Eco. Env. & Cons. 26 (June Suppl. Issue) : 2020; pp. (S108-S112)
Copyright@ EM International
ISSN 0971–765X

The effect of molasses biostimulation on Pertamina Balongan oil sludge biodegradation using bioslurry reactor

Wiwit Sri Yuliastuti[1], Aziz Purnomo Hakim[1], Nadia Safira[1], Agus Supriyanto[1,2], Fatimah[1,2], Hanif Yuliani[3], and Ni'matuzahroh[1,2]*

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2]Research Center for Bio-Molecule Engineering, Universitas Airlangga, Surabaya, Indonesia
[3]Agency for the Assessment and Application of Technology, BPPT, South Tangerang, Indonesia

(Received 27 September, 2019; Accepted 10 March, 2020)

ABSTRACT

Oil sludge is the largest oil waste in Indonesia that is accommodated in various oil companies. Oil sludge contains hydrocarbon compounds such as benzene, toluene, ethylbenzene and xylene (BTEX), and contains metal elements such as Hg, Cd, Ag, Ni, Pb, As, Cr, Sn, Zn and falls into the category of hazardous and toxic wastes (B3). The research aimed to determine the effect of biostimulation of molasses on residual levels of oil sludge TPH (ppm), TPC (CFU/mL), and pH and the best value of each treatment in the biodegradation process by using a bioslurry reactor. pH measurements were carried out using universal pH or paper pH, TPH measurements using the gravimetric method, and measurement of total microbial counts with TPC (CFU/mL). The results showed that the biostimulation of molasses had an effect on reducing oil sludge weight (ppm), total microbial count (CFU/mL) and pH. The best combination was found in DH21 treatment (molasses concentration was added 2% (v/v) for 21 days incubation) with the final residual 209 ± 92 ppm and the average of biodegradation percentage was 73.8%.

Key words: *Biodegradation, Oil sludge, Biostimulation, Molasses, Bioslurry reactor*

Introduction

Petroleum production in Indonesia is around 1.2 million barrels per day and produces 3,120 barrels per day of solid oil sludge. The waste produced in one year is 1.3 million barrels with 285,000 barrels including Hazardous and Toxic Materials (Lasari, 2010). Oil sludge contains hydrocarbon compounds such as benzene, toluene, ethylbenzene and xylene (BTEX), and contains metal elements such as Hg, Cd, Ag, Ni, Pb, As, Cr, Sn, Zn (Asia *et al*., 2006).

The oil and gas refinery industry produces hazardous and toxic waste (B3) from specific sources in the form of oil sludge (Government Regulation, 2014). Hydrocarbons are included in the category of hazardous and toxic waste (B3) which can pollute the surface of the soil, be swept by rain water, and enter the soil and then settle. As a result it can disrupt the soil ecosystem and the water cycle (Karwati, 2009). Based on the Decree of the Minister of Environment No. 128 of 2003 which regulates the procedures and technical requirements for processing biologically contaminated waste and oil, the final value of processed TPH products must be set below 10.000 ppm.

One of many ways to manage and utilize waste

can be done by using a biological agent called bioremediation, which is a technology that utilizes microorganism activities that are quite effective, relatively inexpensive, environmentally friendly, and able to degrade waste with stable and non-toxic final compounds. Bioremediation technology can be applied to restore the environment due to petroleum pollution from mining or refining activities. One kind of waste generated by these activities is oil sludge (Atlas, 1992). Bioremediation uses the addition of microbes to reduce environmental contamination by petroleum waste. Microbial activity produces enzymes and biosurfactants that function as decomposers of oil into compounds that are simpler, harmless, and have added value in environmental improvement. Biosurfactants work using the principle of increasing oil solubility (Ni'matuzahroh *et al.,* 2013).

