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Proceeding

The 2nd International Conference of the Indonesian Chemical Society 2013

IC CS 2013

Research in Chemistry for Better Quality of Environmental

Universitas Islam Indonesia, Yogyakarta, Indonesia
October, 22 - 23th 2013

Abdul Kahar Muzakkir, Conference Hall
Universitas Islam Indonesia (UII), Yogyakarta.
Kampus Terpadu, Jl. Kaliurang KM 14,5 Sleman, Yogyakarta.

Preface

The international conference is an annual conference of the Indonesian Chemical Society (Himpunan Kimia Indonesia, HKI). In the year 2013, the mandate of the organizing committee was given to the HKI Yogyakarta branch and also supported by Department of Chemistry of Universitas Negeri Yogyakarta (UNY), Department of Chemistry of Universitas Gadjah Mada (UGM), Department of Chemistry of Universitas Islam Negeri Sunan Kalijaga (UIN Suka), National Nuclear Energy Agency (BATAN Yogyakarta), and Volcano Investigation and Technological Development Center (BPPTK Yogyakarta). For the year 2013, ICICS 2013 is hosted by Department of Chemistry, Faculty of Mathematics and Natural Sciences, Islamic University of Indonesia, Yogyakarta from October 22 – 23, 2013. This conference was also prepared to celebrate 70th anniversary of Universitas Islam Indonesia.

The Sicentific Programme of ICICS2013 comprises the following:

1. Invited Speaker 11 papers
2. A total 256 paper for parallels sessions
   a. Organic Chemistry 32 papers
   b. Inorganic Chemistry 43 papers
   c. Physical Chemistry 37 papers
   d. Analytical Chemistry 68 papers
   e. Education Chemistry 23 papers
   f. Biochemistry 43 papers

The breakdown of the presentation is as follows:

Session	Oral	Poster	Total
Invited Speaker	11	0	11
Organic Chemistry	25	7	32
Inorganic Chemistry	38	5	43
Physical Chemistry	31	6	37
Analytical Chemistry	61	7	68
Education Chemistry	22	1	23
Biochemistry	34	8	43
Total	**222**	**34**	**256**

Yogyakarta, 25th November 2013

ICICS 2013

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CONTENT

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Biodegradation of Phenol by Native Bacteria Strains Isolated from Polluted and Non-Polluted Sources

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Abstract

The present investigation was undertaken to assess the biodegradation of phenol by native bacteria strains isolated from polluted and non-polluted sources, which are hospital wastewater, wastewater of textile industry, and peat soil. Phenol degrading performance and growth of all the strains was evaluated periodically within 96 hours. Five isolates showed high performance in degrading phenol, they are DL120 and DOK135 which were obtained from hospital wastewater, TU3 from textile industry wastewater, also SG3 and SG1 from peat soil.Isolate DL120 was found to be highly effective for the removal of phenol, which was used as sole carbon and energy source. From an initial concentration of 300 mgL^{-1} it degraded to 96,35%.
Three isolates from hospital wastewater, namely DL120 and DOK135, showed high performance in degrading phenol. Also one isolate from textile industry wastewater (TU3) and three isolates from peat soil (HG1, SG3, and HP3). One of the strains, namely DL120 was found to be highly effective for the removal of phenol, which was used as sole carbon and energy source. From an initial concentration of 300 mgL^{-1} it degraded to 96,35%. On the other side, isolate TU3 show better growth because it still grow exponentially from 48 to 96 hours of incubation, even it has lower performance in degrading phenol (92,29%) than isolate DL120. Therefore, the utilization of bacterial strains from polluted and non-polluted sources has potential for bioremediation. Further study is needed to investigate the best condition for isolates' growth and identification of the isolates.

Keywords: biodegradation, phenol, bacteria, polluted source, non-polluted source

Introduction

Development in industries, especially in the industry of pharmaceutical, textile, copper, petroleum, dye synthesis, and plastic, produce many pollutant in the environment. Mixed pollutant of phenol and phormaldehid is an example of specific wastewater that often founded in the effluent of chemical plant, ironworks, and hospital (Lee et.al., 1997; Tsai et al, 2005; Al-Thani et al, 2007).

