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Oxygen bleaching of kraft pulp with polyoxometalates and laccase applying a novel multi-stage process

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Abstract

A series of manganese substituted polyoxotungstates, $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$ and $[PW_{11}Mn^{III}(H_2O)O_{39}]^{4-}$, and $[SiW_{11}V^{V}O_{40}]^{5-}$ were applied as catalysts for the oxygen delignification of unbleached eucalypt kraft pulp with laccase of *Trametes versicolor*. Unlike to modest results obtained in the Laccase-Mediator System (LMS) at 45–60 °C (lignin oxidation and catalyst re-oxidation occurred at the same stage), a sustainable delignification with removal of about 50% of residual lignin was achieved with $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$ and $[SiW_{11}V^{V}O_{40}]^{5-}$ when the kraft pulp treatment was carried out with polyoxometalate at 110 °C (lignin oxidation stage) and with laccase at 45 °C (catalyst re-oxidation stage) in separate stages. The use of $[PW_{11}Mn^{III}(H_2O)O_{39}]^{4-}$ in this multi-stage process was limited by the low re-oxidation rate with laccase. The best selectivity on the pulp delignification was found with polyoxoanion $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$, whereas $[SiW_{11}V^{V}O_{40}]^{5-}$ was the most effective in the oxidative delignification. The influence of process factors on the POMs re-oxidation, such as the amount of laccase, oxygen pressure and temperature is discussed. UV–vis and ^{51}V NMR studies indicated that POMs maintained stable after redox turnovers during the pulp delignification.

Keywords: Polyoxometalate; Keggin structure; Laccase; Oxygen delignification; Bleaching; Kraft pulp

1. Introduction

Polyoxometalates (POMs) are well known oxidation catalysts for both homogeneous and heterogeneous organic syntheses [1–3]. In the middle of 1990th POMs were proposed as highly selective renewable reagents or catalysts for the kraft pulp bleaching [4–7]. These were claimed as suitable alternative to chlorine based ecologically hazardous bleaching reagents and to the conventional oxygen alkaline bleaching. Unbleached chemical pulps are produced from wood mostly by kraft pulping process and composed mainly of cellulose (80–90%), hemicelluloses (10–15%) and by a small amount of residual lignin (2.5–4.0%), which is mainly responsible for the undesirable dark colour and photoyellowing of the pulp. The bleaching process is applied to reach the pulp brightness required for the papermaking. In the bleaching procedure lignin should de degraded without significant polysaccharides damage, in order to preserve the physical properties of the bleached pulps.

The feasibility of POM-promoted pulp oxygen bleaching was recently confirmed in semi-industrial scale experiments [8]. Applied as catalysts under aerobic conditions, POM oxidises the residual lignin in kraft pulp, and the reduced form of POM is re-oxidised by molecular oxygen at the same process stage [7,8]. The redox turnover of POM is possible when the common thermodynamic condition is fulfilled:

$$E^{\circ}(\text{Lignin}) < E^{\circ}(\text{POM}) < E^{\circ}(O_2) \quad (1)$$

Several POMs were used for the catalytic oxygen delignification, such as α-$[PMo_{12-x}V_xO_{40}]^{(3+x)-}$ ($x = 2$–5), α-$[XW_{11}V^{V}O_{40}]^{n-}$ (X = P, Si, B) and α-$[XW_{11}Mn^{III}(H_2O)O_{39}]^{p-}$ (X = P, Si, B) among others [4–10]. Although most of POMs were effective in the catalytic pulp delignification, they were generally applied under acidic conditions provoking undesirable polysaccharide degradation via the hydrol-

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ysis or by oxidation with species dissociated from catalysts [7,8]. This was particularly dramatic when the experiments were carried out at a very low pH such as those with the α-$[PW_{11}V^{V}O_{40}]^{4-}$ polyoxoanion [4], that is only stable for pH < 2.5 [11,12]. The preservation of pulp polysaccharides was improved by carrying out the experiments under moderate pH (4.0–7.0) with the appropriate POMs. An interesting approach has been proposed using the self-buffering solution of aluminovanadotungstate heteropolyanion [13]. However, some of POMs stable in pH 4-7 aqueous solutions and possessing considerably high $M^{(n+1)/n}$ redox potentials (E = +0.7–0.8 V versus NHE) are hardly re-oxidised by dioxygen [4,10]. This fact limits the application of the later in the oxygen delignification catalysis. This problem can be solved by employing biocatalytic re-oxidation of POMs with laccase [14].

