SCIENTIFIC CONTRIBUTIONS OIL AND GAS
Vol. 39, Number 1, April 2016: 5 of 5

RESEARCH AND DEVELOPMENT CENTRE FOR OIL & GAS TECHNOLOGY LEMIGAS

Journal Homepage:http://www.journal.lemigas.esdm.go.id

OPTIMIZATION OF PHENOLIC WASTES TREATMENT IN ARTIFICIAL PRODUCED WATER BY *PSEUDOMONAS AERUGINOSA*

OPTIMALISASI PENGOLAHAN LIMBAH FENOL DALAM AIR TERPRODUKSI ARTIFISIAL OLEH BAKTERI *PSEUDOMONAS AERUGINOSA*

Syafrizal

"LEMIGAS" R & D Centre for Oil and Gas Technology
Jl. Ciledug Raya, Kav. 109, Cipulir, Kebayoran Lama, P.O. Box 1089/JKT, Jakarta Selatan 12230 INDONESIA
Tromol Pos: 6022/KBYB-Jakarta 12120, Telephone: 62-21-7394422, Faxsimile: 62-21-7246150
E-mail: syafrizal@lemigas.esdm.go.id; & syafrizal.1.saif@gmail.com

First Registered on March 1st 2016; Received after Correction on March 25th 2016
Publication Approval on: April 29th 2016

ABSTRAK

Penelitian ini bertujuan untuk mendapatkan kondisi optimal biodegradasi fenol dalam air terproduksi artifisial dengan memanfaatkan aktifitas bakteri Pseudomonas aeruginosa. Dari hasil seleksi media diperoleh persentase degradasi limbah fenol tertinggi terjadi pada media M5 sebesar 99.85%, diikuti oleh media M1 99.83%, M4 28.47%, M3 27.64% dan M2 0.09% selama waktu inkubasi 24 jam. Tapi media M5 dan M1 relatif mahal bila teknologi ini diaplikasikan ke lapangan, sehingga perlu dilakukan optimalisasi terhadap parameter perlakuan yang mempengaruhi proses biodegradasi fenol dengan menggunakan media yang murah dan mudah didapatkan di pasaran yaitu media M4 terhadap lamanya waktu inkubasi, suhu dan pH media serta melihat pengaruh penambahan minyak bumi terhadap biodegradasi fenol. Dari hasil penelitian ini diperoleh persentase biodegradasi fenol pada kondisi optimal terhadap variasi; waktu inkubasi 2 hari, pH 7 dan suhu 30°C adalah sebesar 99.98%. Dan pengaruh penambahan minyak bumi pada konsentrasi optimal yaitu sebesar 100 mg/L, ternyata menurunkan degradasi limbah fenol menjadi 28.01%.

Kata Kunci: *Biodegradasi, fenol, Pseudomonas. aeruginosa*.

ABSTRACT

The purpose of this research is to obtain the optimum condition of phenol biodegradation in artificially produced water using *Pseudomonas aeruginosa*. The screening of medium in the highest phenol degradation at 99.85% in medium M5, followed by M1, M4, M3, and M2, at 99.83%, 28.47%, 27.64%, and 0.09%, respectively, during the 24 hours incubation time. Medium M5 and M1 are relatively expensive if applied in the field, thus optimization of incubation time, temperature, pH, and the adding of oil is needed in the treatment parameters affecting the phenol biodegradation process using an easy and cheap medium, M4. This research resulted in phenol biodegradation percentage at optimum condition for 2 days incubation, pH 7, and temperature 30°C, was 99.98%.The adding of oil at optimum condition: 100 mg/L could degrade phenol waste to 28.01%.

Keywords: biodegradation, phenol, *Pseudomonas. aeruginosa.*

I. INTRODUCTION

One of industries which is important in Indonesia'economic growth is oil and gas industry, since this industry has contributed high stated income and revenue, in order side they also increase in the environment impact. The oil and gas industries produce not small amount of water during their operation and activities. They are called as produced water (Hendarsa 2013).

Produced water of oilfield is one of the most found waste from oil and gas head sector as by product which is taken to the surface during the production of oil and gas. Particularly for marginal field, water cut production can reach up to 90%. The produced water quantity will increase each year, with the average of 210 million barrels per day in the world. It is 3 times higher than the amount of produced oil (Ishak 2012, Hidayat 2013)

The waste water produced by this oil and gas industry can damage the environment and affect the human health if not handled well because it has dangerous chemical materials such as; hydrocarbon compound, sulfide, ammonia, phenol, and other heavy metals.

