# COMPARATIVE KINETIC ANALYSIS OF A LACCASE–MEDIATOR SYSTEM TREATMENT OF PULP AFTER OXYGEN DELIGNIFICATION AND CHLORINE DIOXIDE BLEACHING

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The effect of Novozyme AS laccase and of the 1-hydroxybenzotriazole mediator on unbleached and oxygen delignified kraft pulp is studied. The kinetic investigations, based on the relatively decreasing kappa number and light adsorption coefficient, show that an exponential kinetic equation valid for processes taking place on uniformly inhomogeneous surfaces can explain the action of the laccase-mediator system (LMS). The activation energy found remains constant during the bleaching processes, while the preexponential factor changes, thus determining the current rate decrease. The correlations derived between the kinetic characteristics depend neither on temperature nor on the reaction time. The investigations carried out indicate that delignification becomes the prevailing process in the LMS treatment, followed by chlorine dioxide bleaching. The process leading to the chromophoric decrease starts to dominate the complex process when the LMS treatment is followed by oxygen delignification.

***Keywords***: laccase-mediator system, oxygen delignification, chlorine dioxide bleaching, exponential kinetic equation.

## INTRODUCTION

In nature, wood degradation is carried out by microorganisms, such as white-rot basidiomycetes. In 1984, Paice and Jurasek reported[1] the first applications of enzymes for the modification of pulp properties. Laccases (E.C.1.10.3.2.), a group of multi-copper oxidases, are the most promising oxidoreductive lignin degrading enzymes.[2] Laccases oxidize the lignin structure into radicals, which are spontaneously degraded. However, due to the steric hindrances, laccases alone are not very efficient in delignification. Furthermore, their oxidation potential provides the oxidation of lignin's phenolic end-groups only.[3] It is recognized that their substrate specificity can be extended to non-phenolic end-groups, as well as by adding oxidized low-molecular mass compounds (mediators).[4] 1-hydroxy-benzotriazole (HBT) can act as a mediator.

Laccase oxidizes this mediator, forming a HBT- radical in the initial stage of the process.[5] The mediator is small enough to penetrate the lignin network and perform oxidations of the non-phenolic end-groups. The application of a laccase–HBT mediator system provides an efficient, selective and completely chlorine-free bleaching stage.[6,7]

It has been found out that pulp treatment with a laccase-mediator system (LMS) leads to high lignin extraction (40-60%) in the subsequent bleaching stages. Along with that, the carbohydrates hydroxyl groups present in the pulp remain unchanged and neither a decrease in viscosity, nor changes in the content of hexeneuronic acids are observed.[8-12] Besides, the enzyme treatment does not directly affect the pulp degree of brightness.[13,14] Generally, oxygen is required in normal delignification processes.

The small-size molecules of the oxidized mediator provide a relatively uniform lignin depolymerisation.[8,12]

Previous investigations of ours evidenced[15-17] the highly selective delignification effect of LMS. A kappa number decrease of 32% is produced after chlorine dioxide bleaching, while it reaches only 17% after oxygen bleaching. In absolute values, the kappa number decrease is of 1.4 and 2.2 units for chlorine dioxide and oxygen bleaching, respectively. It is evident that the enzyme effect on delignification is more pronounced in the case of chlorine dioxide bleaching.[18]

The objective of the present work is to find out the relations between the kinetic characteristics, the LMS effect being determined after oxygen delignification and chlorine dioxide bleaching.

## EXPERIMENTAL

The experiments were carried out with two types of pulp produced at an industrial scale by Svilocell-AD (Bulgaria): the former was kraft unbleached pulp, with a kappa number of 21.02, viscosity of 985 $dm^3 \cdot kg^{-1}$ and 33.50% ISO brightness; the latter was oxygen delignified pulp, with a kappa number of 9.99, viscosity of 900 $dm^3 \cdot kg^{-1}$ and 48.66% ISO brightness.