This enzyme belonging to oxidase family (*p*-diphenol:oxygen oxidoreductase, EC 1.10.3.2) possesses the multi-copper prosthetic group composed by four copper (II) atoms (one T1 copper and a T2/T3 trinuclear cluster) and typically perform one-electron oxidations of phenolic substrates with concomitant re-oxidation of the formed copper(I) centres by molecular oxygen [15,16]. Laccase was found to be a suitable catalyst for the oxidation of several reduced POMs under aerobic conditions even at room temperature and has been proposed for the oxidative catalytic delignification [14]. POMs have been applied as inorganic redox mediators in the Laccase-Mediator System (LMS) for the pulp delignification [14,17]. In the catalytic cycle, POM oxidises the residual lignin in pulp and the reduced POM is re-oxidised by laccase at the same stage. The significant difference in the optimal conditions required for the kraft pulp delignification with POM (90–110 °C) and for the re-oxidation of reduced POM with laccase (30–45 °C) caused a limited pulp delignification degree at compromise temperature (around 60 °C) due to the poor lignin oxidation with POM and to the laccase denaturising [14]. An alternative multi-stage process was proposed using the alterative pulp treatment with POM at high temperature (100–110 °C) followed by the POM re-oxidation with laccase at 30–60 °C in a separate stage [17].

In this paper, the comparative results of the use of manganese and vanadium substituted polyoxotungstates, $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$ ($SiW_{11}Mn$), $[PW_{11}Mn^{III}(H_2O)O_{39}]^{4-}$ ($PW_{11}Mn$) and $[SiW_{11}V^{V}O_{40}]^{5-}$ ($SiW_{11}V$), with laccase of *Trametes versicolor* for the catalytic oxygen delignification of kraft pulp are discussed. The efficiency and the selectivity of pulp delignification with POM and laccase in the LMS and in the multi-stage process are compared.

2. Experimental

2.1. Reagents and methods

The bleaching experiments were carried out with industrial *Eucalyptus globulus* kraft pulp supplied by PORTUCEL (Portugal). The kraft pulp has a kappa number (index related to the amount of residual lignin in pulp) of 12.3 and an intrinsic viscosity (index related to the molecular weight of polysaccharides) of 1185 cm^3/g.

The laccase was produced from white-rot fungi *Trametes versicolor* as previously reported [17]. Laccase activities were measured at 40 °C using 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [18]. No peroxidase activity was detected (test with veratryl alcohol in the presence of H_2O_2).

α-$K_6[SiW_{11}Mn^{II}(H_2O)O_{39}]\cdot 12H_2O$, α-$K_5[PW_{11}Mn^{II}(H_2O)O_{39}]\cdot 12H_2O$ and α-$K_6[SiW_{11}V^{IV}O_{40}]\cdot 6H_2O$ were prepared following the known procedures [11,12,19,20]. Aqueous solutions of $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$ and $[PW_{11}Mn^{III}(H_2O)O_{39}]^{4-}$ were prepared using a method described in literature for the synthesis of its potassium salts, excluding the precipitation step [19]. A solution of $[SiW_{11}V^{V}O_{40}]^{5-}$ anion was prepared by oxidation of α-$K_6[SiW_{11}V^{IV}O_{40}]\cdot 6H_2O$ in water with the equivalent quantity of Br_2 [12]. The purity of these POMs was confirmed by UV–vis and ^{51}V NMR in the case of $[SiW_{11}V^{V}O_{40}]^{5-}$ anion.

Elemental analyses of W, Mn, V and K were performed by ICP. Thermogravimetric analyses were carried out under aerobic conditions between 30 and 700 °C at a heating rate of 5 °C/min, on a Mettler M3 thermobalance equipped with a TC 10A microprocessor. Electronic absorption spectra (UV–vis) were recorded on a Jasco V-560 spectrophotometer.

^{51}V NMR spectra were recorded at 105.2 MHz on a Brucker MSL 400 (9.4 T) spectrometer (298 K). A 45° pulse and a recycle delay of 5 s were used in all experiments. Chemical shifts are referenced against $VOCl_3$ using ammonium metavanadate (NH_4VO_3, $\delta = -576$ ppm at 9.4 T) as a secondary reference.

Cyclic voltammetry measurements ($v = 10$ mV/s) were carried out on a BAS 100BW electrochemical analyser using a conventional three-electrode cell supplied by BAS Inc. The working electrode was a glassy carbon disc electrode (BAS). The reference and auxiliary electrodes were saturated Ag/AgCl and Pt wire, respectively. The measurements were made at room temperature (ca. 25 °C). Solutions were purged with nitrogen for 5 min before the measurements and blanketed with nitrogen gas during the voltammetric scans. Solutions of polyoxometalate (1.0×10^{-3} mol/l) in KH_2PO_4 buffered at pH = 4.5 (0.5 mol/l) were used.

2.2. Reaction of POMs with laccase monitored by UV–vis spectroscopy

Studies on the oxidation of reduced polyoxotungstates by laccase were performed at room temperature under stirring in an Erlenmeyer or under oxygen pressure of 0.3 MPa with mechanical stirring in a PARR reactor model 4842 (0.25 l) equipped with an automatic temperature control system: [POM] = 3.0 mmol/l; [laccase] = 380 U/l or 1330 U/l; 0.1 mol/l phosphate buffer at pH = 4.5. Aliquots of the POM-laccase reaction mixture were sampled for selected periods of time and analysed by UV–vis immediately.

