한국환경위생학회지, 제30권 제2호(2004)
Kor. J. Env. Hlth., Vol. 30, No. 2, pp 79~83(2004)

Reaction Conditions for Laccase Catalyzed Degradation of Bisphenol A

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(Received February 2, 2004; Accepted April 25, 2004)

ABSTRACT

The oxidative degradation of BPA with laccase from *Trametes versiclor* was conducted in a closed, temperature controlled system containing acetate buffer for pH control. The effects of medium pH, buffer concentration, temperature and mediator on degradation of BPA were investigated. The inactivation of the enzyme by temperature and reaction product was also studied. The optimal pH for BPA degradation showed about 5. Buffer concentration did not affect BPA degradation. On the other hand, the enzyme stability was higher at low concentration buffer (25 mM). Temperature rise increased the degradation rate of BPA up to 45°C. The valuable mediator of laccase for BPA was ABTS. Elevated temperature and reaction product irreversibly inactivated the enzyme.

Keywords: bishenol A, 2,2-bis(4-hydroxyphenyl)propane, endocrine disrupting chemical, laccase, *Trametes versicolor*

I. Introduction

BPA (2,2-bis(4-hyroxyphenyl)propane) is widely used as a material for the synthesis of polymers including polycarbonate, epoxy resins, phenol resins, polyester and polyacrylates. BPA has been demonstrated to have endocrine disrupting effects with estrogenic action.[1,2] Animal experiments have proved that BPA is capable of modulating or disrupting the endocrine system in both wildlife and human. This compound has induced proliferation, alternation and *c-fos* gene expression in the female reproductive tract.[3] BPA has brought about development of testicular and prostate cancer, reduction in sperm counts in humans, demasculinization, feminization, alteration of immune functions and decreased fertility in birds, fish and mammals.[4] Therefore, the release of BPA into the environment is of great concern on account of its toxicity.

It has been demonstrated that laccase, manganese peroxidase, and/or lignin peroxidase produced by lignin-degrading fungi are involved in the degradation of pollutants such as chlorophenols and nitrobenzene.[5,6] BPA is a phenolic compound that is good substrate for the above lignolytic enzymes. Laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) is a blue multi-copper containg enzyme that catalyzes one-electron oxidations of a variety of organic substrates coupled the reduction of molecular oxygen to water. This enzyme, with dissolved molecular oxygen for its catalytic activity, may be more advantageous than peroxidases that use hydrogen peroxide for their activities.[7,8]

The objective of the work is to determine the effect of medium pH, buffer concentration, temperature and mediator on laccase catalyzed degradation of BPA and the inactivation of enzyme by temperature and reaction product in terms of BPA degradation rate. This study also aims at development of the preliminary enzymatic method of BPA degradation for industrial application.

II. Materials and Methods

1. Chemicals and enzyme

Bisphenol A was purchased from TCI (Tokyo Chemical Industry, USA). Mediators and laccase (*Trametes versicolor*) were purchased from Sigma-aldrich (Canada). Other chemicals, obtained from

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various commercial suppliers, were of analytical grade purity.

2. Enzymatic assay

Laccase activity was determined by oxidation of ABTS (2,2'-azinobis-(3-ethyl benzthiazoine-6-sulfonic acid). The reaction mixture consisting of 2.5 mM ABTS, 100 mM citrate/phosphate buffer (pH 4.5) and a suitable amount of enzyme in a total volume of 1 m*l* was incubated at 25°C. Oxidation of ABTS was followed by absorbance increase at 420 nm (ε_{420}=3.67 × $10^{-4}M^{-1}cm^{-1}$).[9] One unit of enzyme activity was defined as the amount of enzyme oxidizing 1 μM of ABTS per min.

3. Analytical method

Samples of the reaction mixture were withdrawn and a small amount of concentrated acetic acid was added to stop enzymatic reaction. The acidified samples were filtered with 0.45 μm filter (Millipore, Canada) to remove insoluble products. BPA concentration in the filtrate was assayed by HPLC (Agilent, USA), which was carried out using a ZORBAX SB-C18 column (Agilent, USA) and detected at 277 nm with isocratic elution at 1.0 m*l*/min with a mobile phase composed of water and acetonitrile (40:60; v:v).

4. Bach reactions

BPA (100 μM final concentration) was incubated at 25°C for 1 h in 25 mM acetate buffer (pH 5.0) using laccase (0.15 U/m*l* final activity). This reaction was conducted in a 20 m*l* batch reactor. The medium was mixed with a magnetic stirrer at an approximate rpm during reaction. Before the reaction was started, bisphenol A and oxygen were dissolved in a buffer by vigorously shaking.