The enzyme used, Laccase NS 51002, kindly donated by Novozyme (Denmark), with an activity of 200 LACU/g, was added in a quantity of 4 LACU/g pulp. HBT (1-hydroxybenzotriazole) as mediator was used in a quantity of 1% as reported to the pulp. In fact, these were the optimum amounts established in preliminary investigations.[15]

The enzyme treatment was carried out at an oxygen pressure of 3.5 atm, 10% pulp consistency and a pH value equal to 4. The temperature range investigated was between 30 and 60 °C, while the reaction time was varied from 30 to 180 min.

To establish the real effect of the enzyme treatment, control experiments were carried out without addition of enzyme, but with 1% HBT for 2 h at 50 °C, pH equal to 4, oxygen pressure of 3.5 atm, for both pulp types.

The effect of the enzyme action for the first type of pulp was determined after oxygen delignification, performed at a temperature of 100 °C, 10% consistency, NaOH charge of 2.5%, and oxygen pressure of 6 atm for 60 min.

The oxygen and LMS treatment was carried out in 4 stainless autoclaves rotated in a polyglycol bath previously heated to the desired temperature.

The effect of the enzyme action with the latter type of pulp was determined after subsequent chlorine dioxide bleaching, performed in plastic bags placed in a water bath at 60 °C. The other bleaching conditions were: 10 % pulp consistency, 2% active chlorine charge, reaction time of 45 min, with the addition of $H_2SO_4$ to maintain a final pH = 2.85.

The delignification degree, controlled through the non-dimensional quantity $\alpha_\chi$, indicated the relative decrease in the kappa number in accordance with:

$$\alpha_\chi = \frac{\chi_o - \chi_t}{\chi_o} \tag{1}$$

where $\chi_0$ is the initial kappa number value measured after oxygen delignification of the former type of pulp ($\chi_0$ = 12.23) and after chlorine dioxide bleaching of the latter type of pulp ($\chi_0$ = 4.17). $\chi_t$ is the current kappa number value obtained after the respective reaction time of the enzyme treatment – *t*, and following the bleaching stage.[18,19]

The LMS effect on both oxygen and chlorine dioxide bleaching was also followed by the relative change of the light absorption coefficient, *k*, on considering the change of the chromophore groups content. The non-dimensional quantity $\alpha_k$, used as a kinetic variable, was introduced by:

$$\alpha_k = \frac{k_0 - k}{k_0} \tag{2}$$

where *k* is the current value of the absorption coefficient, and $k_0$ is the corresponding value of this coefficient in the absence of enzyme. The values of $k_0$ are of 0.22 and 0.07 in oxygen and in chlorine dioxide bleaching, respectively.[18,19]

The values of *k* were calculated with the Kubelka-Munk equation (3), using the experimentally obtained value of the light scattering coefficient *s* (*s* = 47.0 $m^2/kg$) and the current brightness values $R_\infty$:

$$k/s = \frac{(1 - R_\infty)^2}{2.R_\infty} \tag{3}$$

The kappa number of bleached pulp was determined according to SCAN–C 1:77, while brightness – according to SCAN–C 11:75.

## RESULTS AND DISCUSSION

The LMS effect on oxygen and chlorine dioxide bleaching has been studied using the degree of delignification, $\alpha_\chi$ (eq. 1), and the relative change in the light absorption coefficient $\alpha_k$ (eq. 2). Thus, lignin extraction and the accompanying variation of the fibrous material brightness were followed.

The well-known Michaelis-Menten mechanism cannot be applied to heterogeneous systems with an indefinite substrate concentration.[20] It has been found out that the exponential kinetic equation (4), valid for processes taking place on uniformly inhomogeneous surfaces, provides a good interpretation of the LMS action:[15,16]

$$v = v_0 e^{-a.\alpha} \qquad (4)$$

where $a$ is the kinetic coefficient of inhomogeneity, $v = \frac{d\alpha}{dt}$ is the current rate and $v_0$ is the initial rate of the investigated processes.