2.3. Bleaching experiments

The bleaching experiments were carried out in a PARR reactor model 4842 (0.25 l) equipped with an automatic temperature control system, pressure control and mechanical stirring. Around 7.0 g of unbleached kraft pulp (o.d. weight) were mixed with 113 ml of phosphate buffer solution (pH = 4.5) containing 3.6–4.0 mmol/l of polyoxometalate. In the multi-stage process the first bleaching stage was performed at 110 °C and $P°(O_2) = 0.6$ MPa; the second bleaching stage (with laccase) was performed at 30–60 °C and $P°(O_2) = 0.3$ MPa. The reactor after the first bleaching stage with heteropolyanion (without laccase) was quickly cooled to the desired temperature, degasified and the laccase solution (380 U/l) was added. The second bleaching stage continued after the oxygen supply. After each bleaching sequence the alkaline extraction of pulp was carried out at 70 °C during 1 h and NaOH charge of 2% o.d. pulp. Finally, the bleached pulp was filtered off and washed with hot water, until neutral reaction on filtrate.

The pulps were characterised using the TAPPI T236 cm—85 standard for kappa number and the SCAN-CM 15:88 standard for intrinsic viscosity.

3. Results and discussion

3.1. Oxidation of polyoxometalates by laccase

Manganese substituted polyoxotungstates $[SiW_{11}Mn^{III}(H_2O)O_{39}]^{5-}$, $[PW_{11}Mn^{III}(H_2O)O_{39}]^{4-}$), and the vanadium polyoxoanion $[SiW_{11}V^{V}O_{40}]^{5-}$ were previously suggested as perspective catalysts for the oxygen delignification of kraft pulps [4,10]. These POMs, possessing the redox potentials in the range +0.7 to 0.9 V, fulfil the formal thermodynamic conditions for the oxidative catalysis (Eq. (1)). However, these POMs are hardly re-oxidised by molecular oxygen even at high (>150 °C) temperatures [4,10]. The possibility of $SiW_{11}V^{IV}$ [13] and $SiW_{11}Mn^{II}$ [17] re-oxidation by laccase under aerobic conditions was demonstrated and these polyoxoanions were tested as mediators in the laccase-promoted oxygen delignification. The facility of POMs re-oxidation with laccase was suggested to be one of the crucial factors determined the sustainable oxygen delignification. In this context, the comparative study on the oxidation of $SiW_{11}V^{IV}$, $SiW_{11}Mn^{II}$ and $PW_{11}Mn^{II}$ with laccase has been carried out aiming to found a relationship between the POMs re-oxidation and their efficiency in the delignification catalysis. Additionally, the stability of catalysts under the re-oxidation stage was evaluated.

The redox turnover of POMs was monitored using UV–vis spectroscopy. Thus, the yellow colour solution of $SiW_{11}Mn^{II}$ changes gradually during the oxidation with laccase to pink solution of $SiW_{11}Mn^{III}$ [19] showing a d–d* transition band with maximum at 490–495 nm in the visible spectrum (Fig. 1A). Similar behaviour was observed for $PW_{11}Mn^{II}$ (spectra not shown). Under similar conditions, the dark-purple solution of $SiW_{11}V^{IV}$ changes to yellow colour solution with the gradual disappearance of the characteristic band with maximum at around 490 nm in the visible spectra (Fig. 1B) indicating the presence of $SiW_{11}V^{V}$ [21]. The oxidation of $SiW_{11}V^{IV}$ to $SiW_{11}V^{V}$ was additionally monitored by ^{51}V NMR spectroscopy confirming the authenticity of the α-$SiW_{11}V^{V}$ Keggin-type structure (Fig. 2) [11,12].

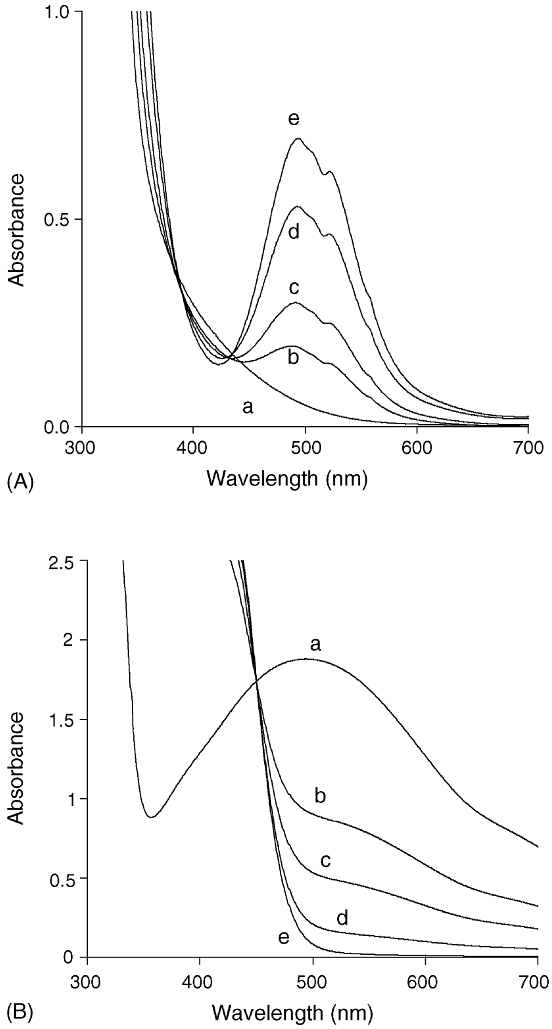

Fig. 1. UV–vis spectra of 3.0 mmol/l aqueous solutions of $SiW_{11}Mn^{II}$ (A) and $SiW_{11}V^{IV}$ (B) at pH 4.5 before (a) and after the addition of laccase (380 U/l) at room temperature and atmospheric pressure: A- 4 h (b); 8 h (c); 24 h (d); 50 h (e); B- 15 min, (b); 30 min, (c); 60 min, (d); 120 min, (e).