The pH value of the medium was varied from 3 to 8 by using universal buffers at low concentrations (pH 3-5; acetate buffer, 25 mM, pH 6-8; phosphate buffer, 25 mM) with 100 μM bisphenol A. The conditions included 25°C and 0.15 U/m*l* laccase activity.

For the assessment of buffer concentration effect, buffer concentration was varied from 25 to 100 mM. This reaction was conducted at constant temperature, 25°C and pH 5.0 in acetate buffer reaction medium using 0.15 U/m*l* laccase activity.

The laccase-catalyzed reaction was repeated at seven temperatures of 25, 30, 35, 40, 45, 50 and 55°C in acetate buffer (25 mM, pH 5.0) medium. Constant 100 μM bisphenol A was used each time as the substrate and laccase (0.15 U/m*l*) was added to start the reaction.

The effect of reaction product was conducted at a constant initial bisphenol A concentration of 100 μM, temperature 25°C and pH 5.0 in 25 mM acetate buffer reaction medium. This experiment was primarily intended for the inactivation by reaction product relative to laccase activity.

The effect of mediators in 25 mM acetate buffer was evaluated as the same concentration as fixed initial concentrations of 100 μM bisphenol A. The experiments were at 25°C, pH 5.0 and with 0.15 U/m*l* laccase activity.

III. Results and Discussion

1. Effect of medium pH

The optimum pH value is about 5 for the oxidative degradation of BPA from the curve of degradation rates versus pH (Fig. 1). This optimum pH value is a little above the reported isoelectric point of laccase.[10] Since the degradation of BPA is highly inhibited at alkaline condition, the enzyme may function optimally with a negative net charge.

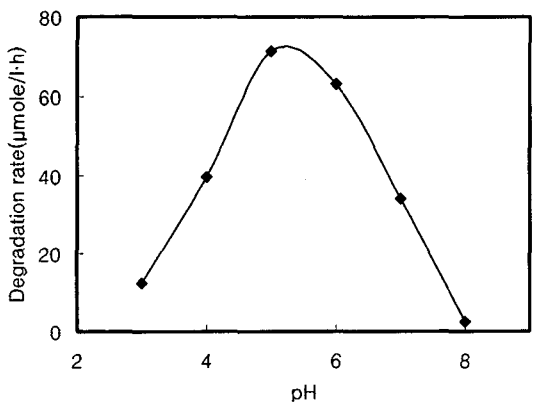

Fig. 1. Effect of pH on degradation of bisphenol A. The used buffers were 25 mM acetate buffer (pH 3-5) and 25 mM phosphate buffer (pH 6-8). Reaction conditions : 25°C, 100 μM BPA and 0.15 U/m*l* laccase.

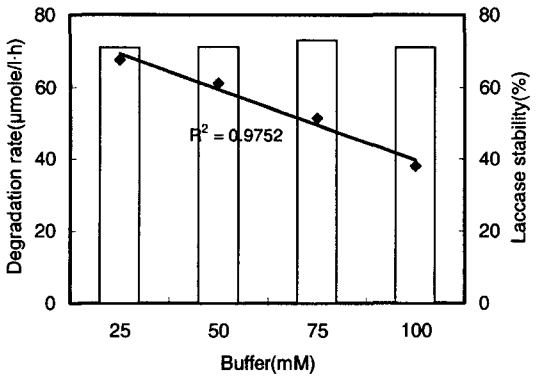

Fig. 2. Effect of buffer concentration on degradation of bisphenol A (□) and laccase stability (◆). Reaction conditions : 25°C, 100 μM BPA and 0.15 U/ml laccase.

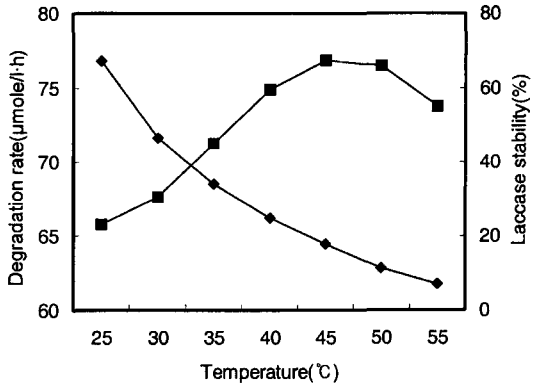

Fig. 3. Effect of temperature on BPA degradation (■) and laccase stability (◆). Reaction conditions : 25 mM acetate buffer (pH5), 100 μM BPA and 0.15 U/ml laccase.