One of the major pollutant from this produced water is phenol. Phenol is a poisonous and dangerous substance which produces odor, is toxic and corrosive to the skin (irritation), detrimental to human health and when reaches particular concentration can cause death in the organisms in the water (Awan 2013)

Therefore, based on the Regulatory of Minister of the Environment Number 19 Year 2010 Dated 30 November 2010 on "Waste Water Quality Standards For Oil and Gas Business And/Or Exploration and Production from The Land Facility", the maximum phenol content is 2 mg/L.

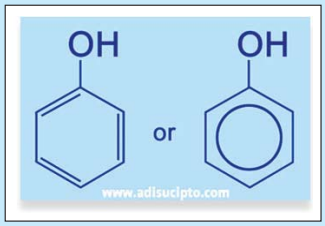

Figure 1
Chemical structures of phenol.

Phenol consists of benzene aromatic basic chain with one or more hydroxyl group as can be seen in Figure 1. Phenol is a compound that can cause odor, is a toxic and corrosive to the skin, causing interference on human health and the death of the organisms contained in the water with a specific concentration value. Phenol is composed of a chain of benzene aromatic base with one or more hydroxyl groups. Phenol toxicity levels vary depending on the number of atoms or molecules attached to its chain of benzene. For chlorinated phenols, the more chlorine atoms bonded benzene chain, the more toxic the chain. Chlorophenol are more toxic to aquatic biota, such as accumulation and more persistent than simple phenols. Simple phenols such as phenol, cresol and xylenol easily soluble in water and is more easily degraded (Dewilda 2012)

In order to remove the polutan from effluent, expensive chemical and physical process like ozonation, adsorption, ion exchange, membrane filtration, chemical oxidation etc are used. Most of these process are high energy consumsing, non economic and release effluent waste water which is impacting to environmental.

Recently biotechnological process have been reported as an alternative to these complex and expensive treatment methods. Bilogical methods are simple as well as ecofriendly and have the potencial to completely reduce and degrade the pollutans under aerobic or anaerobic conditions at relatively low capital and operating cost (Thapa 2012)

Biodegradation is the process of decaying or reduction of different organic materials and toxic metals to their non toxic form with the help of microorganisms. In this process complete mineralization of the starting compound to simpler ones like CO_2, H_2O, NO_3 and other inorganic compounds takes place. Biodegradation is a microbial process in which nutrients and physical conditions plays important role. Temperature and pH are the important physical variables and carbon, nitrogen, oxygen, phosphorus, sulfur, calcium, magnesium, and several metals are the micronutrients that also show a significant impact on degradation behavior is reported (Rajani 2015)

Most of the efficient phenol degrading microorganisms are capable of using phenol as the sole source of carbon and energy for their growth and metabolism. Microorganisms capable of degrading phenol do so with the action of a variety of enzymes. They first convert phenol to catechol with the help of hydroxylase enzyme. Catechol is then degraded to its intermediates via ortho or meta cleavage with the help of another set of enzymes. The enzyme catechol 2, 3 dioxygenase cuts the benzene ring of catechol at the meta position and the enzyme catechol 1, 2 dioxygenase cuts the benzene ring at the ortho position. The intermediates released trough ortho and meta cleavage are finally consumed by the microbes with the help of various enzymes through the TCA cycle resulting in CO_2, metabolites and energies as followoing the reaction: (Debadatta 2012).

$$\text{phenol} + \text{oxygen} \xrightarrow[\text{Oxidation}]{\text{Microbes}} \text{metabolites} + \text{electrons} + \text{energies}$$

Many studies on biodegradation of phenol come from bacteria. The genus Pseudomonas is widely applied for the degradation of phenolic compounds. These bacteria are known for their immense ability to grow on various organic compounds. Phenol biodegradation studies with bacteria species have resulted in bringing out the possible mechanism and also the enzyme involved in the process. There are reports on many microorganisms capable of degrading phenol through the action of variety enzymes. These enzymes may include oxygenase hydroxylases, peroxidases, tyrosinases and oxydases (Nair 2008)

P. aeruginosa is able to degrade aromatic polycyclic hydrocarbon such as toluene, the simple form of methylbenzene, through the oxidation of methyl aldehyde, alochol, and acid, which later is transformed into catechol optimum in room temperature and survive in temperature 10°C – 45°C in salty water and distilled water, also in pH 6.0-9.0 (Mailin 2011).

