STUDY OF TEMPERATURES AND CONCENTRATIONS ON FAST BIODEGRADATION OF TOXIC BPA BY *PSEUDOMONAS AERUGINOSA* NR. 22 ISOLATED FROM MALAYSIAN POND

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***Abstract*— There are more attentions have been focused on environmentally friendly ways on how to solve some of arises problem due to the globally increase in water pollution and aquatic imbalance. There is also incremental in public awareness regarding on this problem. One of the techniques is the use of microorganisms and their aggregates and the biodegradation methods for the treatment of phenol contaminated wastewater are more effective and less costly. Phenolic compound regarding on the pollution, it is one hazardous pollutant that is toxically relative at low concentration which the accumulation of the phenol creates toxicity at both flora and fauna. Bisphenol A is one of the most common toxic environmental pollutants that originate mainly from industrial processes and there is need to decontaminate. The objectives of this work is to study the effect of two variables which are the temperature (30ºC, 35ºC, 40ºC, 45ºC, 50ºC, 55ºC and 60ºC) and the concentrations (100ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm and 400ppm) as to identify the significant effects and interactions in the batch studies. This is because it was found that temperature and concentration significantly affecting the degrading potential of *Pseudomonas aeruginosa* on the toxic BPA. The optimum conditions of the variables for the growth of *Pseudomonas aeruginosa* and for maximum biodegradation of bisphenol A are at the temperature of 30ºC and with the concentration of 100ppm based from the result obtained. These results are useful as to understand the physiological and biochemical properties of *Pseudomonas aeruginosa* before its optimum use in environmental application and these datas will assist in choosing the right bisphenol A degrader for a changeable environment.**

Keywords*— Biodegradation, *Bisphenol A (BPA), Temperature, Concentration, Pseudomonas aeruginosa

I. INTRODUCTION

Recently, the productions of the variety of compounds occur from the massive increase in the synthesis organic chemicals by human being and some of them are xenobiotic. The xenobiotic structures are not easily recognized by the existing degradative enzymes which best describes as xenobiotic characters; hence they accumulate in the environment as a result. (Nakajima N, 2007) Long-range transportation capability, human and animal tissue bioaccumulation and food chain bio magnifications as they persist into the environment. Bisphenol A and its higher homology are aromatic molecules containing hydroxyl group attached to the benzene ring structure which its origin in the environment is both natural and industrial. Forest fire, natural run off from urban area where asphalt is used as the binding material and natural decay of lignocellulosic material are the example of the natural sources, meanwhile the oil refineries, chemical, petrochemical, pharmaceutical, metallurgical, pesticide products, paint and varnish industries, textile, polymer industries, alkylphenols, caprolactums and adipic acid are the example of the industrial sources. (Rabaey, 2005)

Carbolic odor to receiving water bodies has been imparted by the presence of bisphenol A in the water which can cause toxic effects on aquatic lives (Ghadhi, 1995) even at low concentration. This is because phenols or bisphenol A are toxic to human beings which can affect several biochemical functions. (Zeng G, 2006) Bisphenol A is a major pollutant which is included in the list of Environmental Protection Agency (EPA) where the concentration of phenols in waste waters varies from 10 to 300 mg/L. This is essential for phenol-containing effluents need to be properly treated before being discharged and efficient treatment methods are required as to lower down the phenol concentration in waste water to acceptable level which is 5 ppm according to Environmental Protection Agency (EPA) (Fick, 1993)

Chlorination, advanced oxidation process, adsorption, solvent extraction, coagulation, flocculation, reverse osmosis, ozonation, photo catalysis, and electrolytic oxidation are the example of the conventional methods for phenolic wastes treatment in term of chemical and physical. (Narkhede, 2015) These processes somehow led to secondary effluent problems hence the biological treatment for the bulk removal of the pollutants is preferable. Pure and mixed cultures are used for extensive study on biological degradation of phenol. Several studies have been carried out with the bacterium *Pseudomonas aeruginosa s.p* in pure cultures in which phenol is degraded through the meta-pathway. The availability of microbial strains that can mineralize high levels of phenol and withstand adverse conditions to compete under in situ conditions is the factor that determines the success of the bioremediation. High levels of phenol should be tolerated well by an effective bacterial inoculum while maintaining a high level of activity to provide efficient mineralization. The physiological and biochemical properties of phenol degrading bacteria need to be understand before it is been commercialize. (Cai W, 2007)

