BPA DEGRADATION OF ENCAPSULATED *PSEUDOMONAS AERUGINOSA* ON SCOURING PAD, VIABILITY AND SCANNING ELECTRON MICROSCOPE VIEW

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***Abstract*— *Pseudomonas aeruginosa* strain NR. 22 isolated from a lake in Shah Alam, Malaysia was allowed to degrade BPA. The bacteria was known as laccase enzyme producer that is able to degrade BPA. This study was performed by cultivating the bacteria in medium containing low concentration of BPA which is 5 ppm to observe the BPA degradation ability of the bacteria. The bacteria was able to completely degrade 5 ppm of BPA in 24 hours of fermentation as seen from the result obtained from High Performance Liquid Chromatography (HPLC) analysis. This study was also done to determine the viability of a low cost immobilization alternative in which the bacteria was attached to small pieces of scouring pads through 24 hours fermentation. The viability of the immobilization method was validated through the Scanning Microscope Electron view where the bacteria was seen attached on the surface of scouring pad fibers. The efficiency of the BPA degradation for the immobilized bacteria was also determined by adding the scouring pads attached with bacteria in shake flask fermentation for 24 hours. The result obtained from HPLC analysis shows that the immobilized bacteria was able to degrade BPA from 5 to 1.5 ppm. In conclusion, the study on BPA degradation by *Pseudomonas aeruginosa*, the viability of scouring pads as immobilization alternative and the efficiency of the degradation ability of immobilized bacteria has been successfully achieved.**

***Keywords*—** *BPA, Pseudomonas aeruginosa fermentation, scanning electron microscope, HPLC, bacterial morphology.*

I. INTRODUCTION

Bisphenol A(BPA) is an important chemical that are being produced in large scale for various applications for both industrial and consumer products. Bisphenol A has the chemical formula of $(CH_3)_2C(C_6H_4OH)_2$ and are widely used to manufacture resins as well as plastics. According to Pivnenko *et al.* [1], plastic that are produced by using BPA has tough and clear characteristics in which makes it being used in the production of sports equipment and bottles. The epoxy resins that is made up of BPA usually used in water pipes works and as a coating for the beverage cans. BPA has the molar mass of 228.29 $gmol^{-1}$ with a density of 1.20 g/cm^3 and usually appears as white solid. It has a melting and boiling point of 159^{o}C and 220^{o}C respectively. BPA is quite soluble in water and has the vapor pressure of 5 x 10^{-6} Pa at standard room temperature.

The production of polycarbonate plastics uses BPA as the monomer. These plastics that used BPA as the monomer has several advantages such as decent optical clarity, good ductility and durability at room temperature. This is why the BPA is used to produce wide range of consumer products. According to K. Ashberger *et al.* [2], 90% of BPA is utilized in the production of epoxy resins and polycarbonate plastics while the other 10% is utilized as additive in manufacturing or to produce other types of polymers.

As there are a lot of consumer products that utilized BPA as the building block, Geens *et al.* [3] suggested that there are many sources of BPA that have not yet been identified and it is important further study must be conducted in order to identify the exposure from unexpected sources. Pivnenko *et al.* (2015) stated that all of the waste paper samples analyzed showed the existence of BPA in them. Therefore, it can be concluded that there are lots of unexpected sources of BPA that still have not been identified and a reliable method of detection of BPA is required in order to identify more sources of BPA.

The need of continuous search on the unexpected sources of BPA relates to the multiple effects of BPA towards the human health and environment. Welshons *et al.* [4] stated that the human being exposed to BPA is much higher that what is estimated by the industries and that the long term intake of BPA on daily basis results in the bioaccumulation and steady state levels. According to Rubin [5], there are multiple and complex potential actions of BPA which may involve in several pathways related to endocrine. This effect could activate during the exposure or at any given time. Therefore, it is necessary that any source of BPA must be identified and a method to degrade this chemical in order to prevent the harmful effects of this chemical to human and environment.

According to Pacheco et al. [6], biosensors are integrate receptor-transducer devices that are capable to provide specific quantitative analytical information through the utilization of biological recognition element. It was also stated that biosensors are designed to be highly selective while having the ability to provide online and real-time measurements for bioprocess control as well as bioanalysis. There are several types of biosensors such as electrochemical, blood-glucose, potentiometric, conduct metric, thermometric, optical, piezoelectric, whole cell and immuno-biosensor.

Chee *et al.* [7] discussed on the determination of trichloroethylene (TCE) by using novel whole cell biosensor. It was mentioned that TCE is a common pollutant in soil and groundwater that is difficult to be degraded by microorganism. Thus, a novel biosensor was developed by using *Pseudomonas sp.* strain ASA86 and immobilized on a porous cellulose nitrate membrane. It was stated that the response could detect up to 0.05 mg/L TCE concentration with a detection time of 5 minutes at pH 8.0 and 30^{o}C. This biosensor response was reported to be stable for five days and have enough sensitivity for monitoring purpose in environment.

