

## Polyoxometalates as mediators in the laccase catalyzed delignification

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Received 9 March 2001; received in revised form 25 July 2001; accepted 25 July 2001

### Abstract

The polyoxometalate (POM)-laccase catalytic system was applied for the first time to aerobic delignification of kraft pulps at moderate (40–60 °C) temperatures. Laccase was found to readily catalyze the re-oxidation of different kinds of polyoxometalates, including those, which cannot be re-oxidized by dioxygen even at high temperatures (PMo<sub>11</sub>V<sub>1</sub>, SiW<sub>11</sub>V<sub>1</sub>, etc.). This allows a sequence of catalytic redox cycles similar to that in the laccase-mediator system (LMS) where electrons are transferred from the substrate (lignin) via POM and laccase to oxygen. Results obtained showed that the POM-laccase system could decrease  $\kappa$  number of eucalypt kraft pulp from 13.7 to 8.5 though the reaction rate is relatively slow. Among different POM used, SiW<sub>11</sub>V showed the best results. The effect of the process variables on the delignification was studied. The best results in delignification of eucalypt pulp were obtained at 60 °C, oxygen pressure of 5 bar, pH 6.3, SiW<sub>11</sub>V concentration of 4.2 mM and laccase concentration of 0.65 U/ml. The reaction temperature appears to be one of the crucial factors in the achievement of a delignification rate acceptable for practical application. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Laccase; Polyoxometalate; Heteropolyanion; Laccase-mediator system; Lignin; Delignification; Oxidation

### 1. Introduction

Lignin is an aromatic polymer formed in vascular plants by a random dehydrogenative polymerization of the monomeric precursors of cinnamyl alcohol type (*p*-coumaryl, coniferyl and sinapyl alcohols) [1]. The structural units of lignin, so-called phenyl propane units or C<sub>9</sub> units, are linked by different types of ether and carbon–carbon bonds [1].

The delignification, that is the process of degradation and removal of lignin from lignocellulosics, is the basic procedure in the production of fiber material

(pulp) from wood. The main part of lignin is removed from wood during pulping by degradation of the native polymer by the pulping chemicals (for example, NaOH and Na<sub>2</sub>S in the kraft pulping) and solubilization of the resulting fragments in the pulping solution. However, currently used pulping techniques do not allow complete removal of all lignin. Further delignification of unbleached pulps in bleaching process requires application of chemicals, which selectively remove the residual lignin (estimated by reduction of the so-called  $\kappa$  number of pulp) without notable degradation of polysaccharides in pulps (estimated by pulp intrinsic viscosity). Chlorine and chlorine dioxide are traditionally and widely used for pulp bleaching due to their excellent selectivity. However, toxic chlorine-derived organic compounds are produced in these industrial

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processes, which makes these techniques not very attractive from the environmental point of view.

Laccase (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) is a group of enzymes of multi-copper oxidase family found in white-rot fungi. Although white-rot fungi degrade lignin in lignocellulosic materials, isolated laccase is not able to depolymerize lignin without appropriate compounds called as mediators. Catalytic oxygen delignification of kraft pulps with the laccase-mediator system (LMS) or with polyoxometalates (POM) are environmentally benign and selective delignification processes [2–5], which constitute promising alternatives to conventional bleaching. The mechanisms of catalysis with POM and the LMS can be presented in a simplified view as consequences of redox cycles (Scheme 1) where electrons withdrawn from the substrate (lignin) are transferred finally to oxygen, which plays the role of terminal electron acceptor. The lignin oxidation occurs by action of either oxidized forms of POMs or so-called mediators (Scheme 1). The corresponding reduced forms undergo the re-oxidation directly by oxygen in the POM catalysis or by laccase in the LMS. Similar redox cycles occur in the process where anaerobic treatment of pulp with stoichiometric amounts of POM is followed by wet oxidation of the resulting solution of solubilized organic compounds and POM recovery [6].

From the technical point of view, the LMS efficiently and selectively decomposes residual lignin without significant loss in pulp viscosity. However, one of the main drawbacks of the LMS is the low stability of organic mediators used (such as ABTS, HOBT,

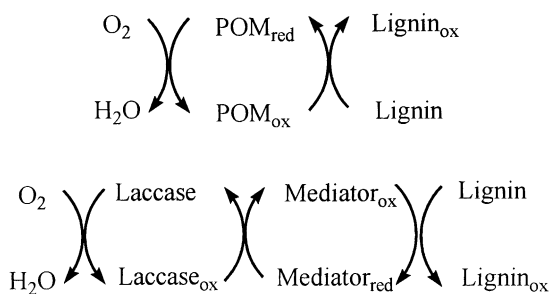
NHAA, etc.) in the reaction system [3] and sorption of some colored mediators on the fiber. Additionally, considering the rather high cost of the mediators, the LMS is not economically viable at the present time. In contrast, most of POMs are very robust, effective as catalysts and can be recycled in the catalytic cycle [4]. POMs of series of  $[\text{PMo}_{12-x}\text{V}_x\text{O}_{40}]^{(3+x)-}$ , particularly  $[\text{PMo}_7\text{V}_5\text{O}_{40}]^{8-}$  (or simply  $\text{PMo}_7\text{V}_5$ ), have been suggested previously as suitable catalysts for aerobic delignification [5,7]. However, the application of these POMs at pH 2–3 and elevated temperature ( $90^\circ\text{C}$ ), required to have sufficiently high rates of pulp delignification and POM re-oxidation, results in undesirable hydrolytic degradation of polysaccharides, which limits the extent of delignification [5]. At the same time, POMs as  $[\text{SiW}_{11}\text{V}_1\text{O}_{40}]^{5-}$  ( $\text{SiW}_{11}\text{V}$ ), effective in terms of delignification and selectivity at pH 5–7, could not be successfully applied in practice because of problems in their re-oxidation with dioxygen [6].

