Evaluating three iron-based biomimetic compounds for their selectivity in a polymeric model system for pulp

Colleen C. Walker, Ronald J. Dinus, Thomas J. McDonough, and Karl-Erik L. Eriksson

ABSTRACT: The ability of fungal enzymes to degrade lignin has suggested the study of simpler compounds to mimic these enzymes. Attempts to bleach pulp with biomimetic compounds have not demonstrated that these compounds are selective catalysts for pulp delignification. This paper evaluates the selectivity of three biomimetic systems: ferrous sulfate, ferrous ion chelated with EDTA (ethylenediaminetetraacetic acid), and hemoglobin, all in the presence of hydrogen peroxide. Lignosulfonate and hydroxyethyl cellulose (HEC) were chosen as polymeric models for lignin and carbohydrate.When these substrates were individually exposed to each biomimetic compound in the presence of hydrogen peroxide, substantial degradation to both lignin and carbohydrate model compounds was observed. Hemoglobin was the most selective for lignosulfonate degradation over HEC degradation.

KEYWORDS: Bibliographies, biobleaching, carbohydrates, depolymerization, EDTA, hydrogen peroxide, hydroxyethyl cellulose, iron compounds, iron sulfates, lignin model compounds, lignosulfonates, selectivity.

Government regulations and increased environmental awareness have provided a strong impetus for the replacement of chlorine-containing chemicals used for the production of fully bleached pulp. The isolation and subsequent characterization of enzymes capable of degrading wood components have created numerous opportunities for application of biotechnology to the production of pulp and paper *(1)*.

In 1983, the enzyme lignin peroxidase was independently isolated from the white-rot fungus *Phanerochaete chrysosporium* in two laboratories *(2, 3)*. Lignin peroxidase has been widely studied *(4, 5)*, and we know that this enzyme possesses a heme structure, or an iron protoporphyrin IX, as a prosthetic group. Ligninolytic enzymes have been used to bleach pulp *(6, 7)*. Limited success has been achieved in enzymatic delignification using lignin peroxidase, possibly due to inaccessibility of the lignin to the enzyme *(6)*.

Biomimetics, the study of mimicking biological processes with nonbiological compounds, is an attractive approach to pulp delignification. Biomimetic compounds are often small, providing increased accessibility to the substrate. In addition, these compounds can, ideally, be designed to perform specific functions.

For biomimetic bleaching systems to be considered commercially feasible, they must demonstrate selectivity for lignin removal. Several studies have evaluated the ability of various natural and synthetic iron porphyrins to degrade lignin model compounds *(8–11)*. Although these studies provide evidence for the ability of biomimetic compounds to act as delignifying agents, the ability of these systems to also degrade car-

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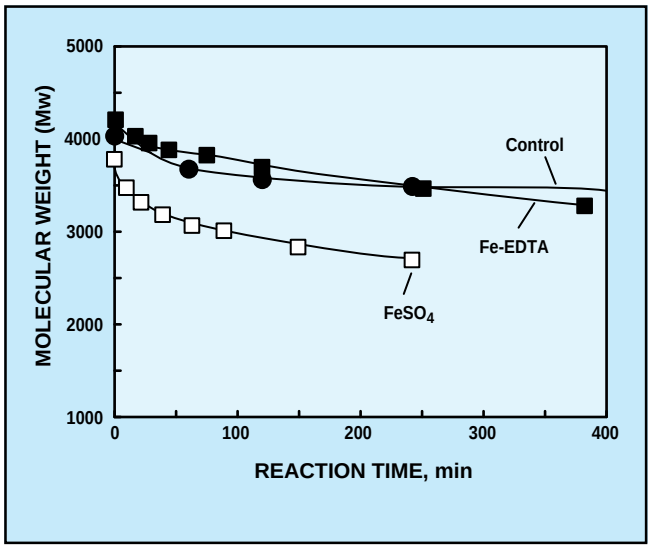
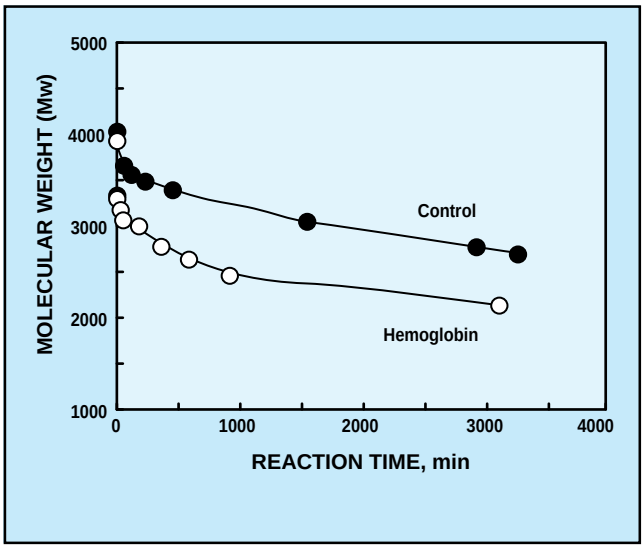
1. Weight-average molecular weight of lignosulfonate vs. reaction time for the control, $FeSO_4$, and Fe-EDTA reactions with hydrogen peroxide