The biodegradation of phenol by *Pseudomonas aeruginosa s.p* which is a potential biodegrade of bisphenol A has been investigated for its degrading potential under different operating conditions. There are two variables that need to be considered which are the temperature and concentration of bisphenol A as to identify the significant effects and interactions in the batch studied. (Fox, 2001)

In the manufacturing of polycarbonate plastics, food cans and other daily used chemicals, Bisphenol A or BPA is one crucial monomer that involved. BPA has been identified as an environmental endocrine disruptor for its estrogenic and hemotoxic activity because of the massive use of BPA-contained products and BPA led to its ubiquitous distribution in water, sediment and atmosphere. BPA degradation is mainly depended on the metabolism of bacteria even though many factors that affect the fate of BPA in the environment. Hence, in this research, the optimum temperature of the BPA biodegradation and the BPA concentration need to be identified by the *Pseudomonas aeruginosa* which been isolated from the pond.

Most of the research works on the biodegradation of phenol was carried out on the isolation of the microorganism from the contaminated site of the industrial effluents which has been mentioned in the last section. Hence based on the available literature, the overall objective of the present study was to evaluate the phenol biodegradation potential of various microbes and the genes present in them which secretes the enzymes responsible for the reaction. The specific objectives of the present investigation are to determine the optimum temperature of the BPA biodegradation, to identify the suitable concentration of the BPA biodegradation as well as to optimize the correlation between the temperature and the concentration for the Bisphenol A biodegradation by *Pseudomonas aeruginosa sp.* (Narkhede, 2015).

METHODOLOGY

A. Materials and microorganisms

The bacterial species *Pseudomonas aeruginosa sp.* was isolated from the local lake in Shah Alam, Selangor, Malaysia. This *Pseudomonas aeruginosa sp.* is a gram-negative, rod-shaped bacterium. This bacterium carries a 16s tRNA gene. Meanwhile, Bisphenol A, BPA (98%) is obtained from R&M Chemicals. The media used for *Pseudomonas aeruginosa sp.* is centrimide agar. This centrimide agar is made with 45.3g of its portion with one liter of distilled water. This centrimide agar is specialized for the *Pseudomonas aeruginosa* culture. Then nutrient broth with the portion of 8g and 1 liter of distilled is used in the fermentation process in the shake flask for the duration of 24 hours.

Fig. 1. Colony of *Pseudomonas Aeruginosa* On The Agar

B. Preparation of media

The growth medium of *Pseudomonas Aeruginosa sp.* is done on the centrimide agar. Centrimide agar with 45.3g is prepared by suspending them in 1 liter of distilled water, the mixture is stirred at heated until it reaches dissolution and been autoclaved at 121ºC for the duration of 15 minutes. The media was poured onto agar plates, sealed and stored in refrigerator in cool temperature when it is cooled. The strain was cultured onto the agar and it is maintained at temperature of 20ºC. Meanwhile, for the nutrient broth, 8g is suspended in 1 liter of distilled water. The mixture is stirred and been heated at the constant agitation for one minute until it is dissolved completely before being autoclaved at 121ºC for 15 minutes. The broth was left to cool at 2-8ºC to be used as the fermentation medium.

C. Inoculum Preparation

The *Pseudomonas aeruginosa sp.* was inoculated in 10mL sterile nutrient broth and was grown for 24 hours at the temperature of 35ºC, pH 7 and agitation rate of 150 rpm. Then, 5% of 5 x 10^{-5} cells/ml of inoculum is then transferred into the sterile fermentation medium for the duration of 24 hours of growth at incubation with the temperature of 35ºC, pH 7 and rotary shaker of 150 rpm. Figure shows the culture condition of *Pseudomonas aeruginosa sp*. in fermentation medium after 24 hours of growth.