Another study that has been conducted that in this experiment efficiency for biodegradation of phenol at different concentrations were checked using three microorganisms like *Pseudomonas putida* NCIM 2650**,** *Pseudomonas aeruginosa* NCIM 2074 and mixed culture (collected from industrial effluent). The above study revealed that *Pseudomonas aeruginosa* is the most efficient host for biological phenol degradation using batch culture (Debadatta 2012)

The present study clearly showed that by using the mixed bacterial consortium which can efficiently degrade phenols as well, temperature of 37˚C and pH 7, maximum percentage of degradation can be achieved. The bacterial cells were able to utilize phenol as the carbon and energy source (Singh 2013).

From the studies above, it can be observed that *P aeruginosa* has a good potential in degrading phenol compound, in nutrition rich medium for bacteria growth. The obstacle, if this is applied in the field needs high operational cost.

The treatment condition highly affects the phenol compound biodegradation process using microorganisms, thus this study needs to optimize the biodegradation process based on medium variation.

Beside that, looking for the easy and cheap medium that containning nitrogen and phosporuos like that urea and NPK. After selecting the medium, advanced optimization of incubation length, medium pH, treatment temperature, and oil with various concentration is conducted.

II. METHODOLOGY

A. Studied Compounds

The studied organic pollutan is phenol A 035506 GR for analysis. It purchased from Merck KGaA. All the solutions/media were made in double destilled water. Bacteriological media were purchased from Difco and Oxoid

B. Culture Mediums

Medium M-1: Medium M-1a solution consists of 1.8 g K_2HPO_4, 1.2 g KH_2PO_4, and 0.01 g $FeSO_4.7H_2O$ dissolved in 500 mL distilled water. Medium M-1b solution consists of 4.0 g NH_4Cl, 2.0 g $MgSO_4.7H_2O$, and 0.1 g NaCl dissolved in 500 mL distilled water. Set the pH of both solution up to 7.4 by adding HCl 10% or NaOH 0.1 N. Both solutions are sterilized at temperature 121°C for 15 minutes. Mix both solutions after cooling down.

Medium M-2: Medium M-2 solution consists of 1.0 g glucose, 1.0 g peptone, 0.01 g $CaCl_2.2H_2O$, 1.0 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.25 g NaCl, and 1.5 g $(NH_4)2SO_4$ dissolved in 1 liter distilled water. Sterilize at temperature 121 °C for 15 minutes.

Medium M-3: Medium M-3 solution has the ratio of Nitrogen-Phosphor 5:1 obtained from urea-NPK.

Medium M-4: Medium M-3 solution is enriched using yeast extract (medium NP 5:1+0.1% yeast extract).

Medium M-5: Medium M-5 is made of 5 solutions, with 100 mL solution A containing 1.0 g K_2HPO_4, 0.5 g KH_2PO_4, and 0.5 g $(NH_4)2SO_4$; 100 mL solution B containing 0.5 g NaCl, 0.02 g $CaCl_2.2H_2O$, 0.02 g $MnSO_4$, 0.02 g $CuSO_4.5H_2O$, and 0.01 g H_3BO_3; 50 mL solution C containing 0.5 g $MgSO_4.7H_2O$; 50 mL solution D containing 0.02 g $FeSO_4.7H_2O$; and 50 ml solution E containing 0.02 g molybdenum powder. All solutions are sterilized at temperature 121°C for 15 minutes. After cooled down, all solutions are mixed and added with distilled water until 1 liter.

C. Microorganism

The strain of *P aeruginosa* was obtained from Microbiology Lab, University of Indonesia in form of slant and was stored at 4°C till futher use as can be seen in Figure 2.

D. Preparation of Inoculum

The bacterial culture are inoculated in liquid nutrient borth medium that containing 10 mg/L phenol and kept in the environmental shaker-incubator ES-20/60 at 120 rpm at 30°C for 3 x 72 hours.

E. Phenol Biodegradation Studies

The experiment studies were carried out in the shake flask as batch reactor under pertinent process variables. 250 mL of bacth volume was taken along with different medium (M1, M2, M3, M4 and M5) in each of the experiment are duplo in which 10% overnight cultred cells were inoculated, 100 mg/L phenol and kept in temperature controlled orbital shaker at 30°C in 120 rpm at pH 7 for 24 hours.

F. Optimization of Various Parameters for Degradation of Phenol

Effects of incubation time, temperature, pH, and adding oil were observed for the degradation of phenol from selected medium.

- **Effect of Incubation Time on Biodegradation of Phenol**

The experiments for degradation of phenol were performed dt different incubation time in shaking incubator at 120 rpm. Incubation time maintained at 1, 2, 3, and 4 days in culture of selected medium at pH 7.