In order to produce a viable and robust whole cell biosensor, many previous studies reported that immobilization approach provides the advantage in the construction of whole cell biosensor. Thus, several latest studies on the immobilization method are reviewed in this study to observe different immobilization

techniques. Liu et al. [8] discussed the immobilization of cell by using polyvinyl alcohol for degrading diesel in seawater. An improvement was made by including activated carbon which increased the degradation efficiency for about 47%. In this study, two design were made by using *Rhodococcus pyridinivorans* CCHCCH11 and *Gordonia alkanivorans* CC-JG39 which achieve degradation effiency of 92% and 88 % respectively. The first design was recommended as a bacterial cell combination with polyvinyl alcohol immobilization to help solving the environment problem. This research will emphasize on the method to degrade BPA through biological route as well as to find a low cost immobilization method for the microorganism used to degrade BPA. The microorganism used for the BPA degradation in this research is *Pseudomonas aeruginosa* isolated from a lake in Seksyen 2, Shah Alam. This species will be immobilized on the scouring pads as a low cost alternative for immobilization of the species for the BPA degradation purposes. The effect of the immobilization on the BPA degradation will be observed in this research to determine the viability of this immobilization method.

II. METHODOLOGY

A. Materials

In this experiment, the *Pseudomonas aeruginosa* were used for the whole experiment. In order to cultivate this bacteria, Cetrimide Agar was used and Nutrient Broth (Merck) was used for the fermentation process. Another chemical that was used in the experiment was the Bisphenol-A (BPA, R&M Chemicals, 98%). A buffer of pH 7 was used to adjust the pH of the Nutrient Broth to pH 7. Several scouring pads were used to study the attachment of this bacteria to the scouring pad.

Figure 1 : *Pseudomonas aeruginosa* stock culture.

B. Preparation of Pseudomonas aeruginosa culture

The *Pseudomonas aeruginosa* stock culture was taken out of the freezer and thawed to room temperature. Then, Centrimide agar was prepared by using the recipe provided at the side of the bottle. The Centrimide agar granules were weighed to 45.3 g and placed into a beaker. After that, distilled water was added into the beaker until the volume reached 1 Liter. The mixture is stirred and heated by using the hot plate until all the of the granules dissolved. The mixture was then transferred into a scott bottle and the bottle was autoclaved at 121ºC for 15 minutes by using the autoclave machine. After the autoclave process, the bottle was taken out and let to cool until it reach around 40ºC before the content is being poured into several petri dishes. After the agar has solidified, the *Pseudomonas aeruginosa* is transferred aseptically from the stock culture to the petri dish by using the streak plate method. The metal loop is re-flamed for each quadrant in order to prevent contamination. The petri dishes was then placed in an incubator at 37 ºC for 24 hours.

C. Preparation of fermentation broth

The Nutrient Broth powder was weighed to 8 grams and placed into a scott bottle. The volume was brought up to 1 Liter by adding distilled water. Then, the bottle was stirred and heated by using a hot plate. After the powder has been dissolved, the bottle was covered with aluminium foil and placed in the autoclave machine. The bottle was autoclaved at 121ºC for 15 minutes. After the autoclave process, the bottle is taken out of the machine and placed in a laminar flow hood and allowed for the temperature to cool down until it reach room temperature before it can be used. For the BPA medium, the nutrient broth will be mixed with BPA at required concentration by using the formula $M_1V_1 = M_2V_2$.

D. Preparation of fermentation broth

The Nutrient Broth powder was weighed to 8 grams and placed into a scott bottle. The volume was brought up to 1 Liter by adding distilled water. Then, the bottle was stirred and heated by using a hot plate. After the powder has been dissolved, the bottle was covered with aluminium foil and placed in the autoclave machine. The bottle was autoclaved at 121ºC for 15 minutes. After the autoclave process, the bottle is taken out of the machine and placed in a laminar flow hood and allowed for the temperature to cool down until it reach room temperature before it can be used.

E. Preparation of Pseudomonas aeruginosa seed culture

Before the fermentation process can be started, it is necessary to prepare the seed culture for the bacteria. The preparation of seed culture begins with the transfer of the bacteria from the petri dish into a shake flask containing 15 ml nutrient broth. By using aseptic technique, one loopful of bacteria colony was taken from the petri dish and placed into the shake flash containing the nutrient broth. The metal loop was used to gently stirred the nutrient broth carefully until all of the bacteria released into the nutrient broth. After that, the shake flask was closed with a cotton plug and placed in a rotary incubator. The rotary incubator was set to 150 rpm and 37ºC. The bacteria in the shake flask was allowed to grow overnight before it can be used for the fermentation process.

F. Morphology of Pseudomonas aeruginosa

The process of morphology identification starts with the preparation of centrimide agar. 45.3 grams of centrimide agar powder was mixed with 1 Liter of distilled water and then heated and stirred on a hot plate. After the powder has been dissolved, the agar was transferred into a scott bottle and then autoclaved at the temperature of 121ºC for 15 minutes. After the autoclaving process, the bottle was let to cool down until around 40ºC before the content was poured into several petri dish. The agar in the petri dish was allowed to cool and solidify. After the agar solidified, one loopful of *Pseudomonas aeruginosa* was taken from a stock culture and streaked into 4 quadrant on the agar. The petri dish was then sealed and incubated at 37ºC for 24 hours. After the incubation process, the petri dish is taken out of the incubator and a little bit of the colony formed on the agar was taken by using a swab and smeared onto glass slide. The glass slide was then passed rapidly over a flame to heat fix the bacteria onto the glass slide. After that, the glass slide was flooded with crystal violet for about 1 minute. Then, the glass slide was rinsed gently with distilled water. The glass slide was then gently flooded with Gram iodine and allowed to stay for about 1 minute. The glass slide was once again gently rinsed with distilled water. Then, the smear was decolorized by dropping 95% ethanol for about 5 to 10 seconds and immediately rinsed with water. After that, the smear was flooded gently with safranin for about 1 minute and rinsed gently with distilled water. The glass slide was then blot dry and placed under the microscope to observe the morphology of the bacteria. This staining method has been adapted from Bartholomew *et al.* [9].