2. Weight-average molecular weight of lignosulfonate vs. reaction time for the hemoglobin and control reactions with hydrogen peroxide



bohydrates has not been investigated in model systems.

Biomimetic compounds have also been used to bleach pulps. In most cases, substantial degradation to the carbohydrate is reported *(12–16)*. It is not clear, however, whether carbohydrate degradation is due to the nonselectivity of the biomimetic compounds or to the hydroxyl radicals present in solution, which are generated from the oxidant (usually hydrogen peroxide).

A solution of hydrogen peroxide and iron, also known as Fenton's reagent, produces hydroxyl radicals *(17)*. Hydroxyl radicals have been measured in solutions containing iron complexes *(18)*, such as Fe-EDTA (ethylenediaminetetraacetic acid) *(19)* and hemoglobin *(20)*. Although hydroxyl radicals react faster with aromatic compounds (lignin-like structures) than with carbohydrate *(21)*, hydroxyl radicals are reactive and generally unselective.

The work presented here represents an evaluation of the selectivity of three biomimetic compounds for lignin over carbohydrate degradation.

Experimental approach

Three biomimetic compounds—ferrous sulfate, iron chelated with EDTA, and hemoglobin—were chosen, the three resembling lignin peroxidase to different degrees. Ferrous sulfate is a simple mimic, containing the metal found in the prosthetic group of the enzyme. Iron chelated with EDTA represents a slightly more sophisticated mimic in which the iron is tightly sequestered by the chelating agent. Hemoglobin resembles the enzyme most closely, as it contains a large amount of protein.

The selectivity of these compounds was evaluated by measuring the relative rates of degradation of lignin and carbohydrate model compounds. Homogeneous reaction conditions in aqueous solution were desired; therefore, water-soluble model compounds representing wood pulp as closely as possible were selected. Lignosulfonate and hydroxyethyl cellulose (HEC) were chosen as models for lignin and carbohydrate, respectively. Changes in the molecular weight of lignosulfonate and HEC were measured by high-performance size-exclusion chromatography and viscometry, respectively.

Lignosulfonate and HEC were individually exposed to each of the three biomimetic compounds in the presence of hydrogen peroxide. Reaction conditions used for the experiments are shown in **Table I**. All reactions were performed at 45°C. Solution pH was adjusted to 3.0, the value at which the enzyme lignin peroxidase exhibits maximum activity *(22)*.

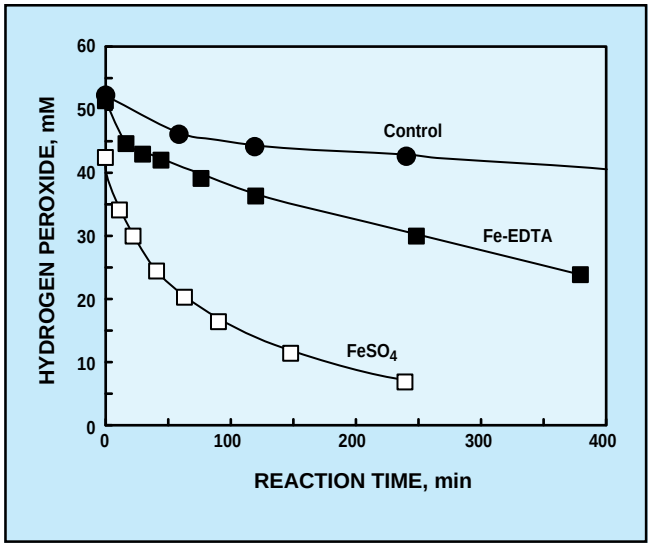
The molar concentration of iron used for the hemoglobin reaction was less than that chosen for $FeSO_4$ and

