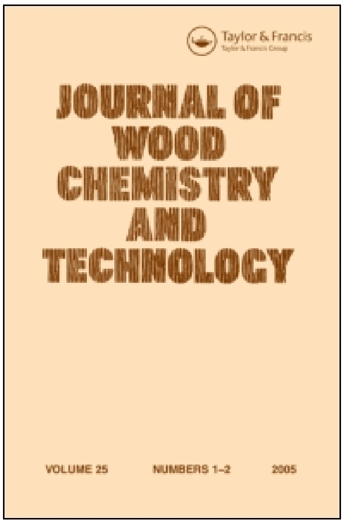

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CATALYTIC MECHANISM OF CU^{2+} AND FE^{3+} IN ALKALINE O_2 OXIDATION OF LIGNIN

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ABSTRACT

The catalytic mechanisms of Cu^{2+} and Fe^{3+} in the alkaline oxidation of lignin are discussed. Best aldehyde yields were obtained by using Cu^{2+} and Fe^{3+} as catalysts. Without these two ions present or in the absence of one or other of them, yields were lower. It is clear that Cu^{2+} and Fe^{3+} significantly influence the reactions. Cu^{2+}, acting as the electron acceptor, accelerates the formation of the phenoxy radical, thus speeding up the radical reaction. In order to interpret the effect of Fe^{3+} on oxidative degradation of lignin and formation of aldehydes, formation of an intermediate, O_2-Fe^{3+}-lignin complex and its catalytic mechanism have been proposed.

INTRODUCTION

It is well known how to produce vanillin via alkaline oxidation of spent sulfite liquors where the lignin is present in the form of lignosulfonate. In the process, $CuSO_4$ (5-10% wt of lignin) is usually added to accelerate the reaction and to increase the yield of vanillin[1].

Little has been reported on the mechanism of the metal-catalyzed lignin oxidation reaction for the production of the aromatic aldehydes. In contrast, the treatment of wood and pulps with oxygen and hydrogen peroxide in alkaline media has received considerable attention, given the need for chlorine-free pulping and bleaching processes. Numerous studies have therefore been conducted to elucidate, in the lignocellulosic matrix, the breakdown of the lignin component by oxygen under alkaline conditions and possible mechanisms have been proposed[2-9].

According to now established mechanisms, the degradation of lignin under alkaline oxidation conditions is preceded by alkali-promoted reactions, i.e. formation of carbanions and conjugated carbonyl structures. The oxidation of the formed carbanions may involve both ionic and free radical mechanisms. The effects of metal ions as catalysts in these processes have been well documented in the literature[10-14].

In our earlier work aimed at the conversion of lignin into aldehydes[15-16], several metal oxides and salts were tested. Based on the preliminary results, we decided to concentrate our efforts on $CuSO_4$, $FeCl_3$ and their mixtures. It was experimentally found that the mixtures are more effective catalysts than each of the salts used alone. In the present study, we concentrate our discussion on the effects of Cu^{2+}, Fe^{3+} and their mixtures on the yields of aldehydes and their mechanistic interpretation during alkaline oxidation of lignin.

EXPERIMENTAL

Feedstock

Wood chips from *Populus tremuloides* were treated in the 4 t/h STAKE II steam treatment facility located at the University of Sherbrooke, at 220°C for a period of 2 minutes. The treated wood chips were first extracted with hot water to remove most of the hemicelluloses, and then extracted with a NaOH solution ($pH \approx 13$, 100°C, 30 min). The alkaline filtrate was acidified to pH = 1.5 -2.0 with H_2SO_4 at 80°C and 10 minutes as contact time. The precipitated residue, acid-insoluble lignin, was washed with water and dried. The exact procedures and yields have been described elsewhere[15,17].

