Bioremediation of Heavy Oil Waste Contaminated Soil by Using Bacterial Consortium with Landfarming Technique

Charlena[*1], M.Yani[2], Zainal Alim Mas'ud[1], Iswandi Anas[3], Yadi Setiadi[4]
[1]Department of Chemistry, Faculty of Mathematics and Sciences, IPB University, Bogor, Indonesia
[2]Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia
[3]Department of Land Resources Management, Faculty of Agricultural, IPB University, Bogor, Indonesia
[4]Department of Silvy Culture, Faculty of Forestry, IPB University, Bogor, Indonesia
[*]charlena@apps.ipb.ac.id

Abstract. Bioremediation of heavy oil waste contaminated soil has been carried out by using bacterial consortium with landfarming technique. Landfarming technique was performed by mixing heavy oil waste, clay, and compost at a various composition with the addition of 10% (v/v) bacterial consortium. The water content, pH, temperature, TPH, and gas production generated during the biodegradation process were observed every week for 4 months. The composition of hydrocarbons in heavy oil waste before and after bioremediation was determined by using GC-MS. The results showed that the percentage of TPH was still quite high at 5:58%. This indicates that the biodegradation process is slow due to the imperfect growth of bacteria, less optimum pH, and low water content. Notwithstanding with the slow process, biodegradation process continues as indicated by production of CO_2 and NH_3 gasses during the observation. This sustainable biodegradation process was also supported by GC-MS data, which showed that after 4 months of bioremediation process, hydrocarbon compounds from C-6 to C-12, which were originally composed of hydrocarbon compounds from C-6 and C-35, were identified.

Keyword: bacterial consortium, bioremediation, heavy oil waste, landfarming technique

1. Introduction

Bioremediation can be carried out by *in-situ* or *ex-situ* techniques. In-situ bioremediation techniques are generally applied at light polluted location, unmovable location, or characteristic of the volatile contaminants. Ex-situ bioremediation is a bioremediation technique in a way that contaminated land or water is removed, then processed at a specific area sets up for the bioremediation process. Such handling is safer for the environment because the agent used is degrading microbes that can decompose naturally (Budianto 2008).

Ex-situ bioremediation can be performed with landfarming and bioslurry techniques. Landfarming technique is one of ex-situ bioremediation techniques in which it could shorten the time required for cleaning contaminated land than those in physical, chemical, and biological processes. Landfarming technique requires excavation and placement in the stacks. Piles were periodically removed for mixing and humidity regulation purposes. Soil pH setting and the addition of nutrients are needed to increase the biological activity (Poon 1996). According to Garcia et al. (2005), landfarming technique is a method that is often chosen for hydrocarbons contaminated soil because it is relatively cheap and has high potential to be successful. Landfarming bioremediation technique has been carried out to

overcome heavy oil waste contaminated soil in the oil industry PT CPI. By using indigen microbes, it takes ± 8 months to reduce TPH to about 4%, which in turn microbes are no longer able to reduce TPH to 1%, according to Minister of Environment Decree No. 128 of 2003.

This study aims at accelerating the bioremediation process in overcoming the heavy oil wastes that are increasingly accumulated by using a bacterial consortium obtained from heavy oil waste and animal droppings.

Animal dropping has an active ingredient containing microbes. Besides rich with decomposer microbes, animal dropping also contains enough nutrients for microbial growth. In general, fresh animal manure contains 70-80% water, 0.3-0.6% nitrogen, 0.1-0.4% fosfor in the forms of P_2O_5, 0.3-1% potassium in the forms of K_2O (Waksman 1957 in Anggraeni 2003). Some species of bacteria contained in cow manure (Bawono 1988 in Srimulyati 2000), among others, *Escherichia coli*, *Citrobacter freundii*, *Pseudomonas putrefasciens*, *Enterobacter cloacae*, *Proteus morganii*, *Salmonella spp*, *Enterobacter aerogenes*, *Flavobacterium*, *Pseudomonas fluorescens*, and *Providencia alcalifasciens*. According to Norman (1985), microbes contained in the cecum, colon, feces of cattle and horses, among others, *Entamoeba caprae*, *Calismatix equi*, and *Entamoeba equi*. Organic matter is important in improving the productivity of land and is the source of life for a variety of microbes. In contrast with cow dung, which tends to have balanced compositions of hemicellulose, cellulose, lignin, total protein, and ash contents, low quantities of protein content but high cellulose and hermicelulose contents are found in the chemical composition of horse manure (Waksman 1957 in Anggraeni 2003).

Landfarming is one of bioremediation technologies that are constantly being developed to date. Landfarming method can reduce the impact of oil pollution since bioremediation is a safe alternative method in which the pollutants (hydrocarbons) can be broken down by microbes into harmless substances such as CO_2 and H_2O. Nevertheless, landfarming has advantages and disadvantages. Because of that it is necessary to study this landfarming technique in handling heavy oil waste. In addition, how effective bioremediation technique with this landfarming remodels hydrocarbons from petroleum wastes in solid phase is a challenge that needs to be developed and improved.

2. Methods

2.1 Materials and Equipment

The materials used in this study were heavy oil waste contaminated soil obtained from the petroleum industry area, microbial consortium derived from heavy oil waste and animal manure (cow and horse) are taken from the Faculty of Animal Husbandry IPB, urea, SP36, glucose, NaOH , technical CaCO3, seawater, marine agar, nutrient broth, hexane, Na_2SO4, silica gel, and distilled water. The equipment used include rotary evaporator, a set of Soxhlet, ovens, centrifuges, autoclave, incubators, Petri dish, micropipette, threaded tubes, Erlenmeyer 500 mL, spectrophotometers, GC-MS and some other glassware.

2.2 Bacterial Consortium

Development of bacterial consortium by using cow and horse manures (fresh) was performed in rich media and minimal media. A total of 400 g of cow and horse manure samples dissolved in 4 L of seawater was mixed with 200 g of sugar, 20 g urea, 2 g of SP36. Samples were stored in the laboratory at room temperature (25-27 °C) and aerated for 1 week. The pH measurement was taken everyday; the pH was neutralized by adding NaOH 6 N or H_2SO_4 6 N when the pH was too acidic or too basic, respectively. This fresh

consortium was poured into fresh minimal medium consisting of as many as 4 liters of seawater and then mixed with diesel oil 5% (v/v), 8 g urea, 0.8 g of SP36 and aerated for 3 weeks. The pH was observed everyday whilst TPH was recorded every week. The bacterial consortium from cow and horse manures (starter) was used in the process of bioremediation with landfarming and bioslurry techniques.

2.3 Bioremediation with Landfarming

The bacterial consortium that has been used in degrading heavy oil waste was tested by using landfarming technique. Landfarming was performed under a closed system using a sealed plastic container. The work was performed in duplicate and the sampling was taken weekly for 4 months of observation. Treatment with landfarming technique was aimed at getting an efficient mixing media with the composition listed in Table 1.