In the present work, we try to combine the high efficiency of laccase in oxidative catalysis with the high stability of POMs by using POMs as mediators for the LMS.

## 2. Experimental

Industrial well-washed eucalypt (*E. globulus*) ( $\kappa$  number = 13.7) and Scandinavian softwood ( $\kappa$  number = 29.5) kraft pulps were used for delignification. Laccase was obtained from Novo Nordisk Co. The activity of the enzyme was determined by ABTS assay [8]. POMs were synthesized according to known procedures for  $\text{PMo}_{12-x}\text{V}_x$  [7] and  $\text{SiW}_{12-x}\text{V}_x$  [9] series.

The pulp (3 g o.d.) was placed in a 230 ml Teflon reactor (Berghof Model BAR 845), a solution (60 ml) of an appropriate amount of a POM and laccase was added and the mixture was thoroughly mixed. The reactor was closed and pressurized with oxygen (0.5 MPa). Time-to-temperature was 40 min and was not included in the reaction time. After the reaction, the pulp was washed on a glass filter. Alkaline extraction was conducted under standard conditions:  $70^\circ\text{C}$ , 2% (o.d. pulp) NaOH, 1 h and 10% pulp consistency. The  $\kappa$  number and viscosity of pulp were determined according to Tappi standards.



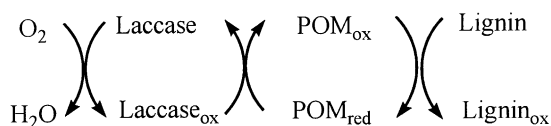
Scheme 1. Redox cycles in POM (top) and LMS (bottom) catalytic oxidation.

### 3. Results and discussion

According to our previous results,  $[\text{PMo}_7\text{V}_5\text{O}_{40}]^{8-}$  heteropolyanion ( $\text{PMo}_7\text{V}_5$ ) and laccase showed a synergetic effect in the aerobic oxidation of hydrazonaphthalene type colorants [10]. It was tentatively suggested that  $\text{PMo}_7\text{V}_5$  could act as a redox mediator in the laccase-catalyzed dye oxidation [10]. To verify this suggestion we tested the ability of laccase to catalyze the oxidation of different kinds of reduced POMs. It has been shown that laccase can readily catalyze the oxidation of the reduced forms of  $\text{PMo}_7\text{V}_5$  and  $\text{PMo}_{11}\text{V}_1$  ( $[\text{PMo}_{11}\text{VO}_{40}]^{4-}$ ) prepared by reduction of the corresponding oxidized forms with hydrazine. The absorption band at 680 nm, characteristic for the reduced form of  $\text{PMo}_{11}\text{V}_1$  and  $\text{PMo}_7\text{V}_5$ , disappeared in a few seconds after addition of laccase at room temperature and atmospheric pressure. The re-oxidation of  $\text{PMo}_{11}\text{V}_1$  by molecular oxygen in the absence of laccase is impossible not only at room temperature, but also under much more vigorous conditions [11]. Moreover, laccase has been found to easily catalyze, in 1–2 min at room temperature and atmospheric pressure, the re-oxidation of POMs such as  $\text{SiW}_{11}\text{V}$  and  $\text{PW}_{11}\text{V}$ , which could not be easily re-oxidized by oxygen. In addition to the change in colors of these POM solutions from dark blue to orange, their re-oxidation has been proved by EPR spectroscopy [12]. The easy aerobic re-oxidation of POM with laccase opens attractive possibilities to use these POMs as catalysts in different redox processes.

The results from the previous experiments show that laccase, catalyzing POM re-oxidation, allows completion of the redox cycle, which can be presented in a simplified view by Scheme 2. Obviously, the thermodynamic conditions of redox reactions occurring may be formulated as follows:

$$E_{\text{substrate}}^0 < E_{\text{POM}}^0 < E_{\text{laccase}}^0 (\sim 0.8 \text{ V}) \\ < E_{\text{O}_2}^0 (1.23 \text{ V})$$



Scheme 2. Redox cycles in the catalytic system substrate (lignin) POM-laccase- $\text{O}_2$ .