Determination of organic acids

The alkaline O_2 oxidation of lignin was conducted as described in our previous work[16]. The conditions employed are listed in Table 1. After oxidation, the reaction product was acidified to pH = 2 with H_2SO_4 and filtered. The residue was dried and weighed to determine the conversion. The determinations of the yields of aldehydes from the ether soluble fraction were carried out as detailed in our

TABLE 1. The data of lignin oxidations

Oxidant		CuO			Oxygen											
Catalyst		Fe_2O_3			$CuSO_4$ +$FeCl_3$				$FeCl_3$			$CuSO_4$			None	
log(Ro/min)		4.63	4.27	3.99	3.08	3.35	3.06	2.56	3.34	3.08	2.65	3.40	2.85	2.57	3.12	2.53
Temperature	°C	220	190	180	170	170	160	160	170	170	170	170	160	150	170	150
Time	min	10	40	40	10	20	20	5	20	10	2	20	10	10	10	10
Input: Aqueous solution	ml	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Contains: Klason lignin	g	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
NaOH	g	4.00	4.00	4.00	17.50	14.70	13.60	10.40	17.00	16.00	10.20	14.60	13.50	12.30	12.20	10.90
O_2	g				7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50	7.50
CuO	g	10.00	10.00	10.00	-	-	-	-	-	-	-	-	-	-	-	-
Fe_2O_3	g	1.00	1.00	1.00	-	-	-	-	-	-	-	-	-	-	-	-
$CuSO_4$	g	-	-	-	0.50	0.50	0.50	0.50	-	-	-	0.50	0.50	0.50	-	-
$FeCl_3$	g	-	-	-	0.05	0.05	0.05	0.05	0.05	0.05	0.05	-	-	-	-	-
Conversion	%	40.90	34.20	28.60	73.00	79.10	71.20	57.50	81.70	73.70	59.00	69.80	59.70	53.70	58.00	50.40
Hydroxybenzaldehyde	%	0.20	0.20	0.20	0.40	0.30	0.40	0.30	0.40	0.30	0.20	0.30	0.40	0.30	0.40	0.30
Vanillin	%	2.00	1.50	1.40	4.70	4.00	4.60	3.10	4.50	4.10	2.40	3.50	3.70	3.30	3.40	3.20
Syringaldehyde	%	7.40	6.00	5.40	9.50	7.80	8.70	6.40	6.90	6.70	0.80	6.50	7.60	5.20	4.70	3.20
Acetovanillone + acetosyringone	%	2.70	1.90	1.80	1.3	0.80	0.60	0.90	0.60	1.10	6.80	1.90	1.20	1.20	0.50	0.70
Total aldehyde	%	9.60	7.70	7.00	14.60	2.10	13.70	9.80	11.80	11.10	8.10	10.30	11.70	8.80	8.50	6.70
Oxalic acid	%	1.80	1.10	0.90	-	17.00	13.50	8.00	18.80	13.50	0.04	15.60	14.10	13.80	11.30	8.60
Maleic acid	%	0.01	0.01	0.00	-	0.05	0.03	0.02	0.03	0.03	0.06	0.04	0.01	0.04	0.01	0.01
Fumaric acid	%	0.01	0.01	0.00	-	0.08	0.07	0.04	0.11	0.10	12.20	0.07	0.07	0.06	0.09	0.07
Formic acid	%	1.60	1.00	0.70	-	15.60	14.10	12.40	21.60	20.00	16.50	16.30	15.60	14.10	13.20	10.50
Acetic acid	%	13.70	8.60	5.10	-	27.40	22.70	18.00	34.50	28.40	4.20	26.50	23.20	22.90	18.90	15.00
Propionic acid	%	1.30	0.80	0.50	-	7.70	5.60	4.80	8.10	6.10	0.20	7.90	5.70	3.60	5.50	3.10
Hydroxybenzoic acid	%	0.50	0.10	0.10	-	0.30	0.20	0.20	0.30	0.20	0.50	0.30	0.30	0.30	0.30	0.30
Vanillic acid	%	0.10	0.10	0.10	-	0.60	0.40	0.30	0.90	0.90	0.10	1.00	0.90	0.90	0.50	0.00
Syringic acid	%	0.30	0.30	0.30	-	0.10	0.10	0.20	0.10	0.20	0.20	1.70	1.20	1.00	0.00	0.00
Benzoic acid	%	0.10	0.10	0.00	-	0.30	0.40	0.30	0.60	0.60	42.20	0.30	0.20	0.20	0.50	0.30
Total acids	%	19.50	12.10	7.70	-	69.20	57.20	44.30	84.90	70.10	42.20	69.70	61.30	57.00	50.30	38.00

% refers to Klason lignin, which represents 84.7% of the dry brut lignin.

previous study[16]. Organic acids content of the aqueous raffinate was determined on a Varian, model 5000, liquid chromatograph equipped with an Aminex HPX-87H column (300 mm x 7.8 mm) and a UV Detector (UV-100).