Table 1 Composition of bioremediation with landfarming technique

Code	Composition (kg)			Remarks
	HOW	Clay	Compost	
K	10	0	0	No bacteria
A	10	0	0	With Bacteria
B	5	0	5	With Bacteria
C	5	5	0	With Bacteria
D	5	2.5	2.5	With Bacteria

Notes: K = Control; HOW = Heavy Oil Waste; A = HOW; B = HOW + Compost; C = HOW + Clay; D = HOW + Compost + Clay

The clay used was not heavy oil waste contaminated and was taken from oil fields around Duri PT CPI. The compost has composition of manure, worm manure, humus soil, rice straw, rice husk, and EM4 fermentation. Aeration was done by using the aerator. Soil sampling (solid) and the air was conducted weekly. Analysis of TPH, TPC, pH, moisture content and composition of hydrocarbon compounds was performed on soil samples, whilst analysis of CO_2 and NH_3 content was performed on the air samples.

2.4 TPH Measurement (EPA 1998)

TPH values were measured by using a gravimetric method and were performed weekly during the ± 2 months. A total of 5 grams of sample was weighed and then rolled-wrapped with filter paper. The rolled-wrapped samples was put into a Soxhlet and extracted with the solvent of ± 125 mL n-hexane for 4 hours. The extracts were dewatered with anhydrous Na_2SO_4 and filtered, and then the long-chain hydrocarbons and functional groups (grease) were removed with silica gel 60 and filtered. The extracts were then emptied into a round flask and concentrated by a rotary evaporator until thick or has been separated with the solvent. The round flask containing the extracted sample was heated in an oven at a temperature of 70 ºC for 10 minutes and then cooled in a desiccators and weighed. TPH levels were calculated as:

$$\text{TPH (\%)} = \frac{m_1}{m_2}\ x\ 100\%$$

where m_1 = extract weight (g)
m_2 = sample initial weight (g)

2.5 Gas Sampling

Sampling equipment was prepared by filling an impinger tube with an absorber solution each of 10 mL. Flow rate was determined by means of a flow meter at 0.2 L/min. Sampling was conducted for 1 hour, and after that the absorber solution containing the gas was put into a film container, then impinger was rinsed with distilled water. Sampling of gas is shown in Figure 3.1.

2.6 CO_2 Analysis (Eaton *et al*. 2005)

Sample in the forms of absorber solution containing gas was emptied into an Erlenmeyer. Titration was performed by using standardized HCl 0.025 N with PP indicator. The same procedure was done on a 10 mL of CO_2 absorber solution. CO_2 absorber solution was Na_2CO_3 0.0245% (w/v) solution used as a blank.

$$W_{CO_2}(\text{mg}) = (\text{A} - \text{B}) \times \text{N HCl} \times 44$$

$$CO_2(\text{mg/m}^3) = \frac{W_{CO_2}}{\text{V}} \times \frac{(\text{t} + 273)}{298} \times 1000$$

where: A = mL of HCl used (blank)
B = mL of HCl used (sample)
V = volume in liter [flow rate x t (minute)]
W_{CO_2} = mg sample obtained

Figure 1. CO_2 and NH_3 sampling during the landfarming bioremediation process.

2.7 NH_3 Analysis (Lodge 1989)

NH_3 was determined by the indophenols method, which is the reaction of NH_3 with phenolic compounds and alkaline citrate producing a blue complex of indophenols. The absorbance of this complex was measured with a spectrophotometer at 635.5 nm. Standard series was made by adding 2 mg/L of 0.05, 0.1, 0.2, 0.3, 0.4, 0.8, and 1. 2 mL standard solution into a 25 mL flask peck. 5 mL NH_3 absorber solution (H_2SO_4 0.1N), 1 mL of sodium phenolics, 1 mL nitroprusside solution, 2.5 mL of oxidizing solution, and distilled water were successively added into the flask peck. The sample was put into a 25 mL flask peck and treated with the same procedure as the standard solution. Standard solution and

samples were left for 1 hour and the absorbance was read at 635.5 nm by using UV spectrophotometer. NH_3 concentration was calculated by the formula:

$$NH_3\ (\mu g/m^3) = \frac{W_{NH_3}}{V} \times \frac{(t+273)}{298} \times 1000$$

where V = volume in liter [flow rate x t (minute)]
W_{NH_3} = µg sample obtained from calibration curve.

3. Results and Discussions

3.1 Bioremediation with Landfarming Technique

pH Change

The pH affects the ability of bacteria in maintaining its cellular activities, cell membrane transport, and equilibrium reactions catalyzed by its enzymes. Based on pH measurements performed weekly, the measured pH ranged from 4 to 7 (Figure 2).

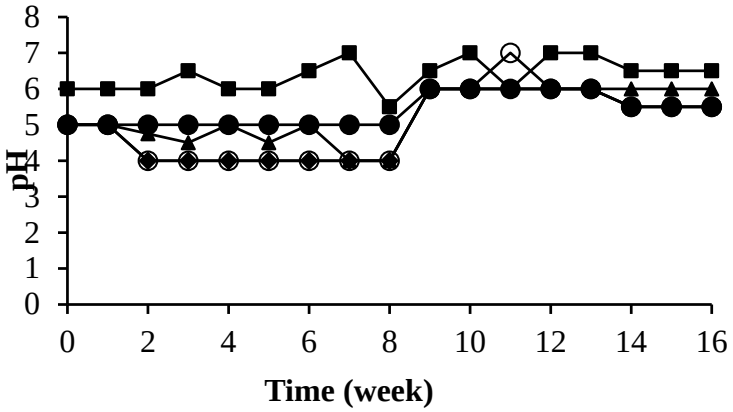

Figure 2. Change in pH during landfarming bioremediation process on HOW (o), HOW + Compost (■), HOW + Clay (▲), HOW + Compost + Clay (●), and Control (♦).

According to Cookson (1995), the optimum pH for bacterial growth is 7 and has a pH range between 4 to 10 while for the oxidation of nitrogen ranges from 6 to 8. Hydrocarbon degradation is faster when performed at pH above 7 as compared with a pH below 5. Thus, if the media solution contains a high concentration of organic materials, which could reduce the alkalinity of the solution, then $CaCO_3$ or another basic material is needed to neutralize the pH.

The tendency of pH decrease was observed in all samples in a similar manner. The decline suggests that the accumulation of organic acids due to metabolism increases with the incubation time. High pH values may be caused by the release of ammonia from the substrate or effects of remaining cation after organic acids metabolism.

Changes in Water Content

Humidity is very important for microbial to live, grow and metabolic activity. Based on the weekly measurements, the water content ranged from 10.10-32.67% as shown in Figure 3.

