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BIODEGRADATION ENHANCEMENT OF OILY WASTEWATER BY OZONATION METHOD

PENINGKATAN KEMAMPUAN BIODEGRADASI LIMBAH CAIR BERMINYAK DENGAN METODE OZONASI

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ABSTRAK

Penelitian ini bertujuan untuk meningkatkan kemampuan biodegradasi limbah cair berminyak dengan metode ozonasi, dimana ozon akan mengoksidasi senyawa-senyawa organik di dalam limbah cair berminyak yang sulit untuk didegradasi oleh bakteri sehingga strukturnya menjadi lebih sederhana. Proses ozonasi dilakukan dengan menggunakan ozonator corona-discharge dan reaktor semi-batch, dimana pada proses tersebut divariasikan nilai dosis ozon dan pH. Proses ozonasi kemudian dilanjutkan dengan proses biodegradasi, dimana bakteri yang digunakan adalah Pseudomonas sp. Pada penelitian ini juga diamati efek penggunaan surfaktan dalam menunjang proses biodegradasi. Indikator keberhasilan penelitian ditunjukkan dengan peningkatan nilai persentase biodegradasi dari sampel yang diikuti dengan peningkatan nilai konstanta laju pertumbuhan bakteri. Hasil penelitian yang didapat menunjukkan bahwa proses ozonasi dan penggunaan surfaktan dapat menunjang proses biodegradasi limbah cair berminyak. Hal tersebut ditunjukkan oleh besarnya nilai persentase biodegradasi dan konstanta laju pertumbuhan bakteri yang didapat, dimana berturut-turut nilainya mampu mencapai 86,787% dan 0,339 pada kondisi operasi optimal (dosis ozon 0,53 g/jam; pH 7; surfaktan Bios-H dengan konsentrasi 500 ppm).

Kata kunci: limbah cair berminyak, ozonasi, biodegradasi, surfaktan, pseudomonas sp

ABSTRACT

This research is aimed at improving the ability of oily wastewater biodegradation through the ozonation method, where ozone will oxidize organic compounds in the oily wastewater that are difficult to be degraded by bacteria so that the structure becomes simpler. The ozonation process is done by using a corona-discharge ozonator and semi-batch reactor, and the process is varied by ozone dose and pH value. The ozonation process is then followed by a biodegradation process, where the bacteria that is used is *Pseudomonas sp*. This study also observed the effect of surfactants in supporting the biodegradation processes. An indicator of research success is the increase in the biodegradation percentage of the sample, followed by an increase in the value of the constant rate of bacterial growth. The research results shows that the ozonation method and the use of surfactant can support the oily wastewater biodegradation process. This is shown by the value of the biodegradation percentage and the constant rate of bacterial growth results, in which respective values are able to reach 86,787% and 0,339 at the optimal operating conditions (ozone dose of 0.53 g/h; pH 7; Bios-H surfactant concentration 500 ppm).

Keywords: oily wastewater, ozonation, biodegradation, surfactant, *pseudomonas sp*

I. INTRODUCTION

Environmental pollution caused by oil spills at sea and produced water that is a by product of oil and gas industry operations and activities, often occurs. The oil pollution that has been dispersed and then mixed with water, results in waste known as oily wastewater (Hendarsa 2013).

Generally, oily wastewater contains hydrocarbons which have components of organic compounds with complex structures such as aliphatic (alkanes and alkenes), aromatics (benzene, toluene, ethylbenzene, and xylene isomers) hydrocarbons and polycyclic aromatic hydrocarbons (PHAs). These compounds are highly toxic and carcinogenic (Atlas 2008).

Oily wastewater can be processed in a number of ways (physical, chemical and biological). Physical processing is the initial stage of processing by localizing the oil spill using a buoy barrier, which will then be transferred by the pump to a receiving facility either in a tank or a balloon and proceed with chemical process by using dispersants. However, it is expensive and can cause new pollutants (Anonymous 1994). The biological way (bioremediation) is by using a bacterium and is recognized as the best method because apart from relatively inexpensive processes it is also environmentally friendly. In this process, the bacteria use the oil as a carbon source (nutrition), therefore the oil component will split into a mixture that has a lower molecular weight (Prakash 2011).