Under the same reaction conditions ($T = 25$ °C, pH 4.5, POM concentration 3.0 mmol/l; laccase concentration 380 U/l) the three POMs showed distinct re-oxidation behaviour with laccase (Table 1). Thus, only 5% of $PW_{11}Mn^{II}$ was oxidised after 24 h reaction with laccase, whereas about 55% of $SiW_{11}Mn^{II}$ was oxidised for the same period. At the same time, more than 50% of $SiW_{11}V$ was oxidised just after

Table 1
Oxidation of polyoxometalates by laccase under aerobic conditions[a]

POM	Laccase concentration (U/l)	Oxygen pressure (MPa)	Temperature (°C)	POM oxidised (%)
$SiW_{11}Mn^{II}$	380	∼0.02	25	16 (55)[b]
$SiW_{11}Mn^{II}$	1330	∼0.02	25	35 (84)[b]
$SiW_{11}Mn^{II}$	1330	0.3	25	48 (88)[b]
$SiW_{11}Mn^{II}$	1330	0.3	45	52 (95)[b]
$SiW_{11}Mn^{II}$	380	0.3	45	36 (69)[b]
$SiW_{11}V^{IV}$	380	∼0.02	25	52 (72)[c]
$SiW_{11}V^{IV}$	1330	∼0.02	25	82 (96)[c]
$PW_{11}Mn^{II}$	380	∼0.02	25	<1 (5)[b]

[a] All experiences were performed with [POM] = 3.0 ± 0.1 mmol/l; in phosphate buffer (0.1 mol/l), pH = 4.5.

[b] Measurements after 4 h or after 24 h (data in parentheses) of the reaction.

[c] Measurements after 15 min or after 30 min (in parentheses) of the reaction.

15 min of the reaction (Table 1). The complete re-oxidation of $SiW_{11}V^{IV}$ was achieved in 2 h at room temperature under the same experimental conditions (Fig. 1B). Hence, the oxidation rate of POMs by laccase should be ordered as follows: $PW_{11}Mn^{II} < SiW_{11}Mn^{II} < SiW_{11}V^{IV}$. The hard oxidation of $PW_{11}Mn^{II}$ with laccase is consistent with the considerably high redox potential of this polyanion (E = +0.91 V versus NHE) [22]. Since the redox potential of laccase is around +0.8 to 0.9 V (versus NHE) [15,16], this POM should be hardly re-oxidised by the enzyme. At the same time, the significant differences in the oxidation behaviour exhibited by $SiW_{11}Mn^{II}$ and $SiW_{11}V^{IV}$ cannot be formally explained by their effective redox potentials, which are very close. The effective redox potential ($E = (E_{pc} + E_{pa})/2$) of the redox couple $V^{V/IV}$ in $SiW_{11}V$ has been measured at +0.64 V (versus NHE, pH = 5) [21] and, in another study, at +0.72 V (versus NHE at pH = 4.5) [23]. Recently, Weinstock with co-workers reported E-value of +0.69 V at pH 5 [4]. Similarly, the redox potential of couple $Mn^{III/II}$ in $SiW_{11}Mn$ has been measured at +0.65 V (versus NHE) [19] and, more recently, as +0.70 to 0.73 V (versus NHE) [22,24,25]. Therefore, a comparison of the redox potentials of these two anions is not straightforward. Moreover, it is known that the redox potentials of polyoxoanions may be very sensitive to factors such as the pH, the counter-cations and others [26].

The differences in the redox behaviour of the $SiW_{11}Mn^{II}$ and $SiW_{11}V^{IV}$ anions were evaluated by cyclic voltammetry under conditions similar to those applied at the delignification experiments (Fig. 3). It was observed that the couple $V^{V/IV}$ in $SiW_{11}V^{IV}$ is electrochemically reversible (Fig. 3a) possessing E = +0.67 V (E_{pa} = +0.70 V, E_{pc} = +0.64 V, $E_{pa} - E_{pc}$ = 60 mV). The corresponding $Mn^{III/II}$ couple in $SiW_{11}Mn$ is quasi-reversible (Fig. 3b) possessing the potential E = +0.76 V with E_{pa} = +0.88 V, E_{pc} = +0.64 V, and E_{pa}–E_{pc} = 240 mV (potentials *vs.* NHE). Therefore, the easier oxidation of $SiW_{11}V^{IV}$ by laccase is related with the higher reversibility and the lower oxidation peak potential (E_{pa}) of the vanadium couple when compared to the manganese one. The kinetic inertness of the $Mn^{III/II}$ couple has been referred to several Mn complexes [27].