G. Fermentation of Pseudomonas aeruginosa

This section covers the fermentation process of the *Pseudomonas aeruginosa* bacteria. The fermentation process starts with the inoculation of the bacteria by using the seed culture. The seed culture was mixed with nutrient broth in 250 ml shake flask aseptically until the volume reach 100 ml. The shake flask was swirled gently to ensure that the bacteria was well dispersed inside the medium. After that, the shake flask was placed in the rotary incubator at 150 rpm and 37ºC for 24 hours. Samples were collected at the interval of 1 hour for the first 4 hours of the experiment. Then, the samples were collected at 2 hours interval until the fermentation is complete at 24 hours. This method for fermentation of *Pseudomonas aeruginosa* has been adapted from LaBauve *et al.* [10]. The samples that were collected will undergo optical density reading by using UV-spectrophotometer and preparation for cell dry weight calculation. For the cell dry weight calculation, 2 ml of samples were placed in eppendorf tube and centrifuged by using refrigerated centrifuge machine at 4ºC and 10000 rpm to separate the biomass from the liquid. Then, the supernatant was removed and the biomass in the tube were dried by using oven at 80ºC in overnight duration. After the drying process, the final weight of the tube was subtracted with the initial weight of the tube. Both of the data obtained from the optical density and cell dry weight were used to produce a microbial growth curve for the *Pseudomonas aeruginosa*.

H. Fermentation of Pseudomonas aeruginosa with scouring pad

The fermentation of Pseudomonas *aeruginosa* was repeated by using the same method but modified by adding BPA at concentration of 5 ppm and also 20 pieces of 5 mm x 5 mm scouring pads. The scouring pads were added to the shake flask for the purpose of immobilization of the bacteria. The fermentation was allowed to proceed for 24 hours and at the end of the fermentation, samples were collected to analysis the degradation of BPA by using High Performance Liquid Chromatography (HPLC) and the scouring pads were collected for viewing under Scanning Electron Microscope (SEM).

I. High Performance Liquid Chromatography (HPLC) analysis.

The samples collected from the fermentation process will be analyzed by using HPLC to determine the degradation of BPA during the fermentation process. This method has been adapted and modified from Aurand [11] and the analysis of BPA for both standard and fermentation samples is summarized in the table below.

Table 1 : Parameters used for the HPLC analysis.

Parameters	Values
Column	Perkin Elmer C18
Mobile phase	Solvent A : Acetonitrile (40%) Solvent B : Water (60%)
Flow rate	0.4 ml/min
Column temperature	35 ºC
Detector	UV at 230 nm
Analysis time	10 minutes
Injection volume	10 µL

J. Crude laccase enzyme and laccase assay

100 ml of nutrient broth was used for fermentation of *Pseudomonas aeruginosa* for 24 hours. The broth was centrifuged at 10000 rpm at 4ºC for 20 minutes after the fermentation process. This was done to separate the biomass from the supernatant which is the crude laccase enzyme. The crude laccase enzyme activity by using UV-spectrophotometer at 465 nm and mixture of 1:1 enzyme and 50 mM phosphate buffer as blank. A standard curve was prepared by mixing 1 ml of crude enzyme, 1 ml of 50 mM phosphate buffer and 1 ml of different concentration of at 1,2,3,4 and 5 ppm BPA solution. This method was adapted from Nik Raikham [12].

III. RESULTS AND DISCUSSION

A. Pseudomonas aeruginosa culture and morphology

For this research, the agar used for the cultivation of *Pseudomonas aeruginosa* is Cetrimide agar. This agar is known as selective agar in which it only allows the *Pseudomonas aeruginosa* species to grow on the agar. Thus, the risk of contamination by other types of microorganism in the surrounding can be prevented and this eliminates the need for subculture and isolation process in order to obtain pure colony of the species. The *Pseudomonas aeruginosa* bacteria is transferred aseptically in laminar flow hood onto the Centrimide agar in the petri dish. This petri dish was then placed in an incubator at 37ºC for 24 hours to allow the growth for the bacteria. Figure 2 shows the comparison between the uncultivated agar and the growth of *Pseudomonas aeruginosa* on the agar after 24 hours of incubation. The cultivated agar shows green pigmentation which indicates healthy growth of the bacteria.