Bioremediation techniques are widely applied to treat oily wastewater because they are relatively inexpensive (low energy consumption) and environmentally friendly (limited use of chemicals). However, these processes have a very long retention time, which is about 20-30 days (Pavlostathis 1986), and also have a fairly low degradation efficiency (20-50%) for a time of degradation of 7 days. One reason is the complexity of the structure of the compounds contained in petroleum, making it difficult to be degraded by bacteria which is used in the degradation process. Therefore, to improve the efficiency of biodegradation, the most logical approach is to simplify or reduce the stability of the structure of aromatic compounds in advance so they can be more easily degraded by bacteria with a mechanical pretreatment, biological, thermal, and chemical prior to bioremediation. The pretreatment of the process of chemical oxidation using ozone as its oxidant is a process that is relatively effective, which has been applied to the treatment process of water and wastewater, particularly used to oxidize organic compounds difficult to be degraded as aromatic compounds, cycloalkanes, as well as long-chain paraffin compounds C18 or more contained in the oily liquid waste (Song 2007).

The results of a previous study shows that the ozonation process is capable of oxidizing compounds that are difficult to be degraded by bacteria contained in the oily liquid waste (Adetyas 2012).

The study obtained the largest declines – as much as 14.7% for aromatic compounds, cycloalkane compound as much as 37.9%, and the paraffin compounds long chain (C18 or more) as much as 17.7% on the operating conditions of the ozone dose of 0.53 g/hour. Based on these results it can be concluded that the ozonation process can be applied to support the process of biodegradation of oily wastewater.

Research on biodegradation of oily wastewater as well as the various petroleum compounds has been carried out around the world. One microorganism proven effective in degrading hydrocarbons is the bacterium *Pseudomonas sp*.

The bacteria are able to adapt and breed well in petroleum environments within high concentrations (up to 50% v/v) and are able to utilize the hydrocarbon component such as aliphatic compounds and mono - aromatics as substrate/food (Prakash 2011).

Another study that has been conducted in Cuba showed that the *Pseudomonas sp* was able to degrade the oily wastewater from oil refineries Huos Diaz in Cuba with the percentage of biodegradation of 50% for a time of degradation for 7 days (Silva 2006).

From these studies it can be observed that the percentage of biodegradation is still quite low.

Another factor that leads to low effectiveness of the biodegradation of oily wastewater using bacteria is the low solubility of oil in water. The addition of nonionic surfactants would increase the solubility of oil in water to facilitate contact between microorganisms and carbon sources of petroleum as its nutrition (Whang 2010, Zhang 2005).

This study was conducted to determine the effectiveness of the implementation of an ozonation process in order to improve the performance of the biodegradation process of the synthesis of oily wastewater made from a mixture of produced water and crude oil. Success is seen by a decrease in the value of the levels of oil and grease from a sample of the results of the process of bioremediation together with the ozonation method and compared with samples of the results of the bioremediation without ozonation. The lower the value of the oil and grease content in a sample means that more hydrocarbon compounds have been degraded. In addition, this study also aims to determine changes in the characteristics of oily wastewater after ozonation and biodegradation process, which were analyzed using GCMS.

II. METHODOLOGY

This research will observe the effect of the ozonation process to increase biodegradation of petroleum waste by *Pseudomonas sp*. Variables used in this research were ozone dose, pH values, as well as the use of surfactants in the biodegradation process. Media treatment that used is produced water enriched by the addition of urea and NPK to obtain the content of NP (5:1) 0.1 Yeast Extract. This research obtained data to allow for the evaluation of the extent to which the effects of ozonation and the use of surfactants increase the efficiency of biodegradation as indicated by the increase in the percentage of biodegradation and constant value growth rate of bacteria. The success of this research is indicated by changes in the characteristics of the sample associated with the changes the composition of aromatic and organic compounds, which will be analyzed using the GCMS method. Bacterial populations are observed using Total Plate Count (APHA 9215 C 2015) by way of counting the number of bacterial cells during the 7-day incubation using the formula:

$$BO = D \times C \quad (1)$$

Where, BO: the number of bacterial cells/mL, D: dilution factor, C: number of colonies bakteri. The calculation of the amount of the population, can be determined coefficient of growth rate of bacteria by using the formula:

$$\mu = 2.3/t \log (a/b) \quad (2)$$

Where: μ: coefficient of growth rate, t: incubation time, a: number of population after t7, b: a population at t0. Biodegradation indicated by differences in the content of oil and grease at the beginning and end of the samples analyzed by gravimetric method which refers to the ASTM D 4281- 2010.