Phenol can be found as soil water pollutan (Bell et al., 2008). Phenol has a toxic effect to human and environment. Phenol is toxic for some aquatic organisms. Phenol also can cause odor and taste problem in drinking water. Phenol can be absorbed by respiration, digestion, and contact with the skin. The exposure of phenol can cause a sistemic toxicity. Phenol is corrosif and can cause chemical burn, has reproductive toxicity, and promote (ATSDR, 2007; Chakraborty et al, 2010).

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The accumulation of phenol in environment can contaminate the environment and cause negative effects to life system. Therefor, technology in wastewater treatment is needed to cope that problem, to reduce the amount or the concentration of phenol and its derivative. Wastewater treatment can be conducted by some methods, whether physically, chemically, or biologically. Biological wastewater treatment (biodegradation) offer an alternative which is much cheaper and environmental friendly, because it is only produce little side product (Al-Thani, *et.al.*, 2007). Microbes can utilize phenol as carbon source and break it into CO_2 (Amro dan Soheir, 2007). Biodegradation is a promising degradation process that is save for the environmen. For that process, we need isolates which have high ability in degrading phenol.

Isolation of microbes which can degrade chemical component, including phenol, is usually started from contaminated source. Isolation of phenol-degrading-bacteria can be obtained from industrial waste and municipal waste (Mailin dan Firdausi, 2006; Khuanmar *et al*, 2007; Ying *et al*, 2007; Movahedyan *et al*, 2009; Chakraborty *et al*, 2010), wastewater tratment plant of petroleum (Ren *et* al, 2008) and soil in industry area or which is contaminated by industrial waste (Tsai *et al*, 2005; Al-Thani *et al*, 2007; Agarry *et al*, 2009; Mohite *et al*, 2010). The isolate of phenol degrading bacteria also can be obtained from the environment that is not contaminated by phenol, such as from sediment of drainage and river (van Schie and Young, 1998) and from uncontaminated soil (Amro dan Soheir, 2007; Wang YD, 2007). Based on El-Sayed *et al* (2003), the information about bacteria and its tolerance to high concentration of phenol and its high metabolic activities are still needed.

This research was aimed to isolate bacteria which have potential ability in degrading phenol. Isolation was conducted from natural source or non-polluted source (peat soil) and from polluted source (wastewater of hospital and textile industry). The obtained isolates were tested for their ability in degrade phenol and their growth. Then, the isolates are predicted can be used to increase phenolic wastewater tratment and to design effective and efficient wastewater treatment system.

Material and Method

a. Isolation of Bacteria

Bacteria were isolated from polluted source (wastewater of hospital and textile industry in Daerah Istimewa Yogyakarta) and from non-polluted source (peat soil from Riau, Sumatera). Isolation was done by enrichment method, using Ramsay medium (2 g

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NH_4NO_3; 0,5 g KH_2PO_4; 1 g K_2HPO_4; 0,5 g $MgSO_4.7H_2O$; 0,01 g $CaCl_2.2H_2O$; 0,1 g KCl) with phenol as sole carbon source. The inocula were incubated for a week at 125 rpm. At the end of each week, the medium was added with phenol in gradual concentration(100ppm, 300ppm, 500ppm).

b. Assessment of Degradation Ability

Isolates were tested for their ability in degrading phenol. Isolates were grown in Nutrien Broth (NB) medium for 24 hours at 125 rpm. Then the number of bacteria was accounted by plate count method. Then, inoculum of bacteria (10^6cell/mL)was added in Ramsay medium with 300 ppm of phenol, incubated for 96 hours at 125 rpm. Every 24 hours, the residu of phenol and bacterial growth were analyzed.