I. Reaction conditions for biomimetic oxidation of lignosulfonate and hydroxyethyl cellulose

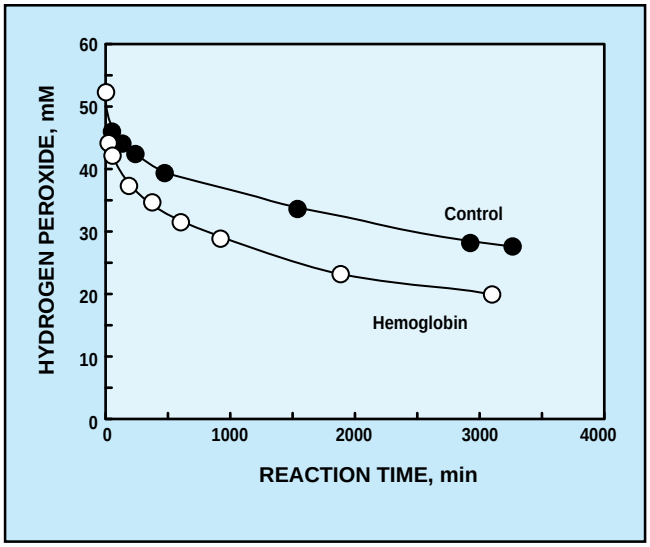
Lignosulfonate:	3.4 g/L
H_2O_2	50 mM
Biomimetic compounds	
$FeSO_4$	0.5 mM
Fe-EDTA	0.5 mM
Hemoglobin	0.062 mM
Hydroxyethyl cellulose:	3.0 g/L
H_2O_2	20 mM
Biomimetic compounds	
$FeSO_4$	0.2 mM
Fe-EDTA	0.2 mM
Hemoglobin	0.025 mM

All reactions performed at pH 3.0 and 45°C.

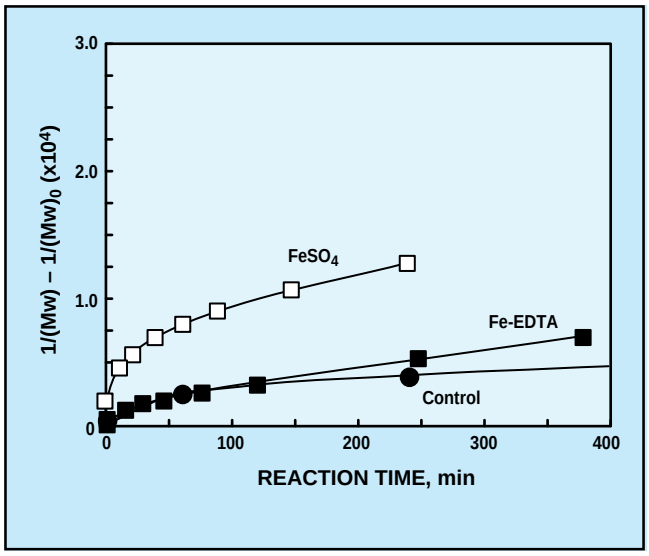
3. Residual hydrogen peroxide concentration vs. reaction time for the control, $FeSO_4$, and Fe-EDTA reactions with hydrogen peroxide