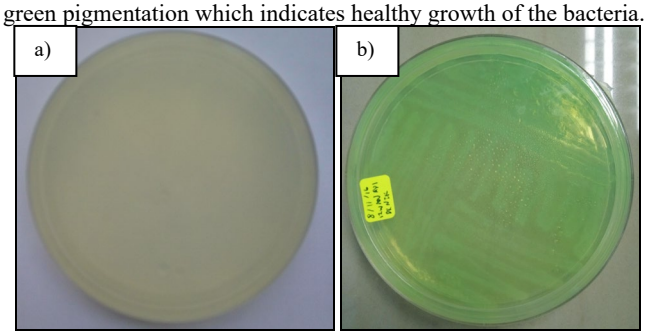

Figure 2 : a) Centrimide agar for *Pseudomonas aeruginosa* cultivation b) *Pseudomonas aeruginosa* growth after 24 hours incubation showing pyocyanin pigment released.

After the cultivation process, a bacterial smear was prepared by using the bacteria grown on the agar. A sterile cotton swab was used to take the bacteria cells at a small amount and smeared on a glass slide. The bacteria cells on the glass slide then went through gram staining process and placed under a microscope to observe its morphology. The magnification of the microscopic view was gradually increased until the cells of the bacteria can be clearly seen.

Figure 3 shows the shape of *Pseudomonas aeruginosa* cells which can be identified as rod-shaped bacteria. From the figure, it can be seen that the bacteria cells were stained in pink color. Thus, it can be concluded that *Pseudomonas aeruginosa* is a gram negative bacteria. As the bacteria was identified as gram negative bacteria, it shows that the bacteria has thin peptidoglycan layer. During the staining process, the bacteria cells were washed with alcohol which dissolves the outer membrane of the cells which results in the cell wall of the cells became porous and allowing the crystal violet solution retained in the cell to diffuse out of the cells. This results in the bacteria cells to retain the pink color when they were counter-stained with the safranin solution.

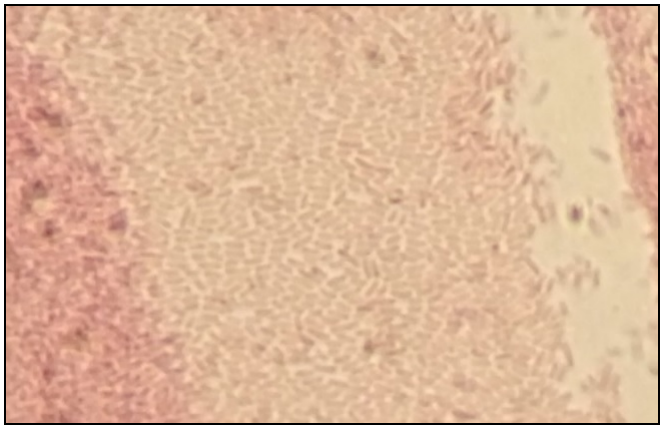

Figure 3 : The microscopic view of *Pseudomonas aeruginosa* cells.

B. Microbial growth curve of Pseudomonas aeruginosa

Each microorganism has its own growth curve but generally, the growth curve of microorganism consist of four different phases which are lag, exponential, stationary and death phase. There are a lot of importance of the growth curve such as to determine the time of metabolites production by the microorganism. For the purpose of constructing the microbial growth curve of *Pseudomonas aeruginosa*, one loopful of the bacteria was taken from the petri dish and transferred aseptically in 10 ml of nutrient broth in order to prepare an inoculum. The inoculum is then placed in incubator for overnight duration to allow the growth of the bacteria. Then, the inoculum is transferred aseptically into a shake flask and the volume was brought up to 100 ml using nutrient broth. The shake flask was placed in a rotary shaker and allowed to incubate for 24 hours. Sampling was done at 2 hours interval and the samples were centrifuged and dried overnight in an oven at 80ºC.

Based on figure 4 below, the microbial growth curve clearly indicates each phase for the growth of *Pseudomonas aeruginosa*. It can be seen that the lag phase for this bacteria starts from 0th hour until 2nd hour. After that, the growth of the bacteria enters the exponential phase starting from the 3rd hour until 18th hour. After 18 hours of fermentation, the growth of the bacteria starts to become stationary and slowly decreasing. This shows that the bacteria has reached the stationary phase and gradually moving into the death phase. Based on the data obtained above, it relates directly to the study by Schleheck *et al.* [13] where it was mentioned that the exponential growth of the bacteria was at 8 to 14 hours of incubation time while the biomass of the bacteria started to decrease at 19th hour of the incubation. There is only minor difference between the study above in which the lag time in this research only last for two hours. This shows that the bacteria in this research adapts quickly to the environment and possess a fast growth.

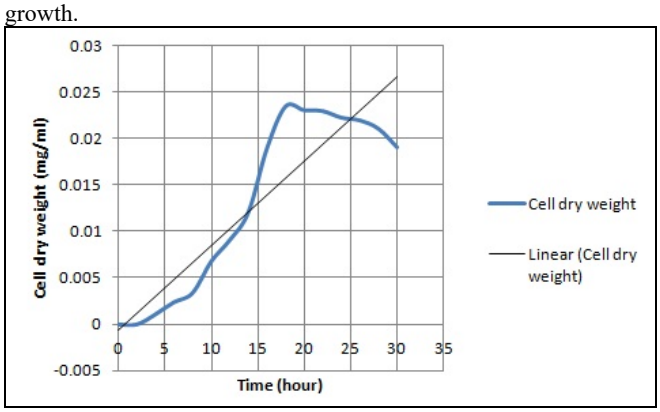

Figure 4 : Microbial growth curve of *Pseudomonas aeruginosa*

C. Degradation of BPA and HPLC analysis

The fermentation of *Pseudomonas aeruginosa* was repeated in duplicate by using BPA medium, one with the presence of small pieces of scouring pads and the other without the scouring pads. The small pieces of the scouring pads were attached with *Pseudomonas aeruginosa* beforehand by adding the scouring pads to shake flask during fermentation for 24 hours. The nutrient broth used for the fermentation with the bacteria was introduced with 5 ppm of BPA and allowed to incubate for 24 hours. A sample was collected before the fermentation starts and labeled as initial concentration of BPA. After 24 hours, another sample was collected from the fermentation broth and was labeled as the final concentration of BPA.