The conditions used were as follows:

Detection:	UV, 210 nm
Eluent:	10% $CH_3CN/0.01N\ H_2SO_4$
Flow rate:	0.7 ml/min
Column Temperature:	65°C

The organic acids in the oxidized lignin solutions were identified by their retention time. Identification of the acids was confirmed by comparing the retention times with those of pure acids using a second eluent, 0.01N H_2SO_4. The relative error involved in the determination of all the acids was ±4.6%.

Some of the results obtained were analyzed and represented as a function of Ro, a reaction ordinate, previously defined and used by Overend and Chornet[18]. It is defined as

$$Ro = t * \exp[(T - 100)/14.75]$$

where t is expressed in min and T in °C. The Ro factor is a useful marker of the severity of the reaction and has been used as a means of combining the effects of temperature, °C and time, t (min), into one variable, which facilitates plotting of the derived yields versus $\log_{10}(Ro/min)$. Unless otherwise stated, all percentages mentioned in the following pages are based on dry lignin.

DISCUSSION

The effect of a particular metal ion in the alkaline oxidation of lignin with oxygen depends on its redox characteristics and its solubility under the conditions employed. The latter also depends upon the presence of soluble wood degradation products having an ability to form soluble complexes with the metal ion[10]. $FeCl_3$ and $CuSO_4$ added in an alkaline solution change to $Fe(OH)_3$ and $Cu(OH)_2$ respectively. Their solubilities are so low that they are almost insoluble in the alkaline solution. For example, at pH ≥ 7 ferric hydroxide will precipitate even with a ferric ion concentration as low as 10^{-15} M. In our study, the ferric ion concentration in the alkaline lignin solution was 10^{-3}M without any precipitation of Fe^{3+}. The same situation occurs in the case of cupric hydroxide. These results led us to believe that the metals can be present in the alkaline lignin solution in other forms, which are not only quite stable, but are also present in relatively high concentrations.

It has been well-known for many years[19-24] that low molecular phenolic compounds react with some metal ions, such as Fe^{3+} and Cu^{2+}, forming complexes. Fe^{3+} complexes are intensely coloured and may be used in the colorimetric determination of iron ions present in solution. Some studies have shown that lignin and its degradation products have an ability to form complexes with the salts of these metals in a similar manner. These complexes accelerate the reaction rate in peroxide bleaching and oxygen pulping[10,13,14].

It seems likely that the aldehyde production by alkaline oxidation of lignin will proceed via the same mechanism proposed for oxygen bleaching and pulping. The oxidation reaction is widely considered to be preceded by alkali-promoted reactions[7,8], since the medium is strongly alkaline (Figure 1). The phenolic structure in the lignin molecule(I_a) is dissociated to a phenolate anion(I_b), which is in resonance with a variety of carbanion structures(I_c - I_f).

After ionization of the phenolic structure, the corresponding cyclohexadienone or keto-hydroperoxide structures form via two different reaction pathways: (1) a direct electrophilic attack by molecular oxygen, i.e. ionic pathway; (2) two consecutive one-electron transfers, i.e. radical pathway. Once formed, these hydroperoxide structures undergo an alkali-induced rearrangement and oxidative degradation into a series of low molecular weight compounds, such as aldehydes and organic acids. Some studies[7,11,14] have shown that these two pathways exist simultaneously in the reaction system. Their ratio depends on the conditions used. The radical reaction is very slow, and its effect can be ignored in the absence of metal ions. Hence, the ionic reaction is predominant[11].

Alkaline copper oxide oxidation

To understand the mechanism of the radical reaction in lignin oxidation, a series of experiments targeted at the alkaline CuO oxidation were designed. In the absence of oxygen, the only oxidant is CuO which is known to be a one electron transfer oxidant. This reaction may be considered as a typical radical reaction[9]. In the process, the cupric ion serves as the electron acceptor. It abstracts an electron from a phenolate anion of the lignin structure, thus forming a phenoxy radical. The corresponding cyclohexadienone forms via another one-electron transfer from a