Eq. (4) is applied in its approximate integral form:

$$\alpha = \frac{1}{a}\ln\left(v_0 a\right) + \frac{1}{a}\ln t \qquad (5)$$

which requires a linear increase of α with ln$t$ at constant temperature. Figures 1-4, illustrating the kinetic curves obtained, show clearly that eq. 5 is valid in all cases under study and especially, when using both kinetic variables, *i.e.* $\alpha_\chi$ and $\alpha_k$.

For the kinetics referring to the kappa number $\chi$, the kinetic coefficients of inhomogeneity $a_\chi$ are calculated while, for the kinetics referring to the light absorption coefficient $k$, the $a_k$ ones are calculated. All data obtained in the kinetic study are summarized in Table 1.

For both kinetic variables, the running rate values are calculated according to expression (6):

$$v = \frac{1}{at} \qquad (6)$$

Figures 5 and 6 show the current rate decrease with time $t$ for both types of sample treatment. It is clear that, irrespective of the high initial rates, the processes start to slow down.

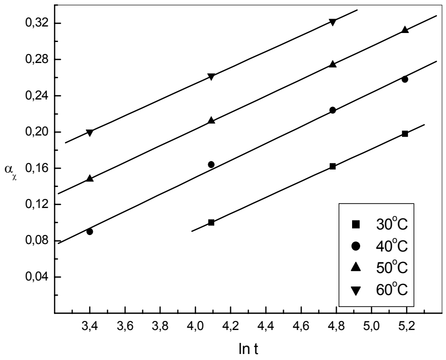

Figure 1: Linear dependencies $\alpha_\chi$ *vs*. ln $t$ for LMS + $ClO_2$

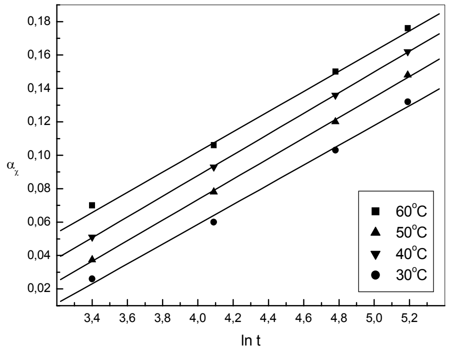

Figure 2: Linear dependencies $\alpha_\chi$ *vs*. ln $t$ for LMS + $O_2$

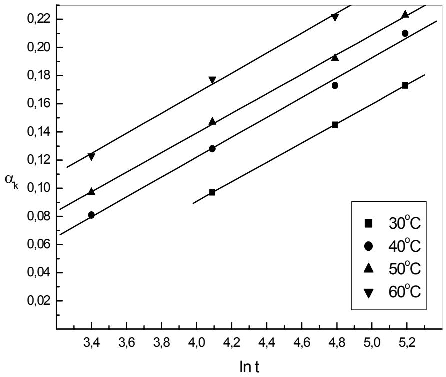

Figure 3: Linear dependencies $\alpha_k$ *vs*. ln $t$ for LMS + $ClO_2$

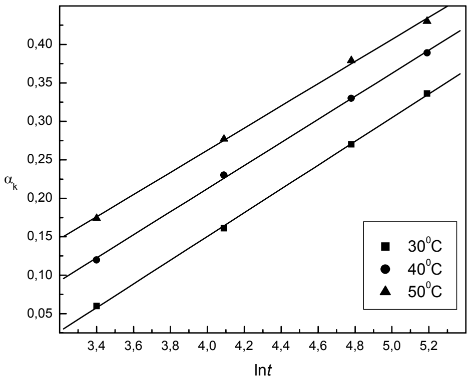

Figure 4: Linear dependencies $\alpha_k$ *vs*. Ln $t$ for LMS + $O_2$

Table 1
Kinetic characteristics of the delignification processes investigated

| Based on kappa number ($\chi$) measurement | | | | Based on light absorption coefficient ($k$) measurement | | | |
|---|---|---|---|---|---|---|---|
