105,5	8,47	73,35

From Table IV shows that the percentage of bioremediation obtained approximately 88%, which occurred in experimental plots No. 4, 9 and 10. This was pointed out that the additional nutrients as much as 8.47% P at a ratio of N: P = 105.5: 1 indicates that the composition of the additional nutreint make the optimum microbial environment, while the addition of other nutrients makes microbes environment not optimum, either additional nutrients are low or high. It is possible for a high additional nutrients become not optimum due to the need of excess nutrients into the cells that cause the biotransformation process to be disrupted thus inhibiting the growth of bacteria, consequently microbes performance is not optimal. To obtain linkages information of decrease percentage in oil concentration (% bioremediation) of nutrients and the ratio of N: P then graphed the response surface as shown in Figure 4.

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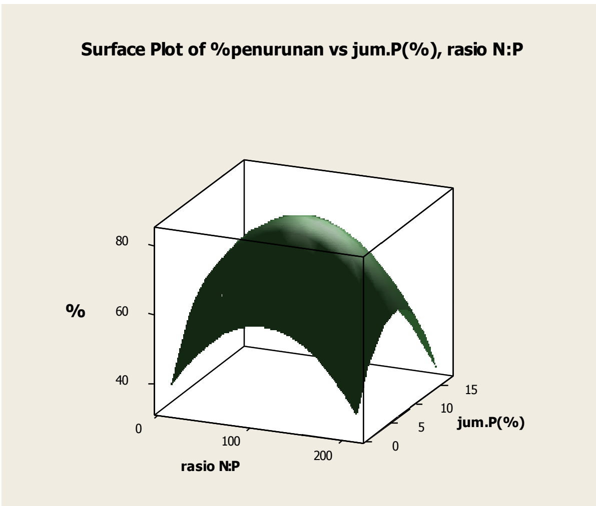

Figure 4. Percentage of Bioremediation to % P and the ratio of N: P

Percentage of bioremediation can also be seen on the graph where the contour line of value 0.8 is in the middle, this means the show is at the optimum position. As shown in Figure 5.

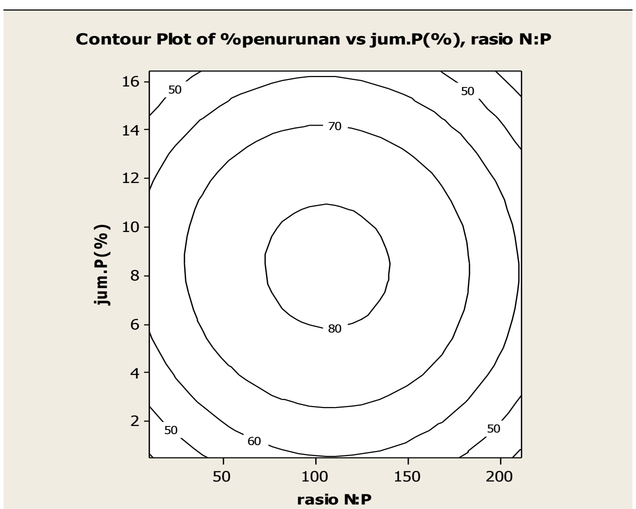

Figure 5. Contour percentage of bioremediation to% P and the ratio of N: P

Laboratory test results obtained total numbers of microbial growth on each plot are presented in Table V.

TABLE V
CONCENTRATION RESULT OF TESTING, MICROBIAL GROWTH RATE COEFFICIENT (M)

No of partition	Number of nutrient (g)	N:P Ratio	P (%)	Value µ/day
1	1370	31	14,11	0,202
2	1370	180	14,11	0,203
3	822	10	8,47	0,202
4	822	105,5	8,47	0,240
5	1597	105,5	16,45	0,201
6	47	105.5	0,48	0,205
7	274	31	2,82	0,202
8	274	180	2,82	0,202
9	822	105,5	8,47	0,240
10	822	105,5	8,47	0,240
11	274	105,5	2,82	0,199
12	822	211	8,47	0,203
13	822	105,5	8,47	0,236

From Table V, it is seen that the highest growth rate coefficient µ microbial 0.240/day occurred in experimental plots No. 4, 9 and 10. This is pointed out that the additional nutrients as much as 822 g with a ratio of N: P = 105.5: 1 cause the microbes environment to be optimum, while the addition of other nutrients makes microbes environment not optimum, either additional nutrients are low or high. It is possible for a high additional nutrients become not optimum due to the need of excess nutrients into the cells that cause the biotransformation process to be disrupted thus inhibiting the growth of bacteria, consequently microbes performance became not optimum.

The ratio of nutrients is one of the environmental resources for the growth of microorganisms, so experts have assumed that the presence of nutrients is a factor to be reckoned with. As reported by Ian M Head and Richard PJ Swannell [7], that the theory of resource-ratio linking structure and function of biological communities to the competition over resources that are necessary in growth. When the quantitative requirements for limiting resources (i.e. limiting resource concentration [resource determining] that supports the growth of zero [zero growth]), the growth and rate of death from a variety of organisms compete, nutrient resource ratio theory would give the possibility to predict the result (outcome) of various interactions.

The implementation of the nutrient resource-ratio theory is chemostat culture experiments where the outcome of competition between two species of bacteria for growth-limiting resources that can be determined from the maximum specific growth rate and the constants of bacteria that grow on the substrate [4]. The underlying principle of bioremediation is the addition of determining nutrient supply (N and P) to stimulate hydrocarbon-degrading microorganisms native competing for nutrients has been widely accepted then the nutrient resource ratio theory can be used to develop a bioremediation process.

IV. Conclusion

From these results, we obtained the optimum values of additional nutrients in the ratio of N: P at 107.63: 1, the amount of nutrients (% P) of 8.4711 and oil concentration reduction coefficient (k) 0.0332/day. Meanwhile, in the experiment with additional nutrients as much as 8.47% P at a ratio of N: P = 105.5: 1, while the percentage of bioremediation obtained approximately 88.47%. The output value of the regression analysis of R-Sq (adj) = 82.2%, this indicates that the expected response of high significance can be interpreted that the ratio of N: P and nutrients were added (% P) is the optimum composition of nutrients in the bioremediation of oil, means that the composition of these nutrients could stimulate soil microorganisms to achieve significant growth. In this condition, the microbes work quickly so that the oil concentrations also decrease quickly. This occurs in experimental plot no 4, 9 and 10. It is possible for a high additional nutrients become not optimum due to the need of excess nutrients into the cells that cause the biotransformation process to be disrupted thus inhibiting the growth of bacteria, consequently microbes performance became not optimum.

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