A. Preparation of Bacteria Culture

The waste petroleum degrading bacteria used is a pure culture of *Pseudomonas sp* originating from the laboratories of Microbiology, Faculty of Medicine UI Salemba. Activated bacterial culture in NB medium (nutrient broth) for 24 hours at room temperature. Once activated, the culture bacteria adapted gradually in 100 ml of produced water as media treatment and enriched with the addition of urea and NPK to obtain N/P (5: 1) extract yeaste + 0.1% (v/v). For process variations by using a surfactant, Tween 40 surfactant is added at a concentration of 29 ppm in accordance with the value of the CMC (critical micelle concentration) (v/v) dissolved in produced water 150 mL. Meanwhile, for process variations by using biosurfactant, added biosurfactant Bios-H with a concentration of 500 mg/L (v/v) dissolved in media produced water 150 mL. In addition, the media was whipped in Shaker Table for 72 hours at room temperature with a speed of 120 rpm. After turning the media three times, that is 3 times the adaptation period, 10% (v/v) culture containing ± 107sel/mL, was ready for use in the process of biodegradation of petroleum waste.

B. Biodegradation Test Sample Preparation

Waste crude oil used as a sample is the result of the mixing of crude oil and produced water which were taken from the oil refinery PPT Migas Cepu, Central Java, with a concentration of crude oil as much as 3% (30,000 ppm) by adding 5 g of crude into 150 mL of aqueous media produced. These samples will be followed by the biodegradation process, which will involve the *P. aeruginosa* bacteria. Therefore, the whole sample including tools should be sterilized beforehand in an autoclave at a temperature of 121^{o}C for 15 minutes before the ozonation process.

For variety, acidic pH 5 N H2SO4 solution is used while for alkaline pH variation used a solution of NaOH 5 N.

Biodegradation process was carried out for 7 days while observations were made on day 0 and the 7 day of the parameters pH , bacterial population and oil and grease.

C. Ozonation Stage

At this stage, the waste processing petroleum -based ozonation advanced oxidation process will be carried out in a semi-batch reactor for 30 minutes and followed by dose variation of ozone and pH values.

Petroleum waste samples that had been prepared previously were introduced into the reactor (150 mL). Ozone is then poured into the reactor for 30 minutes. Variations were made to the feed air flow rate of 100 L / h to 400 L / h and pH variations in the value of 5, 6, 7 and 8 (use strong base and a strong acid H_2SO_4 NaOH).

III. RESULTS AND DISCUSSION

Levels of oil and grease is a parameter used to determine the percentage of biodegradation in the process of biodegradation by bacteria. Determination of oil and grease content was by gravimetric analysis performed on samples with varying doses of ozone and pH predefined. Ozone dosage varied by changing the flow rate of the feed air ozonator. Results of calculation of ozone doses in each variation of the air flow rate that has been used can be seen in Table 1 below:

A. Effect of Ozone Dose Against Percentage of Degradation

Biodegradation process from day 0 to day 7 showed decreased levels of oil and grease and an increase in the value of the percentage of degradation due to ozone dose enhancement. Figure 1 shows a decrease of oil and grease levels after ozonation for 30 minutes and after biodegradation with an incubation period of 7 days, while Figure 2 shows the percentage of degradation at various doses of ozone that were used. The control sample in this study is the initial sample without ozonation treatment.

Table 2 and Figure 1 show that the decline in oil and grease levels is achieved with the highest ozone dose of 0.53 g/h is of 32 997 mg/L on day 0 into 5.420 mg/L on day 7 levels 78.87% of biodegradation.

From Figure 1 can also be concluded that the influence of ozonation on the biodegradability determines the final condition after biodegradation. It can be seen from the sample after ozonation for each of the various doses of ozone. Samples with decreased levels of oil and grease highest after ozonation also decreased levels of oil and grease highest after biodegradation as well as the percentage of degradation. So, from the initial condition after ozonation alone we can already guess the sample that will give the best results (highest degradation).

This is due to the increase in ozone doses that were administered during treatment. The more doses of ozone given the more the ozone is in contact with hydrocarbon components contained in the waste oil. This will lead to enlarged compounds which can not be degraded by bacteria converted by ozone into simpler new derived compounds as shown in Table 2 where there was a decrease in the oil content from 32.997 mg/L to 31.997 mg/L. Other than that, it will also lead to increased growth of the bacterial population in the treatment of media that will ultimately increase the activity of bacteria in degrading petroleum waste. This is because the microbes will degrade hydrocarbons as a carbon source to produce energy for its survival and will produce a gas, organic acids, and biomass (Karwati 2009).