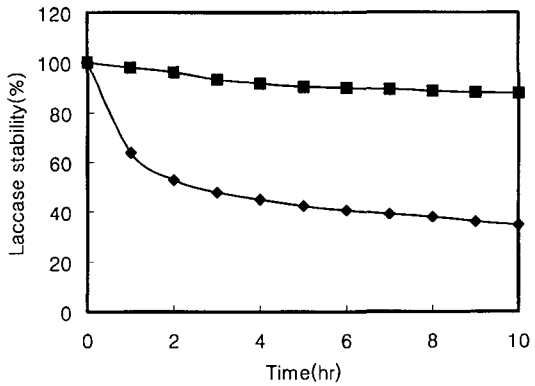

Fig. 4. Effect of reaction product on Laccase stability with BPA (◆) and without BPA (■). Reaction conditions : 25°C, 25 mM acetate buffer and 0.15 U/ml laccase.

2. Effect of buffer concentration

The effect of buffer concentration on enzyme stability and BPA degradation was investigated at various buffer concentrations (Fig. 2). The data indicate that the buffer concentration does not affect BPA degradation ($p>0.05$). The enzyme is more stable at low buffer concentration than at high buffer concentration ($p<0.05$). It has been reported that buffer concentrations for BPA degradation vary in 20-100 mM according to the enzyme source.[11-13] This result suggests that laccase from *Trametes versicolor* may be stable at low buffer concentration. This hypothesis should need further studies.

3. Effect of temperature

The degradation rate versus reaction temperature is plotted in Fig. 3. The data show that the degradation rate increases with temperature up to 45°C, after which the rate is decreasing. The decrease at elevated temperature was attributed to drops of in dissolved oxygen concentration and enzymatic stability.[14] Furthermore, higher temperature adversely affects the enzyme stability. This result suggests that the reaction temperature may be a crucial factor for practical application.

4. Effect of reaction product

BPA was reported to be oxidatively degraded to phenol, 4-isopropenylphenol, 4-isopropylphenol and hexestrol by manganese peroxidase from *P. ostreatus*[15] and to 4-isopropenylphenol and 5,5'-bis-[1-(4-hydroxy-phenyl)-1-methyl-ethyl]-biphenyl-2,2'-diol by laccase from *T. villosa*.[12,16]

From the results of this investigation, the enzyme activity decrease during BPA oxidation was associated with enzyme inactivation by irreversible reactions between the enzyme and reaction products (Fig. 4). Bisphenolic radicals formed by oxidation of BPA may act as inhibiting agent. The above inactivation of oxidoreductase is a common phenomena during the oxidation of phenolic substances.[17,18] Therefore, it is necessary to study on the conservation of enzyme activity and the mechanism of reaction products during BPA degradation.

Table 1. Effect of mediators on BPA degradation

Mediator	Concentration (μM)	Degradation rate (mole/.h)
Control	-	71
HBT (1-Hyroxy-benzotriazole)	100	67
VLA (Violuric acid)	100	89
TEMPO (Tetramethoxypiperidine-N-Oxyl)	100	64
ABTS (2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)	100	115
Syringic acid	100	75

Reaction conditions : 25℃, 25 mM acetate buffer (pH5.0), 100 μM BPA and 0.15 U/m*l* laccase.

5. Effect of mediators

The performance of the laccase/ABST system appears higher than any other laccase/mediator system tested (Table 1). The data show that an electron-transport route of laccase/ABTS system is superior to an ionic oxidation route of laccase/TEMPO system or laccase/HBT system in BPA oxidative degradation. Addition of mediators such as HBT, TEMPO and syringic acid to the reaction gave no acceleration effects on enzyme activity, although these chemicals were efficient as the electron transfer in other compounds.[19] The laccase/ABTS system following an electron-transport of oxidation, a non-N-OH mediator, is valuable for the degradation of BPA.[20] But this laccase/ABTS system could not be directly applied for industry since purple color was developed during reaction in the medium.

IV. Conclusion

Oxidative degradation of BPA was catalyzed by laccase from *Trametes versiclor* in acetate buffer in a closed system at controlled temperature and pH. The optimum pH for the enzymatic oxidative degradation of BPA was around 5. Oxidative BPA degradation with laccase could not be carried out under alkaline condition. Buffer concentration did not affect BPA degradation. However, the enzyme activity was more stable at low buffer concentration than at high buffer concentration. The reaction temperature increased the rate of degradation of BPA but it decreased the enzyme stability. The optimal reaction temperature was found to be about 45℃. During BPA degradation, the laccase was inactivated. The increase of reaction yield by reduction laccase inactivation should be performed by water-soluble polymer such as polyethylene glycol. The laccase/ABST system exceedingly enhanced the oxidative degradation of BPA. The use of mediators in practice should need the detailed investigation because most of mediators are decomposed during the reaction causing deactivation of the enzyme and develop their costs and regeneration problems.

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