#### 3.1. Delignification of pulp catalyzed with laccase and different POMs

POMs of different types were used in the delignification of softwood and eucalyptus kraft pulps by the POM-laccase (PL) catalytic oxidation system (Tables 1–3). The PL stage was followed by the alkaline extraction of pulps (E) to remove oxidized lignin fragments insoluble in the acidic reaction solution. Parallel experiments were performed at the same reaction conditions but in the absence of laccase in order to elucidate the role of laccase in the catalytic system. Experiments on the pulp delignification catalyzed by only laccase in the absence of POM were not performed since it is well known that laccase alone is not able to delignify a pulp [3]. The residual laccase activity was qualitatively estimated by two criteria: (i) by the color of the reaction solution after the delignification: it was deeply blue in the presence of reduced forms of the POMs (the enzyme was not active or absent) or had orange color (color of oxidized POMs) if the laccase was still active after the reaction; (ii) addition of a few mg of ABTS to the solution resulted in a fast appearance of deep blue color indicating the oxidation of ABTS in the presence of laccase; in the absence of laccase the rate of this reaction was rather low. Of course these tests do not provide any direct information on reasons of loss of laccase activity (deactivation) such as inhibition, denaturation or others, but they can be useful in explanation of the delignification results under different reaction conditions.

The application of  $\text{PMo}_7\text{V}_5$  and  $\text{PMo}_{11}\text{V}$  in the PL system for pulp delignification was not very efficient (Table 1). The degree of delignification ( $\kappa$  number reduction) did not exceed that in the experiments with POM in the absence of laccase. The results on the application of the PL system for pulp delignification could be explained by difference in the optimum conditions for the use of these POMs and laccase.  $\text{PMo}_7\text{V}_5$  and  $\text{PMo}_{11}\text{V}$  are effective at  $\text{pH} \leq 3$  [7], whereas laccase is very unstable at this pH, even at a moderate temperature (Table 1). On the other hand, at higher pH, when laccase is more stable, the activity of the POMs is not enough to provide a sufficient reaction rate in lignin oxidation [7].

Obviously, the delignification under mild reaction conditions requires application of a POM with a higher oxidation potential to be able to degrade

Table 1  
Pulp delignification catalyzed by PMo<sub>7</sub>V<sub>5</sub>-laccase and PMo<sub>11</sub>V-laccase<sup>a</sup>

Type of POM	[POM] (mM)	Temperature (°C)	pH	Reaction time (h)	$\kappa$ number <sup>b</sup>	Residual laccase activity
Softwood pulp <sup>c</sup>						
PMo <sub>7</sub> V <sub>5</sub>	2	40	5.45	2	26.2	+
PMo <sub>7</sub> V <sub>5</sub>	2	40	5.25	2	26.9	+
PMo <sub>7</sub> V <sub>5</sub>	2	40	2.45	2	26.1	–
PMo <sub>7</sub> V <sub>5</sub>	2	40	2.95	2	26.8	–
PMo <sub>7</sub> V <sub>5</sub>	2	40	4.0	2	26.8	+
PMo <sub>7</sub> V <sub>5</sub>	2	60	4.0	4	25.8	+
PMo <sub>7</sub> V <sub>5</sub>	2	60	3.5	4	25.7	–
PMo <sub>7</sub> V <sub>5</sub>	2	40	2.93	2	26.3	w/o
Eucalyptus pulp <sup>d</sup>						
PMo <sub>7</sub> V <sub>5</sub>	4.2	60	2.65	8	8.55 (10.44)	–
PMo <sub>7</sub> V <sub>5</sub>	4.2	60	2.65	8	8.26 (10.10)	w/o
PMo <sub>11</sub> V	1	50	4.0	4	12.07	–
PMo <sub>11</sub> V	0.25	50	4.0	8	11.95	–
PMo <sub>11</sub> V	1	50	4.0	4	12.01	w/o
PMo <sub>11</sub> V	4.2	60	1.6	8	8.40 (10.36)	–

<sup>a</sup> Reaction conditions: 10% consistency; laccase concentration of 0.65 U/ml; oxygen pressure of 5 bar. A positive test on the residual laccase activity is designated as “+” and a negative test is designated as “–”; w/o are experiments without laccase.

<sup>b</sup> The values given are after PL-E stage and the values in parenthesis are after PL stage.

<sup>c</sup>  $\kappa$  number of initial unbleached pulp was 29.5.

<sup>d</sup>  $\kappa$  number of initial unbleached pulp was 13.7.

Table 2  
Delignification of eucalypt kraft pulp catalyzed by the POM-laccase system (POM = SiW<sub>11</sub>V, SiW<sub>10</sub>V<sub>2</sub> and PW<sub>11</sub>V)<sup>a</sup>