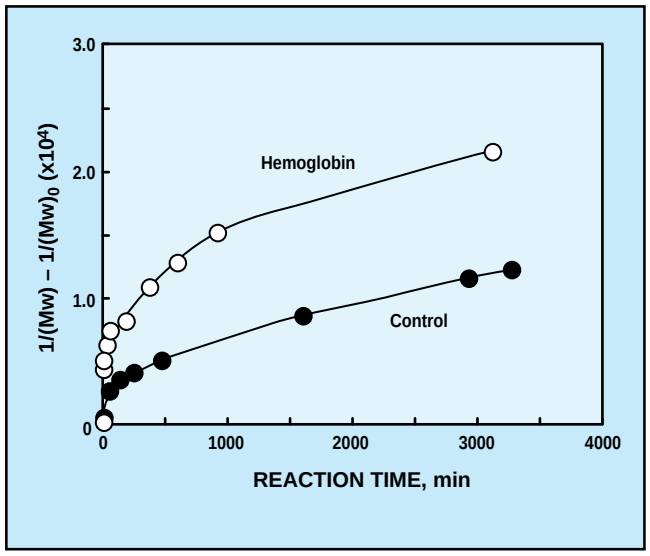
4. Residual hydrogen peroxide concentration vs. reaction time for the hemoglobin and control reactions with hydrogen peroxide



5. Number of chain scissions of lignosulfonate vs. reaction time for the control, $FeSO_4$, and Fe-EDTA reactions with hydrogen peroxide



6. Number of chain scissions of lignosulfonate vs. reaction time for the hemoglobin and control reactions with hydrogen peroxide



Fe-EDTA. To reach, for example, a 0.5 mM concentration of iron using hemoglobin, 8.1 g/L of hemoglobin would be needed in solution. As this would exceed the concentration of lignosulfonate, a smaller value was chosen (1.0 g/L).

Hydroxyl radicals were measured by means of a chemiluminescence assay *(23)*. The results will be described in detail in a subsequent publication.

Results and discussion

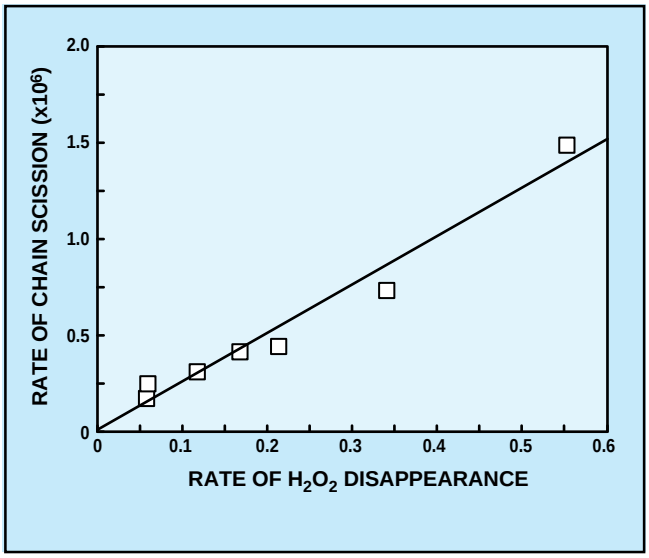
Depolymerization of substrates

Lignosulfonate. Weight-average molecular weights of lignosulfonate are shown as a function of reaction time in **Figs. 1 and 2**. The $FeSO_4$ and Fe-EDTA reactions (Fig. 1) proceeded much more quickly than the hemoglobin or control reactions (Fig. 2), which took nearly 50 hours. In all cases, significant degradation of lignosulfonate was observed.

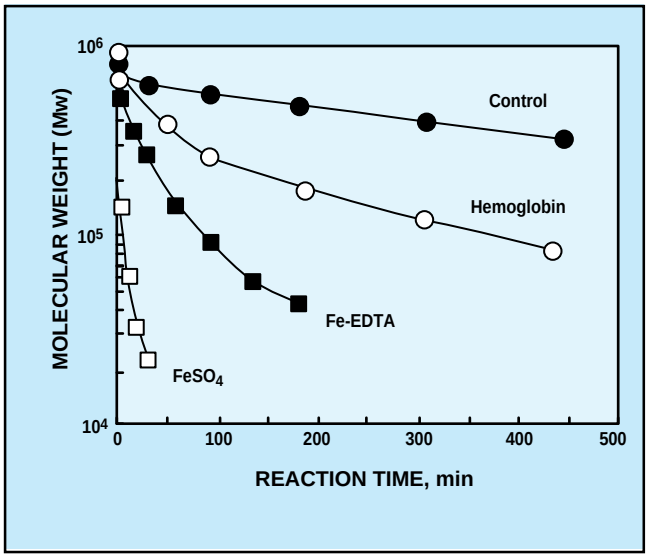
Figures 3 and 4 show the corresponding residual hydrogen peroxide data plotted against reaction time for those reactions shown in Figs. 1 and 2. All exhibit a substantial consumption of hydrogen peroxide throughout the reaction.