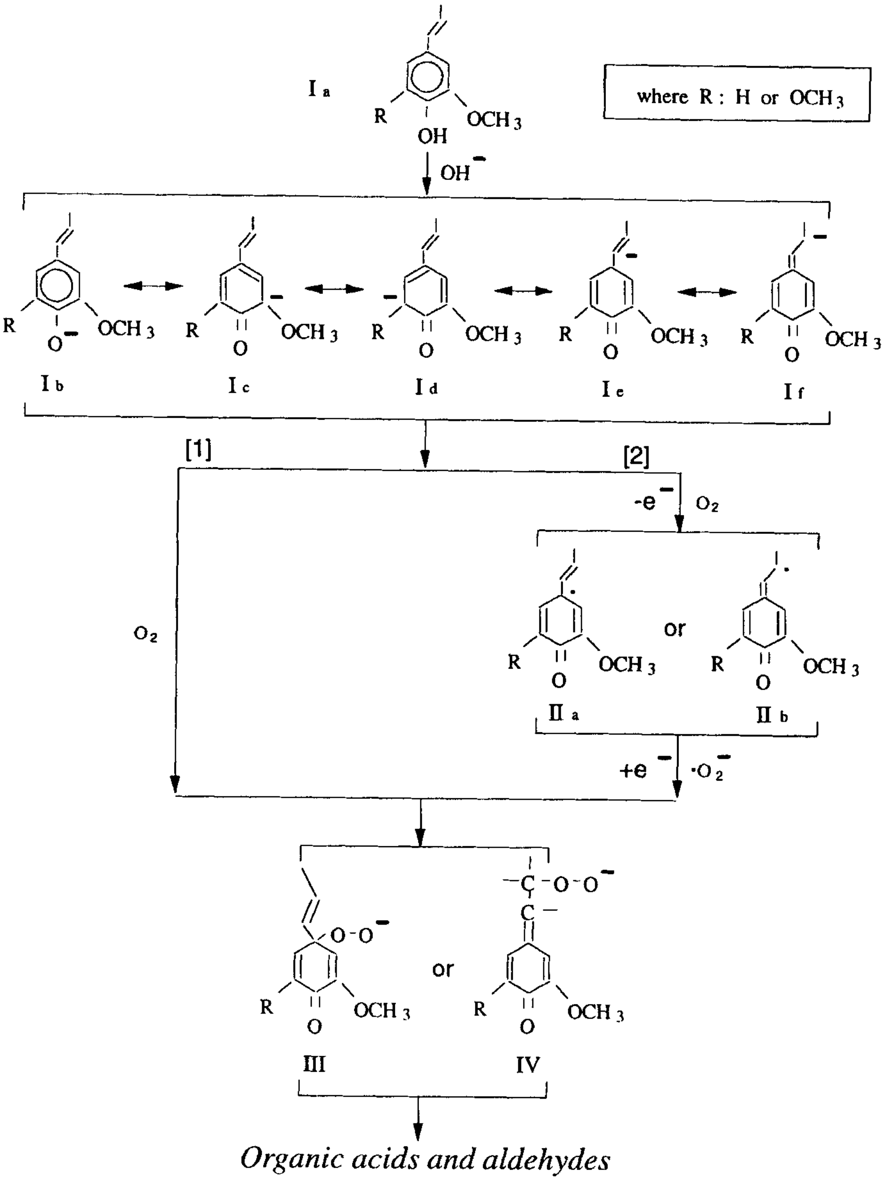

Figure 1. Proposed oxidation scheme for lignin materials under alkaline oxygen conditions.

phenoxy radical to an electron acceptor. The successive electron transfers will produce aromatic aldehydes and degradation products. Since the resulting phenoxy radical is far less stable than the corresponding phenolate anion, the first electron transfer reaction must be much slower than the successive electron transfers. In other words, the formation of the phenoxy radical is the rate-determining step in the overall reaction[11,14].

From the results obtained in our work (Table 1), it is clear that copper oxide is a relatively weak oxidant. The radical reaction promoted by Cu^{2+} gives rise to a low conversion of lignin (30-40%) and a low quantity of acids formed (8-9%). Therefore, one of the characteristics of the radical reaction is that most of the aldehydes produced can remain in the solution without further degradation into acids, under the severity conditions used.

