Kinetics of Adsorbable Organic Halides (AOX) Reduction in Laccase-Aided Chlorine Dioxide Bleaching of Bagasse Pulp

Xueping Song, a,b Yong Pei, a Jingjing Su, a Chengrong Qin, a,b Shuangfei Wang, a,b and Shuangxi Nie a,b,c,*

This paper presents a kinetic model of the laccase-aided chlorine dioxide bleaching of bagasse pulp. The kinetic model was based on the rate of reduction of adsorbed organic halogen (AOX). The effects of the laccase enzyme dosage, the mediator 1-hydroxybenzotriazole (HBT) dosage, and the reaction temperature on the AOX content of the bleaching effluent are discussed. Good fits were obtained for the experimental data obtained from the different laccase enzyme dosages, HBT dosages, and reaction temperatures, indicating the feasibility of the kinetic model as a means of predicting the optimal operation conditions for the laccase-aided chlorine dioxide bleaching of bagasse pulp in the future.

Keywords: Kinetic model; Laccase; AOX; ECF; Bagasse soda pulp

INTRODUCTION

In recent years, elemental chlorine-free (ECF) bleaching technology has undergone rapid development because of new environmental protection standards (Lehtimaa et al. 2010a,b). Chlorine dioxide is the most important bleaching chemical used in the ECF bleaching of pulp (Nie et al. 2013). It has exhibited a number of critical benefits compared with traditional bleaching chemicals (Bajpai et al. 2006; Nie et al. 2014a). ECF bleaching can restrain the formation of adsorbable organic halogens (AOX) and other chlorine compounds relative to other traditional bleaching (Li et al. 2014). AOX level is generally regarded as one of the most important criteria for assessing the composition of a bleaching effluent (Yetis et al. 1996; Nie et al. 2014b). Many governments have set emission standards for these compounds, especially AOX. The Chinese government, for example, has established a limit on AOX discharge, stipulating that the AOX content of a bleaching effluent discharged must not exceed 12 mg/L (Zhang et al. 2012). Conceivably, there is a close relationship between AOX content and chlorine dioxide dosage, as related studies have reported (Bjorklund et al. 2002; Nie et al. 2014b). Reducing the use of chlorinated bleaching chemicals is crucial for decreasing the discharge of AOX within bleaching effluents; therefore, considerable attention has recently been shifted to exploring cost-effective and environmentally friendly bleaching technologies for the reduction of AOX generation (Sharma et al. 2014).

There are many methods of treating organic materials in wastewater. The advanced oxidation processes (AOPs) includes Fenton reagent oxidation, photocatalytic oxidation, supercritical water oxidation, ultrasonic oxidation, ozone oxidation, electrocatalytic
oxidation, and wet oxidation. Advanced oxidation has attracted more and more extensive attention because of its higher ability to remove the organic materials in wastewater (Soares et al. 2016). However, AOPs can cause secondary pollution. In the context of environmentally friendly bleaching technologies, biobleaching with enzymes has shown tremendous potential for decreasing the use of chlorinated bleaching chemicals (Singh et al. 2008; Dai et al. 2016). Laccase is a biological enzyme used for pulp bleaching and an effective lignin degrader (Knezevic et al. 2013a,b). It can selectively degrade the lignin of paper fibers (Call and Mucke 1997; Qiu and Chen 2012; Quintana et al. 2015), imposes little damage on the cellulose of paper fibers (Bourbonnais et al. 1995), and has been used in labs and at the pilot scale (Euring et al. 2011; Goncalves et al. 2014; Martin-Sampedro et al. 2015). Laccase-aided bleaching can reduce the use of chlorine dioxide in ECF bleaching and reduce the formation of organic chlorine compounds in the bleaching effluent (Bajpai et al. 2006). Laccase-aided bleaching also can improve pulp yield and lower capital investment (Bajpai et al. 2006). Sharma et al. (2014) reported a 35% ClO₂ reduction and a 34% AOX content reduction following ECF bleaching with enzymatic pre-treatment of eucalyptus kraft pulp.