| LMS + $ClO_2$ | | LMS + $O_2$ | | LMS + $ClO_2$ | | LMS + $O_2$ | |
| $a_\chi = 11$ | | $a_\chi = 16$ | | $a_k = 75$ | | $a_k = 16$ | |
| | $\ln A_\chi$ | | $\ln A_\chi$ | | $\ln A_k$ | | $\ln A_k$ |
| $\alpha_\chi = 0.18$ | 12.69 | $\alpha_\chi = 0.08$ | 2.00 | $\alpha_k = 0.12$ | 4.80 | $\alpha_k = 0.20$ | 5.03 |
| $\alpha_\chi = 0.20$ | 12.00 | $\alpha_\chi = 0.10$ | 1.67 | $\alpha_k = 0.14$ | 4.25 | $\alpha_k = 0.25$ | 4.63 |
| $\alpha_\chi = 0.22$ | 11.96 | $\alpha_\chi = 0.12$ | 1.37 | $\alpha_k = 0.16$ | 4.00 | $\alpha_k = 0.30$ | 4.42 |
| $\alpha_\chi = 0$ | $\ln A_0 = 14.44$ | $\alpha_\chi = 0$ | $\ln A_0 = 3.30$ | $\alpha_k = 0$ | $\ln A_0 = 6.16$ | $\alpha_k = 0$ | $\ln A_0 = 6.63$ |
| $E_{\chi 0} = E_\chi = 49.65$ kJ/mol | | $E_{\chi 0} = E_\chi = 22.87$ kJ/mol | | $E_{k0} = E_k = 33.30$ kJ/mol | | $E_{k0} = E_k = 29.10$ kJ/mol | |

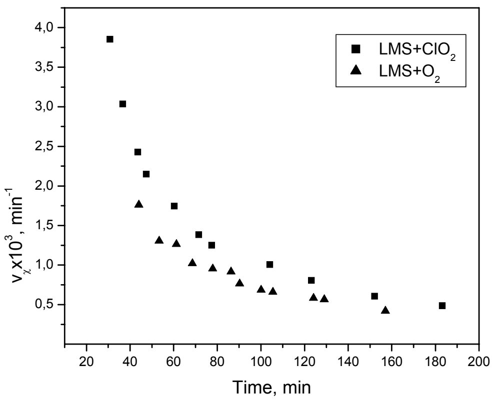

Figure 5: Dependence of current rate $\nu_\chi$ on reaction time

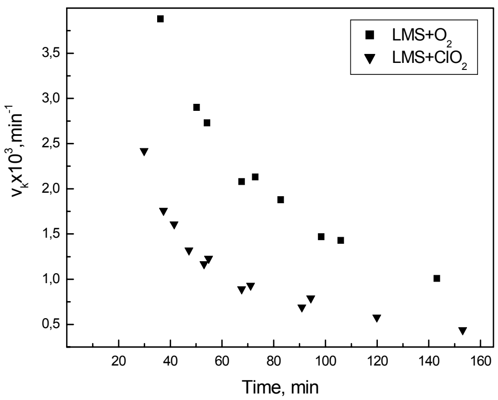

Figure 6: Dependence of current rate $\nu_k$ on reaction time

As to the kappa number decrease, the LMS + $ClO_2$ treatment gives higher $\nu_\chi$ rates, which means that the lignin quantity left in the pulp, as well as the content of chromophoric groups, decrease. This is evidenced by the higher degree of brightness (72.00% ISO), although the relative increase in brightness[18] is of only approximately 4%.

In the LMS + $O_2$ treatment, the delignification rates $\nu_\chi$ are lower, while residual lignin and the corresponding chromophoric groups are higher. This leads to a pulp brightness degree of 60.63% ISO. In this case, bleaching proceeds[18] at a higher rate $\nu_k$, and the relative increase in brightness is of ca 17%.

