LAPORAN AKHIR PENELITIAN

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BIOPROCESS OF OIL CONTAMINATED WATER AT GATHERING STATION PT "X" IN SUMATERA OIL FIELD BY USE OF CONSORTIUM ENDOGENOUS AND EXOGENOUS BACTERIA

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ABSTRACT

Production activities of petroleum have resulted in extensive environmental pollution, due to accidents, leaks, and oil spills during these activities. Gathering Station is one production facility at the oil field that serves as a gathering place for several liquids produced from production wells to measure the production flow rate. Wastewater at the Gathering Station that still contains oil, so before being discharged into water bodies should be treated to match the existing quality standards. One of the promising methods to remove the oil-polluted at gathering stations is the use of bioprocess technology, which is an eco-friendly, cost-effective, and sustainable approach. A laboratory study was carried out on the Bioprocess of wastewater contaminated oil at the gathering station of PT. "X" in Sumatera oil field by use of consortium endogenous bacteria and Bacillus cereus and Pseudomonas aeruginosa as consortium exogenous bacteria. The major objective of this research is to determine the effectiveness of consortium endogenous bacteria and consortium exogenous bacteria (Bacillus cereus and Pseudomonas aeruginosa) in degrading oil at the gathering station of PT "X" in Sumatera oil field. Bioprocess was done by using 10% (v/v) inoculum consortium of endogenous bacteria and 10% (v/v) inoculum Bacillus cereus and Pseudomonas aeruginosa as exogenous bacteria and using oil spill dispersant (OSD) 0.2% (v/v) in media. Oil concentrations were determined by the gravimetric method (SNI 6989.10-2011). The result showed that the mixture of Bacillus cereus and Pseudomonas aeruginosa as exogenous bacteria and consortium endogenous bacteria in media produced the highest value of effectiveness in degradation oil that up to 64.29% during 21 days (from 1062 mg/l to 379.25 mg/l), with a total population 65 x 106 cfu/ml, this indicated a synergistic relationship between Bacillus cereus and Pseudomonas aeruginosa as exogenous bacteria and consortium endogenous bacteria at room temperature (21±1oC), pH range value 8.34-9.68 and Total Dissolved Solids (TDS) range value from 553-167 mg/l.

Keywords: Bioprocess, Consortium endogenous and exogenous bacteria, Gathering station, Oil and grease, Sumatera oil field.

DAFTAR ISI

DAFTAR TABEL

BAB I
INTRODUCTION

Increasing production activities of petroleum at oil fields contribute to increasing wastewater at gathering stations. Gathering station is one production facility at the oil field that serves as a gathering place for several liquids produced from production wells to measure the production flow rate. It should be treated to match the existing quality standards before ditching argued into water bodies. Petroleum contains a complex combination of aliphatic compounds (e.g. alkanes, alkanes, and cycloalkanes), monoaromatic hydrocarbons (e.g. xylenes, toluene, phenol, benzene, ethylbenzene), and polycyclic aromatic hydrocarbons (e.g. anthracene, and naphthalene). In the environment, these compounds could cause damage to living organisms and human health. Contamination of water and soil from various types of petroleum products results in the migration of hydrocarbons to the unsaturated zone, therefore, because of the toxicity of these pollutants, they have become a primary environmental concern over the last decades. (Cabral et al., 2021)[1]. In soil, contamination of hydrocarbon often leads to possibilities of uptake of the contaminants by the plants that are grown in the contaminated sites. Moreover, from those plants, the hydrocarbon contaminants can further be introduced to animal and human populations through the food chain. (Patowary et al., 2018[2]).

The produced water that containing crude oil flow to gathering station caused by the fluid from the drilling process. Oil and water separation process at gathering station currently uses physical chemical processing using a coagulant in large quantities to help the process of settling small particles that cannot settle by themselves. The use of coagulants in large quantities however requires a lot of costs and associated with a range of problems such as environmental toxicity and resistance to biodegradation (Mulligan et al., 2001[9]; Yeung and Gu, 2011[4]). Bioprocess on waste pit at gathering station using microorganisms can treated residue of petroleum in waste water before being discharged into water bodies. One of the promising methods to treat the oil polluted water is the use of bioprocesses, which is an eco-friendly, cost-effective and sustainable approach. Many researchers have conducted studies involving microorganisms to treat oil contaminated

environments. A suitable method that can be adopted to fasten the biodegradation process of such organic compounds (Patowary et al., 2018[2]).