However, the process of laccase-aided chlorine dioxide bleaching is subject to many complex factors that may interact with each other (Paice et al. 2002). Therefore, many transfers and chemical reactions can occur in laccase-aided chlorine dioxide bleaching (Radeva et al. 2009). At present, the most accepted reaction mechanism can be concisely described as a consequence of redox cycles (Balakshin et al. 2001b). Although the reaction mechanism of the laccase-mediator system (LMS) has been researched since laccase was first used for biobleaching (Balakshin et al. 2001b), important problems, including the reaction mechanism and the kinetics of the LMS on AOX reduction, have not been studied thoroughly. Of the number of experiments exploring the reaction mechanism of LMS, few kinetic studies have been conducted (Balakshin et al. 2001b). Macro kinetics can be used to explore the key variables influencing the impact of biobleaching, and can quantitatively describe the effects of these variables (Pei et al. 2016). Balakshin et al. (2001b) constructed a kinetic study on kappa number reduction and the dioxygen uptake of pine-kraft-AQ pulp. They determined that the fast formation of the oxidized mediator species was the critical reason for the fast rate of delignification. Another kinetic investigation of kraft unbleached pulp showed that the action of the LMS could be explained using an exponential kinetic equation (Radeva et al. 2009).

The objective of this work was to establish a macro kinetic model of AOX reduction in the context of the laccase-aided chlorine dioxide bleaching of bagasse pulp. The model will promote the understanding of AOX formation in the laccase-aided chlorine dioxide bleaching of bagasse pulp, which could benefit the pulp and paper industry as well as the natural environment.

**EXPERIMENTAL**

**Materials**

The unbleached bagasse pulp (brightness, 38.97% ISO; viscosity, 1280.86 mL/g; and kappa number, 12.51) used in the bleaching experiments was obtained from the Pumiao paper mill (Guangxi, China). The laccase was supplied by Shanghai Yongye biotechnology Co., Ltd. (Shanghai, China). The laccase had a laccase activity of 2000 U/g. The chlorine dioxide solution, which was stored in a brown bottle, was also obtained from
the Pumiao paper mill (Guangxi, China) (available chlorine concentration, 21.58 g/L). The ceramic wool and activated carbon were procured from Analytik-Jena instrument company (Jena, Germany), and all other chemicals used in this study were purchased from Aladdin reagent (Shanghai) Co., Ltd. All of the chemicals were of analytical grade, unless otherwise mentioned.

Methods

The laccase activity was determined by monitoring the oxidation of 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as increased absorbance at 420 nm. The reaction mixture contained 2.5 mL of sodium acetate buffer (pH 4.5), 0.1 mL of the enzyme sample, and 0.4 mL of 1 mM ABTS, in a final volume of 3.0 mL. 1 U was defined as the amount of enzyme that would convert 1 µmol of substance in one minute under the described conditions at 25 °C (Bajpai et al. 2006).

Unbleached bagasse pulp, an appropriate amount of laccase and HBT, surfactant (Tween® 80), and acetate buffer (pH 4.5) were mixed thoroughly and reacted in a micro-reactor. Other constant parameters needed in this biobleaching treatment were as follows: surfactant (Tween® 80), 0.05%; pulp consistency, 10%; oxygen pressure, 0.4 MPa; and rotating speed, 60 rpm. The laccase enzyme biobleaching treatment of the unbleached bagasse pulp was carried out in a micro-reactor at an appropriate temperature. After the laccase enzyme biobleaching treatment, the pulps were washed with distilled water until reaching a neutral pH. The washed pulps were then used for the chlorine dioxide bleaching.