The LMS treatment and the oxygen delignification concern the same type of lignin structures, which, together with another type of lignin functional groups, are affected during LMS and chlorine dioxide bleaching, determining a different mechanism of the processes and providing a higher delignification effect.[21,22]

The rate–temperature dependence is used to calculate the apparent activation energy $E$ and the pre-exponential factor ln$A$, according to the Arrhenius equation. The values obtained for $E$ are independent of the α increase, but differ significantly not only for both types of sample treatment, but also for both kinetic variables (Table 1).

During the process, the pre-exponential factor $A$ decreases with α increase (Table 1), following the relationship:

$$\ln A = \ln A_0 - a\alpha \qquad (7)$$

The pre-exponential factor is determined by the number and structure of the active reaction centers, which are predominantly of the lignin type. It is suggested that the observed decrease of ln$A$ with the α increase is due to the hampered accessibility of the active centers or to the increased steric hindrances.

A correlation between the kinetic variables $\alpha_\chi$ (referring to the kappa number) and $\alpha_k$ (referring to the light absorption coefficient) is attempted. The approximate integral form of the exponential kinetic equation (5) applied to $\alpha_\chi$ and $\alpha_k$ can be presented as:

$$\alpha_\chi = \frac{1}{a_\chi}\ln\left(v_{0,\chi}a_\chi\right) + \frac{1}{a_\chi}\ln t \tag{8}$$

$$\alpha_k = \frac{1}{a_k}\ln\left(v_{0,k}a_k\right) + \frac{1}{a_k}\ln t \tag{9}$$

The analysis of the latter shows that, at any time following the beginning of the process, a correlation between $\alpha_\chi$ and $\alpha_k$ may be observed, which may be presented by:

$$\alpha_\chi = \frac{1}{a_\chi}\ln\frac{v_{0,\chi}a_\chi}{v_{0,k}a_k} + \frac{a_k}{a_\chi}\alpha_k \tag{10}$$

Figure 7 shows the dependencies between both variables for the processes under investigation, as a verification of the above-stated conclusion.

Figure 7 illustrates the linear correlation between both kinetic variables for both types of sample treatment. The slope of the straight lines obtained is determined by the ratio of the coefficients of inhomogeneity, and does not depend on the temperature as they both ($a_k$ and $a_\chi$) are temperature independent. For LMS + $ClO_2$, this ratio value is of 1.36, while, for LMS + $O_2$, it is of 0.42. One may also observe that both straight lines go through the zero point of the coordinate system, which leads to the conclusion that $\frac{1}{a_\chi}\ln\frac{v_{0,\chi}a_\chi}{v_{0,k}a_k} = 0$. This can be achieved if:

$$v_{0,\chi}a_\chi = v_{0,k}a_k \tag{11}$$

It is evident that this type of correlation between the initial rates and the coefficients of inhomogeneity is displayed during the process. Taking into consideration the equation defining the current rate (eq. 6), the relation above becomes:

$$v_\chi a_\chi = v_k a_k \tag{12}$$

Hence, for the processes studied, it follows that:

$$\frac{\alpha_\chi}{\alpha_\kappa} = \frac{v_{0,\chi}}{v_{0,k}} = \frac{v_\chi}{v_k} = \frac{a_k}{a_\chi} \tag{13}$$

Equation (13) permits to estimate the specific participation of the various reactions in the complex heterogeneous process. For LMS + $ClO_2$, the delignification rates are higher than those of the brightness increase, as $v_x = 1.36v_k$, while, for LMS + $O_2$, where $v_x = 0.42v_k$, the process of chromophoric groups destruction proceeds at higher rates, as illustrated in Figures 8 and 9.

The introduction of the correlation between $\alpha_\chi$ and $\alpha_k$, *i.e.* $\alpha_\chi a_\chi = \alpha_k a_k$, into eq. 7 leads to:

$$\frac{A_\chi}{A_k} = \frac{A_{0,\chi}}{A_{0,k}} = q \tag{14}$$

where $q$ is a constant.