- **Effect of pH on Biodegradation of Phenol**

A sequence of experiments for degradation of phenol was conducted to reveal effect of pH at room temperature at different pH (6; 6.5; 7; 7.5) in shaking incubator at 120 rpm. Samples were collected and were analyzed spectrophotometrically for phenol concentration.

- **Effect of Temperature on Biodegradation of Phenol**

The experiments for degradation of Phenol were performed at different temperatures in shaking incubator at 120 rpm. Temperature maintained at 30°C, 35°C, 40°C and 45°C in culture of selected medium at selected pH. Samples were collected and analyzed spectrophotometrically for phenol concentration.

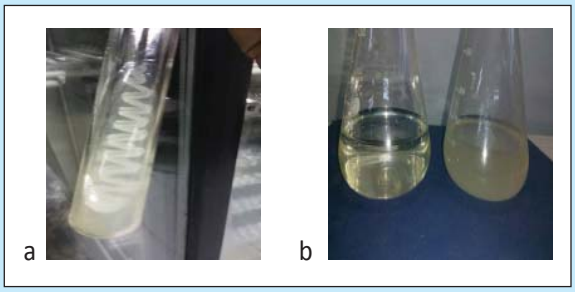

Figure 2
(a). Isolate bacteria.
(b). Left, before inoculation and right, after inoculation.

- **Effect of Adding of Oil on Biodegradation of Phenol**

A series of experiments were performed to know the effect of adding oil on the degradation of Phenol at selected temperature and at pH in shaking incubator. Adding oil maintained to 100, 500, 1500 and 2000 mg/L at 120 rpm in culture of selected medium. Samples were collected and were analyzed spectrophotometrically for phenol concentration.

G. Analysis Method

- **Phenol Estimation by 4-Amino antypirine Method**

The degradation of phenol was estimated at 510 nm in a spectrophotometer using 4-amino antipyrine method. In this method phenolic material reacts with 4-amino antipyrine in the presence of $K_3Fe(CN)6$ at a pH of 10 to produce a purple-red coloured end product whose absorbance is to be checked in the spectrophotometer at 510 nm. The quantity of phenol was calculated with the calibration curved prepare using formula 1. (ASTM D 1783. 2012).

$$\text{Phenol (mg/L)} = \frac{mg\ of\ phenol\ in\ standard\ curve}{mL\ sample}\ x\ 1000 \quad (1)$$

- **Oil and Grease Content Analysis**

The acidified water samples were extracted by n-hexane solvent in a separatory funnel. Then the solvent was evaporated by heated on a hot water bath. The sample which was resulted from the extraction was placed in an oven for 1-2 days to evaporate the remaining solvent. Place the sample in a desiccator for 1 hour, remove and weigh immediately. The exrtract residu was calculated using formula 2. (ASTM D 4281, 2012)

$$\text{Extractalble Residu (mg/L)} = \frac{(B-A)}{mL\ sample}\ x\ 1000 \quad (2)$$

Where, A : tare weight of boliling flask, mg

B : weight of boling flask after removal of extraction solvent, mg.

- **Bacterial Growth**

Bacterial growth was measured spectrophptometrically (Boeco S22 UV/vis Spectrophotometer) by optical density at 600 nm (OD_{600}). The transformation of OD into UFC/mL is made by the relation:

OD = 0.7 →10^8 UFC/mL (Razika. 2010).

- **pH Determination**

Determination of pH in solution is done by using a pH meter Laqua F-71-Horiba.

III. RESULTS AND DISCUSSION

A. Acclimatization Phase

Phenol-degrading bacteria are required to be adapted to the phenol environment. During acclimatization process that certain enzymes in the bacteria are induced so that they are available for taking part in the metabolism reaction. This condition is important when dealing with toxic compound such as phenol with high concentration. In this study, it was envisaged to degrade phenol using P. aeruginosa at concentration up to 100 mg/L. To initiate the acclimatization procedure, 10 mg/L phenol as carbon source in each mediums (M1, M2, M3, M4 dan M5) was used for growth of P. aeruginosa.

Microorganisms growth is highly affected by the availability of important elements which is needed to biologically synthesize of new organism. Nutrition resource deficiency can affect the growth of microbe, even causes death. Table 1 shows the bacteria growth during three times of acclimatization (3 x 72 hours) reaches the average of 10^7 CFU/mL. It indicates the nutrition supply as energy source and cell growth during acclimatization are sufficient, thus the bacteria can grow, reproduce, and do activities well.

