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Submission date: 16-Jan-2020 02:19PM (UTC+0700)
Submission ID: 1242554328
File name: tics_of_Indigenous_Isolated_Bacteria_Bacillus_mycoides_Used.pdf (441.98K)
Word count: 3386
Character count: 17990

Journal of Sustainable Development
www.ccsenet.org/journal.html
Vol. 2, No. 3
November 2009

Kinetics of Indigenous Isolated Bacteria *Bacillus mycoides* Used for Ex-Situ Bioremediation of Petroleum Contaminated Soil in PT Pertamina Sungai Lilin South Sumatera

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Abstract

Bioremediation of petroleum sludge was done by using land farming method in micro scale; the samples were taken from PT Pertamina Musi Banyuasin district of South Sumatra. This process applied *Bacillus mycoides* bacteria which isolated and selected from the contaminated soil. The research aim is to evaluate the performance of the *Bacillus mycoides* bacteria in degrading petroleum sludge pollutants. The initial TPH concentrations of soil contaminated sludge samples were set up at; 4.18, 6.60, 9.82, 10.87 and 13.42%, which were diluted from the main contaminated soil sample with a concentration of Total Petroleum Hydrocarbon (TPH) of contaminated soil was 71.16%. Every sample is inoculated by *Bacillus mycoides* bacteria as much as 10 % v/w and stirred homogeneously. The incubation time was 14 days, and then the samples were analyzed for the TPH content. The results were 3.68, 4.51, 5.91, 6.02 and 8.00% respectively. The rate of the biodegradation process was determined by using differential method. The results of data analyses show that the reaction order is first order. The rate of biodegradation constant was determined by using integral method. The initial concentration of the sample was 9.82 %, and then it had been inoculated by *Bacillus mycoides* bacteria during 14, 17, 22, 26 and 31 days. The TPH concentrations decreased 5.91, 4.59, 4.05, 3.72 and 3.29% respectively. The results of data analysis show that the biodegradation was a first reaction order with a biodegradation reaction constant of 0.0361 day^{-1}. The chemical kinetics model of the bioremediation model is y = -0.0362.x + 2.2448. So, by using this model; the bioremediation process will be completed after 62.5 days. The qualitative analysis was done by using GC-MS to investigate the components of compounds changed during the bioremediation process; the samples were analyzed in the initial and final states of process. The results show that the *Bacillus mycoides* could degrade 99.32% of $C_{19}H_{40}$,$C_{21}H_{44}$,$C_{24}H_{50}$, and $C_{28}H_{58}$ compounds in 31 days.

Keywords: Bioremediation, Degradation, Microbia, *Bacillus mycoides,* Petroleum sludge pollutant

1. Introduction

The amount of oil-contaminated soil generated in the oil production process has been increasing by thousands of tons every year in South Sumatra (Yudono et al., 2006). Parts of the contaminated soil are dehydrated oil sludge separated from the mixture of oil, water and soil. Most of the oil sludge is piled up outdoor next to the production site without any treatment, and poses serious environmental problems. The hydrocarbons in the sludge penetrate from the top soil into the subsoil slowly, presenting a direct risk of contamination to subsoil and groundwater. On the other hand, the light hydrocarbons in the oil sludge vaporize, leaving behind a layer of oil-containing dust of soil which blows upwards to pollute the air. These contaminations of soil, water and air pose serious risks for the environment and human population. Therefore, the oil sludge should be treated to prevent harm to environment. Although burning of the sludge may be simple and easily adaptable, this technique has undesirable hazard in air pollution. Bioremediation of the oil sludge is

believed to be an efficient, economic and versatile alternative to physiochemical treatments if sufficient space and time are available (Jackson et al. 1996; Venosa et al. 1996; Salanitro et al. 1997). Acceptance by the general public is another major advantage of this technology (Skladney and Metting 1993). Indigenous microorganisms can utilize the total petroleum hydrocarbons (TPH) of crude oil as source of carbon and energy and break them down to simpler non-toxic compounds such as CO_2 and H_2O. But bioremediation takes a long time as the degradation efficiency of the bacteria is considerably low under natural conditions (Del'Arco and de Franca,1999; Chaîneau et al., 2003).

