Removal of Endocrine Disruptor Chemical (EDC) from Wastewater with Aided Enzyme Catalysis

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***Abstract* – The occurrence of endocrine disruptor chemicals (EDCs) in water bodies have gain the worldwide attention nowadays. Bisphenol-A that have the significant use in the industry have been identified as one of the EDC that may inhibit the endocrine systems of the living things. In this project, the enzyme catalysis process was studied for its feasibility to remove BPA from the water and also the optimum pH for the process to take places was determined. The purified enzyme from *T. versicolor* was used in this study added to the water containing fixed concentration of BPA at different pH. The reaction was left to take places at different reaction time. High-performance liquid chromatography analysis showed that the highest removal is achieved in the reaction condition of pH 7 with removal of 86% was achieved. This study also reveals that the biodegradation of BPA by Laccase enzyme is feasible for the biotransformation of wastewater in comparison with the previous study.**

Keywords* – *Bisphenol-A, Degradation, Laccase, Wastewater

I. INTRODUCTION

The occurrence of endocrine disruptor chemical (EDC) in water bodies are the major concern of the people around the world today. The industrial wastewater for instance the plastic industry were discharged with certain significant concentration of EDC particularly Bisphenol-A (BPA) that endanger the aquatic life particularly fishes where it cause the gender shift and infertility. In addition, the interference of EDC with the water cycle brings the implication to the human endocrine systems which could lead to human being disorders such as cancer and infertility (Komesli *et.al.,* 2015).

Bisphenol-A (BPA) is heavily used as a plastic monomer and plasticizers in the production of polycarbonate and epoxy products (Lin *et.al,* 2016) and also as a non-polymer additive in plastics such as polyvinyl chloride (PVC) and water pipes (Santhi *et.al,* 2012). Unfortunately, the US Environmental Protection Agency (EPA) and the World Wide Fund for Nature (WWF) has recognized BPA as an Endocrine Disruptor Chemical (EDC) that has been identified to have the ability to interfere with the body's endocrine system which eventually would cause severe health effect such as infertility (Daâssi *et.al,* 2016).

Therefore, the degradation of BPA has been widely studied recently. Various method have been used to degrade the BPA from the water bodies for instance, adsorption by adsorbents (Bhatnagar & Anastopolous, 2017), activated sludge, membrane bioreactor and also anaerobic digester (Komesli *et.al.,* 2015). However, the methods stated above have some drawbacks such as the current physicochemical or biological treatment process still cannot achieve a complete removal of EDCs from the wastewater. Whereas, other oxidation processes such as UV oxidation however, could lead to a higher toxic yield (Lin *et.al.,* 2016).

Recently, bioremediation of wastewater by using enzyme catalysis have gain public attention due to its capabilities in oxidizing, polymerizing and also transforming various substrate such as phenolic and anthropogenic compound to less toxic derivatives (Spina *et.al,* 2015; Majeau *et.al,* 2010). Laccases are a type of lignolytic enzymes that have an exceptional biochemical and catalytic properties can be considered as one of good green biocatalysts. It is used in various applications such as water treatment (Chaterine *et.al,* 2016). Utilization of enzymes such as Laccase would enhance the bioremediation of wastewater. Enzymatic reactions particularly, requires the present of substrate which the substrate for Laccase enzyme would be diverse. For instance, it would be phenols, dyes, polycyclic aromatic hydrocarbons, pesticides and also EDCs that can be oxidized either by extracellular fungal or bacterial Laccase.

In this study, focus was given to evaluate the feasibility of removing EDC of interest which is BPA from the wastewater by using Laccase and to determine the optimum pH for the enzymatic catalysis reaction.

II. MATERIALS AND METHODS

A. Materials

Bisphenol-A (97%) and Laccase enzyme (0.5 U/mg) purified from *T. versicolor* were purchased from Sigma-Aldrich Chemicals. All other chemicals used in this study were of analytical grade.

B. Enzymatic BPA degradation

The reaction mixture containing the mixture of Laccase and BPA solution was prepared. BPA stock solution was prepared by diluting BPA powder with the methanol to concentration of 1000 mg/L. The BPA stock solution then was diluted to the concentration of 1 mg/L using the phosphate buffer (pH 5, pH 7 and pH 8) to create the reaction solution. Laccase (10 U) was added to the reaction solution.

The reaction then takes place for 2 hours at the room temperature. After certain time interval, the samples were collected for analysis. The reaction was stopped by

heating the reaction mixture in the water bath for 20 minutes at 80^0C.

The control reaction mixture was performed exactly the same as the enzymatic degradation procedure by omitting the addition of enzyme into the reaction mixture.

C. Analytical procedure

After reaction was stopped, the BPA concentration was determined by High-Performance Liquid Chromatography (HPLC) TOTALCHROM V.6.2.0.0.1 system with LC instrument control (PerkinElmer Series 200). The mobile phase solution consists of water and acetonitrile (60:40). The sample injection was 20 µL and BPA in the sample were determined by UV absorption (Series 200 UV/VIS Detector) at 230 nm. Identification of the BPA was based on comparisons with the retention time of the internal standard calibration.