All of the samples were analyzed by using HPLC with the parameters provided in the section I of method above. Before the samples were injected into the HPLC machine, a BPA standard was first prepared and analyzed by using the HPLC machine. Based on Figure 5 below, it can be seen that the BPA is detected by the HPLC machine at the retention time of 7.87 minutes. This data however differs from the result obtained by Aurand (n.d.) where the BPA is detected at the retention time of 1.8 minutes. The difference that occur is due to the modification made to the method provided by Aurand (n.d.).

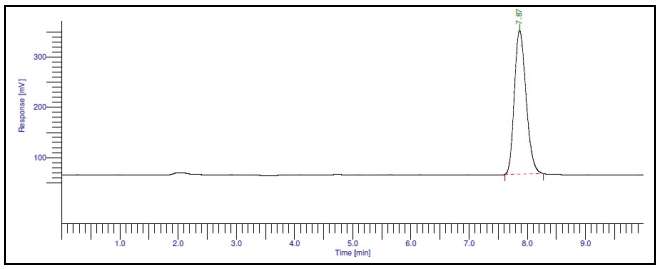

Figure 5 : The graph of BPA standard at 100 ppm analyzed by using HPLC machine.

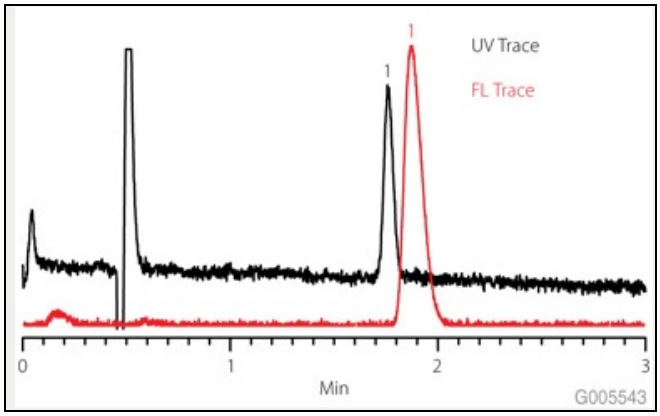

Figure 6 : The retention time of BPA standard. (Aurand, n.d.)

Therefore, for the analysis of samples from the fermentation was expected to detect BPA around the range of 7.87 minutes. The samples for the fermentation without the scouring pads were first analyzed by using the HPLC machine.

Based on the figure 7 below, there are a lot of peaks obtained from the graph produced by the HPLC machine. Therefore, the peak that belongs to the BPA can be determined by looking at the retention time of the peaks. The peak that represents the BPA can be identified by using the standard in the figure 5 where it shows that the BPA peak is obtained at retention time of 7.87 minutes. Thus, the small peak in the Figure 7 at the retention time of 7.87 minutes represents the BPA detected in the sample. The peak however appears much smaller than the one in the standard. This is because the concentration of BPA used differs greatly between the standard and the sample which are 100 ppm and 5 ppm respectively. High concentration of BPA was used in the standard in order to clearly show the peak and retention time of BPA so that it can be used to compare with the samples.

Figure 8 below shows the graph obtained from HPLC machine that represent the sample collected after 24 hours of fermentation without scouring pads. From the figure, it can be seen that there is no peak obtained at the retention time of 7.87 minutes. This shows

that BPA could not be detected from the sample after 24 hours of fermentation. Thus, it can be concluded that the BPA which initially with the concentration of 5 ppm has been completely degraded by *Pseudomonas aeruginosa* in the duration of 24 hours.

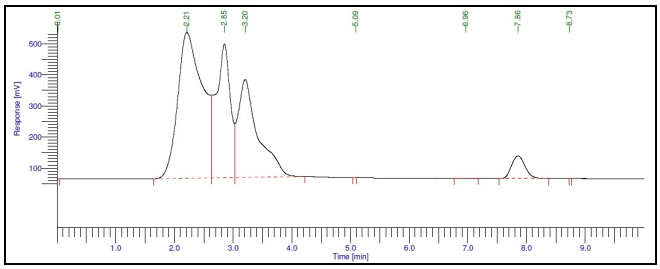

Figure 7 : BPA detected from initial sample of fermentation without scouring pads analyzed by HPLC machine.