Experiment	POM or mediator	[POM] (mM)	Temperature (°C)	pH	Reaction time (h)	$\kappa$ number after PL	$\kappa$ number after PL-E	Residual laccase activity
1	PW <sub>11</sub> V	1	50	4.0	8		11.24	+
2	PW <sub>11</sub> V	1	60	4.0	8		11.17	–
3	PW <sub>11</sub> V	4.2	60	4.1	8	11.73	10.07	–
4	SiW <sub>10</sub> V <sub>2</sub>	1	60	6.3	8		10.87	+
5	SiW <sub>10</sub> V <sub>2</sub>	1	60	6.3	24		9.90	+
6	SiW <sub>10</sub> V <sub>2</sub>	4.2	60	6.3	8	11.67	10.76	–
7	SiW <sub>11</sub> V	0.1	50	4.0	8		11.78	+
8	SiW <sub>11</sub> V	0.5	50	4.0	8		11.55	+
9	SiW <sub>11</sub> V	1	50	6.3	8		11.19	+
10	SiW <sub>11</sub> V	1	60	6.3	8		10.97	+
11	SiW <sub>11</sub> V	1	60	6.3	24		10.05	–
12	SiW <sub>11</sub> V	4.2	60	6.3	24	10.48	8.92	+
13	SiW <sub>11</sub> V	30	60	6.3	24		9.60	–
14 <sup>b</sup>	SiW <sub>11</sub> V	30	60	6.3	24	10.24	9.29	–
15 <sup>c</sup>	SiW <sub>11</sub> V	4.2	60	6.3	8	11.72	10.33	+
16	SiW <sub>11</sub> V	4.2	60	6.3	8	11.21	9.75	+
17	HOBT	5	60	4.0	8	11.30	9.28	–
18	HOBT	5	60	6.0	8	10.41	8.13	Traces

<sup>a</sup> Reaction conditions: 5% consistency; oxygen pressure of 5 bar; laccase concentration of 0.65 U/ml. A positive test on the residual laccase activity is designated as “+” and a negative test is designated as “–”.

<sup>b</sup> Laccase concentration of 2.0 U/ml.

<sup>c</sup> Atmospheric pressure (oxygen).

Table 3  
Delignification of softwood kraft pulp catalyzed by the SiW<sub>11</sub>V-laccase system<sup>a</sup>

Experiment	[POM] (mM)	Laccase (U/ml)	$\kappa$ number after PL	$\kappa$ number after PL-E	Residual laccase activity
1	4.2	0.65	26.07	23.97	+
2	8.4	0.65	24.96	22.46	–
3	8.4	1.3	24.81	22.25	–
4	4.2	0 <sup>b</sup>	27.22	25.36	w/o

<sup>a</sup> Reaction conditions: 5% consistency; 60 °C; pH 6.3; oxygen pressure of 5 bar; 8 h. A positive test on the residual laccase activity is designated as “+” and a negative test is designated as “–”; w/o are experiments without laccase.

<sup>b</sup> Not available.

the residual lignin. Following this strategy, a few POMs with relatively high oxidation potentials, such as [SiW<sub>11</sub>V<sub>1</sub>O<sub>40</sub>]<sup>5–</sup> (or simply SiW<sub>11</sub>V), [SiW<sub>10</sub>V<sub>2</sub>O<sub>40</sub>]<sup>6–</sup> (or SiW<sub>10</sub>V<sub>2</sub>) and [PW<sub>11</sub>V<sub>1</sub>O<sub>40</sub>]<sup>4–</sup> (or PW<sub>11</sub>V) were tested (Table 2). Experiments carried out with these POMs in the absence of laccase under different reaction conditions showed that they could only slightly delignify the pulp. The  $\kappa$  number of pulps after POM-E treatment was about 11.7–11.9 and did not depend notably on the concentration of POMs (0.5–4.2 mM), reaction temperature (50–60 °C) and time (8–24 h) (results not shown). The reason for the poor delignification is obviously the impossibility of POM re-oxidation under the reaction conditions. Introduction of laccase in the reaction system notably increased the degree of delignification (Table 2). Thus, it can be concluded that under the reaction conditions used these POMs are able to oxidize the residual lignin in pulp, and their reduced forms can be in turn re-oxidized by laccase.

To avoid laccase deactivation, the delignification was carried out at pH close to neutral, except for experiments with PW<sub>11</sub>V, since this POM is not stable at neutral pH [13]. This creates some limitation for the use of PW<sub>11</sub>V since the reaction temperature in experiments at pH 4 cannot be increased as much as those at neutral pH without laccase deactivation. For example, the degree of delignification at 50 °C is almost the same in the experiments with SiW<sub>11</sub>V and PW<sub>11</sub>V at pH 6.3 and 4.0, respectively (Table 2, experiment 1 versus 9). However, an increase in the reaction temperature to 60 °C results in increase in the degree of delignification in experiments with SiW<sub>11</sub>V, but not with PW<sub>11</sub>V (Table 2, experiment 2 versus 10) since no laccase activity was detected after 8 h of reaction at pH 4. Among the POMs investigated,

SiW<sub>11</sub>V showed the best results at the POM concentration of 4.2 mM (Table 2, experiments 3, 6 and 16). The efficiency of PW<sub>11</sub>V was slightly lower than that of SiW<sub>11</sub>V, and the degree of delignification was the lowest in the SiW<sub>10</sub>V<sub>2</sub>-laccase system. Interestingly, at POM concentration of 1 mM SiW<sub>10</sub>V<sub>2</sub> has shown the best results among these three POMs (Table 2, experiment 2 versus 4 and 10; experiment 5 versus 11). However, an increase in the SiW<sub>10</sub>V<sub>2</sub> concentration from 1.0 to 4.2 mM does not increase the degree of delignification (Table 2, experiment 4 versus 6) in contrast to delignification with SiW<sub>11</sub>V (Table 2, experiment 10 versus 16) and PW<sub>11</sub>V (Table 2, experiment 2 versus 3). Since no residual laccase activity has been detected in experiment 6 in contrast to the experiments with SiW<sub>11</sub>V and PW<sub>11</sub>V under the same reaction condition, it is likely that deactivation of laccase in the presence of SiW<sub>10</sub>V<sub>2</sub> is more significant than in the presence of the two other POMs, and the optimum concentration of this POM is lower.

