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Kinetics of veratryl alcohol oxidation by lignin peroxidase and in-situ generated H_2O_2 in an electrochemical reactor

이기범, 구만복, 문승현*
광주과학기술원 환경공학과
*TEL (062) 970-2435, FAX (062)-970-2434, shmoon@kjist.ac.kr

Abstract

An electroenzymatic system to oxidize veratryl alcohol on electrodes with in-situ generated hydrogen peroxide was studied. We investigated hydrogen peroxide generation, current efficiency, and veratryl alcohol oxidation in the electrode system at various conditions. The reaction rates of veratryl alcohol oxidation were compared in an electrochemical, an electroenzymatic, and an usual biochemical systems to prove the concept of electroenzymatic oxidation.

Introduction

The lignin peroxidase of the white rot fungus, *Phanerochaete chrysosporium*, has broad substrate specificity and catalyzes the oxidation of a wide range of structurally diverse organic pollutants with H_2O_2. In the system of biochemical oxidation, it is desirable to have convenient procedures for generating H_2O_2 because of loss of oxidant purity, and safety during storage and transportation. The growing appreciation of an indirect electrochemical procedure has been attempted, in which lignin peroxidase and electricity were used. In this work, we used the H_2O_2 in lignin peroxidase activity for oxidation of veratryl alcohol. The enzymatic oxidation of the veratryl alcohol with in-situ generated H_2O_2 were conducted as shown in Fig. 1.

Experimental methods

Phanerochaete chrysosporium (ATCC 24725) was cultured in the same manner as reported by Tien and Kirk[1]. *Phanerochaete chrysosporium* spores were immobilized in polyurethane foam and used for the production of lignin peroxidase in repeated batch cultures using carbon-limited medium. Experimental runs using veratryl alcohol were carried out under various sets of operating conditions. The reactor used in this study was divided into an anode and a cathode compartment (each volume: 30cm^3) with a cation-exchange membrane. Two 4 x 2 cm conductive titanium with platinum coating and reticulated vitreous carbon plates were used as a counter and a working electrode. The potential of the working electrode

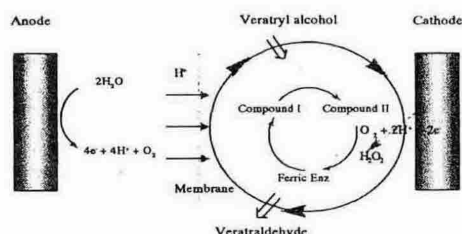

Fig.1 A scheme of direct electrochemical generation of H_2O_2 combined with enzyme reaction.

was measured against a saturated Ag/AgCl reference electrode. Ten milimolar HCl and 0.1 M sodium tartrate at pH 3.0 were used as the anolyte and catholyte, respectively. Pure O_2 gas was sparged into the catholyte. Electrolysis was carried out over a period of 60 min at a constant potential during batch experiments by using a potentiostat (263A EG&G Co.). The experiments of cyclic voltammetry and controlled potential electrolysis were carried out in 0.1 M sodium tartrate. Electrochemical oxidation of veratryl alcohol was studied at a desirable voltage in the absence of enzyme. In the electroenzymatic oxidation, the electrolysis was performed at +0.1 V with a Ag/AgCl reference electrode. Lignin peroxidase, and veratryl alcohol were added to the catholyte. Biochemical oxidation was also performed under the lignin peroxidase, veratryl alcohol, and 0.2 mM H_2O_2. During the experiments, samples were taken periodically to measure veratraldehyde. H_2O_2 was analyzed colorimetrically by the method reported by Zhen-Yue *et al*[2].

Results and discussion

Cyclic voltammetric studies. Figure 2 shows typical cyclic voltammograms obtained for the electroenzymatic and biochemical oxidations with 0.2 mM H_2O_2 in 0.2 mM veratryl alcohol, 100 U/L lignin peroxidase in 100 mM sodium tartrate buffer system at pH 3.0. The scan rate was 20 mV/s. The slightly positive potential must be necessary for oxidizing the veratryl alcohol. The results implied that the biochemical oxidation of veratryl alcohol is similar to that obtained in electroenzymatic oxidation.

The effects of operating parameters by electroenzymatic reaction. The effect of lingin peroxidase dosage was observed based on the initial reaction rate for veratraldehyde formation in the range from 50 to 300 U/L. Positive 0.1 volt with 2 mM veratryl alcohol was applied in this experiment (Figure 3). The effect of pH on the reaction rate was studied at different pH values from 2.5 to 5.0. Positive 0.1 volt with 2 mM veratryl alcohol, and 100 U/L lignin peroxidase was applied in this experiment. From Figure 4, it was found that the highest reaction rate occurred at pH 3.0. The optimum pH was consistent with that of H_2O_2-driven method, indicating that similar enzymatic function is involved in the electroenzymatic method.

Enzyme activity. Lignin peroxidase experiments with electrogenerated H_2O_2 were performed. The electrolyte solution consisted of 100 U/L lignin peroxidase, 0.1 M sodium tartrate buffer (pH 3.0) and 2 mM veratryl alcohol. Experiments were run for 60 min. Samples were taken at 10 to 20 min intervals for veratraldehyde and peroxide analysis. The results show a constant rate of veratraldehyde formation and the H_2O_2 concentration stabilized to a slower rate of increase after an initial rapid rise as shown in Figure 5.