Table 1
Results of the ozone dose calculations

Air flow rate (L/hour)	Dose ozone (g/hour)
100	0,35
200	0,41
300	0,46
400	0,53

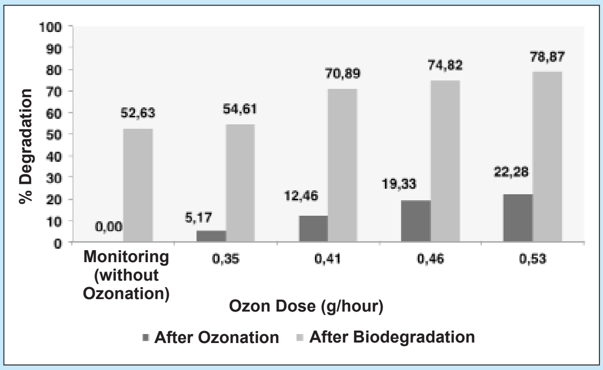


Figure 1
Biodegradation Percentage of hydrocarbons compound to variations in ozone dose

Table 2
Levels of oils and grease for variations in ozone dose

Dose Ozone (g/hour)	Levels of Oils and Grease (mg/L)		
	Initial	After Ozonation	After Biodegradation
0,00	32.997	32.997	15.631
0,35	32.997	31.289	14.201
0,41	32.997	28.887	8.409
0,46	32.997	26.616	6.703
0,53	32.997	25.645	5.420

B. Effect of pH Against Percentage of Degradation

One other variable that was decisive in the ozonation process and biodegradation of petroleum by *Pseudomonas sp* is the pH value of the sample. Table 3 and Figure 2 below shows the levels of oil and grease as well as the percentage of biodegradation after ozonation with ozone remains the dosage of 0.53 g/h for 30 minutes and after biodegradation for 7 days with a variation in pH.

Table 3 and Figure 2 below shows that a decrease in oil content and a fairly high grease occurs at pH 7 and pH control (pH 7.4 initial sample) with successive degradation level of 79.32% and 78.87%.

In contrast to previous results in which the initial condition after ozonation determines the final condition after biodegradation, this was not the case with pH variations. The initial condition after ozonation provides reduced levels of oil and grease although given the same dose of ozone, namely 0.53 g / hour for 30 minutes. This is because the pH value can affect the action of ozone on organic materials.

Decreased levels of oil and grease as well as the highest percentage of degradation after ozonation ware found in the highest pH variation used in this study (pH 8) (Table 3, Figure 3). This is because under alkaline conditions, the solution will be dominated by OH- ions, the ions which will initiate ozone to decompose into hydroxyl radicals. The higher the pH value, the ozone decomposition rate will increase. In effect, the more the ozone converted into hydroxyl radicals. The hydroxyl radical is the strongest oxidizer in water and has a low selectivity, so the more the number of hydroxyl radicals in the reaction system, it will result in more organic compounds attacked/oxidized by the radicals directly (Adetyas 2012).

The effect of pH on ozonation was not the same as the effect of pH on biodegradation. This is evident from a decrease in the levels of oil and grease, as well as the highest percentage of degradation after biodegradation are also not on the highest pH level. Theoretically, pH 8 should provide the largest percentage biodegradation. In fact the percentage biodegradation at a pH value of 8 is not much larger in value than pH 7 and pH control. This is because the pH value of 8 is the maximum pH value that *Pseudomonas sp* bacteria can grow, meaning that pH is not optimal for bacterial growth. Accordingly, although the ozonation process succeeded in reducing the composition of the compound that are difficult to be degraded by bacteria, but if the core agent is in the biodegradation of the *Pseudomonas sp*, it is difficult to grow in media treatment. Therefore, the increase in biodegradation becomes insignificant. This also explains why the values of pH 5 and 6 produce a fairly low level of biodegradation.