Bioremediation techniques for petroleum can be done with biostimulation. Biostimulation is a method that is carried out by adding nutrients or changing habitats which serve to stimulate the growth of indigenous hydrocarbon decomposing microbes (Venosa and Zhu, 2003). Molasses is an easy and inexpensive sugarcane processing waste obtained. Molasses contains high levels of sucrose which can be utilized by biosurfactant-producing bacteria as a carbon source (Onbasli and Aslim, 2009). The addition of nutrients stimulates the growth of indigeneous microbial decomposers present in the oil sludge. Indigenous microbes have become accustomed to live in a toxic oil sludge environment and finally able to synthesize catabolic enzymes for hydrocarbon degradation (Leahy and Colwell, 1990).

The incubation time is the time needed by microorganisms to adapt and multiply themselves until they reach an exponential phase with high activity in remediating pollutants. At the time of incubation is getting longer, the process of microbial growth is increasing which is characterized by an increase in the number of microbial cells so that the bioremediation process runs fast (Pelczar and Chan, 1988). Therefore, further research is necessary to determine the effect of molasses biostimulation in the biodegradation process of oil sludge waste on the TPH residue level in oil sludge (ppm), TPC (CFU/mL), and pH.

Materials and Methods

The materials used in this research were oil sludge from Balongan Oil Refinery with 10% concentration, sterile distilled water, spiritus, 70% alcohol, molasses with a concentration of 0%, 0.5%, 1%, 1.5%, 2% (v/v), organic solvents (n-hexane), ethanol, physiological salts, NA media, glycerol, plastic clips, labels, latex, masks, aluminum foil, cotton, cling wrap, ice cubes, vaseline, and wrapping paper or newspapers.

Composing Molasses Concentration

Initial molasses was considered to be 100% concentrated, then dilution was carried out to 50% so that the nutrient content and impurities present in molasses can be settled. At reactor A, molasses with 0.5% concentration (v/v) were given, which means that molasses with 50% concentration were added as much as 1.35 mL with distilled water as much as 133.65 mL. The others reactors have same treatment carried out depending on prescribed concentration with dilution formula:

$$M1.V1=M2.V2 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Bioslurry Reactor Preparations

The reactor used was a closed tube made of glass with 500 mL capacity (working volume of 150 mL). The tube volume contained 150 mL of slurry and 350 mL of free space. The reactor cover was made of cotton with a hole in the middle for the hose, then coated with aluminum foil. The slurry formed was a mixture of oil sludge added with sterile distilled water mixed with a predetermined variation of molasses concentration, in a ratio of 1: 9 (15 mL of oil sludge and 135 mL of sterile distilled water mixed with molasses). The reactor was equipped with an aerator to supply oxygen for microbial needs and so that stirring or agitation occurs within 12 hours per day at a speed of 0.5 L/min. Sampling was carried out on days 0, 3, 7, 10, 14, 18, and 21 with three repetitions. The bioslurry reactor design can be seen in Figure 1.

pH Measurement

pH measurements were carried out using universal pH or pH paper. The pH measurement process was carried out by inserting pH paper into the reactor then the process lasted for 1-2 minutes. On pH paper the color changes will be seen, then the color of pH paper was matched to color scale found on universal pH package. The pH measurement was carried out three times so that the data obtained can be more accurate.

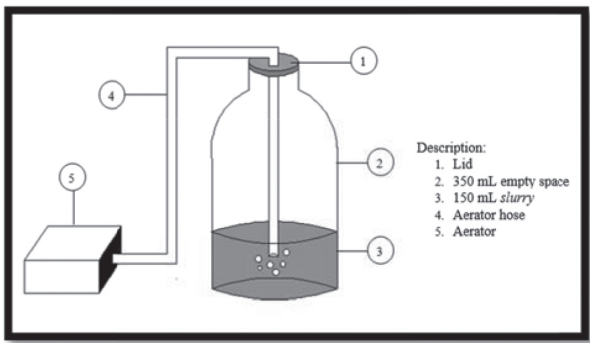

Fig. 1. Bioslurry Reactor Design

TPH Measurement

Total Petroleum Hydrocarbons were measured by the Gravimetric method. Samples were taken as much as 30 mL using a volume pipette, then the samples were put into separate flasks. Samples were extracted using 15 mL n-hexane, then homogenized by shaking for 15 minutes, until two phases formed, namely a clear brown solution (top) with a solution that was black or dark brown (bottom). The bottom solution was put into a 100 mL bottle, while the top solution was put in a dark bottle, the top solution was a mixture of n-hexane and oil residue. The solution which was in a 100 mL bottle is put back into a separate flask to be extracted again. If there was no two phases formed, then ethanol was added as an emulsifier.

