SCIENTIFIC CONTRIBUTIONS OIL AND GAS
Vol. 41, Number 3, December 2018: 4 of 5
RESEARCH AND DEVELOPMENT CENTRE FOR OIL & GAS TECHNOLOGY LEMIGAS
Journal Homepage:http://www.journal.lemigas.esdm.go.id
ISSN: 2089-3361, e-ISSN: 2541-0520

KINETICS OF BATCH MICROBIAL DEGRADATION OF PHENOL BY *PSEUDOMONAS AERUGINOSA* AND DEEP-SEA SEDIMENT BACTERIA: SCALE-UP

TINJAUAN KINETIKA BIODEGRADASI FENOL PADA REAKTOR *BATCH* DENGAN BAKTERI *PSEUDOMONAS AERUGINOSA* DAN BAKTERI SEDIMEN LAUT DALAM: *SCALE-UP*

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.1970@esdm.go.id & syafrizal.1.saif@gmail.com

First Registered on January 27th 2018; Received after Correction on April 6th 2018
Publication Approval on: December 31st 2018

ABSTRAK

Minyak bumi yang diproduksi dari beberapa sumur minyak (oilwell) menghasilkan fluida yang mengandung campuran minyak bumi, gas bumi dan air terproduksi. Air terproduksi yang dihasilkan biasanya mengandung bahan-bahan kimia berbahaya seperti senyawa hidrokarbon, sulfida, ammonia, fenol dan logam-logam berat lainnya. Salah satu polutan yang tinggi kandungannya dalam air terproduksi adalah fenol. Melalui suatu proses biodegradasi, kandungan senyawa fenol dalam air terproduksi diharapkan bisa direduksi sehingga memenuhi baku mutu air limbah kegiatan eksplorasi dan produksi Migas. Penelitian ini merupakan pengembangan dari hasil penelitian sebelumnya dengan mengunakan bioreaktor dengan skala yang lebih besar yaitu 3 L. Proses degradasi senyawa fenol dilakukan pada kondisi optimal; pH 7, suhu 30°C, dan pada media sederhana merupakan media terpilih; NP (5:1) yang berasal dari urea dan NPK + yeast extract 0,1%. Dari hasil penelitian ini menunjukkan bahwa P. aeruginosa dan konsorsium dapat mendegradasi senyawa fenol dengan sangat baik yaitu 5,3 kali lebih cepat dibandingkan dengan penelitian sebelumnya. Persentase biodegradasi masing-masing sebesar 98.40% pada P. aeruginosa dan 99.03% pada konsorsium. Pendekatan model kinetika Monod berhasil dilakukan dan memberikan harga parameter-parameter yaitu μ_{max}, K_m, $Y_{S/X}$, dan μ_d masing-masing sebesar 0.6305 jam^{-1}, 0.0280 mg/L, 7×10^{-7} mg/L/CFU/mL, dan 0.00575 jam^{-1} pada P. aeruginosa dan 0.3272 jam^{-1}, 0.0355 mg/L, 6.63×10^{-7} mg/L/CFU/mL, dan 0.00279 jam^{-1} pada konsorsium. Berdasarkan harga parameter tersebut maka P.aeruginosa memiliki affinitas dan pertumbuhan yang lebih baik.

Kata Kunci: *biodegradasi, air terproduksi, fenol, pseudomonas aeruginosa,kinetika monod*

ABSTRACT

Petroleum that is produced from several oil wells produces a fluid containing a mixture of petroleum, natural gas and produced water. The produced water usually contains hazardous chemicals such as hydrocarbons, sulfides, ammonia, phenols and other heavy metals. One of the high pollutants in the water produced is phenol. Through a biodegradation process, the contents of phenolic compounds in the produced water are expected to be reduced so that it meets the quality standards of waste water for oil and gas exploration and production activities. This research is development of the results of previous studies using a bioreactor with a larger scale, namely 3 L. The degradation process of phenolic compounds is carried out in optimal conditions, namely: pH 7, temperature 30^{0}C, and selected simple media: NP (5: 1) derived from urea and NPK + 0.1% *yeast extract*. The results of this study indicated that *P. aeruginos* and bacterial consortium may degrade phenolic compounds very well, which was 5.3 times faster than the previous studies. The biodegradation percentage was 98.40% in *P. aeruginosa* and 99.03% in bacterial

consortium respectively. The monod kinetics model approach was successfully carried out and gave the value of parameters μ_{Max}, K_m, $Y_{S/X}$, and μ_d respectively of 0.6305 hours^{-1}, 0.0280 mg/L, 7×10^{-7} mg/L/CFU/mL, and 0.00575 hours^{-1} in *P. aeruginosa* and 0.3272 hours^{-1}, 0.0355 mg/L, 6.63×10^{-7} mg/L/CFU/mL, and 0.00279 hours^{-1} in bacterial consortium. Based on the valuesof these parameters, *P. aeruginosa* has better affinity and growth.