B. Biodegradation Study of Phenol

Several external factors can limit the rate of biodegrdation of organic compounds. These factors may include various of mediums, incubation times, pH, temperature and physical properties of contaminant like presence of oil. Each of these factors should be optimized for the maximum degradation of phenolic compounds of choice.

C. Effect of Mediums on Phenol Biodegradation

Screening of mediums in phenol biodegradation is pacticularly important since it inhibits the growth of the organism at mediumwith lean nutrition. Biological degradation of phenol has been studied using various mediums.

Figure 3 shows that the bacteria population increase in each medium, especially medium M2 which increase 10% after 24 hours of biodegradation process. It can be caused by the glucose and peptone which have simple molecular structure, thus easier to be metabolized by the bacteria. While the composition of the nutrient in other mediums only consisted of minerals, thus the carbon needed as energy source was supplied by phenol only. This phenomenon caused medium with low carbon source but high minerals resulting in the highest phenol biodegradation; medium M1 and M5, which are 99.83% and 99.85% respectively, after 24 hours of incubation.

The results were then analyzed statistically using completely randomized design, least square

Table 1
Bacterial growth after acclimatization in variuos mediums

Mediums	Cells x 10^7 (CFU/mL/72 hours)		
	Acclimate I	Acclimate II	Acclimate III
M1	4.02	4.04	1.21
M2	4.20	20.08	19.17
M3	3.77	3.81	0.54
M4	4.20	6.91	3.48
M5	3.57	4.21	1.13

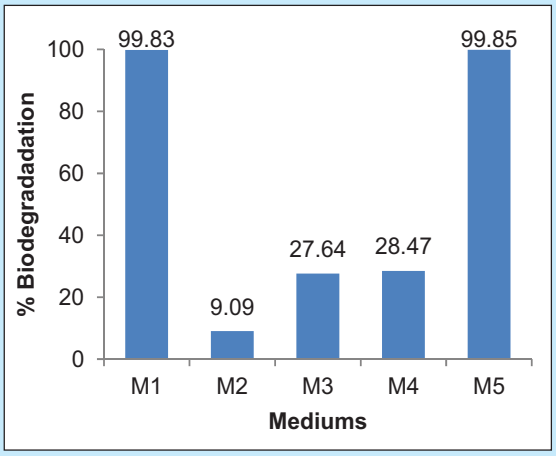

Figure 3
Effect various of mediums on phenol biodegradation.

differences, and Duncan test to find out the medium variation effect; M1, M2, M3, M4, and M5. The results show medium M1 was not significantly different from medium M5, proven by the Sig value > 0.00 which was 0.319 and the subset in Duncan test was found in the same subset. However, the LSD and Duncan results from M1, M2, M3, and M4 give different effect to biodegradation percentage, shown by the Sig value = 0.00 and the subset was found in different location.

Basha, et.al reported that the presence of glucose attenuated the rate of phenol removal by phenol consuming cells (Basha, 2010). Studies on *P. aeruginosa* with peptone and glucose as additional nutrients showed the highest phenol degradation. The rate of phenol degradation was improved when peptone was supplemented at the concentration between 0.25 and 1.0 g/L, with an optimum of 0.25 g/L. Peptone at low concentration influence the rate of degradation; however above 1.0 g/L peptone was inhibitory.

In medium M3 and M4, as well, nutrition enrichment with limited composition and minimum content resulted in biodegradation ability under 50%. But this condition was a challenge for this research, to find out how this simple medium has the potential in yielding higher biodegradation presentation, because this technology is not expensive and the materials are easy to find. Thus, the future research has to optimize the environment of bacteria growth using medium M4.

D. Effect of Incubation Time on Biodegradation of Phenol

Incubation length affects the bacteria growth pattern which is mass increase pattern and total cell number. Figure 4 shows bacteria growth phase, begun with lag phase or adaptation phase, which after the acclimatization, bacteria was inoculated to the treatment medium until they reached 3.48 x 107 CFU/mL. At the beginning, bacteria was adapting to the environment and medium, but after 24 hours the number reached 3.88 x 107 CFU/mL and phenol biodegradation was still low, 28.47%.