Table 3
Levels of oils and grease for variations in pH

pH	Levels of Oils and Grease (mg/L)		
	Initial	After Ozonation	After Biodegradation
Kontrol (7,4)	32.997	25.645	5.420
5	32.997	29.099	18.107
6	32.997	27.279	15.836
7	32.997	25.749	5.326
8	32.997	24.866	10.140

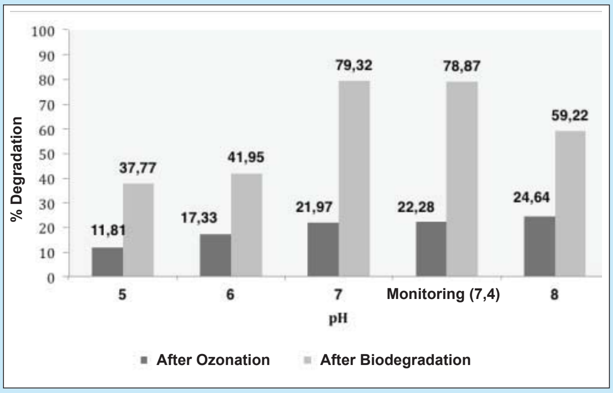


Figure 2
Percentage of degradation to variations in pH.

The pH value is associated with the activation of destructive enzymes petroleum existing in bacteria. Biodegradation by microbes can occur with the growth of bacteria and enzyme activity of the microorganisms. Through enzymatic process, bacteria can transform hydrocarbon substance into simpler forms (Atlas 2005). At the optimum of pH value, these enzymes will be more activated as indicated by the higher percentage of biodegradation (pH 7).

Hydrocarbons oxidized because of the specificity of the enzyme of a microbe is quite extensive, as well as monooxygenases enzymes involved in the oxidation of n-alkanes to alcohols primary, oxygenize enzymes involved in the degradation of cycloalkanes and enzymes deoxygenize the degradation of benzene and catechol. The peroxidase enzyme stimulates aerobic oxidation reactions on compounds pyrene, asenapten, antrasen, dibenzotiopen, and 9 methyl antrasen (PAHs) (Zeynalov 2015).

C. Effect Concentration Surfactant Against Degradation Percentage

One type of surfactant used in this study is Tween 40, where the concentration is varied to determine how it affects the process of biodegradation. Before being given surfactant, the ozonated samples were first given an ozone dose of 0.53 grams/hour for 30 minutes at pH 7.

Figure 3 shows that the highest percentage of biodegradation was obtained on the value of the concentration of 30 mg/L which is the value of CMC (Critical Micelle Concentration) of Tween 40 surfactant. Overall, the value of the percentage of degradation increases with the amount of the concentration of surfactants that have been used. However, when used in concentrations of 40 mg/L (higher than the value of CMC surfactant Tween 40 were worth 30 mg/L), the percentage of biodegradation was only 84.739% or lower than the percentage biodegradation at CMC concentration (30 mg/L), that is 85.009%.

Theoretically, the addition of surfactant in solution will cause a decrease in the surface tension of the solution. Surface tension will decrease until the CMC is reached. Once the CMC is reached, the surface tension will be constant which indicates that the interface becomes saturated and formed micelles that are in dynamic equilibrium with the monomer (Burlatsky 2013).

Therefore, the addition of surfactant which exceeds the CMC will not decrease the surface tension and will only add to the complex structure of organic compounds in the sample that can reduce the rate of bacterial growth and the percentage of oil degradation.

D. Effect of surfactant types Against Degradation Percentage

In this experiment the effect of the addition of chemical surfactants Tween 40 and biosurfactant Bios-H, which is a product of biological surfactants developed by Lemigas, can be seen on the performance of biodegradation. Before being given surfactant, ozonated samples were first given an ozone dose of 0.53 grams/hour for 30 minutes at pH 7. The concentration values used for each surfactant is the value of CMC, where the surfactant Tween 40 was 30 mg/L and for surfactants Bios-H is 500 mg/L.

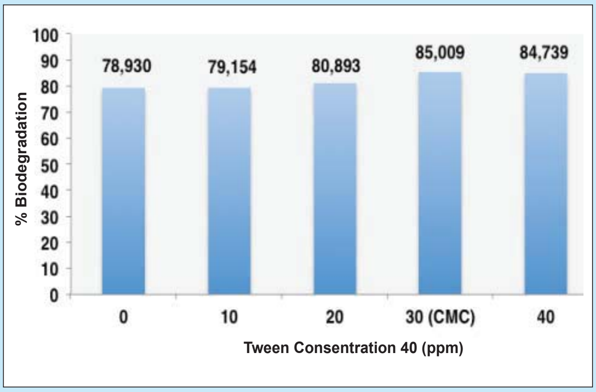


Figure 3
Percentage of biodegradation for variations in the concentration of surfactant.