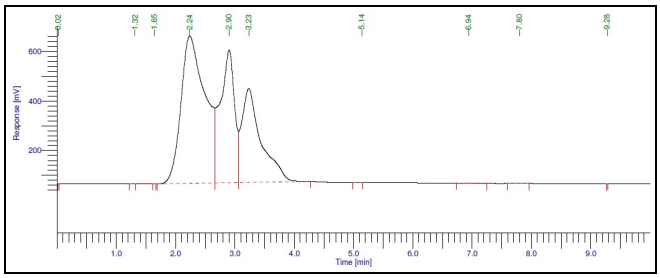

Figure 8 : BPA detected from the final sample after fermentation without scouring pads analyzed by HPL machine.

Another set of fermentation was performed by including the scouring pads in order to compare the BPA degradation of the immobilized *Pseudomonas aeruginosa* to the suspended one. The fermentation started with the same amount of initial BPA concentration which was 5 ppm. All of the samples collected from the fermentation process were analyzed by using HPLC machine.

From Figure 9 below, it can be seen that the graph for fermentation with scouring pads produced by using HPLC machine is exactly the same as the one without the scouring pads. The graph shows that the BPA was detected at the same retention time which was 7.87 minutes similar to both standard and fermentation without scouring pads. Thus, it can be concluded that the initial BPA concentration used for this fermentation was at the same level which is 5 ppm.

Figure 10 below shows the graph obtained from HPLC machine for the sample after 24 hours of fermentation of this bacteria with scouring pads included. It can be seen that the peak area obtained for BPA is smaller than the ones obtained from the initial sample. This shows that there is degradation activity that occur during the fermentation with the immobilized bacteria. However, the BPA was only partially degraded by the immobilized bacteria in 24 hours fermentation. The BPA was unable to be completely degraded is probably due to the fermentation time used for the attachment of the bacteria to scouring pads was only 24 hours. Thus, there was not much bacteria that could be immobilized on the scouring pads. However, this data still shows that the immobilized bacteria on the scouring pads were capable of degrading BPA.

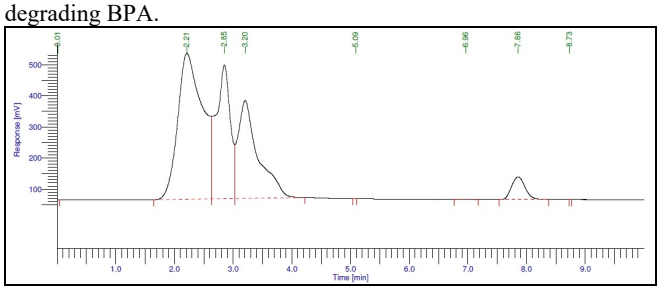

Figure 9 : BPA detected from initial sample of fermentation with scouring pads analyzed by HPLC machine.

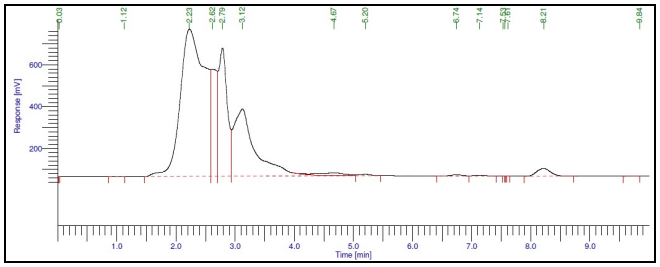

Figure 10 : BPA detected from the final sample after fermentation with scouring pads analyzed by HPL machine.

Based on the result obtained above, it can be concluded that *Pseudomonas aeruginosa* has the ability to degrade BPA. This findings correlates with the study reported by Mita *et al.* [14] in which *Pseudomonas aeruginosa* has a very versatile metabolic pathways and has the ability to degrade BPA.

D. Laccase enzyme assay

Laccase enzyme assay must be performed in order to observe and determine the BPA degradation activity. According to Nik Raikhan *et al.* (2016), one unit of enzyme activity was defined as the amount of enzyme that increased the absorbance by 0.001 units per min at 37°C; the activities were expressed in U/ml.

In order to determine the BPA degradation ability of the *Pseudomonas aeruginosa*, a sample was collected after 24 hours of fermentation of this bacteria with initial BPA concentration of 5 ppm. The sample was then centrifuged at 10000 rpm and 4°C for 20 minutes to remove the cellular debris. After that, 1 ml of the sample was mixed with 1 ml of crude laccase enzyme and 1 ml of 50 mM phosphate buffer. The mixture was allowed to react for 30 minutes before absorbance reading was taken by using UV-spectrophotometer. The absorbance reading that was obtained from the sample was 0.043 which corresponds to BPA concentration of 1.5 ppm based on the interpolation made on the standard curve. From this result, it can be seen that there is a BPA degradation activity during the 24 hours fermentation as the concentration of BPA decreased from 5 ppm to 1.5 ppm after the fermentation process.