### 3.2. Effect of the process variables on the pulp delignification catalyzed with SiW<sub>11</sub>V-laccase system

#### 3.2.1. Kinetics of the delignification

Since the maximum degree of delignification, among the POMs tested, has been achieved in the SiW<sub>11</sub>V-laccase system, further studies were conducted with this POM. SiW<sub>11</sub>V has relatively high  $E_p$  (about +0.65 V), which remains constant in a wide range of pH (2–8) [6]. Previously, good results have been reported in pulp delignification with SiW<sub>11</sub>V at 50 °C and pH close to neutral [6]. However, the main problem, which had not been overcome, was the very difficult re-oxidation of the reduced form of SiW<sub>11</sub>V after lignin oxidation. In contrast, our results show

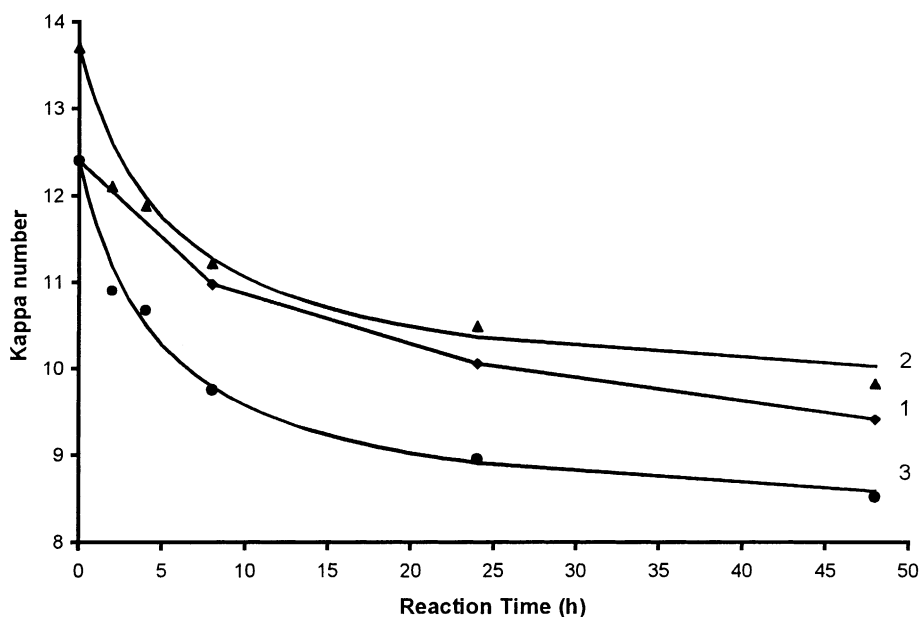


Fig. 1. Kinetics of eucalypt kraft pulp delignification with the  $\text{SiW}_{11}\text{V}$ -laccase system at POM concentrations of 1 mM (curve 1) and 4.2 mM (curves 2 and 3) after PL (curve 2) and PL-E stage (curves 1, 3). Curves 2 and 3 were calculated according to the kinetic parameters obtained. Reaction conditions: 5% pulp consistency; 60 °C; pH 6.3; 0.65 U/ml laccase; 0.5 MPa oxygen pressure.

that laccase can re-oxidize  $\text{SiW}_{11}\text{V}$  very easily, in 1–2 min at room temperature.

Application of the  $\text{SiW}_{11}\text{V}$ -laccase system has showed that a selective delignification of eucalypt kraft pulp could be achieved though the reaction rate was relatively low (Table 2 and Fig. 1).

The alkaline extraction (E) of pulps after the (PL) treatment additionally decreases  $\kappa$  number in about one unit. The maximum degree of delignification was close to 40%. The kinetics of the pulp delignification (Fig. 1) shows that only a part of the residual lignin ( $\kappa_0 - \kappa_\infty$ ) can be removed from the pulp under the conditions employed. The kinetics of  $\kappa$  number reduction formally follows a second-order reaction law with reaction constants ( $k$ ) of  $1.36 \times 10^{-5} \kappa^{-1} \text{ s}^{-1}$  and delignification limit ( $\kappa_\infty$ ) of 9.6 and 8.2 for the PL and PL-E stages, respectively.