Keywords: biodegradation, produced water, phenol, pseudomonas *aeruginosa*, monod kinetics

How to cite this article:

Syafrizal, 3, 2018, KINETIC OF BATCH MICROBIAL DEGRADATION OF PHENOL BY *PSEUDOMONAS AERUGINOSA* AND DEEP-SEA SEDIMENT BACTERIA: SCALE-UP, *Scientific Contributions Oil and Gas*, 41 (3) pp, 145-154. DOI: 10.29017/SCOG. 41.3.145-154.

I. INTRODUCTION

Oil and gas production are a complex series of processes involving various petroleum industry activities, from upstream to downstream. The upstream activities include exploration (searching) and exploitation (lifting) activities, while the downstream activities include processing from refineries, marketing and distribution to storage activities. Petroleum is produced from several oil wells which produce a fluid containing a mixture of petroleum, natural gas and produced water. (Hendarsa 2013, Hasiany 2015).

The produced water is water that rises with petroleum to the soil surface through gas wells or petroleum wells, and produced when the gas and petroleum production processes take place. The produced water usually contains hazardous chemicals such as hydrocarbons, sulfides, ammonia, phenols and heavy metals. One pollutant which is high in the produced water is phenol. (Nasiri. M., Jafari. I. 2016)

Phenol is included in aromatic hydrocarbon compounds with benzene structure with one of H atoms replaced by OH. Phenol is one of the Toxic and Hazardous Substances (THS). Because the solubility of phenolic compounds in water is high enough, these compounds easily and quickly enter the aquatic environment, which disrupt the biological balance of waters resulting in a decrease in water quality and disruption to aquatic ecosystem. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna. (V, Rajani 2015)

The amount of pollution effect depends on the size of phenol concentration contained in the waters. At concentration of 0.002 mg/L, it may change the color and taste of water. While at concentration of 0.1 mg/L, it may cause death in fish. In humans, it may affect health, especially its liquid form which is very corrosive to the skin. The contact between phenol and body tissue will cause burns or tissue death if not cleaned. (Awan 2013).

Based on the Regulation of Minister of Environment No. 19 of 2010 concerning quality standards for business and /or oil and gas exploitation and production activities,the maximum permissible limit of phenol is 2 mg/L.

Various technologies have been used to treat this produced water, including *gravity-based separation - flotation, separation technique based on filtration, and biological process treatment*. The biological process treatment is one of the technologies commonly used in the wastewater treatment (Hidayat 2013).

The success of biological waste water treatment is highly depending on the activity and ability of microorganisms to degrade organic matter in the waste. The principle is utilization of activities of microorganism such as bacteria, fungi and protozoa. These microorganisms remodel the organic waste into simple organic compounds and convert them into CO_2 gas, and H2O. In aerobic microbial growth, the general equation may be written as follows (Doraja 2012).

$$a\,(\text{substrate}) + bO_2 + cNH_4 \rightarrow \text{biomass} + dCO_2 + eH_2O$$

Some types of bacteria that may degrade aromatic hydrocarbons are *Pseudomonas, Bacillus*, and *Nocardia*. However, the bacteria that may degrade phenol is *Pseudomonas*sp. Some *Pseudomonas* species that have been studied include *P. aeruginosa*, *P. fluorescence*, and *P. putida*

The *Pseudomonas* genus includes one of the most diverse and ecologically significant groups of bacteria. Members of this genus are found in large numbers in the terrestrial and marine environment

and in plants and animals. *Pseudomonas* is widely used in various biological processes such as biodegradation of organic components because it is able to adapt and reproduce well in its environment. Pseudomonas sp is most efficiently useful in the degradation of xenobiotics such as aromatic and aliphatic hydrocarbon of oils. (Sign. 2017)

P. aeruginosa is a gram-negative bacterium, rod shaped, and may grow in temperature of 42°C with optimum temperature of 37°C. *P. aeruginosa* is known for its rapid growth and ability to metabolize with a number of substrates (Neves et al. 2014).

Source:Textbookofbacteriology.net (a)

Source:ventyshi.wprdpress.com (b)

Figure 1
***Pseudomonasaeruginosa* on petri dishes (a) and under a microscope (b).**

The present study clearly showed that by using the mixed bacterial consortium which may efficiently degrade the phenol in temperature of 37°C and pH 7, a maximum percentage of degradationwas achieved. The bacterial cells were able to utilize phenol as carbon and energy source (Singh 2013).

Lemigas also observed that *Pseudomonas aeruginosa* had good potential in degrading phenol compounds using a simple and cheap medium with sufficient nutrition, namely M4: N/P (5:1) + 0.1% yeast extract, which could result in 99,98% phenol biodegradation in optimum condition for 2-day incubation, that is, pH 7, and temperature 30°C (Syafrizal 2016).