Endogenous microorganisms that live in locations contaminated with petroleum can be used as degrading microbes because they have the ability to degrade contaminants and reduce the degradation power of petroleum (Patowary et al., 2018[2]; Madonna, S et al., 2007[6]). Beside that exogenous bacteria can also used to degrade petroleum contaminants. There are two approaches in the application of the bioprocess: bio stimulation and bioaugmentation. Bio stimulation uses the metabolic capacity of indigenous microbial populations, in this way bioprocess can be carried out by modifying the supporting environment by carrying out bio stimulation to overcome limiting factors that can limit the rate of hydrocarbon biodegradation by indigenous microbial populations. or electron donors, to enhance the biodegradation of contaminants by native microbes. The second approach is in the form of bioaugmentation, namely by adding exogenous microbes that have special abilities to degrade contaminants. A number of factors can inhibit the bioprocess, including limited physical interactions between microorganisms and substances (bioavailability of contaminants and/or bio accessibility) (Lima et al., 2017[3]).

A laboratory study was carried out on the Bioprocess of wastewater contaminated oil at the gathering station of PT. "X" in Sumatera oil field by use of consortium endogenous bacteria and *Bacillus cereus* and *Pseudomonas aeruginosa* as consortium exogenous bacteria. The major objective of this research is to determine the effectiveness of consortium endogenous bacteria and consortium exogenous bacteria (*Bacillus cereus* and *Pseudomonas aeruginosa*) in degrading oil at the gathering station of PT "X" in Sumatera oil field.

BAB II
MATERIALS AND METHODS

2.1. Preparation Media

Nutrient Agar (NA) and Nutrient Broth (NB) media using for culture media. Nutrient Agar (NA) contained the following: 1 g of beef extract, 1 g of peptone, 15 g of agar in 1 L distilled water. Nutrient Broth (NB) contained the following: 1 gram of meat extract, 1 gram of peptone, oil spill Dispersant (OSD) Aquaquick 2000 with a concentration of 0.2% and crude oil of 0.1% (v/v) in 1 L distilled water.

2.2. Preparation Endogenous Bacteria and Exogenous bacteria

Endogenous bacteria were isolated from water at gathering station of PT. "X" in Sumatera oil field. The consortium of exogenous bacteria in this study was *Bacillus cereus* and *Pseudomonas aeruginosa* from the Microbiology and Bioprocess Laboratory of Research and Development Center of Oil and Gas Technology "LEMIGAS", Jakarta.

2.3. Activation and Preparation of inoculum

Activation and Preparation of inoculum were carried on erlenmeyer flasks contained 250 ml Nutrient Broth (NB) media on shaker incubator at 100 rpm for 24 hours.

2.4. Bioprocess

The bioprocess was carried out using 32 erlenmeyer flasks containing 200 ml produce water and 10 % (v/v) bacterial suspension was inoculated at 27±1^{o}C and 150 rpm at shaker incubator for 21 days. Periodically (0 day, 7 day, 14 day, and 21 day) measurements of oil & grease concentration, Total population of Bacteria, pH, and Total Dissolved Solid (TDS) was carried out.

2.5. Oil & Grease Test

Oil & Grease contain was carried out based on SNI 6989.10 in 2004[7] with observations on days 0, 7, 14, and 21. Calculation of oil & grease residue (mg/l) using the formula: ((W1-W0) × 1000)/V

Where:

W1 = weight of flask filled with oil (mg)

Wo = weight of empty pumpkin (mg)

V = sample volume (200 ml test medium)

The efficiency value of the bioprocess in reducing oil & grease is calculated by the following formula: ((A-B))/B x 100

Where:

A = early bioprocess (mg/l)

B = end of bioprocess (mg/l)

2.6. Total Population

Total population was carried out using the Total Plate Count (TPC) method based on SNI 2897, 2008[5]. Every 1 ml of the bioprocesses product in an Erlenmeyer flask was diluted up to 10^{-7} by inserting the 1 ml into 9 ml sterile distilled water. The solution was homogenized using a vortex, then from a dilution of 10^{-3} to 10^{-7}, each was taken as much as 0.1 ml to be inoculated on the solid NA agar medium. This inoculation was carried out twice (Duplo), incubated at 36^{o}C for 48 hours.