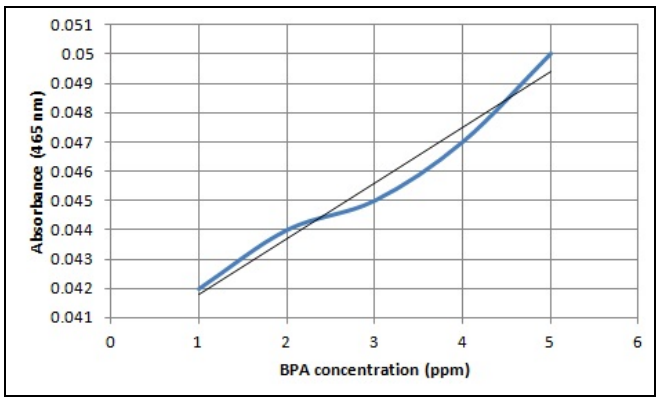

Figure 11 : A standard curve of absorbance at 465 nm against BPA concentration (ppm)

E. Scanning electron microscope (SEM) view

In this research, an alternative for the immobilization of *Pseudomonas aeruginosa* was studied. According to Palleroni [15], this bacteria is very versatile and possess the ability to utilize wide range of organic compound as carbon and energy sources through its wide metabolic pathways. This results in the bacteria being extensively studied by researchers. Therefore, an alternative to immobilized this bacteria is required for industrial and commercialization purpose. Thus, in this study the scouring pads were selected as a mean for low cost immobilization.

In order to immobilize the bacteria to the scouring pads, small pieces of 5 mm x 5 mm scouring pads were included in the shake flask for 24 hours fermentation. After the fermentation process, the

scouring pads were taken out the shake flask and viewed under Scanning Electron Microscope (SEM).

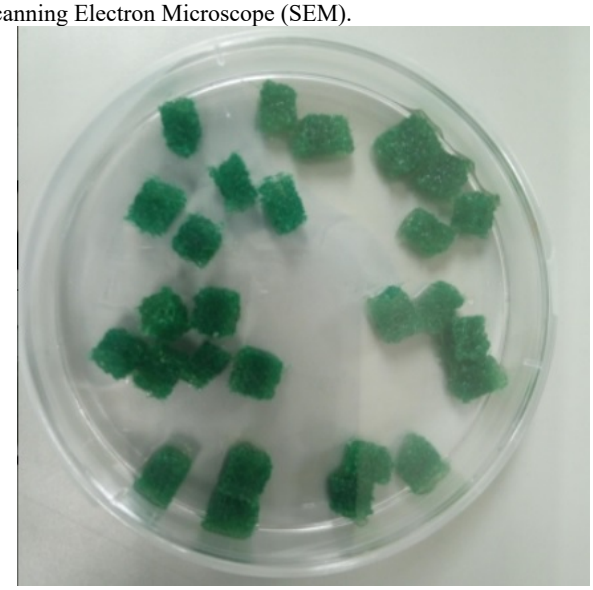

Figure 12 : The scouring pads after 24 hours fermentation.

Figure 13 below shows the image of a scouring pad under the Scanning Electron Microscope view. This image is at 500x magnification where the fibers of the scouring pad can be clearly seen. This image shows the scouring pad fibers without any attachment of bacteria and serve as a control to compare with the ones with bacteria attachment.

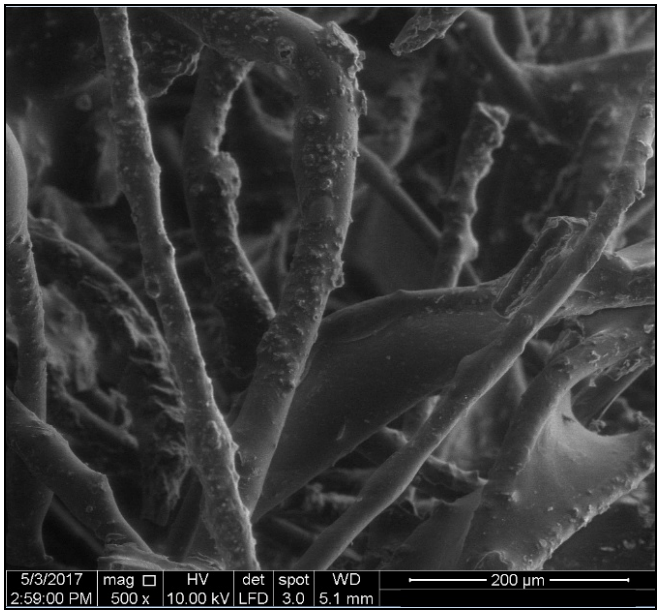

Figure 13 : The image of scouring pad fibers without attachment of bacteria as control under Scanning Electron Microscope view.

After the fermentation process, the bacteria was expected to attach to the fiber surface of the scouring pads. Therefore, in order to confirm the attachment of the bacteria to the scouring pad, the samples of scouring pad obtained after the fermentation process were viewed under the Scanning Electron Microscope and compared with the control. Based on the Figure 14 below, this image was viewed at 250x magnification and it can be seen that the scouring pad fibers were covered by bacteria on the surface of the fibers. In order to confirm the attachment of the bacteria to the surface, a higher magnification is required to obtained a closer look at the attachment of the bacteria. From the figure, it can be seen that there are some surface of fibers that are clear from the attachment of bacteria. This shows that there are more rooms for attachment of bacteria and it is expected that it can be done by increasing the fermentation duration longer than 24 hours to allow more attachment of the bacteria to the scouring pad fibers.