This study was intended to find out the ability of *P. aeruginosa* and bacterial consortium from deep-sea sediments to degrade phenolic compounds with a working volume of 3 L, which was a scale up of the previous research, using the selected media in optimum temperature and pH. In addition, these bacteria have certain mechanisms so that they are able to adapt and survive in extreme environments and are not environmentally friendly; live under high pressure, with limited nutrient content and oxygen (Poly. A 2017).

II. METHODOLOGY

A. Microorganism

The strain of *Pseudomonas aeruginosa*was obtained from the Microbiology Lab, Universitas Indonesia, in the form of slant and in 4°C until further use.

B. Media composition and Cultural Condition

The following media were used for microorganism growth.

i. Nutrient agar

The nutrient agar consists of the following composition:

0.5 g/100 ml Peptone, 0.3 g/100 ml Beef extracted, 1.5 g/100 ml Agar and 100 ml distilled water.

ii. Broth media

The broth media consists of the following composition: 100 ml distilled water, 0.5 g Peptone, 0.3 g Beef extracts and 1 g NaCl.

iii. Simple Media

The simple media consists of following composition:

0.153 g NPK, 0.06 g urea, 0.1 g yeast extract and 100 ml distilled water.

C. Preparation of Inoculum

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

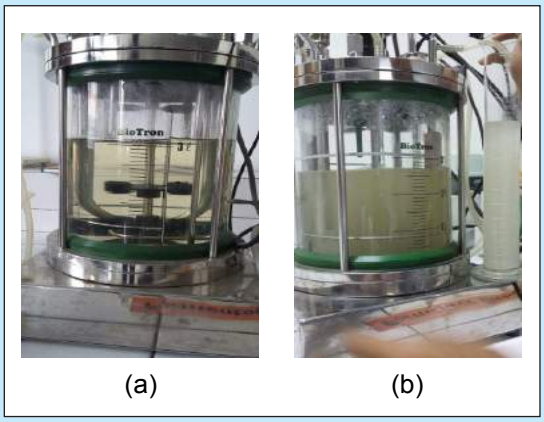
D. Adaptation of Bacteria

The suspension of activated isolates of *P. aeruginosa*and bacterial consortium were then adapted for 2 weeks into media of NP 5: 1 + 0.1% yeast extract with 10 mg/L phenol and shaken in the ES-20/60 shaker incubator at 120 rpm at 30°C. The bacterial growth rate was observed using optical density (OD) measurement at a wavelength of 600 nm.

E. Experimental Design to Study Phenol Biodegradation system

The experimental studies were carried out in the Biotron-LiFlus GX bioreactor with 3 literworking volume.

The adapted bacteria were then put into NP 5: 1 + 0.1% yeast media containing phenol extract with 100 mg/L concentration which had been put into a 3-liter bioreactor aseptically. They were then incubated at 30°C, aerated at a speed of 2 L/min, and agitated at a speed of 120 rpm. Their pH, phenol level, and VSS were further measured with optical density (OD) at the hour 0; 3; 6; 9; 12; 15; 18; 21; 24; 27; and 30.



(a) (b)

Figure 2
The media of treatment looks clear before biodegradation process (a), the media of treatment is more turbid when the biodegradation process is underway (b).

F. Distillation Procedure

A 150 mL sample is measured in a beaker. The pH of sample is adjusted between 0.5 and 4 with H_2SO_4 solution (1 + 9). The mixture is then transferred to the distillation apparatus. A 250mL graduated cylinder is used as a receiver. A 100 mL sample is then distilled. Afterward, the distillation is stopped and when it stops boiling, a 50 mL water is added to the distillation flask. The distillation is continued until a total of 150 mL water is collected. (ASTM D1783: 2014)

G. Phenol Determination by 4-Amino Antipyrine Method

The degradation of phenol was determinated at 510 nm in a spectrophotometer using the 4-amino antipyrine method. In this method the phenolic material reacts with the 4-amino antipyrine in the presence of K_3Fe (CN) 6 at a pH of 10 to produce a purple-red colored 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 (ASTM D 1783: 2014).

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

F. Analysis of Volatile Suspended Solid (VSS)

Sample analysis: the filtration unit is setup with a dried glass fiber filter disk. The well stirred sample volume is applied in the center of the filter. The filter is washed with 10 ml reagent grade water and continued with suction for about 3 min after the filtration is complete. The filter is then carefully removed from the filtration apparatus and transferred to aluminum weighing dish as a support.

The sample is dried for at least 1 h at 505 °C in an oven, and cooled in desiccator to balance the temperature, and then weighed. The cycle of drying, cooling, desiccating, and weighing is repeated until a constant weight is obtained or until the weight change is less than 4% of the previous weight or 0.5 mg, whichever is less.

Calculation:

mg VSS/L = (A-B) x 1000/sample, mL

where:

A: glass fiber filter disk + dried residue, mg

B: weight of glass fiber filter disk, mg.

H. Bacterial Growth

Bacterial growth was measured spectrophotometrically (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:

$OD = 0.710^8 \rightarrow$ UFC/mL (Razika. 2010)

I. pH Determination

The pH in solution is determined with Laqua F-71-Horiba pH meter.