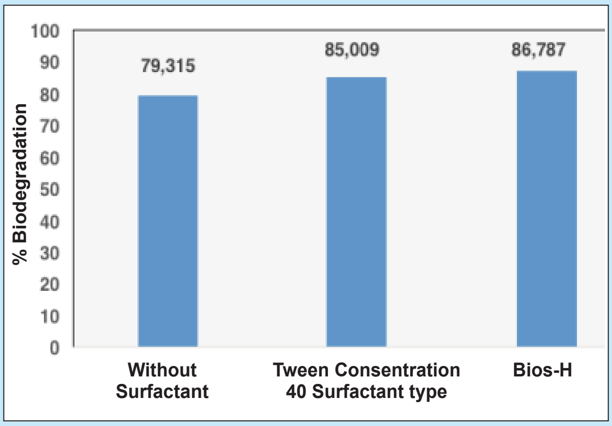


Figure 4
Percentage variation of biodegradation for surfactant.

Results showed that the highest percentage of biodegradation was obtained on the use of biological surfactants Bios-H (Figure 4). Biodegradation percentage of the value obtained for the use of both types of surfactant are in fact not much different, where the surfactant Tween 40 percentage biodegradation obtained a value of 85.009%, whereas surfactants Bios-H obtained a value of 86.787%. When assessed using the percentage of biodegradation, the best type of surfactant is Bios-H. However, the value of the CMC of the surfactant Bios-H differ considerably from the value of CMC surfactant Tween 40 (500 mg/L versus 30 mg/L), so that the surfactant Bios-H used more chemicals than Tween 40 to generate the percentage biodegradation which was only 1.778% different. Although more extravagant in the use of materials, bio-surfactant has a lower level of toxicity and is biodegradable biologically, making it more environmentally friendly than Tween 40.

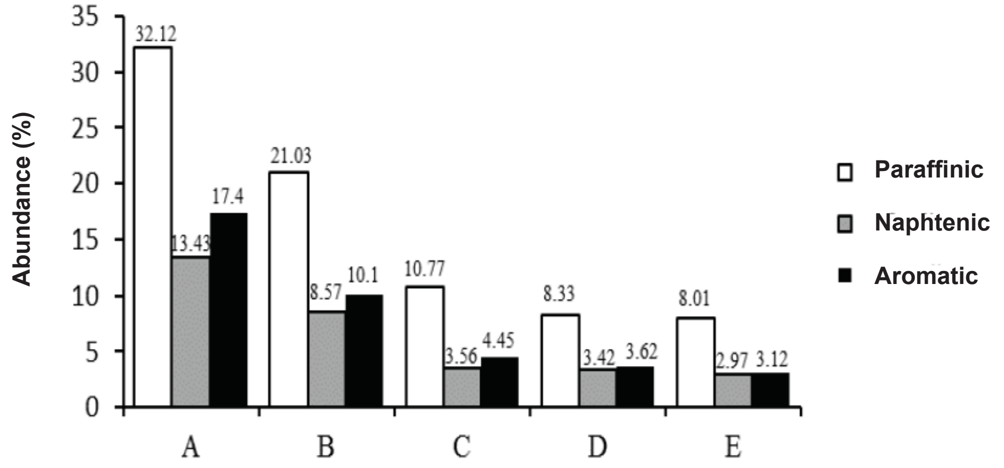
In addition, the biosurfactant is relatively easier than the chemical surfactant to synthesize because it is made from biological raw materials.

Based on these considerations, further study is needed to determine the best type of surfactant that is able to optimize the process of biodegradation of oily wastewater with bacteria *Pseudomonas sp*, especially in terms of manufacturing costs and the effects on the environment.

E. Analysis of Components of Crude Oil

Structural changes of crude oil before and after ozonation and biodegradation can be determined by using GCMS. In this experiment only 5 samples were taken for GCMS analysis, namely pure crude oil, the sample after ozonation with ozone dose of 0.53 g/h and a pH of 7, the sample after ozonation (ozone dose of 0.53 g/h and pH 7) and biodegradation without surfactants, as well as the sample after ozonation (ozone dose of 0.53 g/h and pH 7) and biodegradation with Tween 40 surfactant and Bios-H surfactant. This was done to assess which samples can give the best results and are the most economical.