Oxygen oxidation without any catalyst

In this reaction system, the ionic reaction is the dominant one, since no significant concentration of metal ions exists in the solution. During this process, molecular oxygen dissolving in an alkaline solution reacts with lignin and its derivatives via an ionic route, e.g. pathway (1) in Figure 1, resulting in the degradation of lignin and formation of aldehydes and organic acids. Our previous work[16] has shown that the oxygen concentration in the solution plays a central role in the reaction. The oxygen concentration increases with the oxygen partial pressure, thus increasing the reaction rate, i.e. the degradation of lignin.

The direct attack of molecular oxygen on lignin causes lignin to be degraded rapidly. Under mild severity conditions (170°C and 10 minutes), 58% of lignin is degraded into low molecular compounds, whose concentration is two to three times higher than the amount observed under CuO oxidation (Table 1). Any oxidation intermediates, such as aldehydes, are rapidly oxidized into acids and CO_2, due to the continuous attack by oxygen. The yield of total acids is as high as 50%, an amount that is at least 6 times that obtained during CuO oxidation (Table 1). It is obvious that higher yields of aldehydes can be reached if a method can be found that forces the rapid conversion of lignin into the desired intermediate products while preventing their further oxidation into acids.

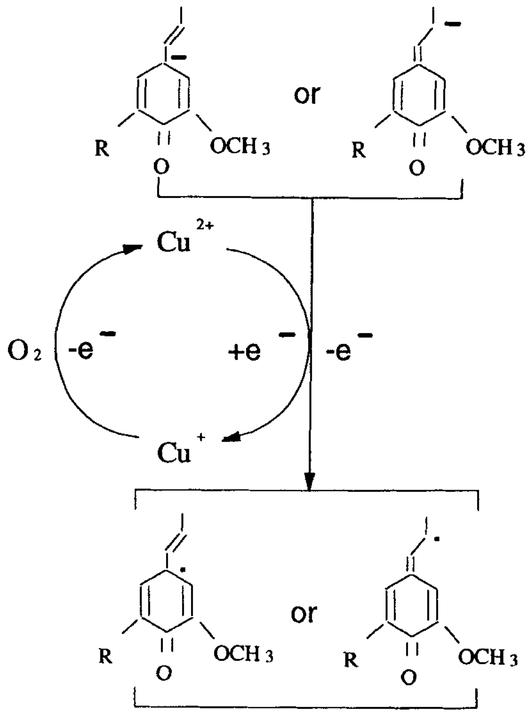

Figure 2. Proposed oxidation scheme with Cu^{2+} as catalyst.

Oxidation with O_2 in the presence of Cu^{2+}

Other than the ionic reaction, which is favoured in the presence of O_2, the radical reaction is also an important reaction when Cu^{2+} is present. A more probable mechanism is represented in Figure 2 and a similar mechanism has been proposed by Landucci[11]. In the process, the oxygen dissolved may not only be used to oxidize lignin and its derivatives, but also used to reoxidize the formed monovalent copper, since the reduced forms of copper are readily oxidized by molecular oxygen dissolved in the alkaline solution[11]. This process speeds up the redox catalytic cycle and increases the content of Cu^{2+} in the solution, thus increasing the rate of the radical pathway. Since both reaction routes, ionic and radical reactions, exist simultaneously, the overall rate of lignin oxidation is higher than in the case where only the ionic reaction exists. As compared with the control without catalyst, the conversion of lignin increases by 3-10% and the yield of total aldehydes increases by 1-4%. The increase in the yields of aldehydes is mainly caused by the radical reaction. However, further degradation of the aldehydes into acids due to the presence of O_2 limits the total yield of aldehydes.

Oxidation with O_2 in the presence of Fe^{3+}

The data obtained from our previous work[15] and from other investigations[11, 25] have revealed that Fe^{3+} is not capable of oxidizing lignin and other phenolic compounds in alkaline media even at high temperatures (220°C). This suggests that Fe^{3+} cannot act as an electron transfer reactant to abstract an electron from the phenolate anion and thus does not accelerate the radical formation.

In our experiments, however, it is quite interesting to observe that under alkaline conditions, Fe^{3+} has an obvious effect on the reaction rate. The effect is different from that of Cu^{2+}. Since Fe^{3+} cannot accelerate the radical reaction, the only reaction Fe^{3+} can influence is the ionic one. The effect of Fe^{3+} on the reaction seems to depend on the treatment severity. Our previous work[15] has shown that at low severity ($\log(Ro/\min) < 2.7$), Fe^{3+} has little effect on the process, since almost all the data obtained, except conversion, are similar without the catalyst. This strongly suggests that the main reaction pathway to cyclohexadienone is still the ionic reaction. However, the oxidation rate increases greatly with the increase in the treatment severity, giving rise to both increases in the conversion of lignin and in the yields of oxidation products, mainly aliphatic acids (Table 1).