J. Analysis of Pile Reactor Kinetics

Bacterial growth kinetics is determined with the Monod equation in which the value of μ_{Max} and K_m will be found by linearizing the Monod equation (Equation 1) into the *lineweaver-burk*equation (Hidayat & Kardena 2013) (Equation 2):

$$\mu = \frac{\mu_{max} S}{K_S + S} \quad (1)$$

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{1}{S}\frac{K_S}{\mu_{max}} \quad (2)$$

Other parameters, namely maximum yield of cells to substrate and specific mortality constant, or respectively Ym,x/s and kd, are determined by applying the mass balance equation in the batch reactor for cells/biomass (Equation 3) and substrate (Equation 4) that is:

$$\frac{dX}{dt} = X\mu - Xk_d \quad (3)$$

$$-\frac{dS}{dt} = \frac{X\mu}{Y^M_{X/S}} \quad (4)$$

Theoretically, with perfect stirring conditions and uniform microorganisms, these equations 3 and 4 may be applied to various volume scales. The equation 3 and 4 are calculated with routine order at 23 in MATLAB R2016a to get the value of $Y_{S/X}$ and μ_d and the construction of growth model and substrate reduction based on the determination coefficient that is closest to 1. The results obtained are in the form of curve that describes the time needed to degrade phenol.

III. RESULTS AND DISCUSSION

3.1 A. Adaptation of Bacteria

The *P. aeruginosa* and bacterial consortium were added to NP 5: 1 + 0.1% yeast extract and 10 mg/L phenol solution, and they are then shaken for 3 × 72 hours. This stage needs to be done to ensure that the microorganisms used may grow well in the media and are expected to have potentials to increase their ability to degrade phenolic compounds. Thisis because these bacteria have ability and physiological activities to develop on the media containing phenol so that the bacteria have a higher growth rate (Liu 2016).

On Table 1, itcan be seen that the adaptation made for three times has very good growth, which is above 108 CFU/mL. This adaptation is carried out in simple media derived from urea fertilizer and NPK as source of nitrogen and phosphorus as well as yeast extract as stimulant for bacterial growth. In addition to being a source of carbon and energy, 10 mg/L phenol are added into the adaptation media to conditionate theused bacteria so that they are accustomed to living in an environment containing toxicity to be degraded. (Debatta & Rajdeep 2012).

Table 1
Bacterial population after adaptation in NP media (5: 1) + 0.1% yeast extract

Adaptation	Cell Number × 10^8 (CFU/mL)	
	P. aeruginosa	Bacterial consortium
1	1.297	1.184
2	0.873	0.690
3	0.820	0.780

B. Biodegradation Study of Phenol

Biodegradation of phenolic compounds by utilizing the activity of *P. aeruginosa* and deep-sea bacterial consortium showed the highest yield of 98.40% and 99.03% during the 9-hour incubation period, meaning that the types of isolates obtained could potentially degrade phenolic compounds to concentration <2 mg/L (the maximum permissible limit based on the Regulation of Minister of Environment No. 19 of 2010). The degradation ability of the phenolic compounds is 5 times faster than previous studies. From the results of research conducted by Syafrizal. 2015, it was found that *Pseudomonasaeruginosa* bacteria was able to degrade phenolic compounds by 99.98% for 2-dayincubation period with s of limited aeration. Another study reported that phenol degradation reaches 70%, dominated by bacterial consortium, especially those from *Pseudomonassp., Bacillus sp., Alcaligenes sp.* and *Corynebacterium sp*. (Partila 2013).

The acquisition of several types of bacterial consortium from deep sea sources and their ability to degrade phenolic compounds that are higher than single cultures may be because these microbes have activities which playan important role in the elemental cycle globally during the geological time span. In addition, these bacteria have certain mechanisms so they are able to adapt and survive in an environment which is extreme and unfriendly; living under high pressure, with limited nutrient and oxygen level.

In Figure 3, itcan be seen that the process of phenol biodegradation by *P. aeruginosa* begins to occur at the hour 3 and continues until the hour 6 of 12.10% and 31.77%, respectively, while at the hour 9 the optimal biodegradation process occurs of 98.40%. Meanwhile, the process of phenol biodegradation by bacterial consortiumbegins to occur at the hour 3 and continues until the 6th hour of 21.10% and 38.85%, respectively, while at the hour 9 the optimal biodegradation process is 99.03. %.