The degradation of lignosulfonate can be treated as random chain scission of a polymer. The number of

7. Rate of chain scission of lignosulfonate vs. the rate of hydrogen peroxide disappearance for the $FeSO_4$ reaction with hydrogen peroxide



8. Viscosity-average molecular weight of HEC vs. reaction time for the $FeSO_4$, Fe-EDTA, hemoglobin, and control reactions with hydrogen peroxide



II. Rate of chain scission for lignosulfonate in 40 mM H_2O_2

Compound	Conc. of Fe, mM	Rate
Control	...	0.36×10^{-7}
$FeSO_4$	0.50	20.0×10^{-7}
Fe-EDTA	0.50	1.90×10^{-7}
	0.06	0.63×10^{-7}
Hemoglobin	0.06	1.73×10^{-7}

III. Rate of chain scission for HEC in 40 mM H_2O_2

Compound	Conc. of Fe, mM	Rate
Control	...	0.04×10^{-7}
$FeSO_4$	0.2	13.8×10^{-7}
Fe-EDTA	0.2	1.22×10^{-7}
Hemoglobin	0.025	0.23×10^{-7}

chain scissions (N) is proportional to the difference in the degree of polymerization (DP):

$$N \propto 1/DP_t - 1/DP_0 \qquad (1)$$

where

DP_t = the DP at time t

DP_0 = the DP at t = 0 min.

As the DP is proportional to the molecular weight (M_w), Eq. 1 can be rewritten to give:

$$N \propto 1/Mw_t - 1/Mw_0 \qquad (2)$$

Figure 5 shows the number of chain scissions versus reaction time for the control, $FeSO_4$, and Fe-EDTA. **Figure 6** shows data for the hemoglobin and control reactions. The rate at which chain scission occurs is equal to the slope of these curves.

For these reactions, the concentration of hydrogen peroxide is not constant (Figs. 3 and 4). The changing rate of chain scission is due to the changing hydrogen peroxide concentration. Plots of the rate of chain scission versus the rate of hydrogen peroxide reacted are linear. (**Figure 7** shows an example plot for the $FeSO_4$ reaction with hydrogen peroxide.)

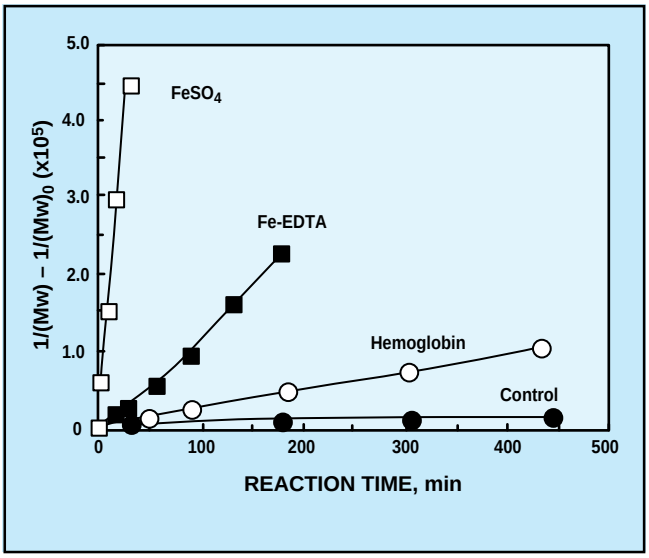
To compare the ability of these compounds to degrade lignosulfonate, the rate of chain scission at 40 mM H_2O_2 was determined. This rate was obtained by determining the slope from the plots in Figs. 5 and 6 at the appropriate time as determined from Figs. 3 and 4. These values are shown in **Table II**.

The $FeSO_4$ catalyzed reaction results in the greatest rate of chain scission at 40 mM H_2O_2. This is obvious from a comparison of the slopes of curves in Fig. 5. This high rate is related to the high rate at which hydrogen peroxide reacts in the presence of $FeSO_4$ as opposed to the other biomimetic compounds.