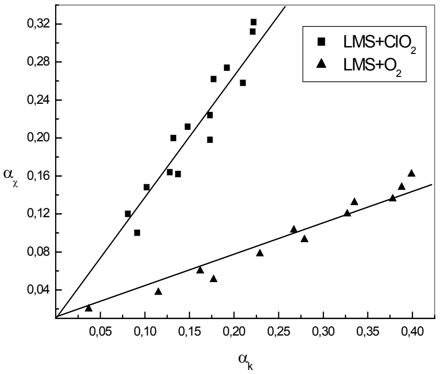

Figure 7: Linear correlation between $\alpha_\chi$ and $\alpha_k$

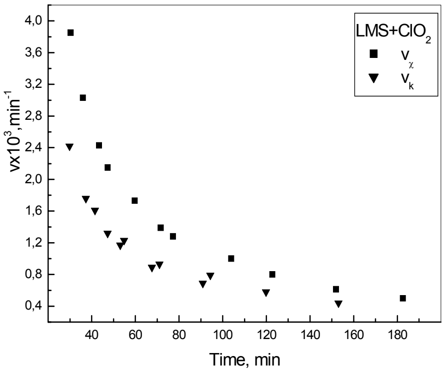

Figure 8: Comparative presentation of the time dependencies of $v_\chi$ and $v_k$ for LMS + $ClO_2$

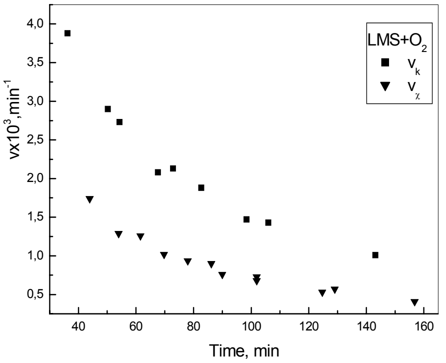

Figure 9: Comparative presentation of time dependencies of $v_\chi$ and $v_k$ for LMS + $O_2$

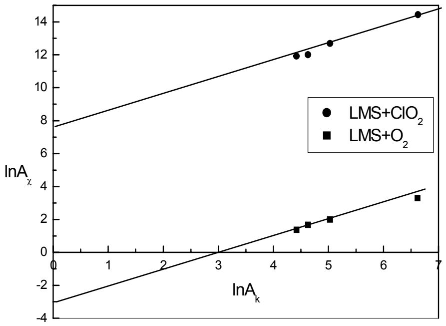

Figure 10: Correlation between ln$A_\chi$ and ln$A_k$ for LMS + $ClO_2$ and LMS + $O_2$

This means that the ratio between the pre-exponential factors $A_\chi$ and $A_k$ remains constant during the process, being determined by the corresponding value at $t$ = 0. This can be attributed to the entropy inhomogeneity of the surface formed during the very first contacts between the reactants.

The slope of both straight lines in Figure 10 is equal to 1, while the intercept permits to determine ln $q$. It has been found out that the values of the latter are of 7.80 and 3.00 for LMS + $ClO_2$ and LMS + $O_2$, respectively, which supports the conclusion that delignification is the dominating process in the first case, while chromophoric groups decreasing plays the same role in the second one.

## CONCLUSIONS

The combination of LMS with $ClO_2$ leads to the domination of the delignification reaction, while the combination of LMS and $O_2$ leads to the decrease in the content of chromophoric groups. The correlations between the kinetic characteristics of delignification and of the brightness increase are established, permitting to estimate the specific participation of the various reactions in the complex heterogeneous process. Such correlations also permit to establish the delignification degree on the basis of the experimentally determined coefficient of light absorption. From a practical point of view, the combination of the laccase-mediator system with $ClO_2$ leads to better results with respect to lignin extraction, and to a higher final brightness.

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