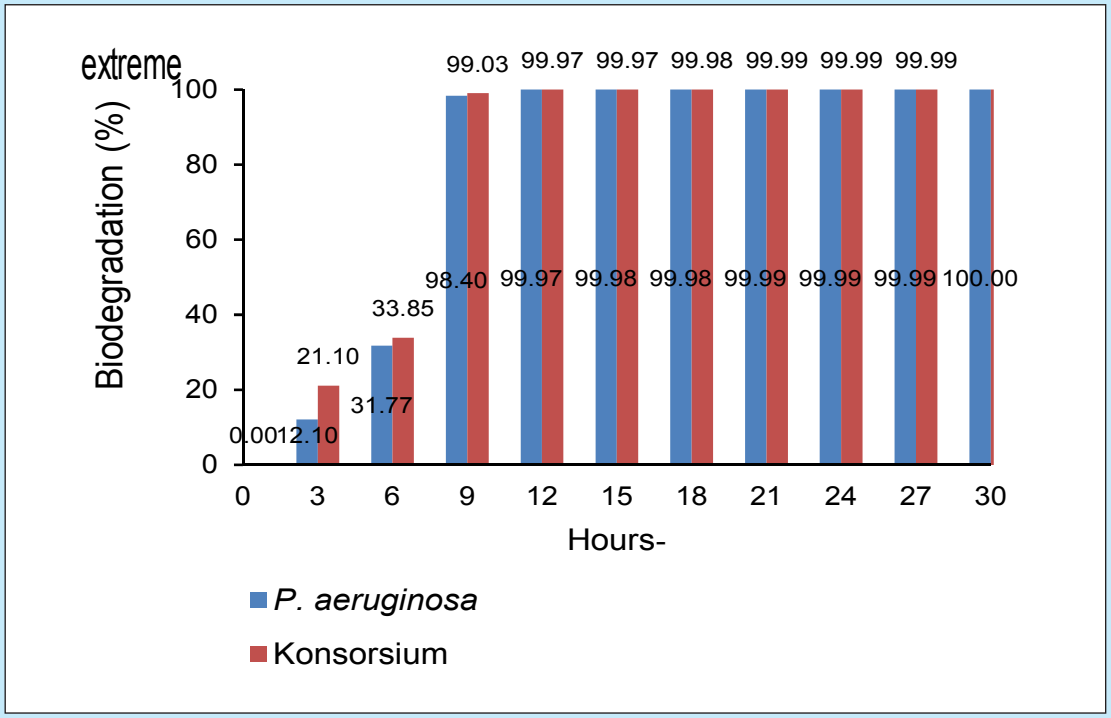


Figure 3
The relationship between the length of incubation period and the percentage of phenol biodegradation by utilizing the activity of *P. aeruginosa* and bacterial consortium.

The time factor used for this biodegradation process occurs in the exponential phase and lag phase. The exponential phase, according to (Cisneres 2014), comprises of 2 stages;in the first stage,the bacteria uses the carbon source from yeast extract and they produce enzymes that are utilize to decompose the carbon sources derived from phenol.

The increased bacterial population is an indication of bacterial growth by utilizing nutritional sources during the treatment period. The growthcan be observed from the increasing cell number or cell mass (dry weight of cells). In general, the bacteria may multiply by binary division, that is, from one cell dividing into two new cells, then the growthcan be measured with the increased number of cells. The time needed to divide from one cell to two perfect cells is called *generation time*. The time taken by a number of cells or the mass of cells to be twice the number/mass of the original cell is called *doubling time*. The doubling time is not the same between various microbes, from a few minutes, several hours to several days depending on the speed of growth. The speed of growth is a change in the number or mass of cells per unit of time.

In its growth, every living creature needs sufficient nutrition and environmental conditions that support the growth process, including bacteria. Bacterial growth is generally influenced by environmental factors. The influence of this factor will provide an overview that shows an increase in the number of different cells and ultimately gives an overview of the growth curve.

The relationship between increasing cell numbers is directly proportional to the increasing percentage of biodegradation of phenolic compounds. The greater the number of carbon atoms derived from phenolic compounds metabolized by bacteria, the better the growth of bacteria, the higher the percentage of biodegradation. The results of observation of bacterial population are shown in Figure-4, as itcan be seen that at the hour 0, the number of population of *P. aeruginosa*is 0.03 x 108 CFU/mL and the number of population of bacterial consortium is 0.20 x 108 CFU/mL. The bacterial population tends to increase until the hour 9 where the population of *P. aeruginosa* is 1.47 x 108 CFU/mL and the population of bacterial consortiumis 1.67 x 108 CFU/mL. In this condition, the bacteria are optimal in metabolizing phenolic compounds as carbon sources for growth.

In general, bacteriaare able to grow well at optimal pH 6.50 - 7.50. Most microorganisms cannot tolerate pH values <4.0 or> 9.0 for growth. In alkaline conditions the cell tends to be in an uncoordinated form and the electrostatic force generated cannot protect the cell wall. This also affects the activity of the enzymes needed by some bacteria to catalyze reactions associated with bacterial growth (Cisneres 2014). And according to Debadatta et al. (2012), the percentage of optimal phenol biodegradation is obtained at pH 7.