### 3.2.2. Effect of temperature

Results of pulp delignification in the  $\text{SiW}_{11}\text{V}$ -laccase system show that an increase in the reaction temperature from 50 to 60 °C notably increases the degree of delignification (Fig. 2) due to increase in

the reaction rate between the POM and lignin. However, further increase in the reaction temperature to 70 °C decreases the degree of delignification because the rate of laccase deactivation increases. After 8 h of reaction at 70 °C, the reaction solution does not show any laccase activity and has blue color characteristic for the reduced form of the POM. Deactivation of laccase does not allow POM re-oxidation and consequently terminates the delignification. Thus, the reaction temperature appears to be one of the crucial factors in the POM-laccase catalyzed delignification. On the one hand, the reaction of  $\text{SiW}_{11}\text{V}$  with lignin requires elevated temperature to occur with a rate acceptable for practical application. On the other hand, the reaction temperature is limited by laccase stability. One of the possible solutions could be engineering of a thermostable laccase to increase the temperature of the delignification. In this case, the optimal laccase concentration could be also decreased.

### 3.2.3. Effect of oxygen pressure

Solomon et al. [14] have shown that the rate of laccase re-oxidation with dioxygen is very fast. More-

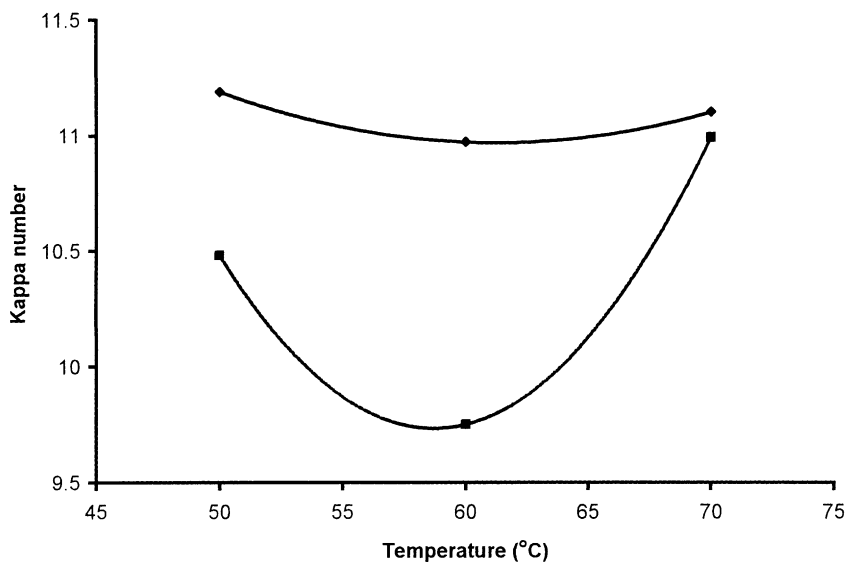


Fig. 2. Effect of the reaction temperature on the delignification of eucalyptus kraft pulp at concentration of SiW<sub>11</sub>V of 1 mM (◆) and 4.2 mM (■). Reaction conditions: pH 6.3, 0.65 U/ml laccase concentration, 8 h.

over, as it has been mentioned above, the rate of POM re-oxidation by laccase is also rather high as compared with the rate of delignification, which is supposed to be the rate-determining step of the process. Therefore, the dioxygen concentration (or correspondingly the oxygen pressure in the gas phase) should not affect the rate of delignification since dioxygen does not participate in the rate-determining step. However, the experimental results show that the use of pressurized oxygen increases the degree of delignification (Table 2, experiments 9 and 10). One of the possible reasons of the unexpected results could be the better reagent diffusion at higher oxygen pressure, similarly to the effect of oxygen pressure on the reaction rate in pulp delignification with the LMS [15].

### 3.2.4. Effect of pH

In acetate buffer solution at pH 4, laccase is less stable than at neutral pH and does not survive during the reaction time of 8 h at 60 °C. Therefore, the effect of pH on the delignification efficiency was studied at higher pH values, in the range of 5.3–7.0. An increase in pH from 5.4 to 6.3 significantly increases the degree of delignification (Fig. 3). However, further increase in pH to 7.0 results in a slight decrease in the delignification degree.

### 3.2.5. Effect of the concentration of POM and laccase

The increase in the SiW<sub>11</sub>V concentration from 0.1 to 4.2 mM appreciably increases the degree of delignification (Table 2, experiment 7 versus 8, and Fig. 1). Further increase in the POM concentration to 30 mM resulted in increase in  $\kappa$  number (Table 2, experiment 12 versus 13). Apparently, high amount of SiW<sub>11</sub>V results in complete laccase deactivation because no laccase activity was detected after the reaction. Increase in the laccase concentration three times, from 0.65 to 2.0 U/ml, at the SiW<sub>11</sub>V concentration of 30 mM increases the degree of delignification, but, anyway, it is lower than in the experiment at the SiW<sub>11</sub>V concentration of 4.2 mM (Table 2, experiment 14 versus 12 and 13). Thus, the degree of delignification cannot be increased by the increase in the POM concentration over the optimal value, at least without significant increase in the laccase concentration.