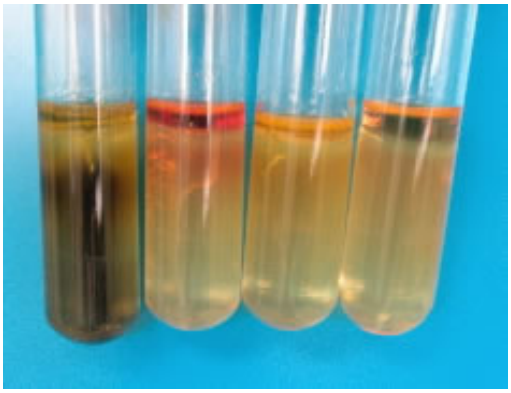

Fig. 2. Culture Condition of *Pseudomonas Aeruginosa sp.* in Fermentation Medium after 24 Hours

D. Influence of temperature of the medium on Bisphenol A degradation

Pseudomonas aeruginosa cells were grown in Centrimide agar medium with 100ppm of bisphenol A (BPA) at different temperature values (30ºC, 35ºC, 40ºC, 45ºC, 50ºC, 55ºC, 60ºC, 65ºC) at pH 7 and inoculum size 8%(v/v). This mixture was contained in 250 mL Erlenmeyer flasks. The cultures were placed on a shaker (150rpm) at the above temperatures. At different times, growth and bisphenol A degradation were measured. (Fick, 1993)

E. Influence of concentration of BPA on phenol degradation

The effect of Bisphenol A (BPA) concentration (100ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm and 400ppm) on bisphenol A degradation were tested. Cells were grown as shake cultures at 32^0C in Centrimide agar medium supplemented with a pH 7 and inoculum size 8% (v/v) in 250 mL Erlenmeyer flask. At different times, growth and bisphenol A degradation were measured. (PALMER R.B., 2008)

F. Estimation of Bisphenol A (BPA) degradation.

Bisphenol A (BPA) degradation was determined quantitatively by the high performance liquid chromatography (HPLC) method using the wavelength (λ max: 230nm) according to (Bureau of Chemical Safety, July 2009)

G. Growth determination

Bacterial growth was determined in terms of cell mass by measuring optical density at a wavelength of 560nm. (Narkhede, 2015).

II. RESULTS AND DISCUSSION

A. Identification of BPA degrading bacteria

The bacterial species isolated from the polluted lake area of Shah Alam have been used for the first time in remediation of waters polluted by BPA. The aim of our work is to improve the knowledge regarding the methods for the cleaning of the environments polluted with BPA. To identify the isolated bacterial species an almost complete sequence (about 1.5 bp) of the gene encoding 16S rRNA of LCS1 and LCA3 strains was determined. Based on a BLASTN search of GenBank, the closest matches for LCS1 strain was P. aeruginosa (nucleotide identity 97%), whereas the strains are gram-negative and facultative anaerobes and able to use different arrays of electron acceptors for cellular respiration. The identified strains were deposited in the Microbial Strain Collection of Latvia, Kronvaldablvd, 4, RIGA, LATVIA, LV-1586. The accession numbers given by the International Depository Authority were P1444 for LCS1 and P1446 for LCA3. The figure below indicated the bacterium, *Pseudomonas aeruginosa sp*'s growth profile within 24 hours/1 day.