The results of the extraction process were then carried out steam point separation using a rotary evaporator at a temperature of 60°C-70°C. TPH residual levels can be measured by calculating the difference in initial weight and final weight of the evap flask after the evaporation process. Oil levels raised in each treatment sample can also be expressed in percent of oil degraded during the biodegradation test (Badan Standarisasi Nasional, 2004). Percentage value of oil sludge degradation from each treatment can be calculated using the formula:

$$\% \text{ Biodegradation} = (W0 - Wn)/W0 \times 100\% \quad .. (2)$$

Microbial Total Number Measurements

Calculation of total bacterial colonies was done by using Total Plate Count method, which was 1 mL of slurry put into a test tube containing 9 mL of physiological water and then dilution (10^{-1}, 10^{-2}, 10^{-3} and so on according to requirement), but on day 0 it was not diluted because there were too few indigenous microbes in the oil sludge. Homogenisation was used for each dilution using vortex.

In last dilution, 1 mL suspension was taken using a micropipet and put into a Petri dish, then NA media + glycerol 2% were added to a Petri dish as much as 10-15 mL and homogenized by turning the Petri dish in 8-shaped, then incubated for 24 - 48 hours. The calculation of bacterial population number was carried out on a Petri dish with the number of bacterial colonies between 30-300 colonies in the last three dilutions.

The number of bacterial populations expressed by the number of growth colonies that are eligible to count, was multiplied by the serial dilution factor and expressed in the population of bacterial colonies that grow subsequently was calculated using a colony counter. The results of the calculation of the total number of bacterial colonies (CFU/mL) were then multiplied by 1/dilution factor (Edward, 2006).

Data Analysis

Data on the reduction of TPH residual levels in oil sludge (ppm), the value of the total amount of microbes (CFU/mL), and pH were analyzed descriptively.

Results and Discussion

The average results of TPH residue levels in oil sludge (ppm), TPC (CFU/mL), and pH based on variations in molasses concentration can be seen in Figure 2.

Based on Figure 2, it is known that the K treatment has the highest TPH residual content as much as 604 ppm. In the K treatment, molasses were not added so that nutrients and carbon sources were only obtained from the oil sludge. This causes the number of indigenous microbes that can grow in the K treatment is as much as 5.80 (CFU/mL). That number is the least amount compared to other treatments. The amount of TPC in treatment affects the degradation process that occurs, so that TPH residual level in K treatment remains high. Existence of carbon and nutrient sources in the Balongan oil sludge can be seen in Table 1.

Based on Table 1, it is known that in Balongan oil sludge there are carbon and nutrient sources needed by microbes for metabolic processes such as N, P, and K, but the percentage is very small so that the TPH residual level remains high with average amount of 604 ppm and the percentage of biodegradation was only 26.3%. In treatment D, the lowest

Table 1. Data of C organic, N, P, and K *oil sludge* Balongan

Test Parameters	Unit	Test Results	Test Methods
C Organic	%	37.38	Titrimetry
Phospor	%	0.24	Spectrophotometry
Potassium	%	0.04	AAS
Nitrogen	%	0.17	Kjeldahl

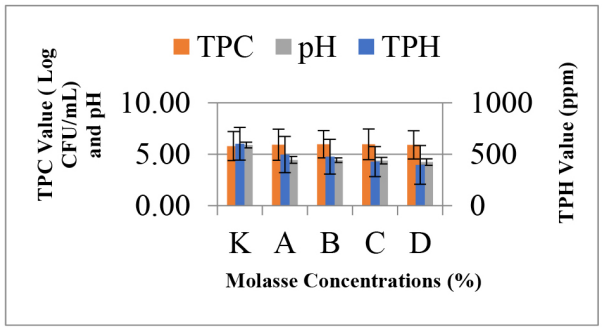

Fig. 2. TPH residual levels in oil sludge (ppm), TPC (CFU/mL), and pH based on variations in molasses concentration