### 3.2.6. Delignification of soft- and hardwood kraft pulps

Delignification of softwood kraft pulp with the SiW<sub>11</sub>V-laccase system occurs more difficult than with eucalypt pulp since the degree of delignification (taking in account the control experiment) of the softwood pulp is lower than that for the eucalyptus pulp

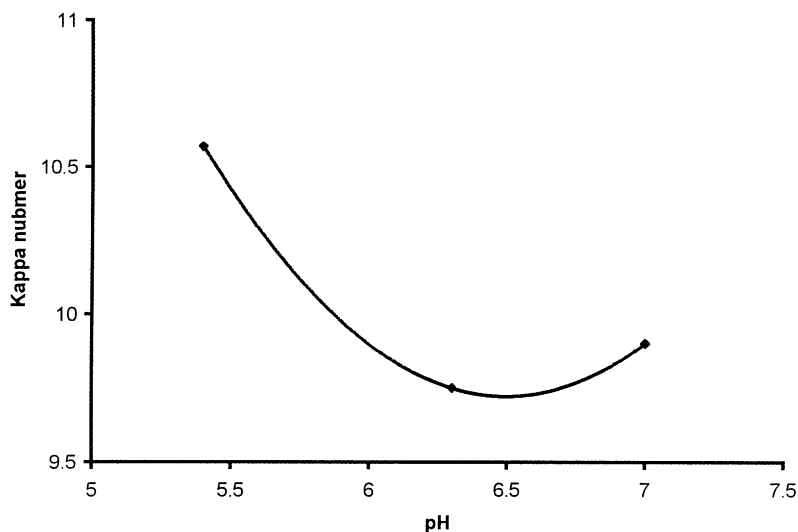


Fig. 3. Effect of pH on the delignification of eucalyptus kraft pulp with the  $\text{SiW}_{11}\text{V}$ -laccase system. Reaction conditions: 60 °C; 4.2 mM  $\text{SiW}_{11}\text{V}$ ; 0.65 U/ml laccase concentration; 8 h.

under the same reaction conditions (Tables 2 and 3). However, in contrast to the delignification of eucalyptus pulp, double increase in the POM concentration from 4.2 to 8.4 mM was rather effective in the delignification of the softwood pulp (Table 3, experiment 1 versus 2), even though it resulted in complete laccase deactivation during the reaction time of 8 h. In contrast, double increase in laccase concentration almost did not affect the delignification (Table 3, experiment 2 versus 3).

One of the reasons of the lower delignification efficiency of softwood pulp compared to eucalyptus one could be the lower reactivity of the softwood residual lignin, compared to eucalyptus lignin, particularly in the POM catalyzed delignification [16]. The POM-laccase system provides removal of approximately the same amount of residual lignin (in absolute values) from pulps with different  $\kappa$  number. However, the delignification degree is higher in the case of pulp with lower  $\kappa$  number. Since, generally, residual delignification of pulps with low  $\kappa$  number is rather difficult, this suggests that a more reasonable application of the POM-laccase system would be in the delignification of semi-bleached pulps taking into account the high selectivity of this system (see the next section).

### 3.3. Pulp viscosity

As it was mentioned above, the delignification/bleaching of pulp must be not only efficient but also selective, that is the reaction system should not appreciably decompose polysaccharides during the removal of lignin. The stability of polysaccharide molecules to depolymerization is estimated via viscosity of pulp in cupriethylenediamine solution. The results obtained show that the viscosity of the eucalypt pulps after the delignification is high enough when  $\text{SiW}_{11}\text{V}$ ,  $\text{SiW}_{10}\text{V}_2$  and  $\text{PW}_{11}\text{V}$  are used, i.e. the delignification of the pulp catalyzed by the POM-laccase system is rather selective (Fig. 4). A linear correlation between viscosity and  $\kappa$  number of pulps has been found for all experiments with these POMs. It means that the selectivity of the delignification does not depend significantly on the reaction conditions (kind of POMs, reaction time, reaction temperature, pH) used in this research.

In contrast, delignification of the pulp with  $\text{PMo}_7\text{V}_5$  and  $\text{PMo}_{11}\text{V}$  (60 °C, POM concentration of 4.2 mM, pH 1.6–2.65) results in a strong viscosity drop (Fig. 4). This can be explained by acidic hydrolysis of polysaccharides in the reaction solution with rather low pH value.