2.7. pH

Measurement of pH is based on SNI 06-6989.11 of 2004[8] with observations on days 0, 7, 14, and 21. Performed using a pH meter that has been calibrated with a buffer solution of pH 4, 7, and 10.

2.8. Total Dissolved Solid (TDS)

Total Dissolved Solid (TDS) testing was carried out at the beginning and the end of the observation time, using a calibrated OEM digital TDS meter. Recording the results of reading the scale or numbers on the TDS meter display.

BAB III
RESULT AND DISCUSSION

3.1. Preliminary Treatment

Before the bioprocess, the initial condition of the water is measured, the results of the measurement of these parameters are listed in Table 3.1.

Table 3.1. Parameters Value Before Bioprocess

Treatment	Oil & Grease (mg/l)	Total Population of Bacteria (cfu/ml)	pH	TDS (mg/l)
A	1067.75	43×10^7	9.36	553
B	1053.5	40×10^7	9.25	261
C	1062	39×10^6	8.86	190
Control	1000	30×10^4	8.76	167

Note:

A = 10% (v/v) consortium endogenous bacteria;

B = 10% (v/v) consortium exogenous bacteria;

C = 10% (v/v) consortium exogenous and endogenous bacteria;

Control = without addition bacteria

Based on (Table 3.1), it is known that the oil & grease content ranges from 1000 mg/l to 1067.75 mg/l. The initial content of oil & grease still exceeds The Quality Standards for Wastewater Produced for Oil and Gas Exploration and Production Activities from New On-shore Facilities in 2010[4] which is 25 mg/l, so a bioprocess is needed to reduce oil & grease levels by using consortium endogenous and exogenous microorganisms. Meanwhile, the total population ranged from 30×10^4 cfu/ml – 43×10^7 cfu/ml. The pH value of the media ranges from 8.76 to 9.36, the pH value which tends to be high is influenced by the addition of alkaline (alkaline) Oil Spill Dispersant (OSD). Total

Dissolved Solid (TDS) levels in each treatment were influenced by the oil degradation process that occurred, where TDS levels ranged from 553 – 167 mg/l.

3.2. Bioprocess Oil & Grease

Observations on the decrease in oil & fat during bioprocessing were carried out every 7 days for 21 days, observations were made on days 0, 7, 14, and 21 days. The decrease in oil & fat (mg/l) in each treatment is shown in Table 3.2.

Table 3.2. Oil & Grease Concentration (mg/l) and Efficiency Value

Treatment	Observation Time (Days)				Efficiency
	0	7	14	21	Value (%)
A	1067.75	888	685.5	509	52.33
B	1053.5	915.5	670	495.75	52.94
C	1062	873	547.75	379.25	64.29
Control	1000	916	797.25	678.75	32.13

Note:

A = 10% (v/v) consortium endogenous bacteria;

B = 10% (v/v) consortium exogenous bacteria;

C = 10% (v/v) consortium exogenous and endogenous bacteria;

Control = without addition bacteria

Based on Table 3.2 show that decreasing concentration of oil & grease at each treatment. The highest efficiency value of oil & grease biodegradation was 64.29% which degraded from 1062 mg/l to 379.2 mg/l, occurred in treatment C with the addition of an exogenous consortium (10%) to the wastewater containing endogenous bacteria, it is known that there is a synergistic mutualism relationship between the consortium of exogenous bacteria and the consortium of endogenous bacteria in the media. In treatment A using a consortium of endogenous bacteria and B using a consortium of exogenous bacteria, it can be seen that the value of efficiency on reducing oil & grease is not much different in the two treatments, which are 52.33% and 52.94%. This shows that consortiums of exogenous bacteria and endogenous bacteria can degrade oil & grease. In the control that was not given the addition of a bacterial consortium, the efficiency value of reducing the

concentration of oil & grease was the lowest of all treatments at 32.13%. This decrease occurred due to the influence of evaporation that occurred during incubation and due to the presence of endogenous bacteria present in the media, as well as the presence of OSD in the treatment which could cause oil & grease to be dispersed into smaller lumps into the water.