Therefore, some engineering processes such as addition of nutrients, watering, tilling and addition of suitable microbial flora are necessary to improve the rate of biodegrading of hydrocarbons (Jorgensen et al. 2000; Vasudevan and Rajaram 2001; Barathi and Vasudevan 2003; Kuyukina et al. 2003). Many efforts had been done to remedy oil containing sludge by the mixed culture of microbial in South Sumatra in an industrial scale (Yudono, et al. 2006). The time needed was too long around 240 days

1.1 Research Objectives

Land farming is one of bioremediation method for treating the oil sludge was conducted on small scale. The samples were taken from a South Sumatra oil-containing pit disposal station, PT Pertamina oil fields. The research aim is to evaluate the performance of the *Bacillus mycoides* bacteria in degrading petroleum sludge pollutants The *Bacillus mycoides* bacteria was isolated and selected from the contaminated soil. This bacterium is one of the major microorganisms responsible for biodegradation of petroleum hydrocarbon (Atlas and Cerniglia 1995; Alexander 1999; Boonchan et a. 2000).

The environmental Indonesian affair called UU no. 23/1997 and PP No. 18/1999 stated that the petroleum sludge pollutants are included hazardous material. It could not be kept too long, at least 90 days; these materials should be treated into non hazardous material (Mursida, 2002).

Handling petroleum pollutants can be done in many ways; physics by using burning process, however it will produce air pollution. Chemistry; the pollutants are attracted by using dispersant such as non ionic detergent. The pollutants will be bounded with dispersant then percolated into the water basin. This precipitation is difficult to degrade, so it will be hazardous for natural life. Chemically and physically pollutants handlings are suitable to reduce petroleum pollutants in water surface (Kadarwati *et al*. 1996).

Bioremediation is alternative technology for treating petroleum pollutant which the most accepted environmentally and should be considered for the future. Bioremediation process uses the ability of microbiology to degrade organic substances into simple substances or demineralization process. It will be an important remediation process for contaminated land, so it will save for living things (Udiharto, 1992:1).

This research evaluated the performance of microbial *Bacillus mycoides* in degrading petroleum sludge pollutant. It is calculated as the decreasing rate of TPH concentration per time unit. Furthermore, the degraded components of petroleum oil sludge were investigated by using GC-MS.

2. Materials and Methods

2.1 Site and Experiment Scale

Bioremediation experiments of petroleum oil sludge were undertaken on small scale of 25 Kg, the ratio is 1: 100 from the actual bed of field scale process. The thickness of the dehydrated sludge in the prepared bed was 10 cm.

2.2 Pretreatment of the Oil Sludge

The petroleum oil sludge collected from the storage pit was put into the prepared bed. The oil sludge had heavy clay texture and low oxygen diffusivity. In order to enhance aeration and water-holding capacity of the sludge, organic and inorganic bulking materials (wood particles and sandy soil) were added. The content of wood particles in the sludge was 10.0% (w/w) and that of sand was 10% (w/w). Urea was provided as a nitrogen source, and potassium dehydrogenate phosphate as a phosphorus source. The ratio of C, N and P in the oil sludge was 100:10:1 after the fertilizers had been added. The initial TPH concentrations were 4.18, 6.60, 9.82, 10.87 and 13.42%, which were diluted from the main contaminated soil sample. The concentration of Total Petroleum Hydrocarbon (TPH) of contaminated soil was 71.16 %.

2.3 Bioremediation Process

Microorganism obtained from petroleum oil sludge contaminated soils in petroleum oil sludge pit PT Pertamina South Sumatra Indonesia (indigenous bacteria). The *Bacillus mycoides* bacteria was isolated and selected from the mixed culture, purified and enriched in BHMS medium which consist of $Mg_2SO_4.7H_2O$ 0.2 g/L, $CaCl_2$ 0.02 g/L, KH_2PO_4 1 g/L, K_2HPO_4 1g/L, NH_4NO_3 1 g/L, $FeCl_3$ 0.05gr/L dissolved in 1 L aquadest.