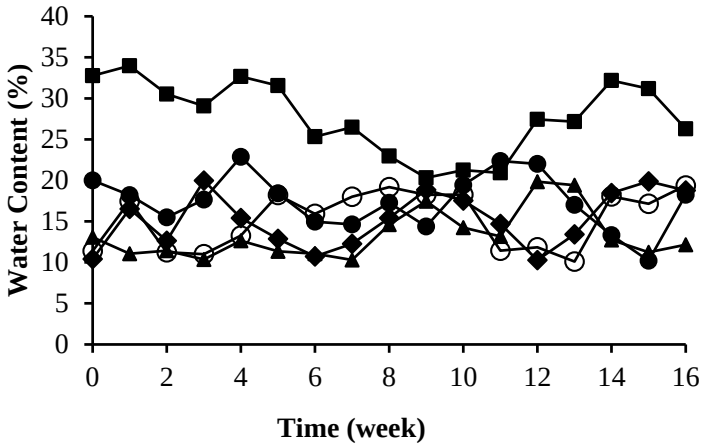

Figure 3. Change in water content during landfarming bioremediation process on HOW (o), HOW + Compost (■), HOW + Clay (▲), HOW + Compost + Clay (●), and Control (♦).

The variation in water content is expected to be due to the different treatment in each sample in which the water was supplied every week regularly. According to Fletcher (1992), during bioremediation, if the water content is too high, the oxygen will be difficult to enter the land whilst without water the microbes cannot live in the waste oil. According to Dibble and Bartha (1979), the water content required for the metabolism of bacteria in degrading hydrocarbon ranges from 30-90%.

Change in Temperature

In a process of degradation, the temperature will affect the physical properties and chemical components of oil, the speed of degradation by microbes, and microbial population composition. Based on temperature measurements conducted weekly, the measured temperature ranges between 27-51 ° C (Figure 4).

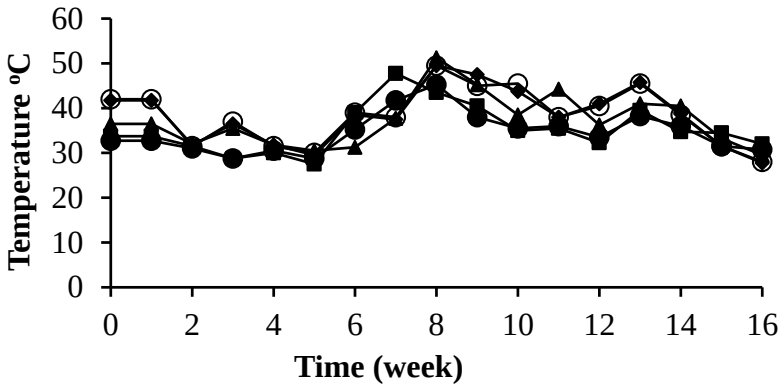

Figure 4. Change in temperature during landfarming bioremediation process on HOW (o), HOW + Compost (■), HOW + Clay (▲), HOW + Compost + Clay (●), and Control (♦).
According to Leahly and Colwell (1990), the optimum temperature for degradation of hydrocarbons is 30-40 ° C. In this study, the temperature fluctuates (Figure 4), because, in

the biodegradation process, breakage of hydrocarbon chains will generate energy results in the temperature rises. The temperature will back to room temperature if the biodegradation process stalled or running very slow. At low temperatures, oil viscosity will increase resulting in volatility of short-chain alkane that is toxic to decline and its solubility in water will increase so that the biodegradation process will be hampered. Inhibitory effects can also be caused by a decrease in microbial enzyme activity.

Change in TPH

TPH is an important parameter that indicates the success of biodegradation process of petroleum hydrocarbons. Measurements were conducted every 2 weeks for 4 months of observation. In addition the bacterial consortium to the treatment of heavy oil waste (HOW), the initial and final measured TPH was 15:32% and 12.61%, respectively. For the treatment without the addition of bacterial consortium (control), the initial and final measured TPH was 15.84% and 13:43%, respectively.

Waste treatment of heavy oil by mixing compost and the addition of bacterial consortium can lower TPH values from 11.96% to 5:58%. The addition of compost will improve the process of biodegradation. The treatment of heavy oil waste mixed with clay and the addition of bacterial consortium has the initial TPH value of 8.73% and 5.78% at the end of measurement. Waste treatment of heavy oil by mixing clay and the addition of bacterial consortium has the initial TPH of 6:52% and 4.87% at the end of measurement. TPH measurement can be seen in Figure 5.

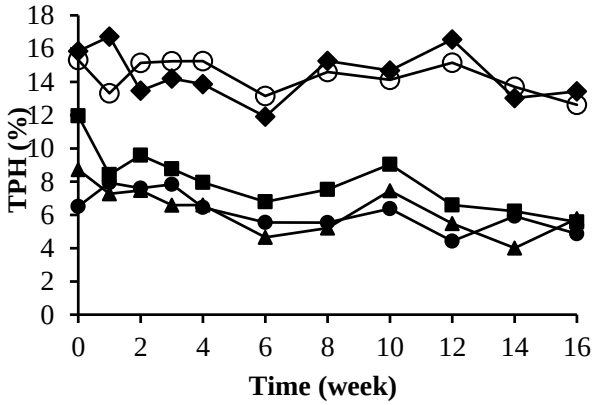

Figure 5. Change in TPH during landfarming bioremediation process on HOW (o), HOW + Compost (■), HOW + Clay (▲), HOW + Compost + Clay (●), and Control (♦).

TPH measured in all treatments showed a fluctuation. Decline in TPH value is suspected to be due to the biodegradation process produces short-chain hydrocarbons that are volatile, while increasing the value of TPH might be due to the insertion of O_2 and H_2O into organic compounds producing organic compounds that have high molecular weight. The fluctuation of TPH is suspected to be caused by the different work of microbes. Biodegradation of petroleum by microbes may occur under aerobic and or anaerobic conditions, and the degradation activity is a common reaction that occurs in nature. Different environmental conditions will affect different microbial activity in degrading pollutant agents. There is a difference between the results of treatment with the addition of bacteria and control, indicated by the slope resulting from the regression equation (Table 2). Although the difference is not significant, nevertheless, the addition bacterial

consortium results in smaller TPH than that of without the addition of bacterial consortium. This occurred in all treatments, as can be seen in Table 1.

Table 1 Regression equation from the decreased of TPH from various treatments in the landfarming process

Treatment	Regression Equation	Slope
Control	y= -0.124x + 15.21	-0.124
HOW	y= -0.162x + 15.68	-0.162
HOW + Compost	y= -0.277x + 9.748	-0.277
HOW + Clay	y= -0.143x + 7.897	-0.143
HOW + Clay + Compost	y= -0.179x + 6.773	-0.179

From the resulting slope, treatment on the mixture of HOW and compost has a steeper slope compared with other treatments, as an indication that the TPH difference at the beginning and end is large enough so that the degradation is quite high.