Analysis of phenol residu was done based on Vermerris and Nicholson (2006). Sampel was centrifuged at 10.000 rpm for 10 minutes. Supernatan was added with 0,3 mL $NaCO_3$ (200 g/L) and mix, then added with 0,1 mL Folin-ciocalteau reagent. Allow the sample stand for 30-60 minutes in room temperature. Read the absorbance in a spectrophotometer at 750 nm.

c. Assessment of Growth

Sample of bacteria from Ramsay medium with phenol 300 ppm was dilluted into $10^{-4} - 10^{-6}$. Bacteria were grown using spread plate method. Sample (1 mL) from each dillution level were drop on Nutrient Agar (NA) medium and spread over the medium using drigalsky spatel. Then, incubated for 24 - 48 hours. Bacterial growth was determined by the number of its colony (cfu/mL).

Result and Discussion

Bioremediation or a method to eliminate or make harmless pollutants in an environment using microbial activities can be conducted by bioaugmentation or biostimulation. Bioaugmentation is making use of organisms already found in the environment, whether indigenous microbes from contaminated area or microbes from other sources which are non contaminated by the toxic substance. Biostimulation is the method that change the environment condition for enhancing the rule or activities of microbes that already found in the environment (Vogel and Michael, 2002; Narin, 2003). In this research, we obtained the bacteria from wastewater of hospital and textile industry as the contaminated source, and from peat soil as non-contaminated source.

Ramsay medium with phenol as sole carbon was used to isolate phenol degrading bacteria. So, only the bacteria which can use phenol as solely carbon source that can grow on that medium. The obtained isolates (182 isolates) were then tested for their tolerance to phenol. Isolates were grown in medium with various concentraion of phenol, which are from 100 ppm – 1.000 ppm. Then we choose 15 isolates which shown high tolerance to phenol. The category ofhigh tolerance was based on the ability of isolates to grow until 1.000 ppm of phenol. Those 15 isolates then were analyzed for their ability to degrade phenol.

Tabel 1. Phenol degrading ability of isolates from polluted and non-polluted sources

No	Isolate	Source	Phenol reduction (%)
1	DL120	Hospital wastewater	96,350
2	DOK135	Hospital wastewater	94,67
3	TU3	Textile industry wastewater	92,29
4	HP3	Peat soil	90,88
5	HG1	Peat soil	89,31
6	SG3	Peat soil	86,87
7	SG1	Peat soil	86,500
8	ATA6	Hospital wastewater	79,030
9	AS1	Hospital wastewater	75,300
10	TP1	Textile industry wastewater	49,000
11	AS3	Hospital wastewater	28,440
12	HG2	Peat soil	26,930
13	ATA2	Hospital wastewater	23,500
14	ATA3	Hospital wastewater	14,940
15	DOK144	Hospital wastewater	9,500

The result showed that 9 isolates can reduce phenol 75% and up (table 1). Four isolates are from hospital wastewater (DL120, DOK135, ATA6, and AS1), 1 isolate from textile industry wastewater (TU3), and 4 isolates from peat soil (HP3, HG1, SG3, and SG1). It can be seen that not only bacteria from polluted source which can use or degrade phenol, but also bacteria from non-polluted source. Beside analyzing the ability in reducing phenol, those isolates were also analyzed their growth (figure 1 – 9).

Those 9 isolates show that while the number of bacteria increase, the concentration of phenol decline. Almost all isolates reduce the phenol dramatically at 48 hours of incubation, except isolate HP3, HG1, and ATA6 which reduce phenol fastly at 72 hours of incubation (figure 4,5,8) and isolate AS1 at 96 hours incubation (figure 9). Among 5 isolates that can

ISBN: 978-979-96595-4-5

reduce phenol fastly at 48 hours of incubation, and means that they are potential as phenol degrader, isolate SG3 has optimum growth at 48 hours of incubation (figure 6); isolate DL120, DOK135, and SG1 has optimum growth at 72 hours of incubation (figure 1, 2, 7) ; while isolate TU3 still grow exponentially at 96 hours of incubation (figure 3). Isolate DL120 show the highest ability in degrading phenol (96,35%) among those 5 potential isolates, however isolate DL120 only grow optimally until 72 hours of incubation. On the other side, isolate TU3 show better growth because it still grow exponentially from 48 to 96 hours of incubation, even it has lower ability in degrading phenol (92,29%) than isolate DL120. Therefor, further research is needed to get the best degrading phenol bacteria and the best condition for its growth.