The rate of the POM oxidation is dependent on the amount of laccase, the oxygen pressure and temperature of the reaction medium (Table 1). Overall, the increase of the laccase concentration and the oxygen pressure (up to 0.3 MPa)

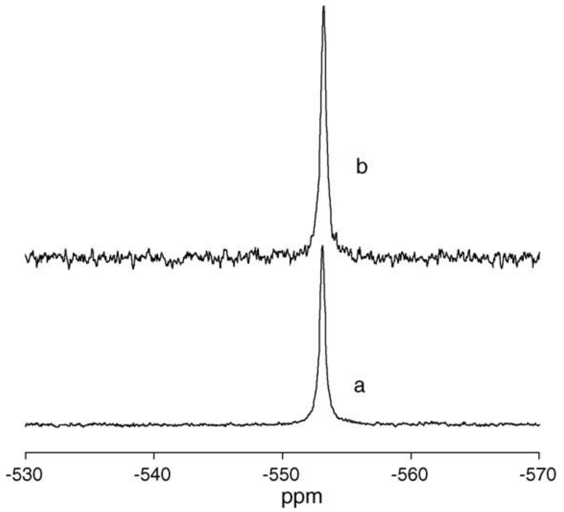

Fig. 2. ^{51}V NMR spectra of authentic α-$SiW_{11}V^{V}$ aqueous solution (a) and the solution obtained after the reaction of α-$SiW_{11}V^{IV}$ (3.0 mmol/l) with laccase (b).

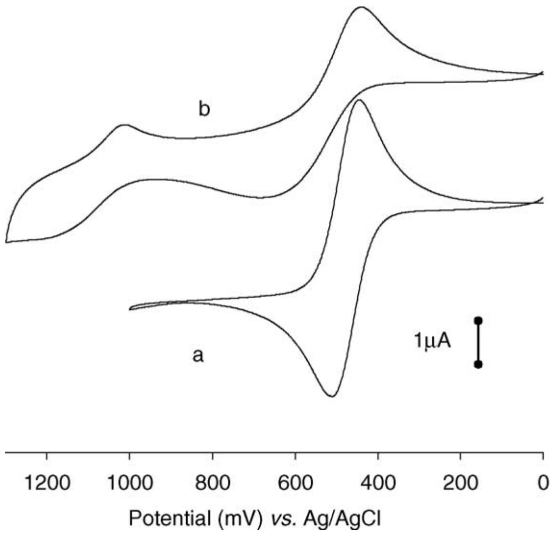

Fig. 3. Cyclic voltammetric plot of α-$SiW_{11}V^{IV}$ (a) and α-$SiW_{11}Mn^{II}$ (b) ([POM] = 1.0 mmol/l, pH = 4.5, 25 °C).

Table 2
Results on eucalypt kraft pulp oxygen delignification catalysed by laccase in the presence of polyoxometalates as inorganic mediators

Bleaching system	Kappa number	Intrinsic viscosity (cm^3/g)	Delignification degree (%)	Intrinsic viscosity loss (%)	POM oxidised (%)
Kraft pulp (control)	12.3	1185	–	–	–
Laccase	10.9	1170	11	1	–
Laccase*	11.1	1155	10	2	–
$SiW_{11}Mn^{III}$/Laccase	10.3	1155	16	2	47
$SiW_{11}Mn^{III}$/Laccase*	10.5	1120	15	5	33
$PW_{11}Mn^{III}$/Laccase	10.2	1160	17	2	4
$PW_{11}Mn^{III}$/Laccase*	10.7	1135	13	4	3
$SiW_{11}V^{V}$/Laccase	10.7	1145	13	3	100
$SiW_{11}V^{V}$/Laccase*	10.4	1130	15	4	79

Experimental conditions: pulp consistency 6.0%; $[XW_{11}Mn^{III}] = 4.0$ mmol/l; laccase (380 U/l); pH 4.5; $P°(O_2) = 0.3$ MPa; 45 °C (* denotes 60 °C); 4 h.

favoured the re-oxidation of POMs. The temperature had a moderate effect on the oxidation reactions. The maximum reaction temperature was limited to 45 °C in these experiments since the diminishing of the laccase activity at temperatures >50 °C was detected. The amount of added laccase had more pronounced effect on the oxidation of $SiW_{11}Mn^{II}$ than on $SiW_{11}V^{IV}$. Roughly, the increase of laccase concentration around 3.5 times originated more than twice conversion of $SiW_{11}Mn^{II}$, whereas the conversion of $SiW_{11}V^{IV}$ increased only around 50% (Table 1).