In Figure 5, itcan be seen that the pH value at hour 0 is 6.82 for the treatment using *P. aeruginosa* and 6.75 for the treatment using bacterial consortium. This pH value continues to increase in line with the increasing percentage of phenol biodegradation up to the hour 9 of 7.86 and 7.51 for the treatments respectively. And after the hour 9, the pH value tends to increase until the end of incubation period at the hour 30 of 8.24 and 8.23 for the foregoing treatments respectively and they are not the optimal pH for bacterial growth.

The decrease in phenol concentration is influenced by the number of bacteria in the treatment with adequate nutrition and suitable environmental conditions, microbes will grow and develop well. The greater number of bacterial cells, the more degraded phenols will be. The number of bacteria may be determined based on the VSS value. The VSS value on *P. aeruginosa* at the hour 0 was at 270 mg/L and at the hour 30 it decreased, reaching 65 mg/L. The VSS value of bacterial consortium also decreasedfrom 415 mg/L at the hour 0 to 50 mg/L the hour 30. This was because the administration of nitrogen in large quantitiesmight affect the biodegradation process (Hidayat & Kardena 2013).

C. Biodegradation Kinetics

Kinetics was reviewed to predict the size and period of biodegradation process in the large-scale application. The model was developed based on the initial

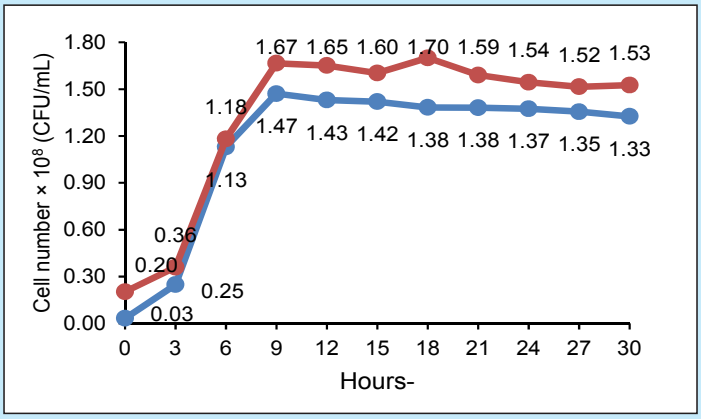


Figure 4
Relationship between length of incubation period for *P. aeruginosa* and bacterial consortium population.

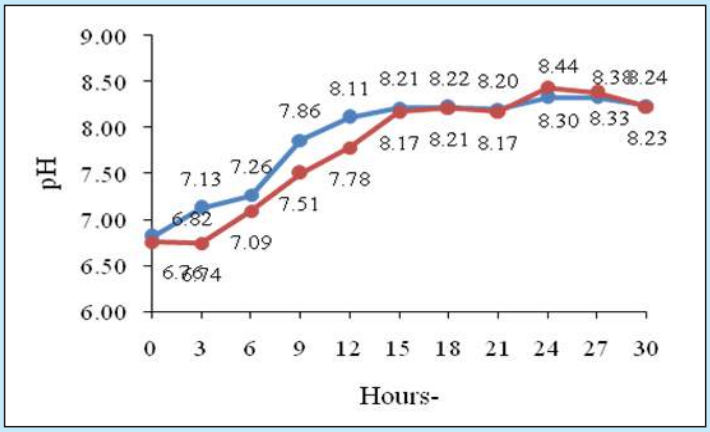


Figure 5
Relationship between the length of incubation period and pH by utilizing the activity of *P. aeruginosa* and bacterial consortium.

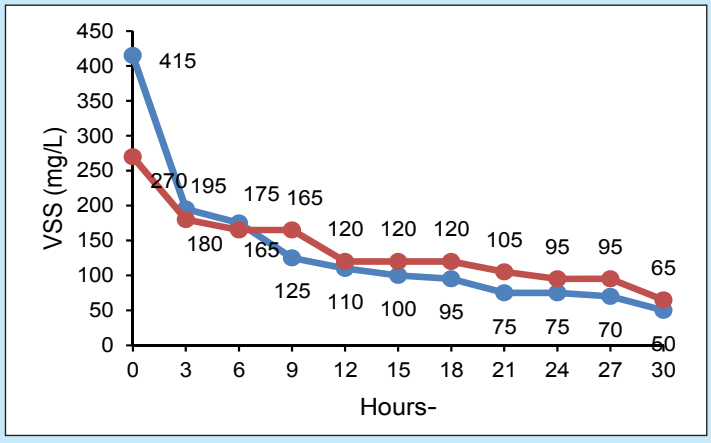


Figure 6
The relationship between the length of incubation period and VSS value by utilizing the activity of *P. aeruginosa* and bacterial consortium.

assumption that the distribution of microorganisms in the system was relatively uniform and perfectly mixed (Kurniawan and Effendi 2014). The results of calculated kinetic parameters are displayed on Table. 2

Table 2
The μ_{max} and K_m values of *P. aeruginosa* and bacterial consortium

	P. aeruginosa	Consortium
μ_{max} ($Hour^{-1}$)	0.6305	0.3272
K_m (mg/L)	0.0280	0.0355
k_d ($hour^{-1}$)	0.0058	0.0028
Ys/x (mg/L/CFU/mL)	$7x10^{-7}$	$6.63x10^{-7}$