Some bacteria that already known involve in phenol degradation are *Achromobacter, Alcaligenes, Acinetobacter, Arthrobacter, Azotobacter, Bacillus cereus, Flavobacterium, Pseudomonas putida, P. aeruginosa, Nocardia, Brevibacterium fuscum* (Bitton, 2005; Essenberg, *et.al.*, 2008), *Acinetobacter* PD12 (Ying *et al*, 2006), dan *Streptococcus epidermis* (Mohite *et al*, 2010).

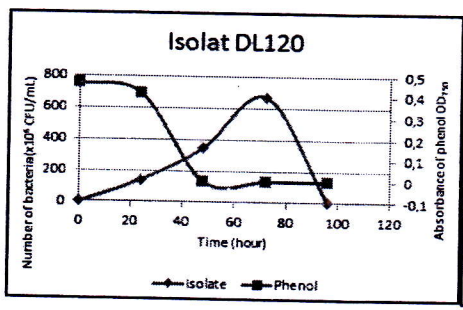

Figure 1. Reduction of phenol by isolate DL120 and its growth

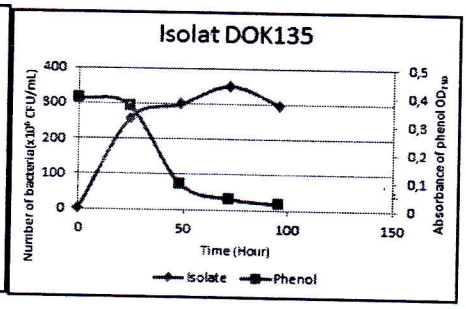

Figure 2. Rreduction of phenol by isolate DOK135 and its growth

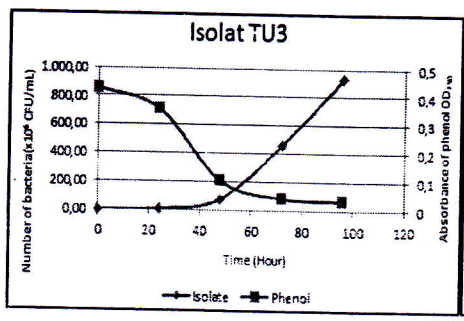

Figure 3. Reduction of phenol by isolate TU3 and its growth

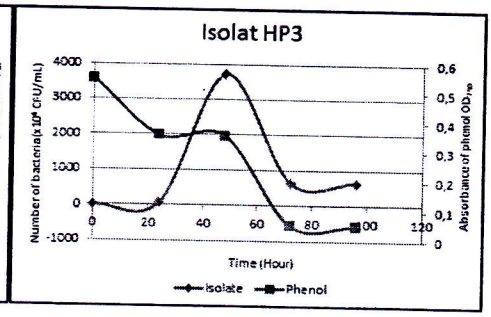

Figure 4. Rreduction of phenol by isolate HP3 and its growth

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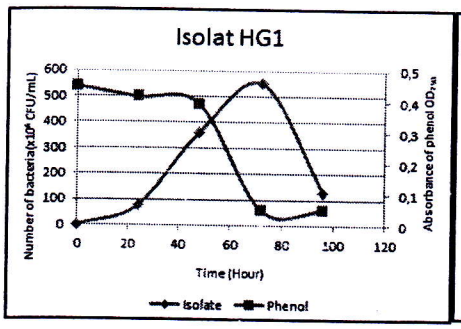