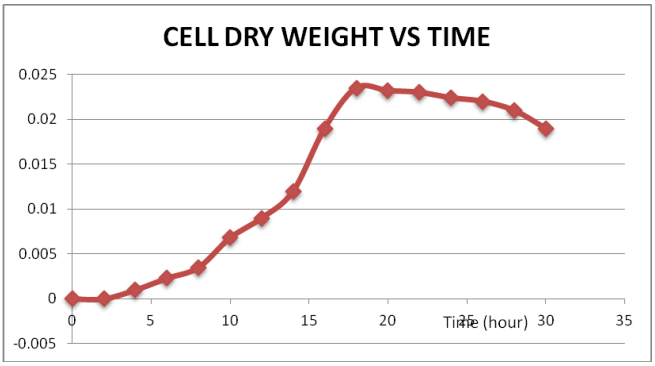

Fig. 3. The Growth Profile

B. The use of High Performance Liquid Chromatography (HPLC) as for the BPA biodegradation analysis.

A comprehensive sample preparation and analytical procedure was developed for determining BPA in drinking water. This fast procedure employed materials and techniques selected in part for speed, but also those that would not contribute unwanted artifacts. The use of SPE allowed BPA to be extracted plus concentrated, which may result in greater method sensitivity compared to simple headspace or direct injection methods. The conditions of HPLC are including the SPE tube, supelclean ENVI-18, 500 mg, 6 mL glass tube, PTFE frit (Product No. **54331-U**). Meanwhile the condition is 1 mL 1% formic acid in acetonitrile, 1 mL DI water; sample addition, 5 mL spiked water, sample elution, 2 mL 1% formic acid in acetonitrile eluate, post- treatment: 1 mL evaporated, then reconstituted to 0.5 mL with acetonitrile. (United States Food and Drug Administration (US FDA, accessed 10-Jan-2012.). The figure 4 shows the standard curve of BPA at the concentration of 100ppm. This standard curve is a reference as to examine the biodegradation by the *Pseudomonas aeruginosa.* From this standard curve, peak at min 8 is where the degradation can be seen. The smaller the area under curve, the greater the biodegradation has been done by the *Pseudomonas aeruginosa.* The figure 5 represents the lowest temperature in this research which is 30ºC meanwhile; figure 6 represents the highest temperature which is at 60ºC. The figure 7 and figure 8 represent the