The washed pulps, chlorine dioxide solution, and sulfuric acid solution were mixed in a plastic bag at a pulp consistency of 10%. Other parameters used during the chlorine dioxide bleaching were a chlorine dioxide dosage of 3.7% and a sulfuric acid solution pH value of 4.5. Then, the bags were heated in a water bath at 70 °C for 1 h with kneading every 15 min. After bleaching, the pulps were washed with distilled water until they reached a neutral pH. The bleached pulps were placed in a lab environment held at constant temperature and constant humidity for 24 h and then subjected to characterization. In the control experiment, the laccase enzyme biobleaching treatment of the unbleached bagasse pulp was replaced with the buffer solution alone, and the subsequent conditions used for chlorine dioxide bleaching were the same as those for the experimental samples. All of the experiments were conducted in triplicate, and the mean values were recorded.

The AOX content was measured using a Multi X 2500 halide analyzer (Jena, Germany). The process was as follows: first, the diluted bleaching effluent was passed through an activated carbon column, where the organic chlorine of the bleaching effluent was adsorbed by the activated carbon; second, the adsorbed inorganic chlorine in the activated carbon column was washed with sodium nitrite; finally, the activated carbon column was burned in a combustion furnace, after which the AOX content was calculated using the micro Coulomb titration method (Nie et al. 2013, 2014b, 2015).

RESULTS AND DISCUSSION

Mathematical Model of the Kinetic Equation

The relationship between the actual AOX reduction and the reaction time is shown in Fig. 1. The reaction was conducted at a laccase enzyme dosage of 15 U/g, a HBT dosage of 1.5%, and a reaction temperature of 323 K. Notably, the AOX reduction calculated represented the relative AOX reduction, and was calculated as follows,
where \( AOX_i \) is the AOX content of the biobleaching experiments and \( AOX_0 \) is the AOX content of the control experiment.

**Fig. 1.** Relationship between AOX reduction and reaction time

From Fig. 1 it can be seen that the AOX reduction increased as the reaction progressed. The rate of AOX reduction, however, decreased as the reaction progressed. This was expected, as, if the reaction time is too short, the residual lignin will not be as thoroughly degraded, whereas if the reaction time is too long, the reaction results in dehydrogenative polymerization of the degraded residual lignin molecules (Balakshin et al. 2001a). These results are similar to those of the sequential enzymatic treatment studied by Sharma et al. (2014). Kinetics has been used to investigate the effects of key variables on the reaction rate (Balakshin et al. 2001b; Roncero et al. 2003). The kinetic model describing AOX reduction in the context of laccase-aided chlorine dioxide bleaching of bagasse pulp was represented as Eq. 2,

\[
dW/dt = k \cdot [\text{Laccase}]^a \cdot [\text{HBT}]^b \cdot W^c
\]  

(2)

where \( dW/dt \) is the rate of AOX reduction, \( W \) is the AOX reduction, \( t \) is the biobleaching time (min), \( k \) is the reaction rate constant of AOX reduction, \([\text{Laccase}]\) is the dosage of the laccase enzyme (U/g), and \([\text{HBT}]\) is the dosage of the mediator HBT (%). The constants \( a, b, \) and \( c \) are the reaction orders of laccase, HBT, and AOX reduction, respectively.

To facilitate the calculation, Eq. 2 was transformed into its logarithmic form (Eq. 3):

\[
\log (dW/dt) = \log (k) + a \cdot \log [\text{Laccase}] + b \cdot \log [\text{HBT}] + c \cdot \log (W)
\]  

(3)
Supposing that \( M_1 = \log(k) + a \cdot \log[\text{Laccase}] + b \cdot \log[HBT] \) when the laccase enzyme dosage of 15 U/g, the HBT dosage of 1.5\%, and the reaction temperature of 323 K were held constant, Eq. 3 could be used to explore the changes in AOX reduction over time, so it was rewritten as Eq. 4,

\[
\log(dW/dt) = M_1 + c \cdot \log(W)
\]  

(4)

where \( \log(dW/dt) \) is the y-coordinate, \( M_1 \) is the intercept, \( c \) is the slope, and \( \log(W) \) is the x-coordinate.