The large degradation resulting from the treatment of heavy oil waste mixed with the compost of around 53.35% is expected to result from good work of the bacteria. In the treatment using a mixture of compost, compost can be used as a good medium for bacterial growth because of its nutrients. The results showed that treatment with the addition of compost has the best performance in storing water content (Figure 5).

According to Daubaras and Chakrabarty (1992), the changes in environmental conditions will affect the microbial activity. Activity is increasing because of the expression of specific genes to produce the appropriate enzymes. Thus, in the degradation of oil where 90% of its components are composed of hydrocarbons, the enzymes that have many roles are oxygenase enzymes.

There are two types of oxygenase enzymes, i.e. monooxygenase and dioxyigenase. Monooxygenase enzyme has many roles in the degradation of aliphatic hydrocarbons while the dioxygenase enzyme has the role in the degradation of cyclic hydrocarbons. Both function in the early stages of degradation, i.e. during the insertion of molecular oxygen into the hydrocarbon structure. In the n-alkane molecules, oxygen insertion into the structure of hydrocarbons occurs in terminal or sub-terminal methyl group. n-alkanes are oxygenized into alcohol and then into carboxylic acid, which then would be the separation of two carbon units continuously known as beta-oxidation sequence (Cookson 1995) as a reaction described by Hurtig and Wagner (1992) (Figure 6 and Figure 7) .

The mechanism of alkane hydrocarbon degradation through oxidation of the terminal will experience a phase change successively into primary alcohols, aldehydes, and fatty acids. In the final stages fatty acid is converted into acetic acid (fatty acid with two carbon atoms) via β-oxidation reaction, which will be further degraded in the cell to produce carbon dioxide and energy. Oxidation sub-terminal alkanes will experience a phase change in succession into a secondary alcohol, methyl ketone, acetyl ester, primary alcohols, aldehydes and fatty acids or carboxylic acid. These fatty acids will be degraded to produce carbon dioxide and energy via β-oxidation reaction (Atlas and Bartha, 1998). Acyl coenzyme A will convert long chains of fatty acids into acetyl-CoA and short chain fatty acid that has reduced its carbon clusters as two units of CO_2 through the cycle of tricarboxylic acid (TCA) repeatedly (Atlas and Bartha 1998; Bailey and Ollis 1988).

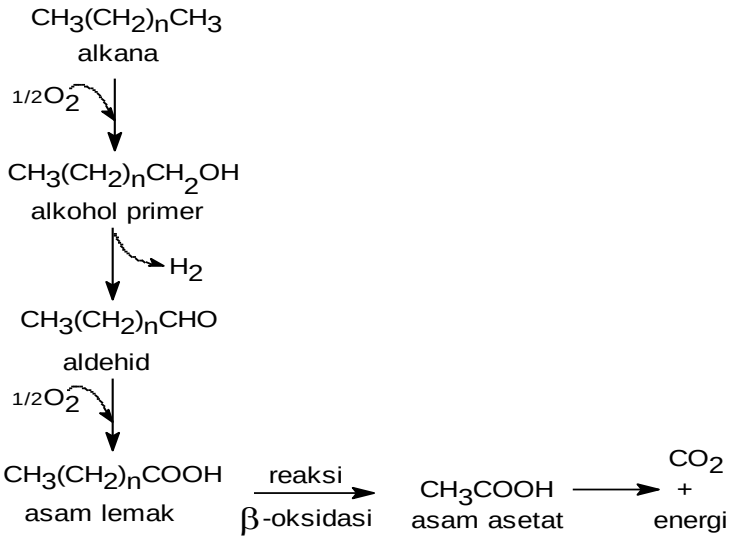

Figure 6. Alkenes hydrocarbons degradation through a terminal oxidation (Hurtig and Wagner 1992)

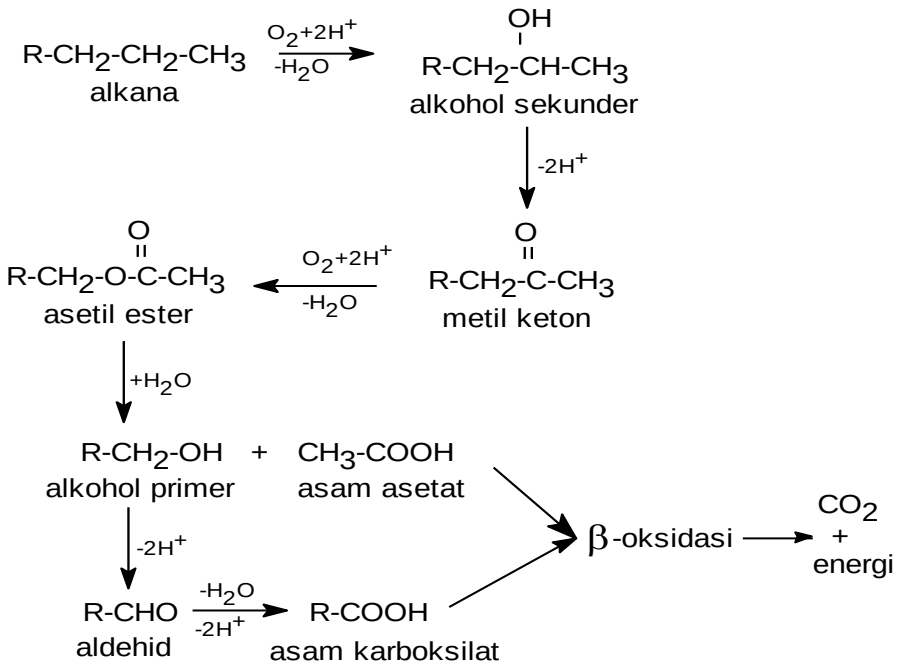

Figure 7. Alkenes hydrocarbons degradation through a sub-terminal oxidation (Hurtig and Wagner 1992)

The degradation of aromatic compounds contained in heavy oil fraction is mostly determined by the type, number, and position of substituted groups on the aromatic ring. The substituted groups in the aromatic ring such in benzene are usually -OH, -CH_3, -COOH, -CH_2OH, -NH_2, and -SO_3H. In the process of degradation of aromatic compounds, intermediate compounds will be produced, where the type is depending on the origin of the compound to degrade. However, in general, intermediate compounds formed can be

grouped into three, i.e. katekol, protokatekuat acid, and acid gentisat. Some of the degradation reactions of aromatic compounds with one, two, and three rings for benzene, naphthalene, and phenantrene will produce an intermediate form of katekol (Alexander 1977). The mechanism of benzene, naphthalene, and fenantren becomes intermediate of katekol can be seen in Figure 3.13, 3.14 and 3.15, respectively.