Comparison of H_2O_2-driven, electrolytic and electroenzymatic oxidations. The conversions of veratryl alcohol into veratraldehyde under different conditions were compared : (1) electrolysis and oxygen supply, (2) electrolysis, enzyme and oxygen supply, (3) no electrolysis, enzyme and H_2O_2 supply. Reaction products with 0.2 mM H_2O_2 used for the oxidation of veratryl alcohol show a rapid increase. Even though the electrochemical enzyme did not give the same oxidation rate compared to that with H_2O_2, about 45 % of the oxidation rate was achieved under an electrical field of +0.1 V without H_2O_2 (Figure 6). Since the initial H_2O_2 concentration in the electrochemical reactor was not high enough to initiate the enzyme reaction, the electroenzymatic method can be further enhanced by improving the control scheme for the H_2O_2 generation.

Conclusions

From the experimental results following conclusions were drawn. The optimum conditions for the production of H_2O_2 were +0.1 V, 25℃, 1000 rpm, and oxygen sparging rate 25 ml/min. A comparison of the biochemical and electroenzymatic oxidations of veratryl alcohol indicated that the two oxidation processes followed a similar mechanism. The electroenzymatic concept for oxidation of veratryl alcohol was found to be feasible for replacement of the conventional enzymatic oxidation with H_2O_2 supplied externally.

Reference

1. Tien, M. and Kirk, T. K. Methods Enzymol. 161 (1988) 238-249.
2. Zhen-Yue Jiang., Hunt, J. V. and Wolf, S. P. Analytical Biochemistry. 202 (1992) 384-389.

Acknowledgement

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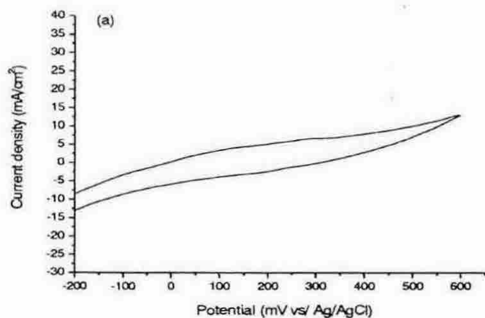

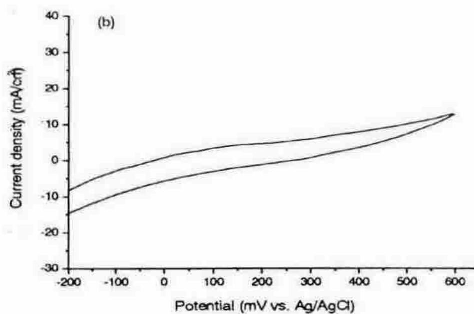

Fig.2. Cyclic voltammogram for oxidation of 0.2 mM veratryl alcohol in 0.1 M sodium tartrate on a RVC electrode at a voltage scan rate 20 mV/s. (a) Electroenzymatic oxidation (b) Biochemical oxidation.

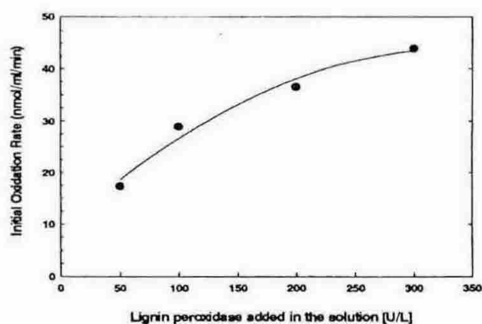

Fig.3. Effect of enzyme dosage on the initial veratraldehyde formation.

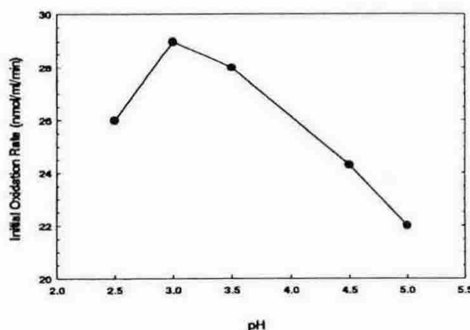

Fig.4. Effect of pH on the initial veratraldehyde formation.

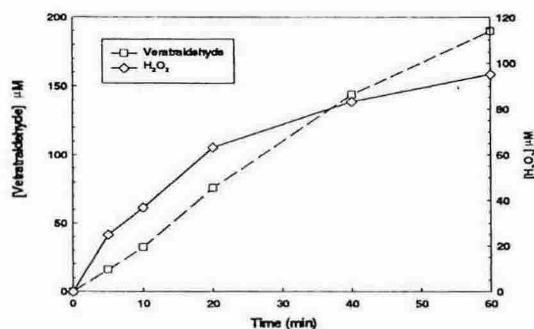

Fig.5. Concentrations of veratraldehyde and peroxide during oxidation.

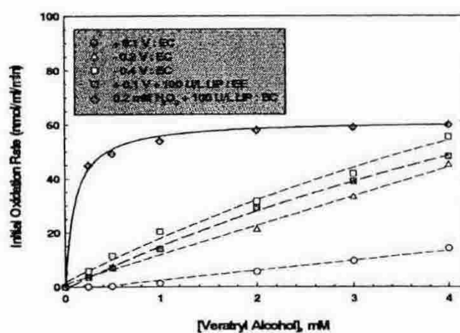

Fig.6. Initial oxidation rates of veratryl alcohol at different procedures.