lowest and the highest concentration. They are 100 ppm and 400 ppm

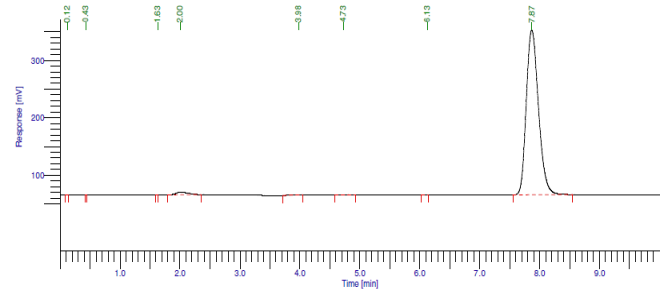

Fig. 4. The Standard Curve of BPA

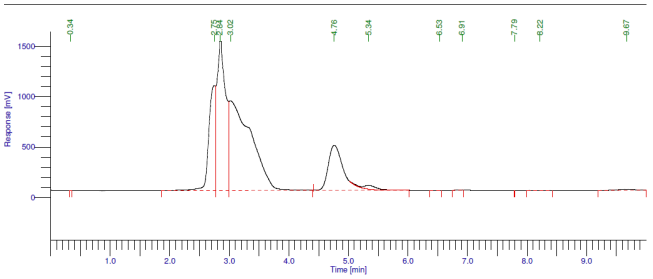

Fig. 5. Temperature of 30ºC

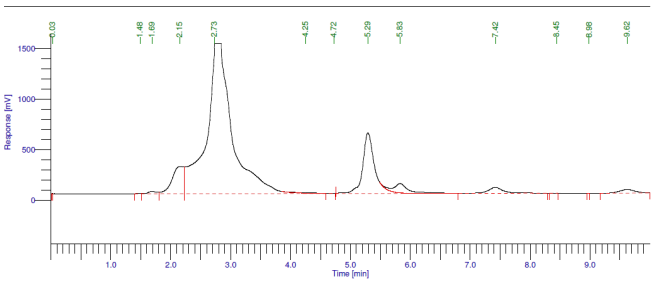

Fig. 6. Temperature of 60ºC

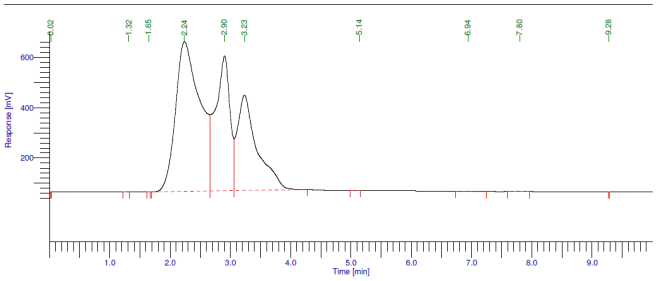

Fig. 7. Concentration of 100ppm

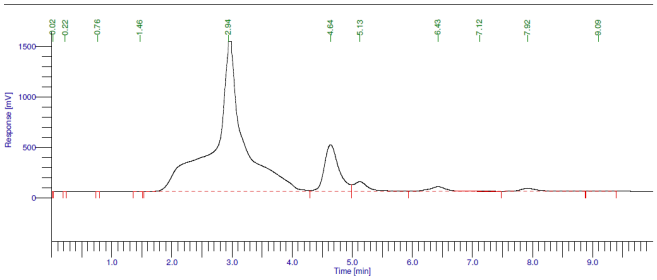

Fig. 7. Concentration of 400ppm

C. Influence of temperature of the medium on phenol degradation

The rate of metabolism is affected by the temperature as it exerts an important regulatory influence on it then little work has been done on the microbiological activity of the organisms present in the water treatment plants operating at lower temperatures. (Mandich A, 2007) Conventional biological waste treatment processes however can operate at low temperature provided sufficient time is allowed for these organisms to degrade in organic wastes. Temperature in an unexpected manner affects the microbiological degradation of bisphenol A in industrial waste water and the efficiency of treatment by microbiological activity on phenol and other contaminants were significantly fine. (Gehring M., 2004)

Bisphenol-A polycarbonate usually has either phenyl or hydroxyl as an endgroup which initial degradation begins at the end group which reacts with any free hydrogen present such as water or free hydroxyl groups. Regardless of the end group presents, the product formed will be the same. The chains with $PhCO^{3-}$end groups give off quantitative amounts of phenol and diphenylcarbonate while those with hydroxyl endgroups evolve only trace amounts of phenol. The carbonate group is the major point of degradation at temperatures below 400°C, while at higher temperatures; the isopropylidene group is alsosusceptible to loss of a methyl radical according to the report. According to the journal of (Sridevi, 2009)

There is seven temperature values from (30ºC, 35ºC, 40ºC, 45ºC, 50ºC, 55ºC and 60ºC) were investigated in Figure 5. Bisphenol A was degraded rapidly at 30ºC. At this temperature value, bisphenol A degradation was high compared to the other values. However, the bisphenol A degradation at temperature 60ºC, 55ºC, 50ºC was slower.These results showed that *Pseudomonas aeruginosa* degraded more bisphenol A per day at 30ºC than at any other temperature value. This is because *Pseudomonas aeruginosa sp.* might not function well at the higher temperature.