3.2. *Oxygen bleaching of kraft pulp using polyoxometalates as mediators*

The three POMs were tested as mediators under similar conditions in the aerobic eucalypt kraft pulp delignification with laccase (Table 2). The delignification conditions were selected taking into consideration the stability/activity of laccase and POMs discussed previously [14,17]. The reaction time was limited to 4 h, because the pulp delignification in LMS, following the second order kinetics, is rather slow after 4–6 h [14]. All POMs showed catalytic activity in LMS (Table 2). However, the poor delignification observed did not allow distinguishing the performance of the different polyoxotungstates as mediators.

The pulp delignification with $SiW_{11}Mn^{III}$ and $PW_{11}Mn^{III}$ was faster at 45 °C than at 60 °C reflecting the partial laccase denaturation at raised temperature. The lower catalysts turnover after the treatment at 60 °C than at 45 °C also supports such a proposition. In contrast, the better delignification results with $SiW_{11}V^{V}$ were obtained at 60 °C, which is in agreement with previous results obtained on the pulp delignification in LMS [14]. This fact may indicate that the temperature dependence on the $SiW_{11}V^{IV}$ oxidation prevailed upon the diminishing of laccase activity at raised temperature.

It is noteworthy that the re-oxidation of $SiW_{11}Mn^{III}$ and $PW_{11}Mn^{III}$ was faster in the presence of kraft pulp (Table 2) than in the reference experiments without pulp (Table 1). This is especially surprising taking into account that POMs are constantly reduced in the reactions with residual lignin and lignin fragments removed in the solution. One of possible explanations might be a fact of laccase stabilisation/activation with phenolic compounds removed from the partially degraded residual lignin. Mai with co-workers reported that the presence of phenolic compounds or the lignin derivatives enhance the stability and the activity of laccase [28]. Authors suggested that this stabilization effect could be due to binding of phenolics to the active centre of the enzyme or to another part of the protein chain.

In spite of the high selectivity of pulp delignification in LMS (defined as the ratio of the delignification degree and the percentage of the intrinsic viscosity loss in pulp) the relatively low degree of the delignification indicates the low efficiency of this catalytic system (Table 2). It can be suggested that in the case of $SiW_{11}V^{V}$ (and possibly with $SiW_{11}Mn^{III}$) the delignification rate is limited by POM reactivity with substrate rather than by POM re-oxidation with laccase.

3.3. *Oxygen bleaching of kraft pulp using polyoxometalates and laccase in multi-stage process*

Aiming to improve the pulp delignification efficiency with POM and laccase, these two catalysts were applied in a multi-stage process. This approach consists in an alterative pulp treatment with POM (POM stage) and with laccase (L stage) in separate steps at different temperatures.

It should be noted that unlike in LMS, in the POM stage of multi-stage process carried out at relatively high temperature and weakly acidic conditions the removal of hexeneuronic acid residues (HexA) also contribute to the decrease of a kappa number of pulp. HexA residues are derived from 4-*O*-methylglucuronic acid residues of xylan during the kraft pulping and contribute of around 3–4 units to the total kappa number of pulp [29]. However, regarding the kappa number decrease under the conditions of POM stage, the contribution of HexA is of minor importance comparing to those of the lignin oxidation.

The POM stage with $SiW_{11}Mn^{III}$ showed pronounced delignification results (Table 3). At the end of the first stage $SiW_{11}Mn$ was found predominantly in the reduced state (Fig. 4). After the second stage at 45 °C (laccase added) 68% of $SiW_{11}Mn$ was re-oxidised (Fig. 4), i.e. about 20% more than after POM/L treatment (Table 2) and almost two times more than after the POM re-oxidation by laccase in the absence of pulp (Table 1). Overall, the POM-L treatment

Table 3
Results on the eucalypt kraft pulp delignification catalysed by $SiW_{11}Mn^{III}$ (POM stage) and laccase (L stage)

Bleaching system	Kappa number	Intrinsic viscosity (cm^3/g)	Delignification degree (%)	Intrinsic viscosity loss (%)	$SiW_{11}Mn^{III}$ oxidised (%)
Kraft pulp	12.3	1185	–	–	–
POM	9.5	1130	23	5	4
POM-L	8.9	1080	28	9	68 (98)[a]
POM-L-POM	6.9	1075	44	9	7
POM-L-POM-L-POM	6.0	1020	52	14	11
POM-L (30 °C)-POM	7.1	1065	42	10	–
POM-L (60 °C)-POM	7.5	1065	39	10	–
$PW_{11}Mn$-L	9.0	1085	27	8	4

Experimental conditions: pulp consistency 6.0%; $[SiW_{11}Mn^{III}] = 3.6$ mmol/l; pH 4.5; $P°(O_2) = 0.3$ MPa; 2 h at 110 °C in POM stage with $SiW_{11}Mn^{III}$ and 4 h at 45 °C in L stage with laccase (except the specified experiments).