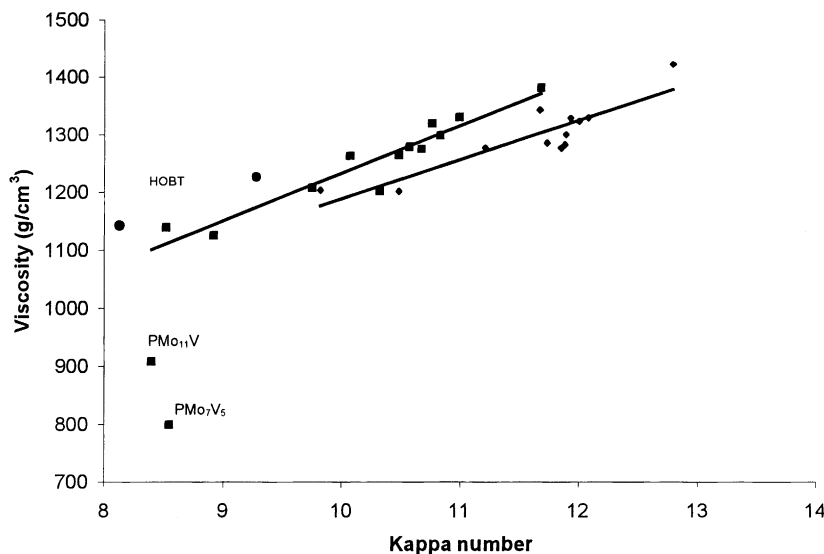


Fig. 4. Correlation between  $\kappa$  number and viscosity of eucalyptus kraft pulps after PL stage ( $\blacklozenge$ ), PL-E stages ( $\blacksquare$ ) and HOBT-E treatment ( $\bullet$ ).

### 3.4. POM versus HOBT

For comparison, the experiments on the pulp delignification with HOBT-laccase were conducted at pH 4 commonly used in the LMS [2,3] and pH 6 used in our experiments with the POM-laccase system. The HOBT-laccase system is more effective so far in terms of the delignification rate and the pulp viscosity than the POM-laccase systems (Table 2 and Fig. 4). In addition, the reaction temperature used (60 °C) is probably not optimal for the HOBT-laccase system and better delignification results might be obtained at a lower temperature [3]. However, as it has been mentioned above, HOBT is decomposed during the reaction, and the reaction solution cannot be recycled. It makes the use of suitable POMs more attractive in terms of process cost if an acceptable delignification rate can be achieved.

In spite of the apparent similarities between the POM-laccase system and the LMS, the mechanism of lignin oxidation in these two systems appears to be different. In the LMS, non-phenolic lignin moieties are believed to be responsible for the lignin degradation [17]. In spite of the high reactivity of phenolic lignin moieties, their role in the delignification is not definitely determined yet since they can contribute not

only to fragmentation of the lignin macromolecule but also undergo polymerization [17,18]. In contrast, the oxidation potential of the POMs appears to be not sufficient to oxidize non-phenolic lignin moieties with appreciable rate in the POM-laccase system under mild conditions. Our experiments [12] have shown that SiW<sub>11</sub>V-laccase system cannot catalyze the oxidation of a non-phenolic lignin model compound, veratryl alcohol (60 °C, 2 days), which is readily oxidized by the LMS [8]. The lignin degradation in the POM-laccase system occurs likely via oxidation of phenolic lignin moieties similarly to the oxidation catalyzed by different kinds of POM in the laccase absence under the required conditions [19,20].

### 3.5. Wet oxidation

Since the reaction of the POMs with lignin requires rather high temperature, and the critical temperature for laccase is 60 °C, one of the alternatives to fit these demands could be the separation of the reaction system by oxidation of lignin with a large amount of POM at higher temperature and then re-oxidation of the POM at a temperature suitable for laccase. For this, anaerobic delignification of eucalypt kraft pulp with 30 mM SiW<sub>11</sub>V has been conducted at 125 °C for 2 h. The

$\kappa$  number of pulp after the POM treatment was 11.2 (10.4 after consequent E stage). The solution of the POM after this treatment was submitted to laccase catalyzed oxidation at 60 °C. However, the regeneration of the POM after the reaction also required wet oxidation of soluble organic material. Unfortunately, this process does not go easily and requires rather large amount of laccase. Interestingly, introduction of 3 ml of laccase by small portions (100–300  $\mu$ l) in intervals of a few hours did not result in successful reaction at 60 °C for 1 day. However, introduction of 1.5 ml of laccase at once resulted in regeneration of the POM within 30 min. Probably, an excess of the reduced form of the POM strongly deactivates the enzyme.

The use of immobilized laccase for the wet oxidation and POM regeneration did not allow overcoming these difficulties either.

#### 4. Conclusions

The most important finding of this report is that laccase catalyzes readily the re-oxidation of the reduced forms of different POMs, especially those, which can not be re-oxidized by molecular oxygen even under vigorous conditions. This opens new attractive possibilities to use these POMs for oxidative catalysis of various substrates. Application of the SiW<sub>11</sub>V-laccase system for pulp delignification allowed the removal up to 40% of residual lignin though the reaction rate is relatively low. A possible reaction mechanism could be presented as a sequence of redox cycles resulting electrons transferring from a substrate to oxygen via the POM and laccase. The reaction of the POM with lignin appears to be the rate-determining step of the overall process. Since re-oxidation of the POM catalyzed by laccase is a very fast reaction compared to the rate of the delignification, laccase concentration should not affect the total reaction rate. However, deactivation of laccase under too vigorous reaction conditions (high temperature, low pH, high POM concentration) results in termination of the delignification. The reaction temperature appears to be a crucial factor to achieve a reaction rate reasonable for practical application. Since increase in the reaction temperature is limited by laccase stability, one of the most important directions for future development of the POM-laccase

system is the engineering of a thermostable laccase. In addition, further optimization of the reaction system in terms of the process variables should be done.

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