Fig. 2. Linear relationship between \( \log(dW/dt) \) and \( \log(W) \)

Through linear fitting, a regression equation was obtained, as shown in Fig. 2. The equation was in strong accordance with the experimental results, with \( R^2 = 0.9947 \). The value of \( M_1 \) was -4.3927, and the value of \( c \) was equal to -1.0442.

When the laccase enzyme dosage of 15 U/g and the reaction temperature of 323 K were held constant and the HBT dosage was changed, Eq. 3 was rewritten as Eq. 5 to explore the AOX reduction changes over time under different HBT dosages,

\[
\log(dW/dt) + 1.0442 \cdot \log(W) = M_2 + b \cdot \log[HBT] \]  

(5)

where \( M_2 = \log(k) + a \cdot \log[\text{Laccase}] \).

Through linear fitting, five regression equations under five different reaction times were obtained, as shown in Fig. 3. Each equation was in strong accordance with their experimental results, with \( R^2 \) values corresponding to 0.9751, 0.9819, 0.9813, 0.9858, and 0.9927, respectively. The slope average of the five fitted linear models was recorded as the value of \( b \), so the value of \( b \) is equal to 0.4910.
When the HBT dosage of 1.5% and the reaction temperature of 323 K were held constant, while the laccase enzyme dosage was changed, Eq. 3 was rewritten as Eq. 6 in order to explore the changes in AOX reduction over time under five different laccase enzyme dosages,

$$\log(\frac{dW}{dt}) + 1.0442 \cdot \log(W) = M_3 + a \cdot \log[Laccase]$$  \hspace{1cm} (6)

where $M_3 = \log(k) + b \cdot \log[HBT]$.

Using univariate regression, five regression equations were obtained under five different reaction times, as shown in Fig. 4. Each equation was in strong accordance with the experimental results, with all $R^2$ values greater than 0.9. In the same way as before, the slope average of the five fitted linear models was recorded as the value of $a$, so the value of $a$ was equal to 0.6946. In accordance with the Arrhenius law, the relationship between the reaction rate constant of the AOX reduction and the reaction temperature can be represented as follows,

$$k = A \cdot e^{-E/RT}$$  \hspace{1cm} (7)

where $A$ is the pre-exponential factor, $E$ is the apparent activation energy for the AOX reduction (KJ/mol), $R$ (equal to 8.314 J/(mol • K)) is the ideal-gas constant, and $T$ is the thermodynamic temperature (K).

Equation 3 can be represented as Eq. 8:

$$\log(k) = \log(\frac{dW}{dt}) - a \cdot \log[Laccase] - b \cdot \log[HBT] - c \cdot \log(W)$$  \hspace{1cm} (8)

In this case, the reaction temperature was changed, while the laccase enzyme dosage of 15 IU/g and the HBT dosage of 1.5% were held at a constant value. Figure 5 shown the relationship between AOX reduction and reaction time when the temperatures ranged from 303 to 323 K.
**Fig. 4.** Linear relationship between $\log(\frac{dW}{dt}) + 1.0442 \cdot \log(W)$ and $\log[\text{Laccase}]$

**Fig. 5.** Relationship between AOX reduction and reaction time at five different temperatures

In accordance with the relationship between the AOX reduction and the reaction time (Eq. 5), the calculated values of $a$, $b$, and $c$, as well as the laccase enzyme dosage and the HBT dosage, were substituted into Eq. 7. Similarly, the AOX reductions at five different times for five different temperature (Eq. 5) were also substituted into Eq. 8. Finally, under the five different reaction temperatures, the value of $\log(k)$ was obtained. In addition, Eq. 7 was transformed into its logarithmic form (Eq. 9):
A fitted linear model, \( y = -2.9391 - 435.0967x \), was obtained using linear regression analysis in accordance with the relationship between \( \log(k) \) and \( 1/T \) (Fig. 6). The value of \( \log(A) \) was equal to -2.9391, which was obtained from the intercept of the fitted linear model. Similarly, based on the slope of the fitted linear model, the value obtained for \(-E/RT\) was -435.0967. Finally, the value of \( A \) (pre-exponential factor) was calculated to be 0.001151, and the value of \( E \) (apparent activation energy) was calculated to be 3.6157 kJ/mol. Thus, the final value of the reaction rate constant of the AOX reduction, \( k \), was 

\[
0.001151 \times e^{-435.0967/T}
\]