The application amount was approximately 10 % of treated soil. Over the course of the experiment, the land farming cells were tilled twice a week to maintain high level of oxygen in the sludge. Water was added after tilling to maintain a moisture level of 40% in the sludge.

2.4 Analytical Methods and Data Analysis

Oil sludge was sampled at different stages of bioremediation. Five samples were taken for treatment; it is described diagrammatically in the Figure 1. The oil content in sludge samples were determined gravimetrically in amount of TPH extracted by diethyl ether (Christofi et al. 1998; Capelli et al. 2001).

The differential method of data analysis was used to determine the reaction order, and the integral method of data analyzes to determine the constant of reaction rate. Oil fraction analyses were performed using a Gas chromatography-Mass Spectrometry (GC-MS).

The method of dilution plating on agar plates was used to monitor the number of bacteria in the sludge samples (Mesarch and Nies 1997).

The method of most-probable-number was used to count the number of hydrocarbon degrading microorganisms and that of aromatic hydrocarbon degrading microorganisms (Wrenn and Venosa 1996).

3. Results and Discussions

3.1 Kinetics Approach of Biodegradation of Petroleum Contaminated Soil

Total Petroleum Hydrocarbon (TPH) represents measurement to calculate the total of hydrocarbon content in the sample. The initial concentrations of samples were set as 4.18, 6.60, 9.82, 10.87 and 13.42 % w/w. The TPH analysis was conducted by using extraction and gravimetric methods.

Nutrients were added to the samples to support the initial growth of bacteria *Bacillus mycoides* before the bacteria could degrade the hydrocarbon as its carbon metabolism resource. The C: N: P ratio for the optimal biodegradation process was 100:5:1.

Before the bacteria were inoculated into samples, the conditions were set up at pH 5.8-6.0, soil humidity 40 %, and the temperature 25 ^{o}C. The samples were stirred twice a week to maintain oxygen diffusivity.

After 2 weeks inoculation, the TPH concentrations of each sample were measured, the results were shown in the Table 1. The data was studied to determine the reaction order of biodegradation of petroleum oil sludge pollutants. The data was analyzed by using differential method.

Microbial growth on pollutant mixture is an important aspect of bioremediation treatment. However, efforts to develop mathematical models for mixed substrate kinetics have been limited. When individual microbial species are considered, simple competition for the growth substrate is the only interaction included (Reardon et al. 2002). The general formula of the first-order kinetic that can describe the rate of TPH reduction is:

$$r = \frac{dC}{dt} = -kC^n \quad (1)$$

where:

r : reaction rate (concentration unit/time unit)

t : time (day)

C : remaining TPH concentration (mg/l) at any time

n : reaction order

k : first order kinetic constant (1/day)

In Eq. 1, it is assumed that the microbial concentration remains constant over the entire experimentation period. Therefore, the effect of microbial concentration on the kinetics constant can be neglected.

The linearization results of the experimental data are graphically presented in Figs. 2. and is derived from Equation 1

$$\ln r = \ln k + n \ln c \quad (2)$$

If Equation 4 ln r vs ln c are plotted, it will be straight line graph with the slope is n and the intercept is ln k. The rate reaction constant will be more accurate when it is determined by using the integral method, this will be discussed further.

The data was plotted into graph described as Figure 2, the graph shows that the slope is 1.037and the regression constant square is 0.93. It proved that the biodegradation process of petroleum oil sludge by using bacteria *Bacillus mycoides* is a first order reaction.

3.2 Determination of rate reaction constant by using Integral Method

The integration of Eq. 5 leads to the known formula of the first-order kinetics

$$C = C_o e^{-kt} \quad (3)$$

where C_0 is the initial concentration (mg/l) or TPH_0

In order to experimentally calculate the kinetic constant k, Eq. 2 is linearized using the following equation

$$\ln \frac{C_0}{C} = kt \quad (4)$$

$$\ln TPH = -kt + \ln TPH_0 \quad (5)$$

The initial concentration of sample was 9.82 %; it was inoculated by using *Bacillus mycoides* bacteria. It had been observed during 14, 1 22, 26 and 31 days, the decreasing concentration of samples were 5.91, 4.59, 4.05, 3.72 and 3.29 % respectively. The first-order kinetics is said to be valid if a linear relationship is achieved upon plotting the logarithmic part of Eq. 7 versus time. Analysis of the rates of hydrocarbons removal showed that most compounds obeyed first-order kinetics (Greene et al., 2000). The slope of the line represents the first-order kinetic constant k. By using the first order reaction equation; $\ln TPH = -kt + \ln TPHo$, the data was plotted ln TPH vs t. The graph is shown in Figure 3.