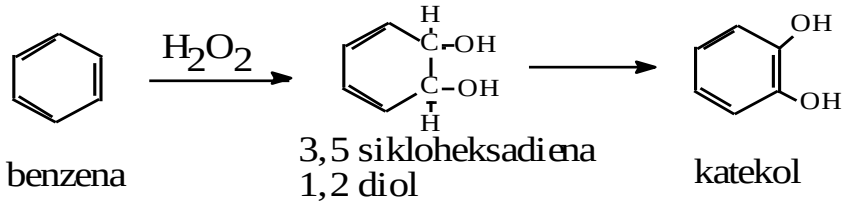

Figure 8. Degradation of benzene into katekol through an aromatic hydroxylized reaction (Alexander 1977)

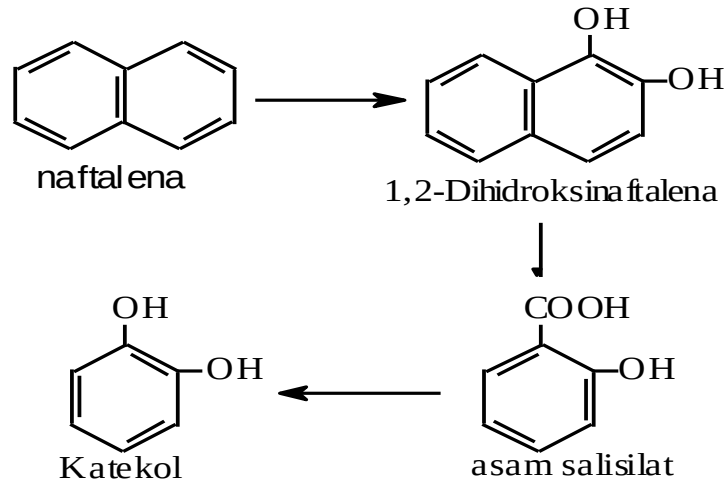

Figure 9. Degradation of a two-ring aromatic compound (naphtalene) into katekol (Alexander 1977)

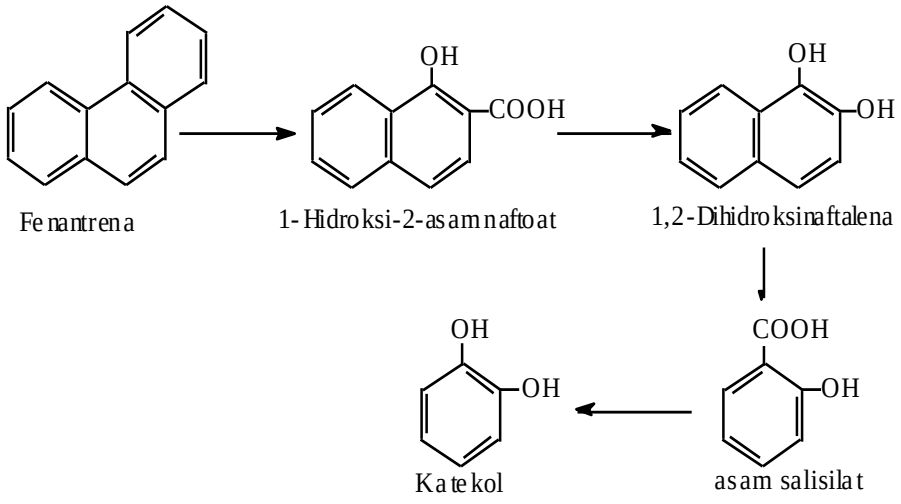

Figure 10. Degradation of a three-ring aromatic compound (phenantrene) into katekol (Alexander 1977)

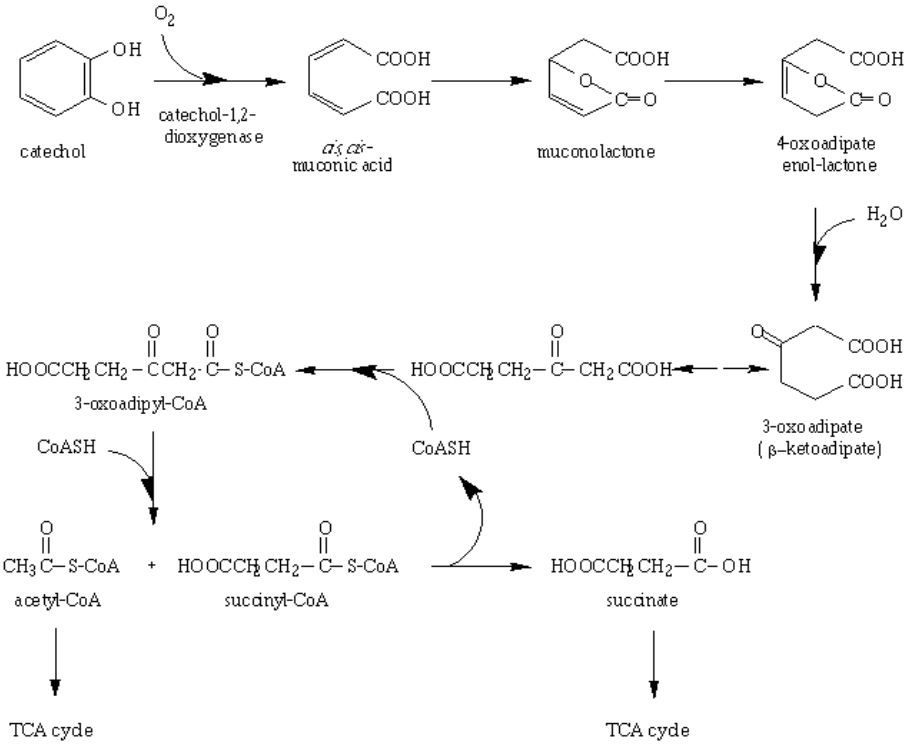

Figure 11. Orto-splitting for katekol breakage through catabolism (Doelle 1994)

Katekol ring can be broken through a catabolism process, which causes the aromatic ring rupture. Breakage of the ring can also occur through two ways, namely ortho-splitting where the aromatic rings divided into two carbon atoms resulting hydroxyl groups and meta-splitting where the benzene ring is split between hydroxylized carbon and the adjacent carbon atoms, or a benzene ring breakage occurs at the meta position.