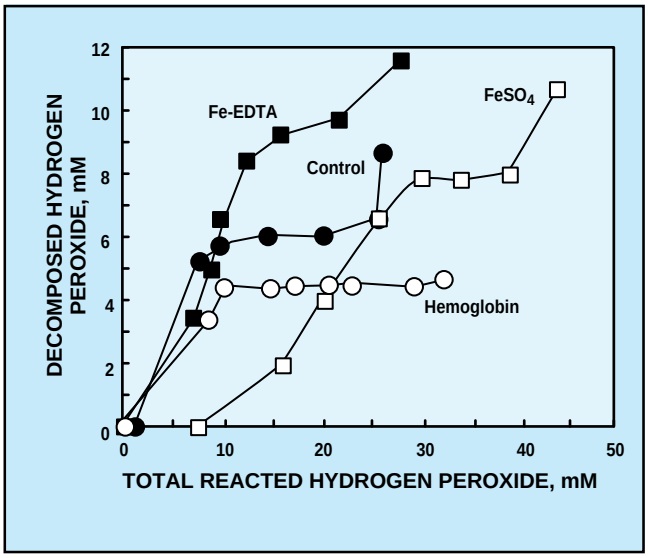
As mentioned previously, the hemoglobin concentration was significantly less than that used for the $FeSO_4$ and Fe-EDTA reactions. The Fe-EDTA concentration was decreased to an equivalent level, and the rate of chain scission was determined at 40 mM H_2O_2. This value, for the reduced Fe-EDTA concentration, is also shown in Table II.

When the concentration of Fe-EDTA is reduced, the rate of chain scission (at 40 mM H_2O_2) is consid-

9. Number of chain scissions of HEC vs. reaction time for the $FeSO_4$, Fe-EDTA, hemoglobin, and control reactions



10. Amount of hydrogen peroxide decomposed to oxygen vs. the total amount of hydrogen peroxide which has reacted



IV. Selectivity of biomimetic compounds for lignosulfonate over HEC at 40 mM H_2O_2

Compound	*Conc. of Fe, mM*	*Selectivity*
$FeSO_4$	0.200	1.4
Fe-EDTA	0.200	1.6
Hemoglobin	0.025	7.4

erably less than that for hemoglobin. This demonstrates the ability of hemoglobin, even at low concentrations, to degrade lignin.

Hydroxyethyl Cellulose (HEC). Viscosity-average molecular weights for HEC are shown in **Fig. 8**. In the presence of $FeSO_4$, HEC is quickly degraded. Substantial degradation is also observed in the presence of Fe-EDTA and hemoglobin as compared to the control.

For these experiments, little change in the hydrogen peroxide concentration was observed. It was therefore difficult to obtain information on the relative amounts of hydrogen peroxide consumed by reaction and decomposed to oxygen.

Figure 9 shows the number of chain scissions (Eq. 2) versus reaction time for these reactions. The straight lines in Fig. 9 indicate that the rate of chain scission is constant throughout the reaction. This constant rate of chain scission is most probably due to the relatively small change in the hydrogen peroxide concentration during reactions. In this case, it is difficult to compare the rates of hydrogen peroxide disappearance because little was consumed.

Rates of chain scission for HEC are shown in **Table III**. The control reaction gives the slowest rate, while $FeSO_4$ gives the quickest, followed by Fe-EDTA and then hemoglobin. This ranking is also readily apparent from the slopes of the curves in Fig. 9.

Selectivity. To compare the selectivity of these biomimetic compounds, one must take into consideration the differing reactivities of these systems. For all biomimetic compounds, the rate at which hydrogen peroxide reacts is different.

A measure of selectivity is achieved by comparing the rates of chain scission of lignosulfonate to that of HEC. **Table IV** shows selectivity values determined from the rate of chain scission obtained for each biomimetic compound at 40 mM H_2O_2 (using data from Tables II and III). Hemoglobin exhibits a substantial preference for degradation of lignosulfonate over HEC as compared to the other biomimetic compounds. In fact, $FeSO_4$ and Fe-EDTA exhibit almost no selectivity for lignosulfonate over HEC degradation.

Combined Substrate Experiments. To provide a more realistic model for pulp, lignosulfonate and HEC were combined and reacted with each of the biomimetic compounds in the presence of hydrogen peroxide. During these reactions, samples were analyzed by both chromatography and viscometry.