The Table 3.2 also shows that the consortium of endogenous bacteria (Treatment A) on the 7th day of observation degraded faster than the consortium of exogenous bacteria (Treatment B) with the concentration of decreasing oil & grease that occurred was 888 mg/l at treatment A and 915.5 mg/l at treatment B. This shows that endogenous bacteria was adapted more quickly because these bacteria come from the wastewater environment at gathering station, while exogenous bacteria are still adapting on the 7th day. On the 14th day of observation, the consortium of exogenous bacteria had adapted to the new environment and was able to degrade oil & grease faster than endogenous bacteria with the decreased concentration of oil & grease degradation that occurred was 685.5 mg/l (Treatment A) and 670 mg /l (Treatment B).

3.3. Total Population of Bacteria

Total Population of Bacteria was observed every 7 days for 21 days to find out how many populations of bacteria degraded oil & grease in this study. Total Population Bacteria of each observation are shown in Table 3.3.

Table 3.3. Total Population of Bacteria (cfu/ml) During Bioprocess

Treatment	Observation Time			
	0 Day	7th Day	14th Day	21st Day
A	43 x 10^7	57 x 10^7	30 x 10^7	85 x 10^6
B	40 x 10^7	39 x 10^7	44 x 10^7	30 x 10^6
C	39 x 10^6	61 x 10^7	43 x 10^7	65 x 10^6
Control	30 x 10^4	34 x 10^4	41 x 10^4	31.5 x 10^6

Note:

A = 10% (v/v) consortium endogenous bacteria;

B = 10% (v/v) consortium exogenous bacteria;

C = 10% (v/v) consortium exogenous and endogenous bacteria;
Control = without addition bacteria

Based on Table 3.3, it is known that in each treatment there was an increase in the number of populations. In treatments A and C the population tends to increase in the same way on the 7^{th} day and decrease on the 14^{th} day. In treatment A with the addition of the endogenous consortium the initial population was 43 x 107 cfu/ml, on the 7th day the bacterial population increased by 57 x 10^7 cfu/ml, and on the 14^{th} day, it decreased with the bacterial population as much as 30 x 10^7 cfu/ml, then on day 21, it decreased again to 85 x 10^6 cfu/ml. In the initial population in treatment C with the addition of a consortium of exogenous bacteria containing endogenous bacteria, the initial bacterial population was 39 x 10^6 cfu/ml, the same as treatment A increased on the 7^{th} day with a bacterial population of 61 x 10^7 cfu/ml and decreased on the 14^{th} day of 43 x 10^7 and the 21^{st} day of 65 x 10^6 cfu/ml. Treatment B showed different fluctuations. Treatment B with the addition of an exogenous consortium at the beginning of the observation (day 0) the initial population was 40 x 10^7 cfu/ml, on day 7 it decreased by 39 x 10^7 cfu/ml and increased with a population of 44 x 10^7 cfu/ml , and on day 21 it decreased again by 30 x 10^6 cfu/ml. This shows that exogenous bacteria are still in the adaptation phase to the environment and then begin to enter the log phase on the 14^{th} day.

The control media that contained water from the gathering station and only contained endogenous bacteria in water with an initial population of 30 x 10^4 cfu/ml and there was a not increase too high on the 7^{th} to 14^{th} day of observation, but on the 21^{st} day the population increased rapidly that is 31.5 x 10^6 cfu / ml. This indicates that the endogenous bacteria on the control medium undergoes adaptation phase until the 14^{th} day and increased population growth rapidly at the end of the observation on 21^{st} day. The population increases due to that bacteria using hydrocarbons as carbon source for bacteria nutrient.

The results of this study indicate that during the 21 days of the oil & grease biodegradation process it was known that at the end of the observation the bacterial consortium was in

the death phase which was indicated by a decrease in population in treatments A, B and C this was due to lack of nutrients and competition between bacteria for nutrients. The increase in pH value occurs due to the decomposition process of nitrogen compounds carried out by a consortium of endogenous and exogenous bacteria during biodegradation.