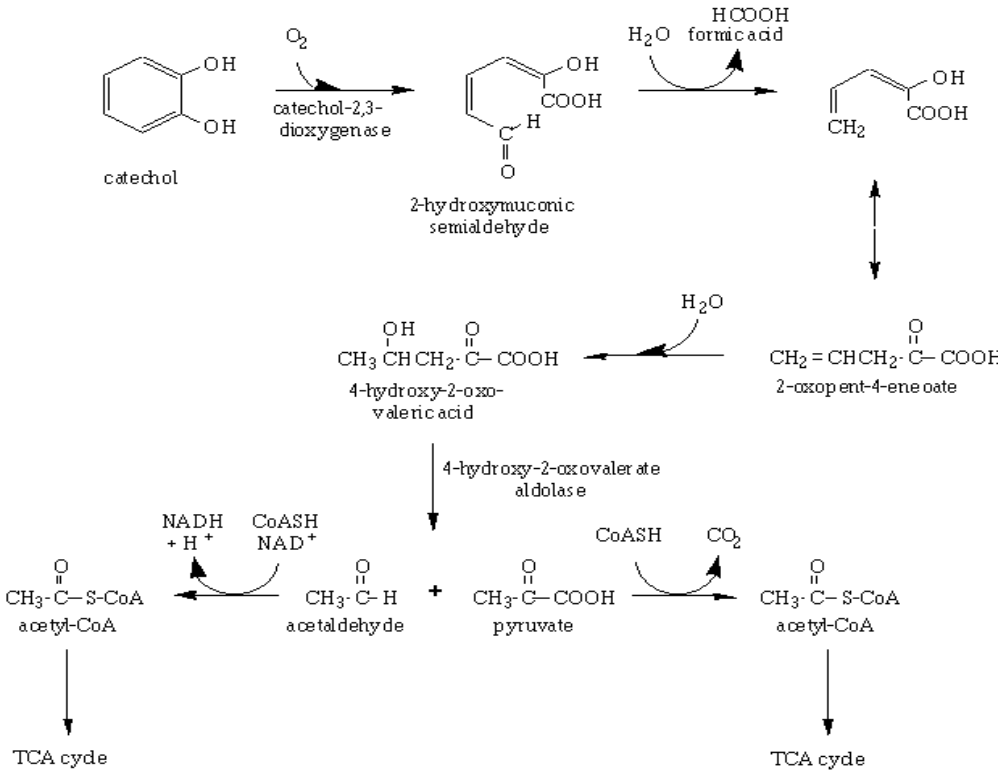

Figure 12. Meta-splitting for katekol breakage through catabolism (Doelle 1994)
Each ring breakage reaction is catalyzed by dioxygenase enzymes.

Subsequent metabolic process is very different but both of the reactions are headed to the intermediate cyclic TCA (acetate and succinate) or to a substrate that can easily be converted into the TCA cycle intermediate (pyruvate and acetaldehyde). Ortho-splitting (also called ketoadipat reaction) is shown in Figure 11 and meta-splitting reaction is shown in Figure 12. Higgins *et al*. (1984) in Pritchard et al. (1993) stated that various species of bacteria that oxidize short-chain hydrocarbons (methane, ethane, propane, butane, ethylene, propylene, and acetylene) would not oxidize long-chain alkanes. This condition is particularly true in the group of methanotrophic bacteria. Degradation of other short-chain hydrocarbons can be accomplished through a co-metabolism process. Cookson (1995) provided examples of microbe that is able to perform a short-chain hydrocarbons co-metabolism namely Pseudomonas methanic. Pseudomonas methanic uses methane as a primary substrate in addition to the secondary substrates such as ethane, propane, and butane into alcohol, aldehydes, and acids.

Nitrogen compounds also changed during the enrichment process. According to Chayabutra and Ju (2000), nitrogen compounds derived from protein remnants and amino acids in animal waste and fertilizer are also changing. In an anaerobic reaction, ammonium ions will be utilized by the anaerobic population, but if the amount of ammonia ions is too much it will inhibit the organic acids, fatty acid production, and methanogenesis.

Although variations in microbial populations in animal feces is relatively high, many of the microbes will die during the decomposition process, which will later be replaced by other microbes that are better suited to the chemical composition exist in the environments (Waksman 1957).

Change in Hydrocarbons

Changes in hydrocarbon compounds are based on the measured area in GC-MS chromatogram. Determination of hydrocarbon compounds is based on the data contained in the library using the CAS Number. Identification of sample compounds is selected from libraries that have more similarities than 90. Changes in hydrocarbon compounds on the overall sample can be seen in Appendix 3.8.

GC-MS chromatogram at the beginning of treatment (Figure 13) shows the number of hydrocarbon compounds contained in heavy oil waste, both aliphatic and aromatic hydrocarbon compounds. According to Lester (2003), crude oil contains hundreds of various components depending on region of origin: aliphatic, alicyclic, aromatic and non-hydrocarbons such as naphthenic compounds, phenols, thiols and sulfuric compounds. Suardana (2002) stated that heavy oil fraction at Duri area contains aromatic compounds, paraffin, naphtenic, and asphaltene and non-aromatic compounds such as compounds of N, S and O. At the initial measurement hydrocarbon compounds from C-6 and C-35 were identified. In all samples, the process of biodegradation is quite diverse (chromatogram can be seen in Appendix 3.5). GC-MS chromatogram at the end of the treatment with the addition of compost (Figure 14) shows that a number of hydrocarbon compounds are lost indicated by decreasing the peaks. The addition of compost will accelerate the biodegradation process.

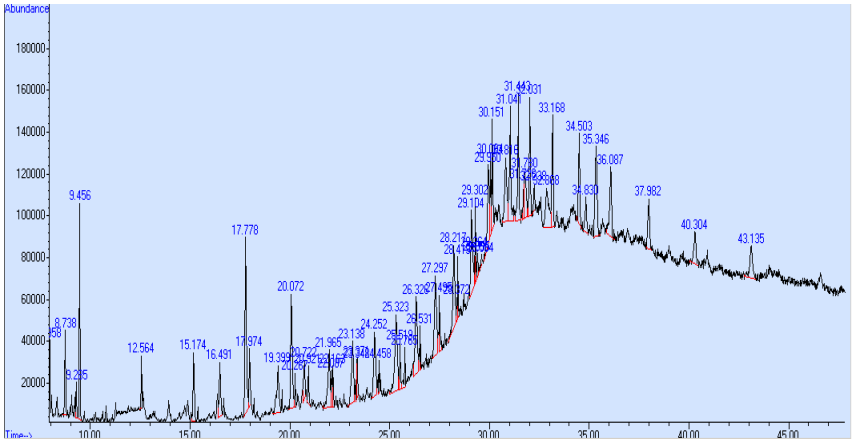

Figure 13. GC-MS chromatogram of the heavy oil waste at the beginning of treatment.

