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Effects of Metal Ions on Laccase Activity

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The effects of five metal ions (Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}) on 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) oxidation catalyzed by laccase were studied by spectrophotometer. The results show that Fe^{2+} ion has obvious effect on the activity and the nature of inhibition is competitive type. It is found that the inhibition is realized through the reduction of 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) by Fe^{2+} ion. Other metal ions have slight influence on laccase activity.

Key Words: Metal ions, Laccase, Activity.

INTRODUCTION

With the development of biotechnology, more and more attention is paid on the biobleaching in pulp and paper industry. There are two kinds enzymes used in pulp bleaching: hemicellulase and ligninase. Among these biotechnologies, laccase mediator system (LMS) biobleaching is the most potential technique to put into practice[1-8]. Laccase activity is the key factor of laccase mediator system biobleaching. There are certen content of metal ions in pulps. The effects of five metal ions (Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}) were studied on 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) oxidation catalyzed by laccase in this paper.

EXPERIMENTAL

Laccase produced by submerged fermentation of a genetically modified *Aspergillus* was supplied by Novozymes (China) Corporation; ABTS (Sigma Corp.); CH_3COOH/CH_3COONa buffer. UV-2201 Visible Spectrophotometer.

Laccase activity assay: Laccase activity was measured by spectrophotometer. Add 1 mL of laccase (diluted 5×10^6 times), 1 mL buffer and metal ions into the colorimeter tube. Then add 1 mL (0.5 mmol L^{-1}) of ABTS to start the reaction and begin to record the time. Record the absorbency every minute at 420 nm. Definition of the unit of laccase activity (U) is the quantity of the laccase, which can oxide 1 μmol of ABTS within 1 min ($\varepsilon = 3.6 \times 10^4\ cm^{-1}M^{-1}$)[9].

Effect of Fe^{2+} on absorptive spectra of laccase: Absorptive spectra of lacccase from 800 to 290 nm was recorded and the values of absorption at 614 and 330 nm were compared control sample with Fe^{2+} addition.

Influence on Fe^{2+} addition time on activity: Add 1 mL laccase and 1 mL buffer into the colorimeter tube. Then add 1 mL (0.5 mmol L^{-1}) of ABTS to start the reaction and record the absorbency by time course. After 1 min, add a certain amount of Fe^{2+} into the vessel and observe the change of the absorptive spectra.

RESULTS AND DISCUSSION

Effect of pH value: Normally, every enzyme has its optional pH range, because enzymes are composed of some acidic or alkaline groups. These groups have different ionization conditions at different pH value. The activity of laccase at different pH is shown in Fig.1. The results indicate that pH has a great effect on activity of laccase. When pH value is 2, activity is nearly 120 U/mL. While pH value is 6.5, laccase almost lose activity.

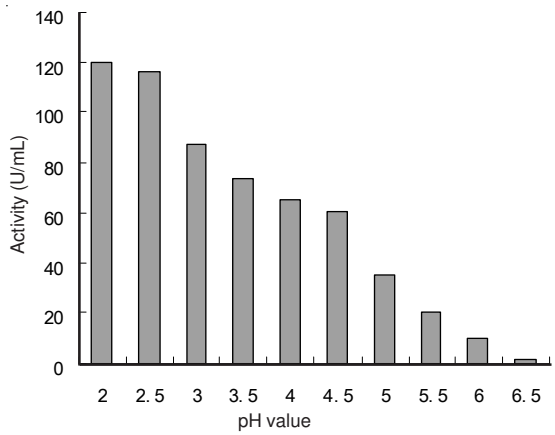

Fig. 1. Laccase activity at different pH value

Effect of metal ions: The effects of metal ions on the laccase activity were studied at pH 4.5. This pH value is common used in biobleaching. Most of the mental ions concentration in pulp is about 5-10 mg/L. So five species of metal ions (Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}) with a concentration of 10 mg/L were used in the study.

As shown in Fig. 2, Fe^{2+} has obvious inhibition on laccase activity. Ca^{2+} has a slight effect on the activity, but other ions almost have no impact on it. So we studied the effect of Fe^{2+} in detail.

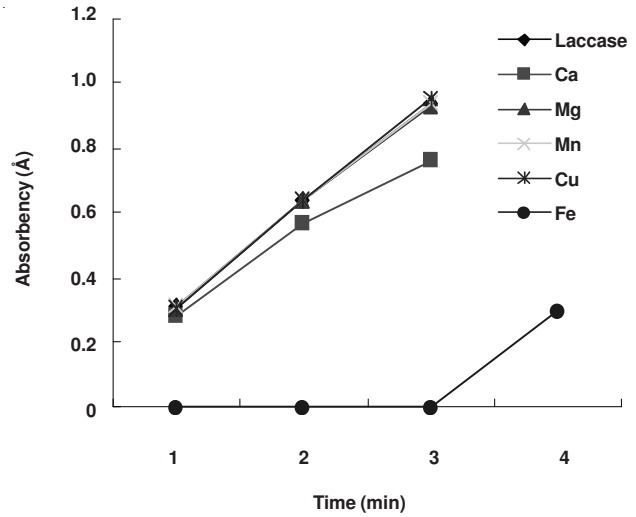

Fig. 2. Effect of metal ions on activity of laccase (absorbency at 420 nm)

Effect of Fe^{2+}: The effect of Fe^{2+} on laccase activity at different concentration with the time was shown in Fig. 3. It was revealed that Fe^{2+} could make the absorbency become zero under 420 nm spectrum at the Fe^{2+} concentration from 1 to 10 mg/L. In addition, it was also found that the inhibition of Fe^{2+} was temporary. After a period of time laccase resumed its activity and oxidized the substrate again. At the same time, the more Fe^{2+} added the longer time needed to resume. So the inhibition is competitive type. Fox example, when Fe^{2+} concentration was 5 mg/L Fe^{2+} behaved inhibition before 230 s, after 230 s the activity of laccase regained and had the same efficiency as the laccase without Fe^{2+} addition (indicated by the slop as shown in Fig. 3).