On the second day of incubation, bacteria growth increased sharply to 16.31 x 107 CFU/mL, along with significant phenol biodegradation 99.29%. normally, the highest bacteria growth found in exponential phase where bacteria reproduction happens quickly and increases logarithmically along the time. The next phase is stationary phase where the growth and death rate are equal, thus the total number of bacteria is constant. But in the bacteria growth curve, there is no stationary phase. This can be caused by the lengthy range of sampling time. The growth pattern directly entered the death phase, where the death rate is higher than the growth rate. It is caused by the nutrition deficiency in the medium so the reproduction stops and the environment becomes not conducive due to useless metabolite. On the third and fourth day, bacteria entered the death phase and the population decreased to 5.19 x 10^7 CFU/mL and 3.96 x 10^7 CFU/mL, respectively. Different to phenol biodegradation pattern, on the third and fourth day became relatively constant with maximum percentage 99.83% and 99.85% respectively, it indicates that almost every carbon atom from phenol was used by bacteria as energy source.

This result was supported by LSD and Duncan test to find out the effect of treatment time length on day 1, 2, 3, and 4. It shows that the incubation time length on day 1 and 2 had significant effect to phenol biodegradation percentage. This is proven by the Sig value = 0.00. however, on day 2, 3, and 4, the LSD and Duncan test gave the same result to phenol biodegradation percentage, which is shown by the Sig value in LSD test > 0.01 which was 0.791 and the subsets were located in the same position.

E. Effect of pH on Phenol Biodegradation

The medium pH is a factor affecting the growth of bacteria, because each species has different optimum pH for growth and doing activities.

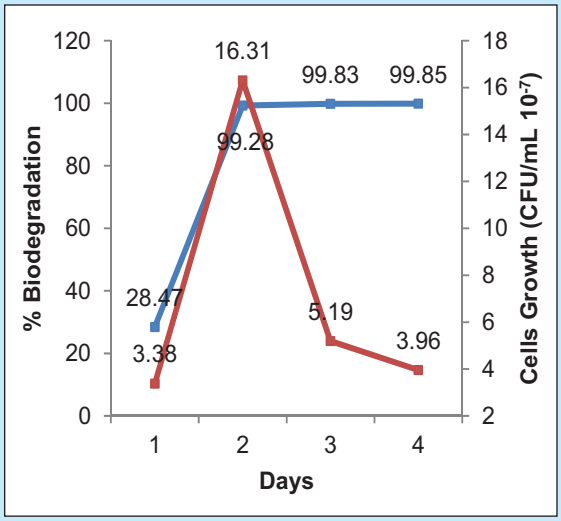

Figure 4
The result of phenol biodegradation (—) and bacterial growth (—) based on incubation time

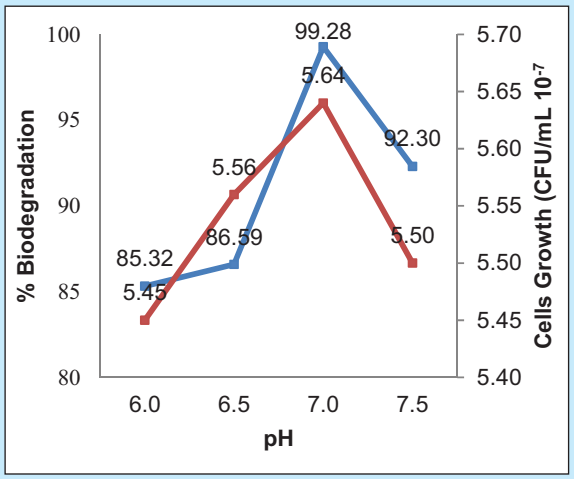

Figure 5
The result of phenol biodegradation (—■—) and bacterial growth (—■—) based oh pH.

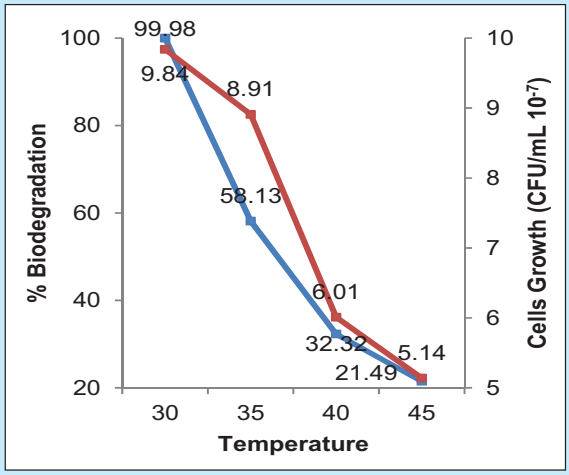

Figure 6
The result of phenol biodegradation (—■—) and bacterial growth (—■—) based on temperature.