Based on the results of GCMS analysis, the content of hydrocarbons found in crude oil is dominated by paraffinic, aromatic, and naftenik which showed a decrease in abundance (concentration) on the initial conditions, after ozonation, and after biodegradation (Figure 5). The composition of paraffinic compounds, and aromatic



Description:
A : Initial
B : After ozonation
C : After Biodegradation (Without Surfactant
D : After Biodegradation (Tween 40)
E : After Biodegradation (Bios - H)

Figure 5
Abundance of paraffinic compounds, naftenik,
and aromatic petroleum waste sample after ozonation and biodegradation process.

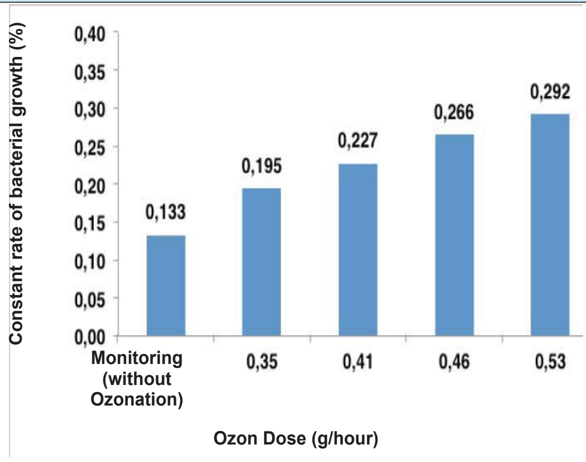


Figure 6
Bacterial growth rate of *Pseudomonas sp*
for variations in ozone dose.

naftenik decreases (changes in characteristics) after crude oil samples undergo a process of ozonation and biodegradesi process (Figure 5). This result is synergistic with previous results, where the ozonation process is able to to reduce the levels of oil and grease from oily wastewater. This shows that the ozonation process can improve biodegradation and *Pseudomonas sp* is able to degrade crude oil. The percentage of degradation also decreases with the use of surfactants. This shows that the surfactant is capable of lowering the surface tension of the sample thus facilitating contact between the bacteria with oil in water media (Burlatsky 2013) and can be used to optimize the biodegradation process of oily wastewater. A decrease in the concentration of non-biodegradable compounds is best obtained on the sample results through the biodegradation of Bios-H surfactants, where the compound paraffinik long chains fell from 32.12% to 8.01%, aromatics fell from 17.4% to 3.12%, and compounds naftaenik down from 13.43% to 2.97%.

F. Observation of Bacterial Growth

Pseudomonas sp has a growth rate that is likely to increase with an increased dose of ozone (Figure 6). The highest growth rate constant value (0.292) was contained in the ozone dose of 0.53 g/h, which is the highest dose used in this study. An increase in the rate of growth in media containing crude oil, indicate that the *Pseudomonas sp* is active in crude oil environments and has the ability to degrade petroleum hydrocarbons.

This increase also occurs because ozone managed to reduce and change the components which are difficult to be degraded by bacteria, such as naftaenik and aromatic compounds, into new compounds that are easier to be degraded. This is because ozone has a tendency to attack naftenik and aromatic compounds compared to paraffin compounds (straight chain) which is more soluble in water and diffuses into the bacterial cell membrane (Atlas 2005).

Observations on the growth of bacteria on the variation of the pH value indicates that pH 7 is an optimal value for the growth of bacteria *Pseudomonas sp* (Figure 7). Generally, bacteria have optimum growth at neutral pH values (pH 7) including *Pseudomonas sp*. The pH value is closely associated with the activity of the enzyme. The ability to degrade crude oil is associated with the presence of hydrocarbons modifier enzymes, such as dehydrogenase, monooxygenases, deoksigenase

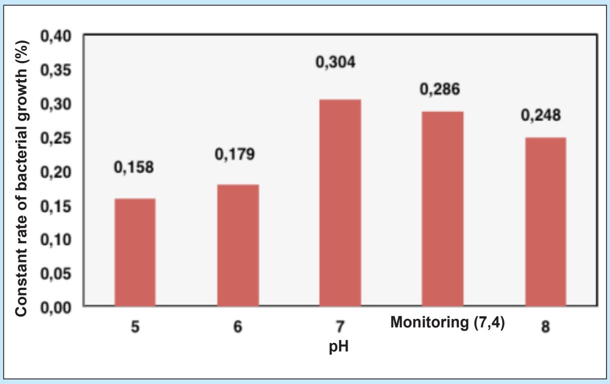


Figure 7
The rate of growth of *Pseudomonas sp* to variations in pH.