[a] The result of measurement after the solution storage for one week at room temperature is presented in parentheses.

originated almost twice the degree of the pulp delignification when compared to the single POM/L stage (Tables 1 and 2) demonstrating, however, less selectivity on the delignification. The loss of the delignification selectivity took place predominantly in the L stage (Table 2). The application of the second POM stage (POM-L-POM sequence) did not reveal the drop in the pulp viscosity when compared to the POM-L sequence (Table 3). It may be proposed that in L stage under aerobic conditions, besides the catalyst re-oxidation, the auto-oxidation of lignin and its phenolic degradation products initiated by one-electron oxidation step with laccase take place [30,31]. The phenoxy radical mesomeric forms participate in propagation reactions reacting with phenolic/non-phenolic substrates [31]. The oxygen-centred radicals formed in auto-oxidation reactions could also be responsible for the pulp polysaccharides depolymerization.

The sequential alternating application of POM and L stages revealed the sustainable highly selective delignification of pulp (Table 3). Thus, in a three-stage bleaching (POM-L-POM) the pulp delignification was 44% with only 9% of viscosity loss (Table 3). After POM-L-POM-L-POM treatment a pulp with kappa number of 6.0 and the intrinsic viscosity of 1020 cm^3/g was obtained (more than 50% of the delignification). The visible spectra of POM solutions after L stages are similar to the spectrum of the initial POM solution before the oxygen delignification (Fig. 4). Hence, the catalyst is stable and can be re-used in several cycles if laccase is applied for its re-oxidation.

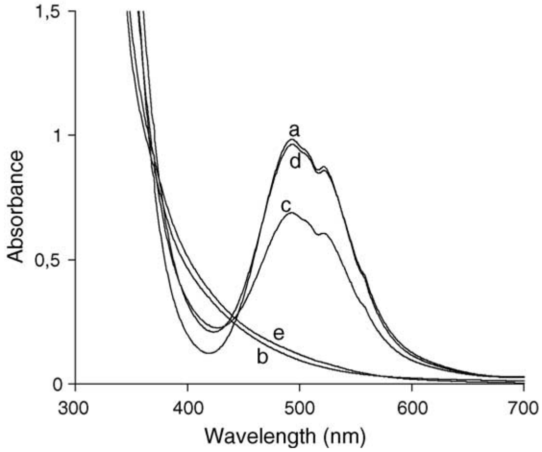

Fig. 4. UV–vis spectra of the $SiW_{11}Mn^{III}$ solution before the delignification (a), after the POM stage (b), after the two-stage POM-L delignification (c) followed by one-week storage of the effluent solution on air (d), and five-stage POM-L-POM-L-POM delignification (e). Reaction conditions are presented in Table 3.

The temperature variation in the L stage from 45 °C (to 30 and 60 °C) did not show benefits in the three-stage pulp delignification (Table 3). This can be explained by less effective re-oxidation of $SiW_{11}Mn^{II}$ at 30 °C than at 45 °C and by partial laccase denaturation at 60 °C.

$PW_{11}Mn^{III}$ was also tested in multi-stage experiments. A two-stage bleaching with $PW_{11}Mn^{III}$ and laccase showed a delignification degree similar to that obtained with $SiW_{11}Mn^{III}$ under the same experimental conditions (Table 3). However, the re-oxidation degree of $PW_{11}Mn^{II}$ after the second L stage was very low (<5%), which limited the use of this catalyst in three- or five-stage bleaching experiments.

The same multi-stage approach was applied to $SiW_{11}V^{V}$. The first POM stage with $SiW_{11}V^{V}$ (Table 4) was more effective for the pulp delignification than with $SiW_{11}Mn^{III}$ though less selective (Table 3). Similar patterns were observed in the second L stage (Tables 3 and 4). In a three stage pulp treatment with $SiW_{11}V$ (POM-L-POM), about 50% of the delignification was reached (Table 4), i.e. almost the same as in five stages (POM-L-POM-L-POM) with $SiW_{11}Mn$ (Table 3). Similarly to $SiW_{11}Mn$, the $SiW_{11}V$ anion was found predominantly in the reduced stage after the POM stage and POM-L-POM experiment (Fig. 5). After the POM-L experiment around 80–100% of $SiW_{11}V$ was found in the oxidised state (Fig. 5). ^{51}V NMR spectra showed the catalyst stability after redox turnovers (Fig. 6).

The less selective pulp delignification in POM stage with $SiW_{11}V$ than with $SiW_{11}Mn$ might be tentatively explained by partial dissociation of the former with release of VO_2^+ ions under weak acidic conditions favouring the polysaccharides degradation [32]. Although VO_2^+ species were not detected using ^{51}V NMR at the end of the bleaching experiments with

Table 4
Results on the eucalypt kraft pulp delignification catalysed by $SiW_{11}V^V$ (POM stage) and laccase (L stage)

Bleaching system	Kappa number	Intrinsic viscosity (cm^3/g)	Delignification degree (%)	Intrinsic viscosity loss (%)	$SiW_{11}V^V$ oxidised (%)
Kraft pulp	12.3	1185	–	–	–
POM	9.0	1055	26	11	6
POM-L (45 °C)	8.2	1035	33	13	98
POM-L (60 °C)	7.7	1010	37	15	80
POM-L (45 °C)-POM	6.9	1005	44	15	15
POM-L (60 °C)-POM	6.2	935	50	21	12

Experimental conditions: pulp consistency 6.0%; $[SiW_{11}V^V] = 4.0$ mmol/l; pH 4.5; $P°(O_2) = 0.3$ MPa; 2 h at 110 °C in POM stage with $SiW_{11}V^V$ and 4 h at 45 °C or 60 °C in L stage with laccase.