Generally, the optimum pH ranges from 6.50 to 7.50. This research conducted optimization of medium pH with the variation; 6; 6.5; 7; and 7.5. The highest phenol biodegradation was found in pH 7 at 99.28%. The internal environment of all living cell is believed to be approximately neutral. Whereas, pH under and above pH 7 were degraded 86.59% and 92.30, respectively. According to Basha, (2010), most organisms cannot tolerate pH values below 4.0 or above 9.0. At low (4.0) or high (9.0) pH values acids or bases can penetrate into cells easily, because they tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells. The optimum pH for phenol degradation is 7.0 for *Pseudomonas putida* NICM 2174.

Statistically, the variation of pH affected phenol biodegradation percentage, indicated by the different position of the subsets and the Sig value = 0.00.

F. Effect of Temperature on Phenol Biodegradation

Another factor affecting phenol biodegradation process is temperature, whereas temperature often fluctuates depending on the environmental condition which can go above 40°C. Therefore, this research conducted temperature optimization within the range of 30-45°C according to general temperature of waste treatment in produced water of oilfield. Figure 6, the highest phenol biodegradation found in temperature 30°C at 99.98%, but along with the increasing temperature, the biodegradation percentage decreased to 21.49% at temperature 45°C.

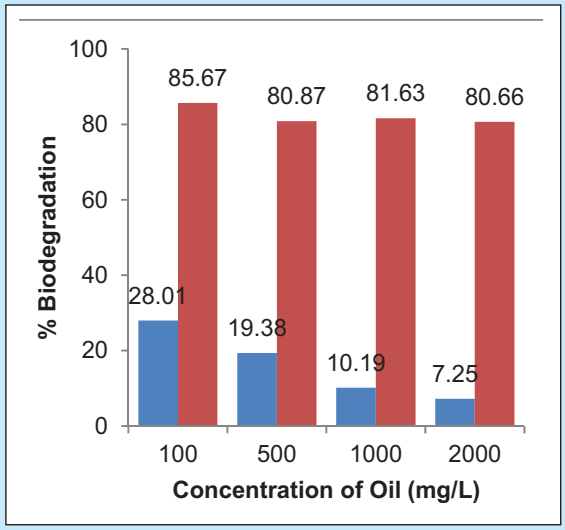

Figure 7
The result of phenol biodegradation (—■—) and bacterial growth (—■—) terhadap pengaruh based on adding of oil.

As well as the bacteria growth during the biodegradation process, the higher the temperature was, the lower the bacteria growth rate was, from $9{,}84.10^7$ CFU/mL at 30 °C to $5{,}14.10^7$ CFU/mL at 45°C. According to (Basha, 2010), (Chakraborty, 2010), temperature plays an important role for the nutrient availability in the degradation of organic pollutants. Phenol biodegradation was significantly inhibited at 30 °C. However, most laboratory studies on phenol degradation have been carried out at an optimum temperature of 30°C and when the

temperature increased from 30°C to 34°C, no phenol biodegradation was observed due to cell decay, which shows that phenol degradation is a temperature dependant process.

However, statistically, the results from temperature variation affected phenol biodegradation percentage, indicated by the different position of the subsets and the Sig value = 0.00.

G. Effect Adding of Oil on Phenol Biodegradation

Figure 7 shows the decrease of phenol biodegradation along with the increase of oil amount. With the adding of oil at concentration 100 mg/L, *Pseudomonas* was able to degrade only 28,01%. The biodegradation percentage kept decreasing along with the increase of oil amount. The lowest phenol biodegradation percentage was 7,25% with the amount of oil 2000 mg/L.

There was an interesting phenomenon in the research. The adding of various oil amount into the treatment evidently interfered the phenol biodegradation process. The biodegradation percentage highly decreased during the adding of oil. Even the oil was easier to degrade than phenol, the main pollutant in this study. This is due to the aliphatic hydrocarbon compound contained in the oil is easier to be degraded by microorganisms than the aromatic hydrocarbon contained in phenol. Figure 7 shows the adding of oil amount varied from 100 mg/L, 500 mg/L, 1000 mg/L, and 2000 mg/L resulted in phenol biodegradation percentage 28,01%, 19,38%, 10,19%, and 7,25%, respectively. While the oil biodegradation could reach 80%. It matched with the research by Udiharto 1996 that generally aliphatic hydrocarbon is easier to degrade than aromatic compound and linear aliphatic hydrocarbon chain is generally easier to degrade than branched-chain hydrocarbon because the branch introduction to the molecule interfere the biodegradation process.