Note:
K = Control (Molasse Concentration 0% (v/v))
A = Molasses Concentration 0.5 % (v/v)
B = Molasses Concentration 1 % (v/v)
C = Molasses Concentration 1.5 % (v/v)
D = Molasses Concentration 2 % (v/v)

TPH oil sludge residual level was obtained with a value of 397 ppm and a degradation percentage of 49.8%. This is allegedly due to the addition of molasses concentration of 2% (v/v) indigenous microbes in the reactor can utilize nutrients for growth, biosurfactant production, and enzymes, so that the process of biodegradation can continue even though the log data the total amount of microbes obtained is 5.92 (CFU/mL).

The difference in molasses concentration added to each treatment was only 0.5% so that the nutrients supporting the increase in the total number of microbes did not differ greatly. It is shown by the TPC values which almost have the same number in each treatment. Examples of TPC results can be seen in Figure 3.

Molasses in the reactor are used by microbes as a support in the process of metabolism and cell division. Indigenous microbes use molasses as an inducer or initial nutrient used for growth and produce biosurfactants and enzymes, and functioning as the process oil sludge biodegradation. The composition of molasses consists of simple sugars

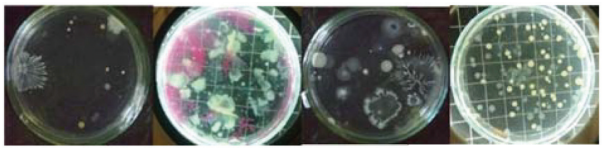

Fig. 3. Total plate count of bacteria on variation of treatments

(monosaccharides and disaccharides), N, P, and K. Microbes will choose to use simple sugars (monosaccharides and disaccharides) because they are easier to degrade. When indigenous microbes use molasses as growth nutrition, they also make an amphiphilic chemical compound in which hydrophilic and hydrophobic properties are present in a single molecule called biosurfactant. Biosurfactants have a structure consisting of a hydroxyl group and a hydrophobic group whose function is to help increase the solubility of oil making it easier for microbes to reach hydrocarbons.

In the treatment, K was not given additional molasses, causing the pH value of the media close to normal pH with a value of 5.9. In treatment A, B, C, and D the pH of the media is acidic because the added molasses has an acidic pH. According to previous research, it is known that the process of molasses sterilization can cause sugar compounds in hydrolyzed molasses due to high temperatures, causing the pH of molasses to become acidic (Pelzar and Chan, 2006). Acidic pH values can also be caused by the activity of bacteria that form acid metabolites. Alkane biodegradation contained in petroleum will form alcohol and subsequently become fatty acids. Fatty acids resulting from alkane degradation will be further oxidized to form acetic acid and propionic acid, thereby they can reduce the pH value of the medium (Rosenberg *et al.,* 1992).

The choice of using molasses as a biosurfactant production substrate due to its low price, easy to obtain, and can be a good substrate for some biosurfactant-producing microbes because it contains high levels of sucrose that microbes can use as a carbon source (Onbasli and Aslim, 2009), in addition to the use molasses is also an effort to utilize

waste and reduce the cost of handling waste (Nitschke *et al.,* 2004).

In this study, the most effective molasses concentration used was 2% (v/v) molasses concentration because only by utilizing *indigenous* microbes in the oil sludge was able to reduce the TPH residue level to 397 ppm with a degradation percentage of 49.8%. Based on the quality standards of the Decree of the Minister of Environment No. 128 of 2003 it is stated that the final value of processed products that is safe to be discharged into the environment as much as 10.000 ppm. In the treatment of additional 2% molasses concentration and 21 days incubation time showed the lowest TPH residue level of 1045 ppm. This value is the TPH residual level of the total reactor volume with degradation percentage of 73.8%. This shows that the results of the treatments that have been carried out successfully meet quality standards and are safely disposed to the environment.