The slope of graph is -0,0361day^{-1}, it represents the rate reaction constant. The intercept of the graph is 2.2448. So the equation of reaction rate is y = - 0.0362x + 2.2448. The progress of bioremediation process can be predicted by using this chemical kinetics equation, for example to reach the TPH c1centration below 1%, the bioremediaton process will take place as long as 62.5 days. These results are well fitted in a great extent with the results achieved in previous studies (Hutchins et al. 1991; Hwang et al., 2001; Antizar-Ladislao et al., 2005).

3.3 GC-MS Analyzes

The changed composition of compounds from initial to final conditions of bioremediation process was identified by using GC-MS. Figure 3 and Figure 4 show initial and final compositions respectively.

Every peak in the chromatogram represents a component of compound in the petroleum oil sludge, and the peak area represents the concentration of the component. The identical retention time in the both chromatograms show the identical compounds. The different shape of the peaks area is caused by the bioremediation process. The predicted compounds were drawn from MS Library. The data analyses were conducted at every identical retention time. The chromatograms show that *Bacillus mycoides* bacteria could almost completely degrade $C_{19}H_{40}$, $C_{21}H_{44}$, $C_{24}H_{50}$, and $C_{28}H_{58}$ compounds as can be seen in the Table 2.

The data show that the *Bacillus mycoides* bacteria could effectively degrade the long chain hydrocarbon compounds. However, it is needed to investigate closely the structure of the compounds which were degraded during the bioremediation process by using more detail separation technique.

4. Conclusions

The following conclusions can be drawn from this research;

2) Bacillus mycoides bacteria could degrade the petroleum oil sludge with initial TPH concentrations 4.18, 6.60, 9.82, 10.87 and 13.42 % to 2.37, 4.51, 5.91, 6.02 and 8.00 % respectively after14 days incubation. The average decreasing TPH concentration was 39,48%.

2) Bacillus mycoides could degrade hydrocarbon compounds; $C_{19}H_{40}$, $C_{21}H_{44}$, $C_{24}H_{50}$, and $C_{28}H_{58}$, the average of decreasing concentration was 99,32% in 31 days.

3) The reaction order of the biodegradation was first order and the constant of rate reaction was 0.0361 day $^{-1}$.

4) The kinetics model of biodegradation is y = - 0.0362x + 2.2448

5) Decreasing the TPH concentration from 9.82 % to below 1 % can be predicted by using chemical kinetics equation in 62.5 days.

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Table 1. Biodegradation of Petroleum sludge after 14 days process

Initial % TPH	After14 days, % TPH	Percent of decreasing concentration
4.18 %	2.37 %	41.18
6.60 %	4.51 %	31.64
9.82 %	5.91 %	39.84
10.87 %	6.02 %	44.59
13.42 %	8.00 %	40.14

Table 2. The GC-MS data analyzes

No	Suggested compounds	Retention Time	Peak Area		% Decrease in Peak Area
			Initial	Final	
1	$C_{19}H_{40}$	19.27	11532410	51086	99.55
2	$C_{21}H_{44}$	20.27	12235678	62664	99.48
3	$C_{24}H_{50}$	21.23	10924657	161593	98.52
4	$C_{28}H_{58}$	23.01	8595758	22005	99.74
Average % Decreasing Peak Area = 99,3225%					