It is supported by LSD and Duncan test that the adding of oil with different concentration affected phenol biodegradation percentage, indicated by the different position of the subsets and the Sig value = 0.00.

IV. CONCLUSION

Using the easy and cheap medium with sufficient nutrition, which was medium M4: N/P (5:1) + yeast extract 0,1%, could result in 99,98% phenol biodegradation by *P aeruginosa* in optimum condition for 2 days incubation, pH 7, and temperature 30°. The adding of oil into the treatment could interfere the biodegradation process and decreased the biodegradation 71,97% and kept decreasing along with the higher oil concentration.

REFERENCES

Awan, Zia Ur Rahman., Shah, Abdul Haleem., Amjad, Mohammad., 2013, Microbial Degradation of Phenol by locally Isolated Soil Bacteria. Global Advanced Research Journal of Microbiology, Vol. 2 (4): 072 – 079.

ASTM D1783,. 2012, Standard Test Method for Phenolic Compounds in Water. American Society and Testing Materials (ASTM) Water and Environmental Technology, Water II 11 (2):1-8.

ASTM D4281., 2012, Standard Test Method for Oil and Grease (Fluorocarbon Extractable Substances) by Gravimetric Determination. American Society and Testing Materials (ASTM) Water and Environmental Technology Water II 11 (2):471-476.

Basha, Khasi Mahammedilyas., Rajendra, Aravindam., Thangavelu, Viruthagiri., 2010, Recent Advances in the Biodegradation of Phenol: A Review. ASIAN J. EXP. SCI., Vol 1 (2): 219 – 234.

Chakraborty, S., Bhattacharya, T., Patel., Tiwari., K.K., 2010, Biodegradation of Phenol by Native Micoorganisms Isolated from Coke Processing Wastewater. Journal of Environmental Biology, 31: 293 – 296.

Debadatta, Das., Rajdeep, Kaur., 2012, Biodegradation oF Phenol by Variuos Indigenous Microorganism. Journal of Environmental Research and Development. Vol.7.2A: 1009 -1015.

Dewilda, Y., Afrianita, R., Iman , 2012, *Degradasi Senyawa Fenol oleh Mikroorganisme Laut*, Jurnal Teknik Lingkungan UNAND 9 (1) : 59-73.

Hendarsa, A.S., Hermansyah, H., Slamet., 2013, A Novel photobiodegradation technology for hydro-carbon wastewater treatment. International Journalof Engineering & Technology IJET-IJENS Vol 13 No. 01: 1- 6.

Hidayat, S., Kardena, E, 2013, *Penyisihan Senyawa Organik Limbah Cair Terproduksi pada Reaktor* Batch *Menggunakan Bakteri Indogenous dan Penambahan Nutrisi*, ITB-Bandung.

Ishak, S., Malakahmad, A., Isa, MH., 2012, Refinary wastewater Biology Treatment: A short review. Journal of Scientific & Industri Research, Vol 71: 251 – 256

Mailin, M., R, Firdausi., 2006, High Performance Phenol Degrading Microorganisms Isolated from Wastewater

and Oil-Contaminated Soil. Malaysian Journal of Microbiology, Vol 2 (2): 32 -36.

Nair, C, Indu., Jayachandran, K., Shasidahar, Shankar., 2008, Biodegradation of Phenol. African Journal of Biotechnology, Vol. 7: 4951 – 4958.

Rajani, V, 2015, Microbial Degradation of Phenol-A Review, IJJR, International, Journal, of, Research, and Review, ISSN. 2349-9788.

Razika, Bared., Abbes, Boucheta., Messaoud, Chaib., Soufi, Kacimi., 2010, Phenol and Benzoic Acid Degradation by *Pseudomonas aeruginosa*. Journal of Water Resource and Protein, Vol 2: 788 – 791.

Singh, Abha., Kumar, Vinay., JN, Srivastava., 2013, Assessment of Bioremediation of Oil and Phenol Contents in Refinery Waste Water via Bacteral Consurtium. Petroleum & Environmental Biotechnology. 4:3, p 1 - 4.

Thapa, B., Ajay, KKC., Ghimire, A, 2012. A Review on Bioremediation of petroleum hydrocarbon contaminant in soil. *J Sci Eng Tech*. 8(1): 16-170.

Udiharto M. 1989. Fenol sebagai Pencemar dan Biodegradasinya. Prosiding Diskusi Ilmiah 6. HasilPenelitian Pusat Penelitian dan Pengembangan TeknologiMinyak dan Gas Bumi (PPPTMGB) “LEMIGAS”. Jakarta, Indonesia.