Conclusion

There is an effect of molasses biostimulation with concentrations of 0%, 0.5%, 1%, 1.5%, and 2% (v/v) on TPH residue levels in oil sludge (ppm) and pH, but there is no effect of variations in molasses concentration against TPC (CFU/mL). DH21 treatment (molasses D concentration (2%) and incubation time of day 21) was the best combination with TPH residue content of 209 ppm and biodegradation percentage of 73.8%.

Acknowledgement

This research was conducted with the support of the STRANAS Research funding, 2017. Therefore, the researchers thanked the "DRPM-Kemenristek Dikti "and "Universitas Airlangga"for its financial assistance and facilities.

References

Asia, I. O., Enweani, I. B. and Eguavoen, I. 2006. Characterization and Treatment of Sludge from the Petroleum Industry. *African Journal of Biotechnology*. 5(5): 461-466.

Atlas, R. M. 1992. *Petroleum Microbiology: Encyclopedia of Microbiology.* Academic Press, Inc. California. page: 39.

Badan Standarisasi Nasional. 2004. Cara Uji TPH dengan Metode Gravimetri. SNI 06-6989.10-2004.

Edward, V. A. 2006. Qualities Control Method-1 Count of Aerobic Microorganism and Spore Count. Alken Murray Corporation. New Hyde Park. page: 1-8.

Karwati. 2009. Degradasi Hidrokarbon Pada Tanah Tercemar Minyak Bumi dengan Isolat A10 dan D8 *Skripsi*, Departemen Kimia, Fakultas Matematika dan Pengetahuan Alam, Institut Pertanian Bogor

Minister of the Environment, 2003. Decree of the State Minister for the Environment Number 128 Concerning Procedures and Technical Requirements for the Treatment of petroleum Waste and Soil Contaminated by Petroleum Biologically. Jakarta. Page 813-829.

Lasari, D. P. 2010. Bakteri, Pengolah Limbah Minyak Bumi yang Ramah Lingkungan. Http://esdm.go.id. Accessed on 24th October 2017.

Leahy, J. G. and Colwell, R. R. 1990. Microbial Degradation of Hydrocarbons in the Environment. *Microbiol Rev*. 54 : 305-315.

Ni'matuzahroh, Fatimah, Surtiningsih, Tini., dan Sumarsih, Sri. 2013. "Biocleanoil" Produk Berbasis Mikroba untuk Pengolahan Sludge Industri Minyak. *Laporan Penelitian Unggulan Perguruan Tinggi*. Departemen Biologi Universitas Airlangga : Surabaya

Nitschke, M., Ferraz, C. and Pastore, G. M. 2004. Selection of Microorganism for Biosurfactant Production Using Agroindustrial Wastes. *Brazilian Journal of Microbiology*. 35 : 81-85.

Onbasli, Dilasad and Aslim, B. 2009. Biosurfactant Production in Sugar Beet Molasses by Some *Pseudomonas sp.. Journal of Environmental Biologi.* 30(1) : 161-163.

Pelczar, J. M. J. and Chan, E. C. S. 1988. *Dasar-dasar Mikrobiologi.* Jakarta: UI Press. Page: 1-14.

Pelczar, J. M. J. and Chan, E. C. S. 2006. *Dasar-dasar Mikrobiologi,* Jilid I. Penerbit Universitas Indonesia, Jakarta.

Government Regulation. 2014. Government Regulation Number 101 Concerning Management of Hazardous and Toxic Waste. Jakarta.

Rossenberg, E., Legmann, R., Kushmaro, A., Taube, R., Ron, E. Z. 1992. Biodegradation In: *Petroleum Bioremidiation a Multiphase Problem.* Page: 337-350.

Venosa, AD. and Zhu, X. 2003. Biodegration of Crude Oil Contaminating Marine Shoreline and Freshwater wetland. *Spill Science and Tecnology Bulletin*. 8(2).