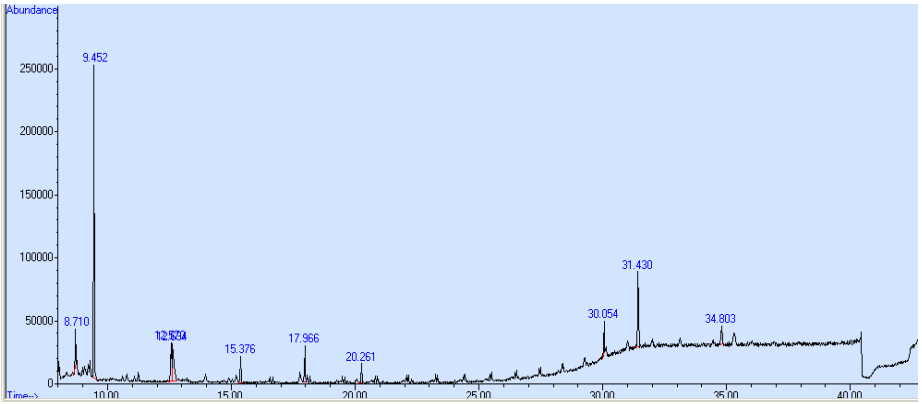

Figure 15. GC-MS chromatogram of the heavy oil waste + compost at the end of the treatment.

After measurements at week 16 many compounds are missing. In chromatogram data it can be seen a decrease of abundance. This might reflect a process of degradation of hydrocarbon compounds. In the degradation of n-alkane, insertion of oxygen molecules into the structure of hydrocarbons occurs in terminal or sub-terminal of methyl group. n-alkanes are oxygenized into alcohol and then into carboxylic acid, which then separated into two carbon units successively and is known as beta-oxidation sequence (Cookson 1995). Long chain of fatty acids are converted by acyl coenzyme A forming acetyl-CoA and short chain fatty acid that has reduced its carbon clusters as two units of CO_2 through the cycle of tricarboxylic acid (TCA) repeatedly (Atlas and Bartha 1998; Bailey and Ollis 1988).

Changes in hydrocarbon compounds on the addition of a consortium to heavy oil waste only are almost the same as treatment with HOW mixture with clay, as well as the treatment of HOW with compost mixture similar to HOW treatment with a mixture of clay and compost. Of all the samples, the least change is that HOW treatment without any mixture. This can be seen from the final result of measurement. In Appendix 3.8, there are still long-chain hydrocarbons, for example dokosane (C-22) with a peak of 0.25%.

Measurements at the end of the week there were a lot of missing compounds. Although on the HOW treatment without any mixture and mixture of HOW treatment with clay there are changes in area, but they are not too significant. At the same time, HOW treatment with a mixture of compost and mixture of HOW treatment with clay and compost, there were significantly changes. The missing compounds at the end of the measurement can be seen in Table 3.3. The missing of these compounds is expected to be due to the degradation processes. In the addition of bacterial consortium to of heavy oil waste and the addition of bacterial consortium on the mixture of HOW and compost there were not too many compounds missing. In the treatment of bacterial consortium to HOW only, there were only 4 missing compounds whilst 8 compounds missing in the treatment of HOW + clay. The most missing compounds (13 compounds) were found in the treatment of HOW + compost, which is the best decrease in TPH. This is expected to be caused by bacteria work better and bacteria that exist in the compost also take part in degrading the hydrocarbons. The un-missing compounds at the end of measurement can be said to be a hydrocarbon that is difficult to degrade by bacteria. For example, in the treatment of HOW + compost, aromatic hydrocarbon compound, dodecamethyl cyclohexasyloxane, which has a peak of 0.20%, in the addition of bacterial consortium to HOW alone with the same compound still has a broad peak area around 1.61% (Table 3).

Table 3 The missing compounds at the end of the measurement

LMB	LMB + Kompos	LMB + Tanah Liat	LMB + Kompos + Tanah Liat
tetrahydro 2-5 dimetil furan	tetrahydro 2-5 dimetil furan	tetrahydro 2-5 dimetil furan	dodekametil sikloheksasiloksan
oktametil Siklotetra siloksan	oktametil siklotetra siloksan	dekametil siklopentasiloksan	tetradekana
pentatriacontana	tetradekana	tetradekana	pentadekana
metil heksadekanoat	pentadekana	pentadekana	heksadekana
	heksadekana	heksadekana	heptadekana
	heptadekana	pentatriacontana	oktadekana
	oktadekana	dokosana	pentatriakontana
	pentatriakontana	1-nonadekana	nonadekana
	nonadekana		metil heksadekanoat
	metil heksadekanoat		eikosana
	Eikosana		heneikosana
	heneikosana		dokosana
	dokosana		

At the end of treatment (week 16) on the mixture of HOW and compost, there were 4 hydrocarbon compounds with peak ranges from 0.18% - 1.77%.

3.2 Gas Analysis during Biodegradation Process

CO_2 Production

The CO_2 formation results from aerobic processes in the biodegradation of oil waste contaminated soil. This process is mainly caused by aerobic bacteria. According to Atlas and Bartha (1987), in the biodegradation process, alkane chains are oxidized to form alcohols, aldehydes and fatty acids, after the formation of fatty acid catabolism process occurs through β-oxidation. Long chain of fatty acids is converted by acyl coenzyme A, which is an enzyme to form acetyl coenzyme A and short-chain fatty acid that has reduced its carbon clusters of two units that take place repeatedly. Acetyl coenzyme A is converted into CO_2 via the tricarboxylic acid cycle.

Table 4 Change in peak area (%) of detected compound with GC-MS at the beginning and at the end of the treatment with HOW and compost mixture.

Senyawa hidrokarbon	C ke-n	Waktu retensi	Area (%)		
			LMB awal	LMB Akhir	LMB + Kompos akhir
Tetrahidro 2-5 dimetil furan	C-6	7.585	0.39	td	td
Toluene	C-7	8.716	td	td	0.20
Heksametil siklotrisiloksana	C-6	9.453	td	0.62	1.77
Oktametil siklotetrasiloksana	C-8	12.565	0.15	td	td
Dekametil siklopentasiloksana	C-10	15.165	0.33	0.25	0.18
Dodekana	C-12	16.455	td	0.08	0.8
Dodekametil sikloheksasiloksana	C-12	17.781	0.82	1.61	td
Tetradekana	C-14	19.438	0.57	0.04	td
Pentadekana	C-15	20.721	1.11	0.32	td
Heksadekana	C-16	21.964	1.28	0.26	td
Heptadekana	C-17	23.138	1.49	0.15	td
Oktadekana	C-18	24.249	0.43	0.34	td
Pentatriakontana	C-35	24.459	0.18	td	td
Nonadekana	C-19	25.324	1.8	0.62	td
Metil heksadekanoat	C-16	25.764	0.32	td	td
Eikosana	C-20	26.325	1.66	0.49	td
Heneikosana	C-21	27.493	1.83	0.59	td
Dokosana	C-22	28.217	0.56	0.25	td

Based on the research of Eris (2006), the formation of CO_2 is a result of bacterial activity in degrading hydrocarbons. Figure 16 shows the result of CO_2 production from week 0 to week 16. From the observation, it can be seen the increase and decrease of CO_2 in each week. On the control and HOW without the mixture, the CO_2 level is not as high as of the others. In general, from each treatment, the first three weeks of CO_2 production increased, but began to decline at week 4, then increased again at week 6 to week 9, and the remaining week has another fluctuation.