3.4. pH

The pH value affects the ability of bacteria to maintain the continuity of cellular activity, cell membrane transport, and the balance of reactions that catalysed by enzymes. The pH value of each treatment during bioprocess can be seen at Table 3.4.

Table 3.4. The pH Value During Bioprocess

Treatment	Observation Time			
	0 Day	7th Day	14th Day	21st Day
A	9.36	9.44	9.24	9.41
B	9.25	9.42	9.19	9.68
C	8.86	8.45	8.83	9.03
Control	8.76	8.34	8.73	8.72

Note:

A = 10% (v/v) consortium endogenous bacteria;

B = 10% (v/v) consortium exogenous bacteria;

C = 10% (v/v) consortium exogenous and endogenous bacteria;

Control = without the addition bacteria

The pH value of treatment A with the endogenous consortium at the beginning (day 0) had a pH value of 9.36 then, on the 7th day it increased to 9.44, and decreased on the 14th day to 9.24 and on the 21st day increased to 9.41. Treatment B with addition exogenous consortium at the beginning of the observation (day 0) the pH value was 9.25 and fluctuated like the endogenous consortium with the pH values being 9.42 (7th day), 9.19 (14th day), and 9.68 (21st day). Meanwhile, the pH value in treatment C with addition consortium exogenous and endogenous bacteria, had a smaller value than

treatment A and B. the pH value at the beginning of the observation (day 0) was 8.86, and than it decreased to 8.45 on the 7th day, and reach to 8.83 on the 14th day and at the end of the observation (21st day) reach to 9.03. The high pH value due to addition of the alkaline OSD in each treatment.

3.5. Total Dissolved Solid (TDS)

The Total Dissolved Solid (TDS) test is carried out as supporting data to determine the solids dissolved in water during the degradation process of oil & grease by a consortium of bacteria, where these solids consist of organic substances, organic salts, and dissolved gases. Data on the reduction in TDS levels are shown in Table 3.5.

Table 3.5. The Total Dissolved Solid (TDS) (mg/l)

Treatment	Observation Time	
	0 Day	21st Day
A	553	263
B	261	259
C	190	170
Control	167	168

Note:
A = 10% (v/v) consortium endogenous bacteria;
B = 10% (v/v) consortium exogenous bacteria;
C = 10% (v/v) consortium exogenous and endogenous bacteria;
Control = without addition bacteria

At the beginning of the observation (day 0) the TDS level in treatment A with the addition a consortium of endogenous bacteria had TDS level of 553 mg/l and treatment B with the

addition a consortium of exogenous bacteria, with a TDS of 261 mg/l, and in treatment C with the addition a consortium of exogenous bacteria and endogenous bacteria with a TDS of 190 mg/l, while the control without the addition of a bacterial consortium had a TDS level of 167 mg/l. The addition a consortium of bacteria to the media causes the dissolved organic matter levels increased.

During 21 days of observation, TDS levels in each treatment decreased, respectively 262 mg/l in the consortium of endogenous bacteria, 259 mg/l on the consortium of exogenous bacteria, 170 mg/l on the consortium of exogenous bacteria containing endogenous bacteria, while the control without the addition of a bacterial consortium increased by 168 mg/l. The decrease in TDS occurred due to the effect of biodegradation of oil & grease by a consortium of bacteria, biomass that occurs in endogenous bacteria which causes the amount of dissolved solids increased.

BAB IV
CONCLUSIONS

The result showed that the mixture of *Bacillus cereus* and *Pseudomonas aeruginosa* as exogenous bacteria and consortium endogenous bacteria in media produced the highest value of efficiency in degradation oil that up to 64.29% during 21 days (from 1062 mg/l to 379.25 mg/l), with a total population 65 x 10^6 cfu/ml, this indicated a synergistic relationship between *Bacillus cereus* and *Pseudomonas aeruginosa* as exogenous bacteria and consortium endogenous bacteria at room temperature (21±1^{o}C), pH range value 8.34-9.68 and Total Dissolved Solids (TDS) range value from 553-167 mg/l.

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