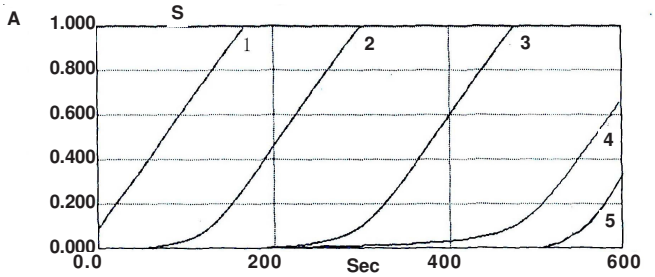

Fig. 3. Effect of Fe^{2+} on the activity of laccase (Fe^{2+} concentration: 1-0; 2-1; 3-3; 4-5; 5-10 mg/L)

Effect of Fe^{2+} on the active part of laccase: As shown in Fig. 4, laccase have the absorptive peaks at 614 and 330 nm. 614 nm is the absorptive spectrum of Cu(I), 330 nm is Cu(III). Those copper ions are the active part of the laccase[10]. The two curves (a and b) in Fig. 4 are almost overlapped. It indicates that Fe^{2+} ion has no influence on the active part of the laccase. So Fe^{2+} doesn't affect the activity of the laccase by changing the active part of the laccase.

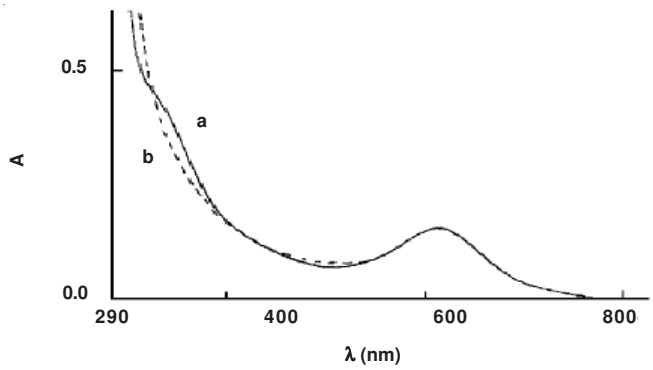

Fig. 4. With and without Fe^{2+} absorptive spectrum of laccase

Effect of Fe^{2+} addition time: An interesting phenomenon was found when Fe^{2+} was added after the enzyme catalytic reaction began. The absorbency increase with the time going at the beginning. And the colour of reaction system become blue from colourless. After 1 min, Fe^{2+} was added into the reactor (Fig. 5). The blue colour disappeared immediately andcalc absorbency become zero. But after 900 s, the colour of the reaction system turned to blue again and absorbency increased as fast as that without the Fe^{2+} addition, which indicated that the catalytic reaction resumed and the inhibition of Fe^{2+} stopped.

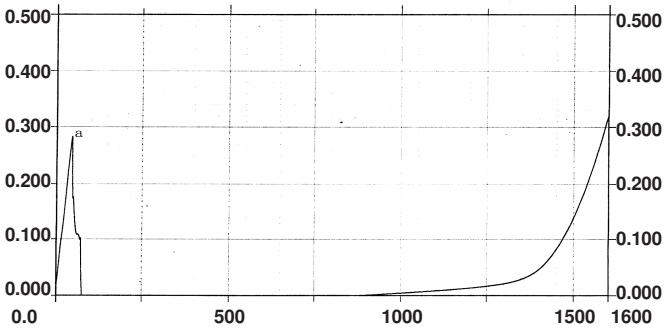

Fig. 5. Effect of Fe^{2+} addition time on laccase activity

Principle of the inhibition: It indicated from the data that the Fe^{2+} doesn't impact the laccase itself and the substrate ABTS. Firstly because the active group of the laccase is comprised of there types of Cu ions[Cu(I),Cu(II) and Cu(III)]. Fe^{2+} ions don't change the structure of the Cu^{2+} ion. Secondly, Fe^{2+} ion doesn't impact the reaction of the substrate and the laccase. Fe^{2+} is a reducing agent, it can be oxidized by the oxidant. While ABTS transferred into $ABTS^{\bullet}$ radical catalyzed by enzyme, $ABTS^{\bullet}$ has strong oxidative ability, which can oxidize Fe^{2+} to Fe^{3+}. The reaction as follows:

$$Fe^{2+} + ABTS^{\bullet} \rightarrow Fe^{3+} + ABTS$$

with this theory we can explain the effect of the Fe^{2+} on laccase activity. When Fe^{2+} has transferred into Fe^{3+} completely, the activity of laccase renewed.

Conclusion

At the experimental conditions, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+} almost have no effect on the activity of laccase. Fe^{2+} has effect on activity of laccase and the inhibition is competitive type. Fe^{2+} has no impact on the active part of laccase. Fe^{2+} can react with ABTS· and be oxidized to Fe^{3+}. When Fe^{2+} transfers into Fe^{3+} completely the inhibition is over and the catalytic reaction continues.

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