Decrease in CO_2 production indicates that the aerobic process decreases. In the control treatment, it seems that CO_2 production fluctuates every week, similar to that in the

treatment with the addition of bacterial consortium on heavy oil waste without mixture. Research of Ramos *et al*. (2009) explained that the gas production in hydrocarbon-polluted soil containing PAHs was not significantly increased.

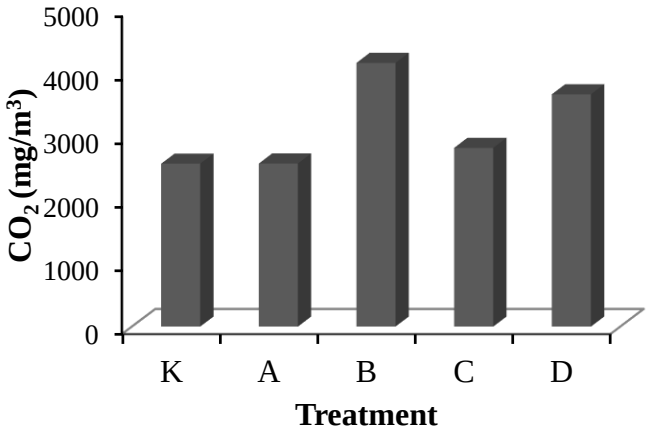

Figure 16. CO_2 production during bioremediation process with landfarming technique on HOW (A), HOW + Compost (B), HOW + Clay (C), HOW + Compost + Clay (D), and Control (K)

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The addition of bacterial consortium on heavy oil waste mixed with compost produces high enough CO_2; this is possible due to the addition of compost. The existence of this compost can be a factor that is very supportive to the ongoing process of degradation by bacteria, because there are nutrients in the compost that can be used as food for microbes. In addition to the nutrients, the compost also contained bacteria that can increase the population of microbes in it.

CO_2 produced from heavy oil waste treatment with a mixture of clay was not high. This might be caused by clay porosity that is in fact less than that of compost, so that the spread of nutrients cannot easily occur. The treatment of heavy oil waste mixed with clay and compost is not too far from the treatment of heavy oil waste mixed with clay. The average value of CO_2 was not that much different for the two treatments.

From all the data, the highest CO_2 was found in the treatment of mixed bacterial consortium addition to heavy oil waste and compost of 4156.3 mg/m3. Baptista *et al*. (2005) explained that the production of CO_2 is an indication the presence of high respiration rates of microbes, which is produced during the process of bioremediation. Kao and Wang (2000), also revealed the same, and explained that CO_2 is the result of all intrinsic bioremediation process. The resulting high gas production could be a clue that the process of intrinsic bioremediation process is taking place. Increased solubility of CO_2 in soil water indicates a biodegradation process. Degradation of hydrocarbons associated with the respiration of microbes and the results are indicated by the formation of this CO_2.

NH_3 Production

NH_3 produced from the degradation of hydrocarbon-containing group N, because petroleum does not just contain the element carbon and hydrogen, but also contain elements of about 0.11-1.70% nitrogen. This detected NH_3 shows that the anaerobic process occurs in the biodegradation process. This research is actually an aerobic process, however, the gases produced through anaerobic processes, such as H_2S and NH_3 were also detected, and this indicates that anaerobic processes was also taken place. Consequently, more oxygen is

needed in order to to keep the aerobic process proceed, because oxygen is also one of the factors that support this biodegradation process.

NH_3 produced was fluctuating in the form of sinusoidal-shaped just as the one produced for CO_2. For the control, the gas produced was high enough as can be seen from the cumulative of 1.9404 mg/m^3. High production of gas can be an indication that the degraded heavy oil wastes contain high content of N. Figure 3.9 shows the graph of NH_3 generated from the process.

Gas produced on the addition of bacterial consortium in the treatment of heavy oil waste without mixture is higher than that of the control, i.e. 2.5658 mg/m^3. Just as in the control, the possibility that the nitrogen content in heavy oil waste is high enough, and with the activity of bacteria, and hence makes the NH_3 output would be high. The graph is presented in Figure 17.

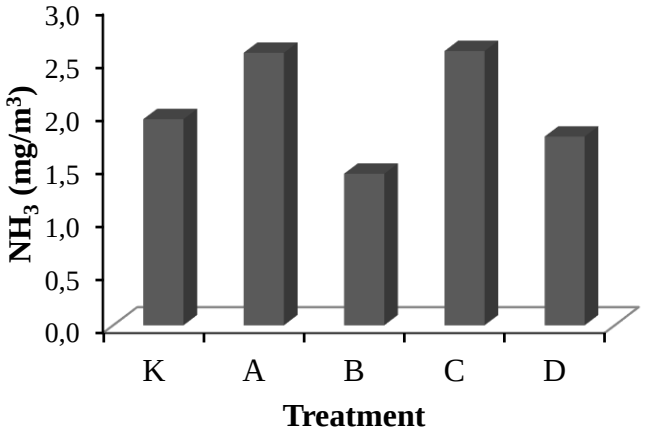

Figure 17. NH_3 production during bioremediation process with landfarming technique on HOW (A), HOW + Compost (B), HOW + Clay (C), HOW + Compost + Clay (D), and Control (K)

The total NH_3 produced in the treatment of mixed bacterial consortium and heavy oil wastes and compost is lower compared with other treatments i.e. with an average of 1426.8 µg/m^3. This can be explained by the fact that with the addition of compost, aeration will go well so that biodegradation occurs aerobically, indicated by high CO_2 content (Figure 3.20), and thus the NH_3 produced will be lower compared with other treatments. The graph is shown in Figure 17.

The total production of NH_3 in the treatment of heavy oil waste mixed with compost and clay is 1.7764 mg/m_3, higher compared with the mixture of heavy oil waste and compost. NH_3 produced in the treatment of heavy oil waste mixed with clay was also higher than that of the treatment of heavy oil waste without any mixture, which is 2.5824 mg/m^3. This was expected to be due to an imperfect aeration that biodegradation occurred anaerobically, and thus resulted in high content of NH_3.

4. Conclussions

Landfarming process conducted for 4 months results in still high TPH of around 5:58%, as an indication that the biodegradation process is slow along with the imperfect growth of bacteria. Less optimum of pH levels and low water content also support this result. Nevertheless, in spite of slow process, biodegradation process continues in the presence of

CO_2 and NH_3 produced during the observation. Sustainability of biodegradation process was also supported by GC-MS data, which showed that after 4 months of bioremediation process, hydrocarbon compounds from C-6 to C-12 that was originally composed of hydrocarbon compounds from C-6 to C-35 were identified.

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