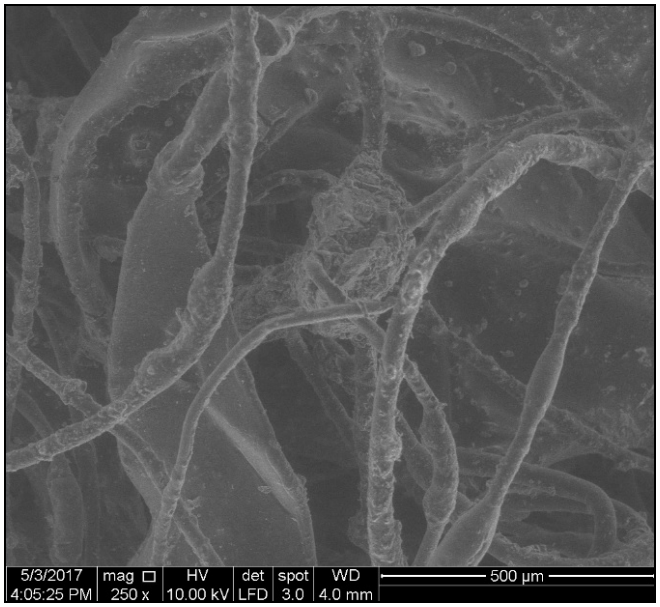

Figure 14 : The image of scouring pad fibers that went through 24hours of fermentation prior to Scanning Electron Microscope viewing.

The image in the Figure 15 was obtained by using 10000x magnification of the Scanning Electron Microscope. It can be clearly seen that the bacteria cells attached to the surface of the scouring pad fibers. From the figure, the bacteria cells were observed to be attached to glue-like substance which acts as the medium of attachment to the surface of the scouring pads. This glue-like substance was identified as biocolloids which was an unexpected finding in this study. The result obtained in this study can be seen similar to the study reported by van Gennip *et al.* [16] in Figure 16 which validates the findings in this study.

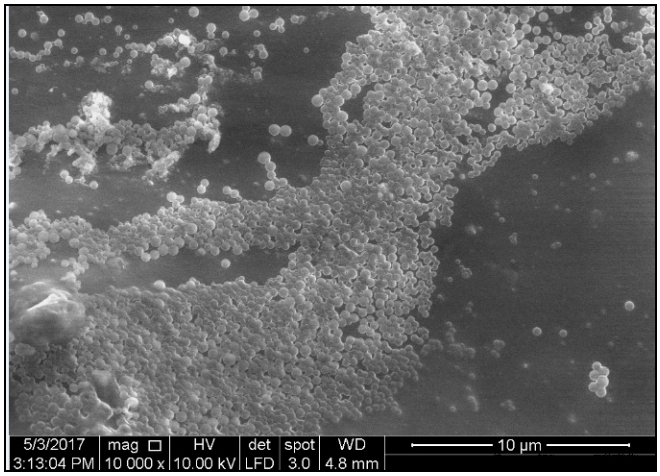

Figure 15 : The bacteria attachment n the surface of scouring pad fibers under Scanning Electron Microscope view.

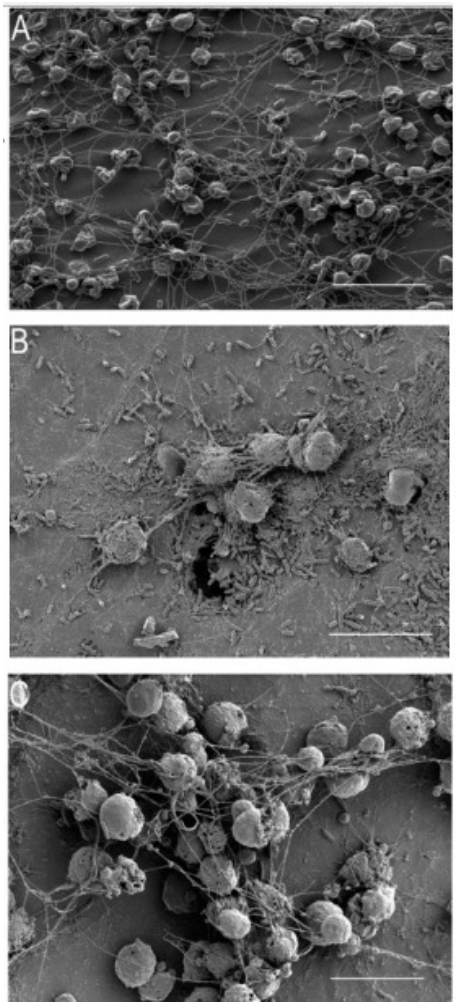

Figure 16 : An image of *Pseudomonas aeruginosa* attached to an implant. (van Gennip *et al.*, 2012)

IV. Conclusion

In conclusion, the objective of this study has been successfully achieved. From the data obtained in the study, it can be seen that *Pseudomonas aeruginosa* possess the ability to degrade BPA whether in suspension or immobilized fermentation. The low cost alternative to immobilize the bacteria shows promising result in which the bacteria has been successfully attached to the surface of the scouring pad fibers in 24 hours of fermentation. The Scanning Electron Microscope view of the attachment validates the viability of the bacteria immobilization by using scouring pads. Other than that, the bacteria was able to degrade BPA efficiently at low concentration of BPA in short amount of time. Both of the immobilization and the efficiency of BPA degradation by immobilized bacteria can be further improved by increasing the fermentation time for the attachment purpose.

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