III. RESULT AND DISCUSSION

A. Removal of BPA by enzymatic catalysis

A quantitative analysis was performed by using HPLC to determine the removal efficiency of BPA by the Laccase enzyme. The results obtained were tabulated in Table 1. The removal efficiency of BPA by Laccase enzyme was calculated by using the formula:

$$Removal\ efficiency, \% = \frac{C_0 - C_i}{C_0} \times 100\%$$

Where:

C_0 = Initial BPA concentration
C_i = Final BPA concentration

Figure 1 shows the concentration of BPA by the Laccase enzyme over reaction time. From the Figure 1, the concentration of BPA is reducing for all reaction time for the reaction condition of pH5 and pH7. However, for the reaction condition of pH8, BPA shows the reduction in concentration for 0.5 hours of reaction. Unfortunately, for the reaction time of 1 and 2 hour there is no further significant reduction of BPA occurs.

BPA was degraded by 86% within 2 hours of reaction at the pH 7, while in other reaction mixture degradation of BPA was 83% and 41% for pH 5 and pH 8 respectively as shown in Fig. 2. Laccase was able to remove BPA from the water since the concentration of the BPA reduced with the incubation duration. A study conducted by Gassara *et.al,* (2013) on the BPA degradation in water by lignolytic enzyme shows that BPA was able to be degraded by that enzyme. Laccase enzyme that is used in this study is a type of ligninolytic enzymes that is produced by the fungus *T. versicolor.* Theoretically, Laccase is able to oxidize all substrate that have the phenolic structure (Catherine *et.al,* 2016). BPA is an essential industrial chemical with two phenol functional group (Chhaya & Gupte, 2013).

The BPA concentration after certain reaction time (0.5, 1, 2 hours) is shown in Figure. 1. From the figure, it can be seen that for reaction that take places in pH 5 and pH 7 the concentration of BPA is reduced in pH 7 conditions and slightly lower reduction occurs in the reaction condition of pH 5.

Table 1: The concentration of BPA and removal efficiency after reaction time (h)

pH	Reaction time, (h)	Concentration of BPA, (mg/L)		Removal efficiency, (%)
		Initial	Final	
pH 5	0.5	1	0.56	44
	1	1	0.42	58
	2	1	0.17	83
pH 7	0.5	1	0.31	69
	1	1	0.29	71
	2	1	0.14	86
pH 8	0.5	1	0.61	39
	1	1	0.59	41
	2	1	0.59	41

A study conducted by Daâssi *et.al,* (2016), shows that BPA is able to be degraded by Laccase enzyme in pH 5 reaction mixtures with the efficiency of 85% after 2 hours of reaction. The chromatography peak obtained from the HPLC analysis indicates that BPA peak at 4.5-5.5 minutes eventually disappeared after the enzymatic treatment with Laccase enzyme for the reaction time.

As for pH 7 reaction mixtures, the BPA concentration reduced significantly after 2 hours of reaction. Nguyen *et.al,* (2014), conducted a study on BPA degradation by Laccase enzyme after incubation for 4 hours in various pH values. From the study, it is found that the maximum BPA degradation (96%) is achieved in pH 7. A complete removal of BPA may be achieved in pH ranging from 5-7, with lower reduction may occurs in acidic condition (Nguyen *et.al,* 2014).

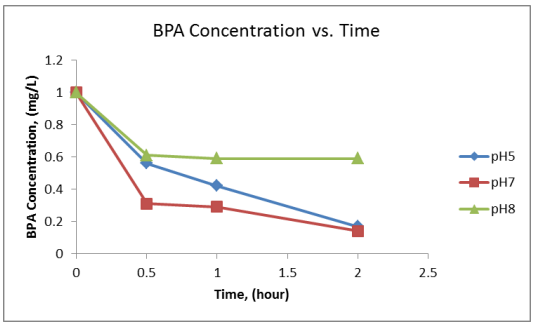

Figure 1: BPA concentration against the reaction time

However, as for pH 8 reaction condition, after 0.5 hours of reaction, the BPA is able to be degraded up to 39%. Unfortunately, there is no reduction of BPA

concentration (Figure. 2) after the reaction take places for 1 and 2 hours. Study from Nguyen *et.al,* (2014) stated that, after 2 hours of incubation, alkaline condition may contribute to the inactivation of enzyme which eventually would cause no further removal of BPA in this study after 1 hour of incubation. In addition, study from Garcia-Morales *et al,* (2015).was performed at pH 5 to degrade the micropollutants since Laccase retain a very high catalytic activity at this condition.

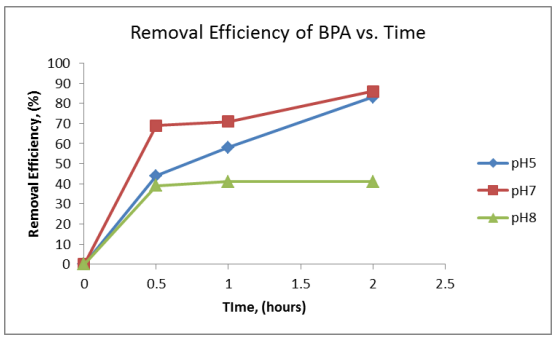

Figure 2: Removal efficiency of BPA against reaction time

A complete degradation of BPA in the water samples would be achieved if the reaction time is prolonged for pH 5 and pH 7 reaction conditions. The addition of mediator or implying the immobilization method should also enhance the degradation of BPA from the water sample. The detection of the degradation compound also can be done by using the mass spectroscopy.

IV. CONCLUSION

Degradation of BPA was successfully achieved by the Laccase enzyme aided catalysis with the highest removal of 86% at pH7 after 2 hours of reaction time. This study also reveals that the biodegradation of BPA by Laccase enzyme is feasible for the biotransformation of wastewater in comparison with the previous study.

ACKNOWLEDGEMENT

The author gratefully acknowledge Universiti Teknologi MARA for the financial support and all the facilities used throughout this research

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