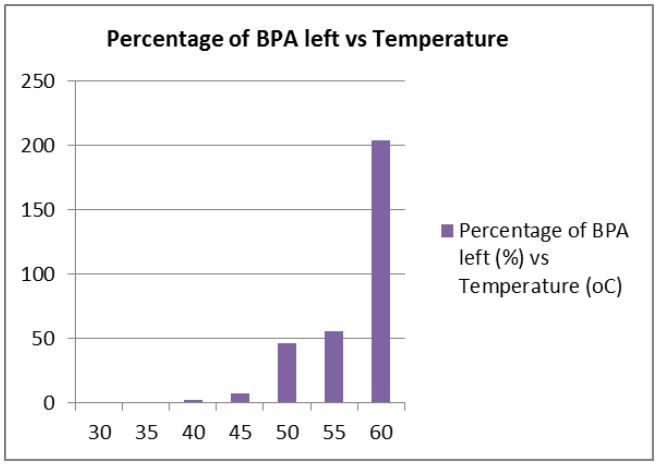

Fig. 7. BPA degradation at different temperatures

D. Effect of BPA concentration on bisphenol A degradation

According to the journal of (Mohanty, 2012), there is afigure that represents the degradation profile of the microbe *Pseudomonas aeruginosa* at various concentration of bisphenol A used as the sole source of carbon and energy as the microbe is able to degrade up to 750 PPM of

bisphenol A in the media but shows an extended lag phase with increase in the concentration of phenol. (Belfroid A, October 2002) The growth of the microbe was inhibited and the microbe was no longer able to degrade phenol above 750 PPM of bisphenol A. It can be concluded that the maximum tolerance of the microbe is 750 PPM. The microbe is able to degrade up to 48% of the initial bisphenol A when the concentration of the substrate is 750 PPM and he microbe degrades 72% of bisphenol A from the media in 144 hours when the initial concentration of the media is 500 ppm.

According to Zheng (2010), he has reported that the microbial strain *Pseudomonas aeruginosa sp.* HSD38 is able to degrade up to 500 ppm of bisphenol A below the detection level but unable to tolerate more than 700 ppm of initial concentration of phenol. Bisphenol A was degraded by *Pseudomonas aeruginosa* (NCIM 2074), at different concentrations of BPA (100ppm, 150ppm, 200ppm, 250ppm, 300ppm, 350ppm and 400ppm) as shown in Figure. 6. The presence of BPA in the culture medium increased the tolerance of the organisms to high bisphenol A concentrations by providing a good source readily metabolisable carbon to support cell growth. Hence, it was concluded that BPA on medium supported bisphenol A degradation. In summary, these results show that *P. aeruginosa sp.* is able to tolerate higher levels of bisphenol A when supplemented with glucose as additional sources. The optimum level of BPA concentration is at 100ppm.

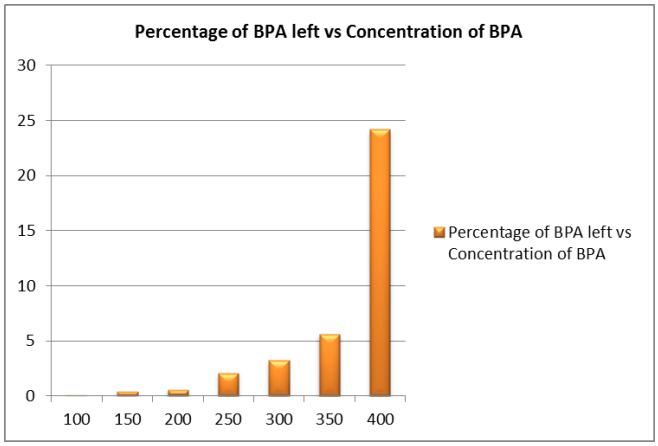

Fig. 6. BPA degradation at different concentrations by *Pseudomonas aeruginosa*

E. The Correlation between the temperature and the concentration for the Bisphenol A biodegradation

The BPA degradation is greater at the temperature of 30°C – 35°C and with the concentration of 100ppm. The optimum condition for *Pseudomonas aeruginosa* is at 32°C hence it can degrade well the Bisphenol A in the pond. Plus, from the HPLC reading , at 100 ppm, BPA has been degraded the most to be compared with other concentration because it affect the *Pseudomonas aeruginosa*'s optimum condition.

III. CONCLUSION

According to the HPLC reading, it can be concluded that the BPA degradation is optimized at the temperature of 30°C with the concentration of 100ppm.

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