Although the mechanism is still unclear, previous work[26] suggests that Fe^{3+} complexes are formed with phenolic compounds and act as oxygen carriers in the oxidation process. This accounts for the observed catalytic effect of Fe^{3+}. As with molecular oxygen in haemoglobin, oxygen is assumed to be linked loosely to the Fe^{3+} complex, thus forming a new complex, an oxo-iron intermediate, $O_2(Fe^{3+}\text{-}Ph)$ (Figure 3). In addition to the molecular oxygen dissolved in the alkaline media, the $O_2(Fe^{3+}\text{-}Ph)$ intermediate attacks the phenolate anion in the lignin structure to form the oxidation intermediate products and further oxidizes them to acids via the ionic reaction route.

The formation of $O_2(Fe^{3+}\text{-}Ph)$ requires energy. The higher the treatment severity, the higher the concentration of intermediates formed. Higher amounts of

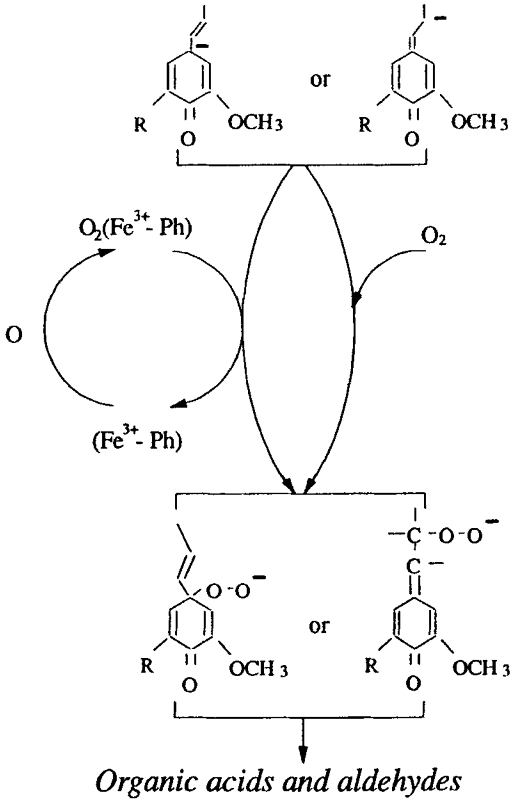

Figure 3. Iron ion as catalyst in the ionic reaction.

intermediates will increase the oxidation rate of lignin. Although this is difficult to prove experimentally, it is strongly supported by the catalytic effect of Fe^{3+} on the reaction. As compared with the control without catalyst, about 20% more lignin is converted into low molecular weight compounds, 20% more organic acids are formed and 2% more aldehydes are produced at high severity (log(Ro/min) > 3). The values of lignin conversion and of total acids are 10% and 15% higher respectively than those using Cu^{2+} as catalyst.

Oxidation with O_2 in the presence of a mixture of Cu^{2+} and Fe^{3+}

From the results obtained in our present work, it is clear that a significant yield of aldehydes is readily obtained when the mixture of Cu^{2+} and Fe^{3+} is employed. The oxidation under 170°C for the period of 10 minutes accounts for 14.6% as total aldehydes, which is at least 3% more than in the cases using other catalysts[15].

As mentioned above, the presence of Cu^{2+} and molecular oxygen results in both radical and ionic reactions. When Fe^{3+} is also present in the reaction system, in addition to Cu^{2+} and oxygen, its catalytic effect is not obvious at low severity. The reaction appears almost the same as that with Cu^{2+} and O_2. A proposed mechanism for interpreting the effects of Fe^{3+} is illustrated in Figure 4. Increasing the severity favours the formation of $O_2(Fe^{3+}\text{-}Ph)$ intermediates, which not only oxidize lignin and its oxidation products (Cycle A in Figure 4), but also oxidize monovalent copper to Cu^{2+} (Cycle B), thus speeding up the electron transfer in the redox catalytic cycle. The rate of radical reaction is then higher than in the absence of Fe^{3+}. The net result is that an increase of 2% total aldehydes can be obtained.