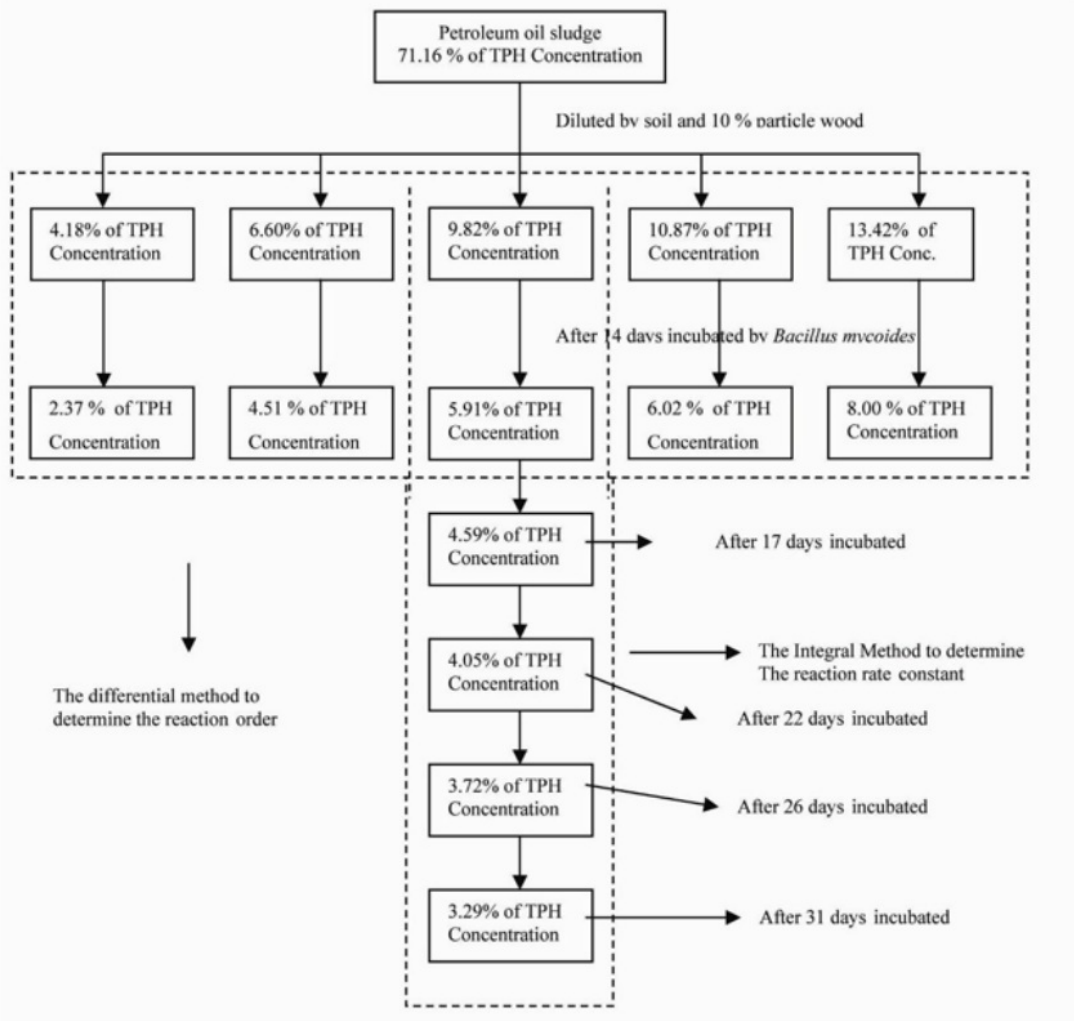

Figure 1. Diagram of The Differential Method and Integral Methods of Kinetics

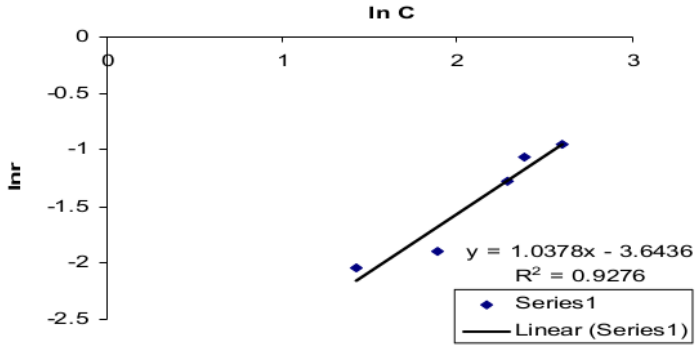

Figure 2. Gaphic ln r vs ln C to determine reaction order

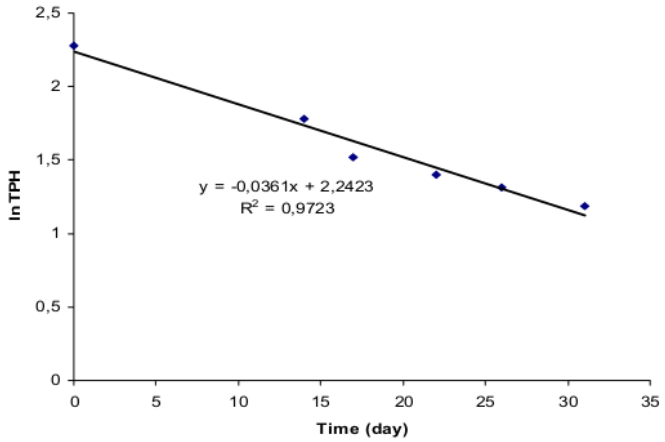

Figure 3. ln TPH vs Time to determine the constant of reaction rate