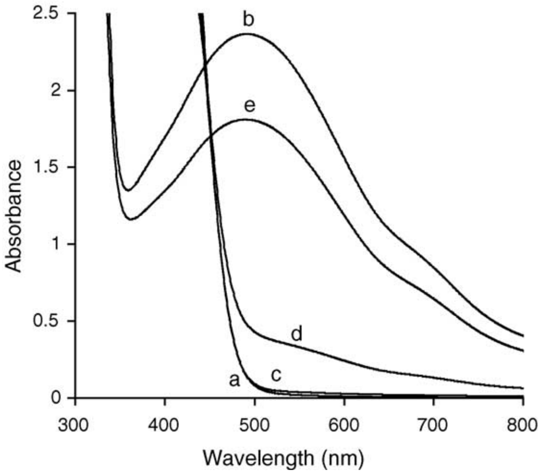

Fig. 5. UV–vis spectra of the $SiW_{11}V^V$ solution before the delignification (a), after the POM stage (b), after the two-stage POM-L (45 °C) bleaching (c), after the two-stage POM-L (60 °C) delignification (d), and after the three-stage POM-L(60 °C)-POM delignification (e). Reaction conditions are presented in Table 4.

$SiW_{11}V$ (only the α-$SiW_{11}V^V$ species was detected after the POM-L bleaching experiment, Fig. 6), their formation in the course of the bleaching is rather probable. VO_2^+ ions possess a significant detrimental effect on polysaccharides being less selective oxidant than parent POM [5,9,33].

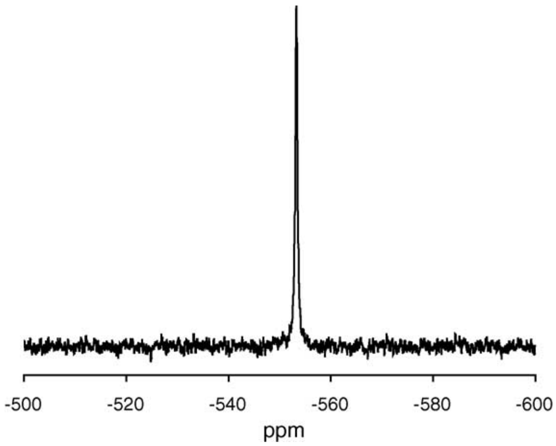

Fig. 6. ^{51}V NMR spectrum of the solution obtained after the two-stage $SiW_{11}V$-L (60 °C) experiment.

It seems, the further improvement of the multi-stage delignification is possible applying the L stage without direct contact of pulp with laccase. In practice, this could be accomplished by continuous withdrawal of reduced POM solution from the bleaching reactor to the laccase containing bioreactor (in solution or immobilised) and supplying the oxidised POM back to the bleaching reactor. These studies are in progress.

4. Conclusions

The study on the oxidation of polyoxometalates $SiW_{11}V^{IV}$, $SiW_{11}Mn^{II}$ and $PW_{11}Mn^{II}$ with laccase under aerobic conditions showed their different reactivity. The facility of POMs oxidation was ordered as follow: $PW_{11}Mn^{II} < SiW_{11}Mn^{II} < SiW_{11}V^{IV}$. High effective redox potential of $PW_{11}Mn$ (around +0.9 V) was the main reason for it poor re-oxidation with laccase, whereas $SiW_{11}Mn$ and $SiW_{11}V$ possessing lower redox potentials (around +0.7 V) were reactive even at room temperature. The higher oxidation peak potential and the lower reversibility of the pair $SiW_{11}Mn^{III/II}$ when compared to $SiW_{11}V^{V/IV}$ were responsible for the lower oxidation rate of $SiW_{11}Mn$. The amount of laccase, oxygen pressure, temperature and the presence of phenolic substances affected the POMs re-oxidation.

All considered POMs showed the catalytic activity as mediators in the laccase-promoted oxygen delignification of the eucalypt kraft pulp. In spite of relatively slow pulp delignification in LMS carried out at moderated temperatures (45–60 °C), this process was rather selective. The delignification rate-determining step was the residual lignin oxidation by the mediator (except in the experiments with $PW_{11}Mn$).

The application of a multi-stage process combining the pulp delignification with polyoxometalates $SiW_{11}M$ ($M = V^V$, Mn^{III}) at raised temperature (110 °C) and the heteropolyanions re-oxidation by laccase at moderate temperature (45–60 °C) carried out in separate stages allowed the removal of more than 50% of residual lignin. $SiW_{11}Mn$ was suggested to be the most selective catalyst, whereas $SiW_{11}V$ was the most effective in the pulp delignification. It was demonstrated that both catalysts, $SiW_{11}Mn^{III}$ and $SiW_{11}V^V$, are stable under reaction conditions, and can be continuously re-used in catalytic cycles.

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