From Figure 4, it is found that the molecular oxygen dissolved is mainly consumed in the formation of $O_2(Fe^{3+}\text{-}Ph)$ intermediate (Cycle A) and the oxidation of Cu^{+1} into Cu^{2+} by molecular oxygen itself (Cycle B) or by the $O_2(Fe^{3+}\text{-}Ph)$ intermediate (Cycle C). These reactions decrease the molecular oxygen content in the solution, which in turn limits the direct attack by molecular oxygen on the intermediates, thus decreasing the formation of acids.

In summary, the formation of $O_2(Fe^{3+}\text{-}Ph)$ intermediates in the system not only increases the rate of oxidation of lignin, but also decreases the rate of further oxidation of the aldehydes by decreasing the oxygen content in the solution. These two factors would then account for higher yields of aldehydes and lower yields of organic acids.

CONCLUSIONS

The present work has provided unambiguous evidence that significant yields of aldehydes from lignin can be achieved by using a mixture of Cu^{2+} and Fe^{3+}. At present, the widely accepted mechanism suggests that in the process, Cu^{2+} acts as an electron acceptor accelerating the formation of the phenoxy radical. Fe^{3+} cannot be a catalyst in the radical reaction, because it has no ability to absorb an electron from the phenolate anion.

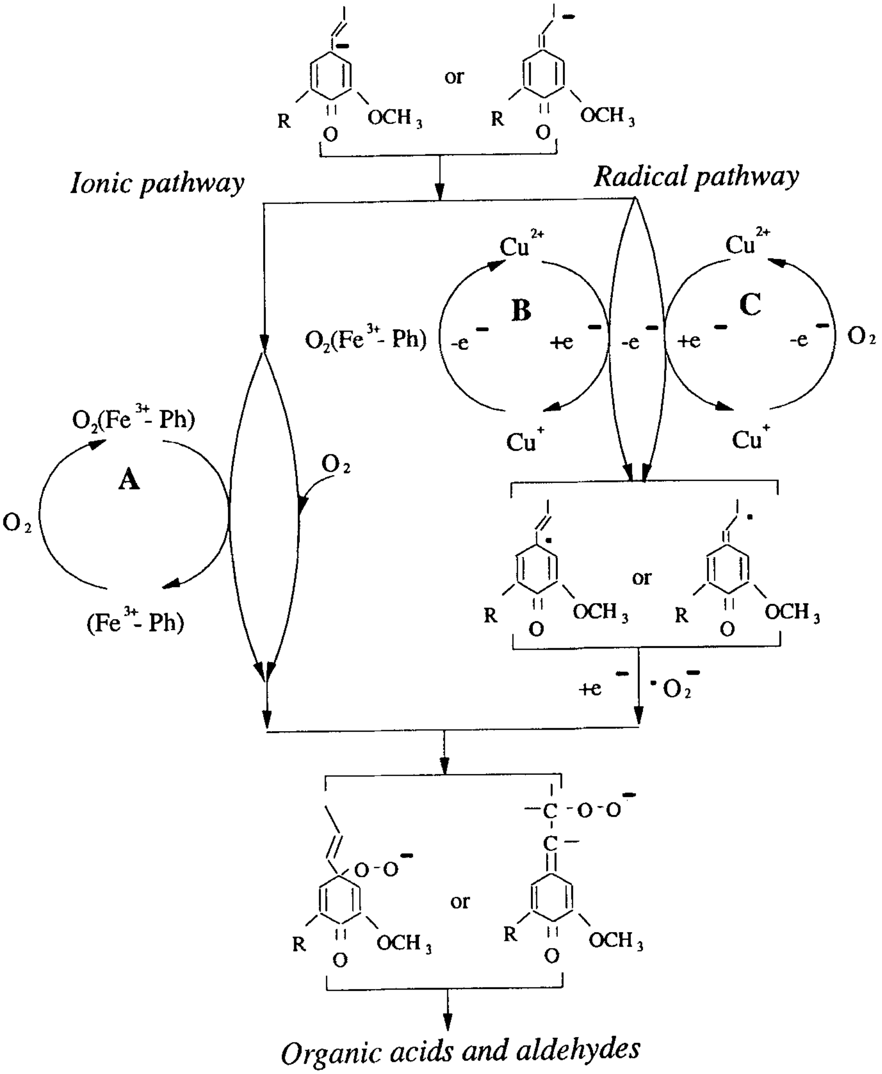

Figure 4. Proposed oxidation scheme under alkaline oxygen conditions with a mixture of Cu^{2+} and Fe^{3+}.