This implies that the apparent activation energy for AOX reduction in the laccase-aided chlorine dioxide bleaching of bagasse pulp was low. In other words, the reaction speed between laccase and bagasse pulp was fast and accelerated the formation of the AOX.

**Fig. 6.** Linear relationship between \( \log(k) \) and \( 1/T \)

Because the reaction orders of the laccase enzyme dosage \( (a) \) and HBT dosage \( (b) \), as well as the pre-exponential factor \( (A) \) and the activation energy \( (E) \), were known, a complete kinetic model of AOX reduction was obtained as follows:

\[
dW/dt = 0.001151 \times e^{-435.0967/T} \times \text{[Laccase]}^{0.6946} \times \text{[HBT]}^{0.4910} \times W^{-1.0442}
\]

Thus, in the future, it will be convenient for paper mills to be able to predict the rate of AOX reduction during the laccase-aided chlorine dioxide bleaching of bagasse pulp by reference to this new kinetic model.

Figure 7 shows the close relationship between the actual values of the AOX reduction and the calculated values from Eq. 10. As can be seen, the points of the scatterplot are distributed in the proximity of the straight line of 45°, indicating that the new kinetic model accurately predicted the rate of AOX reduction in the laccase-aided chlorine dioxide bleaching of bagasse pulp.
**Fig. 7.** Relationship between the actual values and the calculated values of the AOX reduction

### Effect of Laccase Dosage and HBT Dosage on the Rate of AOX Reduction

From the kinetic model (Eq. 10) describing the laccase-aided chlorine dioxide bleaching of bagasse pulp, it is not hard to see that the reaction order values of the kinetic model are not integers. The AOX reduction is a multi-stage and complex reaction, rather than a single simple reaction. Because of the macromolecular structure of lignin, it is too hard to figure out the molecular structure of the lignin clearly. Hence, numerous complex redox reactions occurred on the lignin, and many of the intermediates were formed during the redox reactions.

When the laccase enzyme dosage, the HBT dosage, and the reaction temperature were held at a constant values, the AOX reduction increased from 0.05 to 0.14. The rate of the AOX reduction ($\gamma_{0.14}/\gamma_{0.05}$) was as follows:

$$
\gamma_{0.14}/\gamma_{0.05} = \frac{0.001151 \cdot e^{-435.0967/T} \cdot [\text{Laccase}]^{0.694566} \cdot [\text{HBT}]^{0.491024} \cdot 0.14^{1.04416}}{0.001151 \cdot e^{-435.0967/T} \cdot [\text{Laccase}]^{0.694566} \cdot [\text{HBT}]^{0.491024} \cdot 0.05^{1.04416}} = 0.3413
$$

(11)

Based on Eq. 11, the rate of the AOX reduction was reduced with the increase in AOX reduction in the context of laccase-aided chlorine dioxide bleaching of bagasse pulp. The model also indicated that the LMS oxidation of lignin was very fast at the beginning of the biobleaching. In other words, most of the AOX was formed at the beginning of the biobleaching stage.