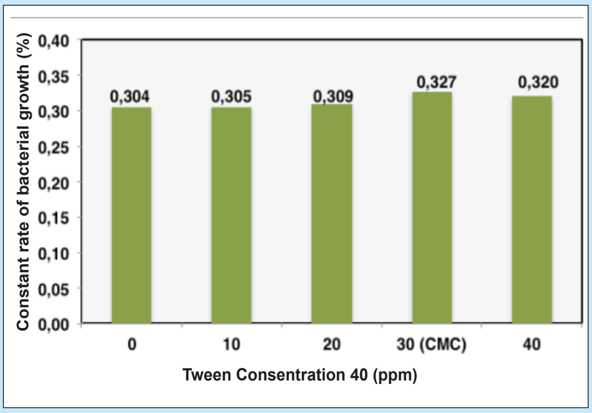


Figure 8
The rate of growth of *Pseudomonas sp* to variations in the concentration of surfactant Tween 40.

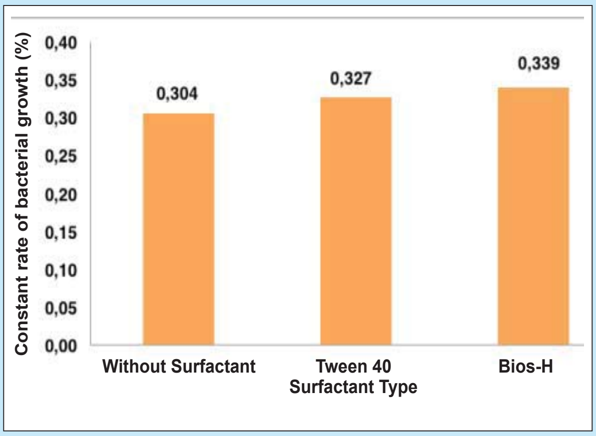


Figure 9
The rate of growth of *Pseudomonas sp* to variations in the concentration of surfactant type.

and others responsible for the overhaul of the hydrocarbon phase that allows the bacteria to grow on crude oil (Atlas 2005). Therefore, at a pH value of pH 7 these enzymes work optimally, increasing the growth of bacteria.

The rate of bacterial growth is influenced by variations in the concentration of a surfactant (Figure 8). The highest growth rate was obtained at 30 ppm concentration value which is the value of CMC of Tween 40 surfactant. Overall, the constant value growth rate of bacteria increases with the level of the concentration of surfactant used. However, when used in concentrations of 40 mg/L (higher than the value of CMC surfactant Tween 40 were worth 30 mg/L), their rate of bacterial growth declined slightly. Profile charts constant bacterial growth rate similar to the graph obtained in the calculation of the percentage of biodegradation. Theoretically, the addition of surfactant in solution will cause a decrease in surface tension of the solution. Surface tension will decrease until the CMC is reached. Once the CMC is reached, the surface tension will be constant which indicates that the interface becomes saturated and formed micelles are in dynamic equilibrium with the monomer (Burlatsky 2013). Therefore, the addition of surfactant which exceeds the CMC will not decrease the surface tension and will only add to the complex structure of organic compounds in the sample so as to reduce the growth rate of *Pseudomonas sp*.

Constant value growth rate of bacterial surfactants (Figure 9) was also influenced by the value of the highest growth rate constants obtained in the use of biological surfactants Bios-H. There is little difference in the value of the bacteria growth rate which was obtained through the use of both types of surfactant.The surfactant Tween 40 obtained a constant value for bacterial growth rate amounting to 0,327, whereas surfactants Bios-H obtained a value of 0,339. This is because the biosurfactant is not toxic and is biodegradable, so there is an optimum bacteria growth rate, and it is very effective in degrading hydrocarbons (Klosowska 2010).

IV. CONCLUSION

Ozonation treatment is able to increase the percentage of biodegradation of hydrocarbons by 2.9 times compared to the biodegradation process without ozonation.

The addition of a surfactant into the treatment increased the percentage biodegradation of hydrocarbons compared with the process of biodegradation without surfactant, of 79.32% (without surfactant) to 85.01% (Tween 80) and 86.79% (Bios-H).

The ozonation process followed by the biodegradation process was capable of changing the characteristics of the compounds that are difficult to be degraded by bacteria, such as long chain paraffinic compounds, and aromatic naftaenik degradation percentage of 34.50%, 36.18%, 41.95% and 66% after the ozonation process, and 47%, 73.49%, 74.42% after the biodegradation process.

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