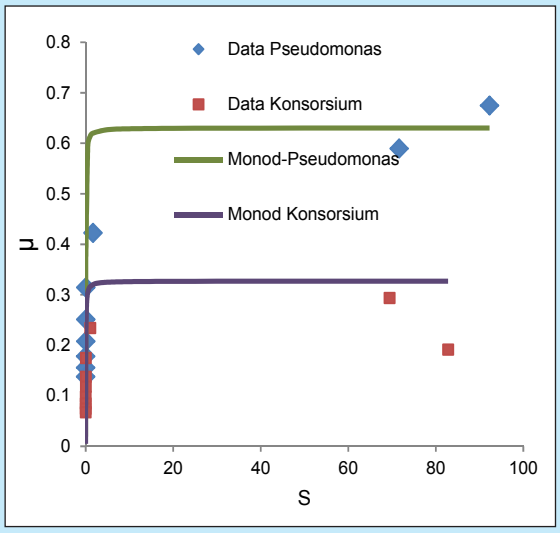


Figure 7
The curve of the monod equation between S vs μ.

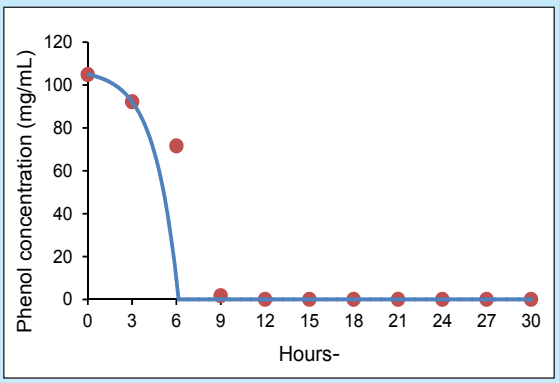


Figure 8
Performance reconstruction between phenol concentration and time in *P. aeruginosa.*

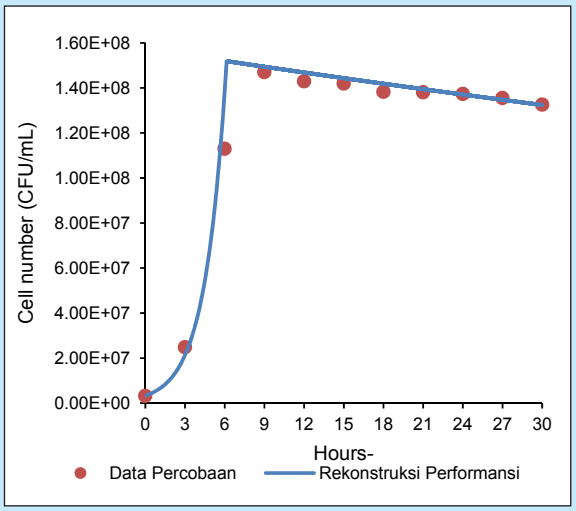


Figure 9
Reconstruction of performance between bacterial populations and time in *P. aeruginosa.*

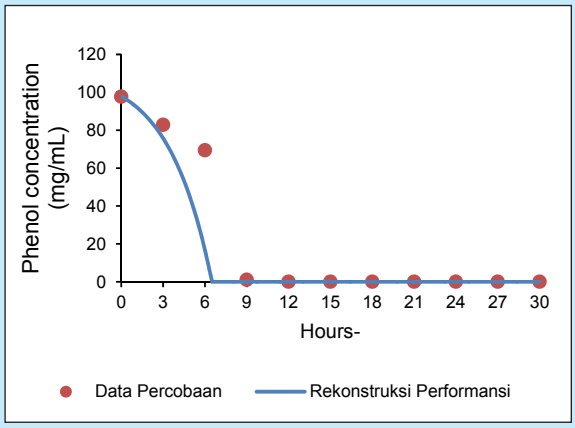


Figure 10
Reconstruction of performance between phenol concentration and time in bacterial consortium.

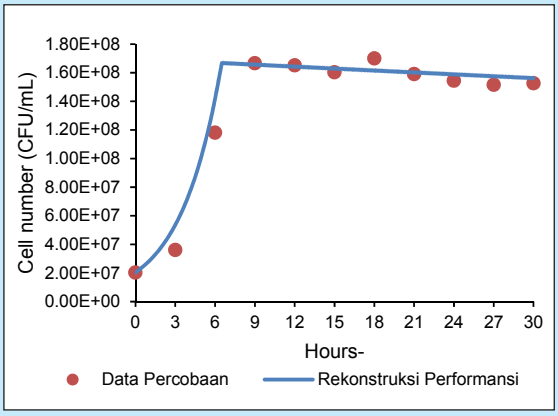


Figure 11
Reconstruction of performance between bacterial populations and time in bacterial consortium.