When the laccase enzyme dosage and the reaction temperature were held at a constant value, the HBT dosage increased from 0.003 to 0.015. The rate of the AOX reduction ($\gamma_{0.015}/\gamma_{0.003}$) was as follows:

$$
\gamma_{0.015}/\gamma_{0.003} = \frac{0.001151 \cdot e^{-435.0967/T} \cdot [\text{Laccase}]^{0.694566} \cdot [\text{HBT}]^{0.491024} \cdot 0.015^{1.04416} \cdot W^{-1.04416}}{0.001151 \cdot e^{-435.0967/T} \cdot [\text{Laccase}]^{0.694566} \cdot [\text{HBT}]^{0.491024} \cdot 0.003^{1.04416} \cdot W^{-1.04416}} = 2.204
$$

(12)
When the HBT dosage and the reaction temperature were held at a constant value, the laccase enzyme dosage increased from 3 U/g to 15 U/g. The rate of the AOX reduction \( \gamma_4 / \gamma_3 \) was as follows from Eq. 13:

\[
\frac{\gamma_4}{\gamma_3} = \frac{0.001151 \cdot e^{-0.0694566 \cdot \frac{T}{435.0967}} \cdot [\text{HBT}]^{0.491024} \cdot [\text{HBT}]^{-0.001151}}{0.001151 \cdot e^{-0.0694566 \cdot \frac{T}{435.0967}} \cdot [\text{HBT}]^{0.491024} \cdot [\text{HBT}]^{-0.001151}} = 3.059
\]

Based on Eqs. 12 and 13, the AOX reduction increased noticeably with the increase in the HBT dosage and with increase in laccase enzyme dosage. Because of its low redox potential (Bourbonnais et al. 1998) and large size (Morozova et al. 2007), laccase alone has little impact on lignin. However, the combination of laccase and mediators resulted in the fast degradation of lignin (Morozova et al. 2007). Therefore, an appropriate laccase enzyme dosage and HBT dosage are essential for increasing the degradation of lignin and the AOX reduction.

**Fig. 8.** Relationship between the rate of AOX reduction and AOX reduction

**Effect of AOX Reduction on the Rate of AOX Reduction**

Based on the relationship between AOX reduction and the reaction time (Fig. 1) and on the kinetic model (Eq. 10), the relationship between the rate of AOX reduction and AOX reduction was obtained (Fig. 8).

Here, the laccase enzyme dosage was 15 U/g, the HBT dosage was 1.5%, and the reaction temperature was 323 K. From Fig. 8, it can be seen that the rate of AOX reduction decreased noticeably when the AOX reduction was less than 0.14. When the AOX reduction was more than 0.14, the change in the rate of AOX reduction was small. This was similar to the results obtained in previous studies (Balakshin et al. 2000, 2001a; Fillat and Roncero 2009), which revealed that the reaction of AOX reduction could be separated into two stages. In the initial stage, when the AOX reduction was less than 0.14, the lignin was degraded rapidly. In the final stage, when the AOX reduction was more than 0.14, degradation of lignin was much slower.
The equation can predict the rate of AOX reduction conveniently under the different LMS condition. It could optimize the level of AOX to meet the regulation limits.

CONCLUSIONS

1. A macro kinetic model of the AOX reduction in the context of the laccase-aided chlorine dioxide bleaching of bagasse pulp was developed.

2. The effects of the laccase enzyme dosage, the HBT dosage, and the reaction temperature on the rate of AOX reduction were illustrated using a macro kinetic model.

\[
dW/dt = 0.001151 \cdot e^{-435.0967/T} \cdot [\text{Laccase}]^{0.6946} \cdot [\text{HBT}]^{0.4910} \cdot W^{-1.0442}
\]

The reaction order values of the kinetic model were not integers, indicating that the AOX reduction was a multi-stage and complex reaction, rather than a single simple reaction.

3. The reaction of AOX reduction was shown to take place in two stages. In the initial stage, when the AOX reduction was less than 0.14, the lignin was degraded rapidly. In the final stage, when the AOX reduction was more than 0.14, the degradation of lignin was much slower.

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REFERENCES CITED


on the reaction kinetics,” *Journal of Molecular Catalysis B-Enzymatic* 16(3-4), 205-215. DOI: 10.1016/s1381-1177(01)00062-5


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