The results obtained in our experiments, however, have shown that Fe^{3+} has a significant effect on the oxidation of lignin with and without Cu^{2+}. Based on the results obtained, we propose that a new reaction intermediate, O_2-Fe^{3+}-lignin complex, forms during the reaction, specially at high treatment severity. This complex acts as an oxygen carrier. Like molecular oxygen, this intermediate can attack lignin to form degradation products, thus speeding up the ionic reaction. Higher conversion of lignin and higher yields of acids can be observed when Fe^{3+} is used alone. When Cu^{2+} is also present, the complex mainly reoxidizes Cu^{1+} to Cu^{2+}, speeding up the redox catalytic cycle, which, in turn, increases the rate of the radical reaction. This accounts for the high yield of total aldehydes observed when a mixture of Cu^{2+} and Fe^{3+} is used.

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REFERENCES

1. H.B. Marshall and D.L. Vincent, Canadian patent 1,040,216 (1978).
2. R.C. Eckert, H.C. Chang and W.P. Tucker, Tappi, 56(6), 134 (1973).
3. K. Kratzl, J. Gratzl and P. Claus, Advances in Chemistry Series. 59, 157 (1966).
4. J. Gierer and F. Imsgard, Acta Chem. Scand. B31, 537 (1977).
5. J. Gierer and F. Imsgard, Acta Chem. Scand. B31, 546 (1977).
6. J. Gierer, F. Imsgard and I. Norén, Acta Chem. Scand. B31, 561 (1977).
7. J. Gierer and F. Imsgard, Svensk Paperstidn. 16, 510 (1977).
8. J. Gierer, Wood Sci. Technol. 20, 1 (1986).
9. H.M. Chang and G.G. Allan, Lignins p 433-486, Sarkanen, K.V. and Ludwig, C.H. Eds., Wiley Interscience, New York (1971).
10. L.L. Landucci and N. Sanyer, Tappi. 58(2), 60 (1975).
11. L.L. Landucci, Trans. Tech. Sect. Can. Pulp Pap. Assoc. 4(1), TR25 (1978).
12. R. Agnemo and G. Gellerstedt, Acta Chem. Scand. B33, 337 (1979).

13. G. Gellerstedt and R. Agnemo, Acta Chem. Scand. B34, 275 (1979).

14. P.K. Smith and T.J. McDonough, Svensk Paperstidn, 12, R106 (1985).

15. G. Wu, M. Heitz and E. Chornet, Proceedings of the 3rd Symposium on Advances in Thermochemical Biomass Conversion, Interlaken, Switzerland. Ed. A.V. Brigwater, Elsevice Applied Science Publ., in press (1992).

16. G. Wu, M. Heitz and E. Chornet, Ind. Eng. Chem. Res., 33(3), 718 (1994).

17. M. Heitz, E. Capek-Ménard, P.G. Koeberle, J. Gagné, E. Chornet, R.P. Overend, J.D. Taylor and E. Yu, Bioresource Technology, 35, 23 (1991).

18. R.P. Overend and E. Chornet, Can. J. Phy. 68(9), 1105 (1990).

19. J.H. Yoe and A.L. Jones, Ind. Eng. Chem. Anal. Ed. 16, 111 (1944).

20. A. Ägren, Acta Chem. Scand. 9(1), 39 (1955).

21. P.H. Gore and P.J. Newman, Anal. Chim. Acta. 31, 111 (1964).

22. J.H. Weber, Syn. React. Inorg. Metal-Org. Chem. 7(3), 243 (1977).

23. M. Tonkovic and O. Hadzija, Mikrochimica Acta. III, 133 (1985).

24. S.S. Kulkarni and M.L. Narwade, Acta Ciencia Indica. 16(3C), 245 (1990).

25. L.L. Landucci, Tappi. 62(4), 71 (1979).

26. R. Riffer, Proc. Sugar Process. Res. Conf., 231 (1984).