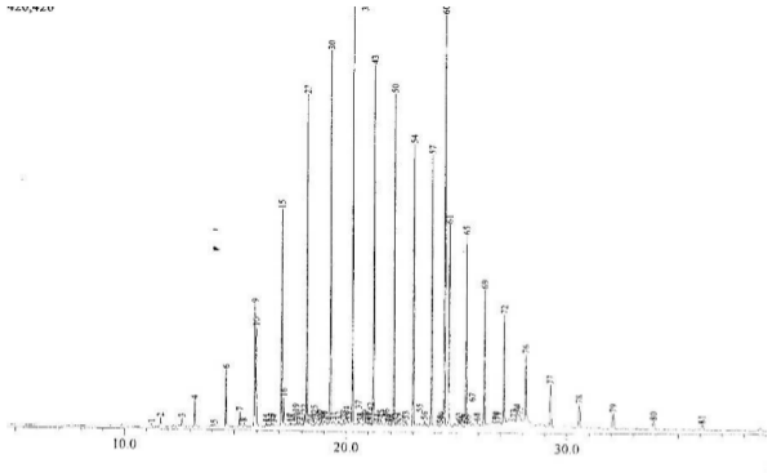

Figure 4. Chromatograph of initial condition before incubated by *Bacillus mycoides* bacteria

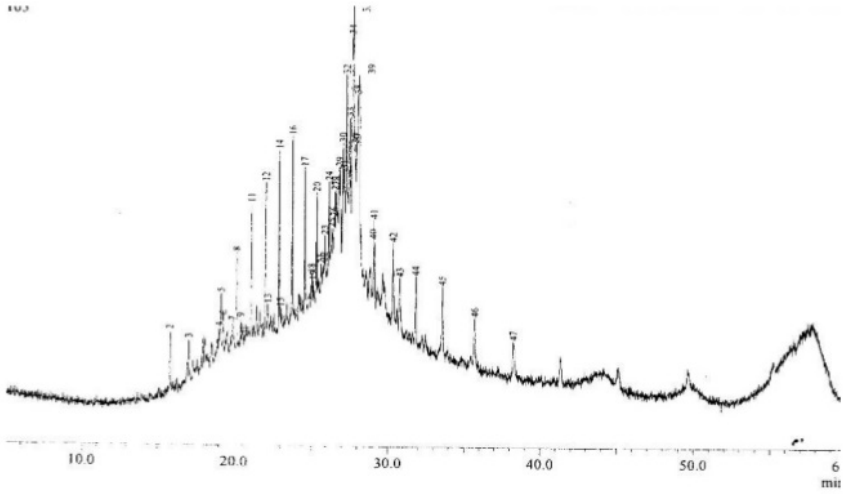

Figure 5. Chromatograph of initial condition after 31 days incubated by *Bacillus mycoides* bacteria

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