The μ_{max} and K_m are determined using the formula above and plotted in the form of curves by linearizing with the Lineweaver-Burk plot. The Monod kinetics plot against experimental datacan be seen in Figure 7.

The obtained values of Monod kinetics parameters showed that the pure culture of *P. aeruginosa* gave a better growth rate compared to that ofbacterial consortium microorganisms, and to achieve a maximum growth rate,*P. aeruginosa* consumed less phenol compared to that ofbacterial consortium microorganisms. The calculation results using the mass balance of batch reactor for cells and phenols (using the equation 1 to equation 3 and 4) provide a performance model thatcan be seen in Figures 7, 8, 9 and 10.

The values of $Y_{S/X}$, and μ_d obtained in *P. aeruginosa*were 7×10^{-7} mg/L/CFU/mL, and 0.00575 hours^{-1} respectively. The time needed to degrade phenol with *P. aeruginosa* based on the results of the model reconstruction was estimated for 6.14 hours and at the same time the maximum cell number was estimated at 1.48 x 10^8 CFU/mL (Figures 7 & 8). The measured results at the hour 6 gave a phenol concentration of 71.6 g/L, and were consumed completely in the measurement at the hour 9.

Acquisition of biomass to substrate in bacterial consortium is lower than that of pure culture of *P. aeruginosa*, but with bacterial consortium mortality rate which is lower, a higher maximum biomass was obtained than that of *P. aeruginosa*. While the time needed to degrade phenol was estimated for 6.5 hours, the phenol concentration measured at the hour 6 was at 69.43 g/L, and consumed completely when measured at the ninth hour.

IV. CONCLUSIONS

The experimental results showed that *P. aeruginosa* and bacterial consortium may degrade phenol with the percentage of biodegradation of 98.40% in *P. aeruginosa* and 99.03% in bacterial consortiumrespectively at 30 °C and initial pH 7. Based on the kinetic parameters obtained, it was known that the pure culture of *P. aeruginosa*has better performance compared to that of bacterial consortium microbes in degrading phenol.

REFERENCES

ASTM D 1783,.2014, 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 D 4281., 2014, '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.

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.

Cisneros. S., Martınez. M.A., 2014, 'Isolation Characterization of a Novel Strain *Bacillus sp* KJ6629314 with High Potensial to Aerobically Degrade Diesel'. Revista Mexicana de IngenieríaQuímica, Vol. 13, No. 2 (2014) 393-403.

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

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.

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

Hidayat, S,. Kardena, E., 2013, 'Penyisihan senyawa organik limbah air terproduksi pada reaktor batch menggunakan bakteri indogenous dan penambahan nutrisi'. Jurnal Teknik Lingkungan, vol 1, ITB.

Hasiany, S., Noor E., Yani M., 2015, 'Penerapan produksi bersih untuk penanganan air terproduksi di industri minyak dan gas'. Jurnal Pengelolaan Sumberdaya Alam dan Lingkungan. 5(1): 25-32..

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

Kurniawan, A,. Effendi, A,J., 2014, 'Biodegradatoin of total petroleum hydrocarbons residues below 1% concentration (w/w) using bioremediation process'. Human and Environmental Journal, Vol 21, No. 3. PSLH, UGM.

Liu. Z., Xie. W., 2016, 'Biodegradation of Phenol by Bacteria Strain *Acinetobacter Calcoaceticus* PA Isolated from Phenolic Wastewater'. Int Journal Environ Res Public Health, 13(3): 300.

Nasiri. M., Jafari. I., 2016, 'Produced Water from Oil-Gas Plants: A Short Review on Challenges and Opportunities'. Periodica Polytechnica Chemical Engineering, 61(2), pp. 73-81.

Poli. A, Finore.I, Romano. I, Gioiello. A, Lama. L, Nicolaus. B., 2017, 'Microbial diversity in Extreme

marine habitats and their biomolecules'. Microorganism Journal, 5 (2) 5, PMCID: PMC5488096.

Partila, A.M., 2013, 'Biodegradation og Polyaromatic Hydrocarbons in Petroleum, Oil Contaminating the Environment'. Microbiology Botany Department, Faculty of Science, Cairo University.

Neves, P, R., 2014, 'Pseudomonas aeruginosa'. Encylopedia of Food Microbiology, Vol. 3, pp 1867-1871.

Singh. R., 2107, 'Biodegradation of xenobiotics away for environmental detoxificatoin'. International Journal of Development Research, Vol. 07, issue 07, pp 14082-14087.

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

Syafrizal, 2017, 'Optimization of Phenolic Wastes Treatment in Artificial Produced *Water by Pseudomonas aeruginosa'. Scientific Contribution for Oil and Gas, Vol. 39, No. 1, Research and Development Centre for Oil & Gas Technology, LEMIGAS.*

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.

V, Rajani, 2015, 'Microbial degradation, of, phenol-A review'. International Journal of Research and Review, Vol. 2, Issue. 2 ISSN:2349-9788.