Figure 5. Reduction of phenol by isolate HG1 and its growth

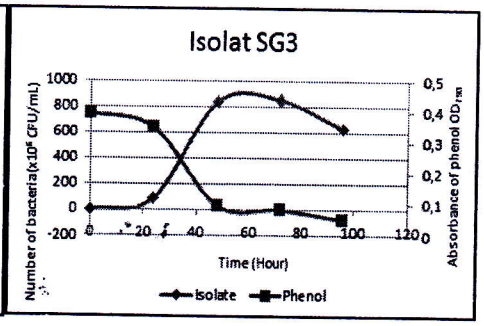

Figure 6. Rreduction of phenol by isolate SG3 and its growth

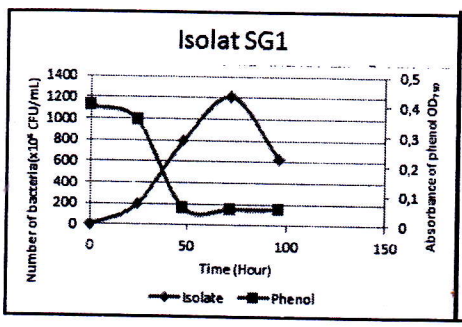

Figure 7. Reduction of phenol by isolate SG1 and its growth

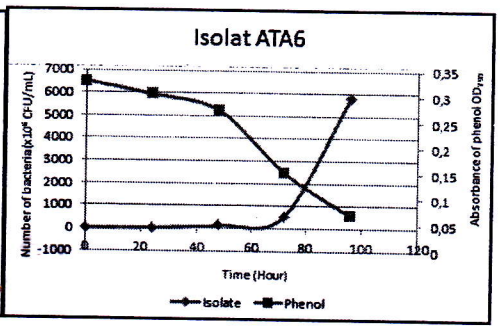

Figure 8. Reduction of phenol by isolate ATA6 and its growth

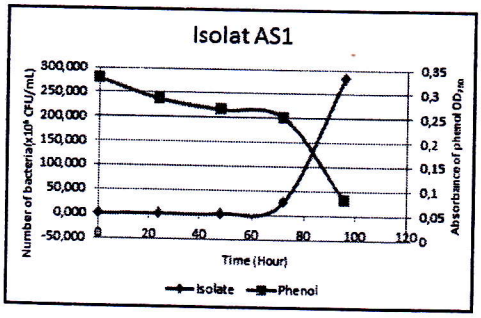

Figure 9. Reduction of phenol by isolate AS1 and its growth

Aerobic degradation of phenol and its derivates can be happened in 2 primer metabolic pathway, which are ortho- and meta-cleavage. Phenol hydroxilase enzyme and cathecol 1,2-dioxygenase or 2,3-dioxygenase will catalyse the degradation at first and second step of degradation. Phenol biodegradation is started with cathecol production, then ring cleavage via ortho- or meta- pathway. At the first step, both pathways use same enzyme that is phenol

ISBN: 978-979-96595-4-5

hydroxilase. At the next step, each pathway use different enzyme, which are cathecol 1,2-dioxygenase for ortho- cleavage pathway, while cathecol 2,3-dioxygenase for meta cleavage pathway (Tsai, *et al*, 2005).

Phenol degradation is more complex than degradation of other chemical compounds. The existence of phenol degradation enzymes makes the microbes posible to degrade many kinds phenolic compound and other compotinds that have similar chemical structure with phenolic compound (Tsai, *et al*, 2005). Therefor, bacteria that can degrade phenol, which is a complex compound, is predicted can degrade other simpler compounds. Then the bacteria can support in solving the environmental problem.

Conclusion

Five isolates showed high performance in degrading phenol, they are DL120 and DOK135 which were obtained from hospital wastewater, TU3 from textile industry wastewater, also SG3 and SG1 from peat soil. Both isolates from hospital wastewater has optimum growth at 72 hours of incubation. Isolates from peat soil, which are SG3 has optimum growth at 48 hours of incubation, while isolate SG1 at 72 hours of incubation. Isolate TU3 still grow exponentially at 96 hours of incubation. Isolate DL120 was found to be highly effective for the removal of phenol, which was used as sole carbon and energy source. From an initial concentration of 300 mgL^{-1} it degraded to 96,35%. However, isolate DL120 only grow optimally until 72 hours of incubation. On the other side, isolate TU3 show better growth because it still grow exponentially from 48 to 96 hours of incubation, even it has lower performance in degrading phenol (92,29%) than isolate DL120.It can be summarized, that isolate from polluted source and from non-polluted source are potential as phenol degrader. Therefor, the utilization of bacterial strains from polluted and non-polluted sources has potential for bioremediation. Further research is needed to study the best condition for isolates' growth, whether the identification of those isolates.

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