In all reactions, including one with hydrogen peroxide alone (no biomimetic compound), analysis of reaction samples by chromatography indicated the formation of a high-

molecular-weight product. This product is believed to be produced by condensation of the lignosulfonate onto the HEC polymer.

A decrease in the molecular weight of this product during reactions indicated that it was degraded. Additional details on this product and its rate of formation will be described in a forthcoming publication.

Consumption vs. decomposition of hydrogen peroxide

For the lignosulfonate reactions, nearly all of the hydrogen peroxide reacted (Figs. 3 and 4). For the HEC reactions, only a small amount of hydrogen peroxide reacted during these experiments. Therefore, the following discussion of the behavior of hydrogen peroxide applies only to the lignosulfonate reactions.

Hydrogen peroxide, in the presence of iron, decomposes to give oxygen via a complex radical mechanism *(24)*:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

As the reaction proceeds, the concentration of dissolved oxygen exceeds the solubility limit of the solution; excess oxygen is then liberated from solution. This oxygen was measured with a gas buret.

Figure 10 shows the amount of hydrogen peroxide decomposed to oxygen versus the total amount of hydrogen peroxide reacted for all three biomimetic compounds and the control (hydrogen peroxide only).

Excessive decomposition of hydrogen peroxide to oxygen is an inefficient use of oxidant. The addition of $FeSO_4$ to reaction solutions causes a decrease in the decomposition of hydrogen peroxide as compared to the control reaction.

Alternatively, the addition of Fe-EDTA results in an increase in the amount of hydrogen peroxide that is decomposed. However, for both the $FeSO_4$ and Fe-EDTA reactions, hydrogen peroxide continues to decompose throughout the reaction.

When hemoglobin is added to reaction solutions, after an initial increase characteristic of all reactions, the amount of hydrogen peroxide decomposed to oxygen levels off. This means that all hydrogen peroxide that is reacted is consumed by oxidation of the lignosulfonate.

Generation of hydroxyl radicals

During these experiments, the generation of hydroxyl radicals was measured using a chemiluminescence assay. Hydroxyl radicals were detected in the $FeSO_4$ and Fe-EDTA reactions. Hemoglobin interfered with the determination of hydroxyl radicals using this technique.

The largest concentration of hydroxyl radicals was detected in those reactions in which $FeSO_4$ was present. This was as expected, as Fenton's reagent is known to generate hydroxyl radicals. Hydroxyl radicals were produced during the initial stages of the reaction. Results will be presented in detail in a subsequent publication.

Summary

The selectivity of three biomimetic compounds was investigated using lignosulfonate and hydroxyethyl cellulose as substrates. All three biomimetic compounds, in the presence of hydrogen peroxide, were able to degrade both lignosulfonate and HEC models. For reactions with lignosulfonate, nearly all of the hydrogen peroxide is consumed as degradation occurs. Alternatively, little hydrogen peroxide is consumed in reactions with HEC.

Rates of chain scission at 40 mM H_2O_2 were compared to determine selectivity. Hemoglobin exhibited the greatest selectivity for lignosulfonate degradation over HEC degradation, when the two substrates were oxidized separately.

Hemoglobin appears not only to be a selective biomimetic compound but is also an efficient one in its use of hydrogen peroxide. When compared to the control reaction, hemoglobin allows more hydrogen peroxide to be consumed by reaction than decomposed to oxygen. Careful selection of biomimetic compounds can increase the efficiency of hydrogen peroxide in a peroxide delignification stage.

When lignosulfonate and HEC were combined in reaction, the production of a high-molecular-weight condensation product was observed in all reactions, including that with hydrogen peroxide alone (no biomimetic compound).

Experimental methods

Chemicals and substrates

Ultrapure water was obtained from a Barnestead reverse osmosis water purifier configured to provide Class Type II (ultrapure) lab water. Hydrogen peroxide (J. T. Baker) and hydrochloric acid were also of ultrapure quality. Hemoglobin (Sigma Chemical Co.) was bovine hemoglobin, unpurified. All other chemicals were of reagent grade.

Lignosulfonate at about 50% solids was obtained from Daishowa Chemical Inc., Rothschild, WI. This solution was diluted, passed through a 0.22-μm Whatman filter, and then ultrafiltered using 30,000 and 10,000 daltons filters to obtain a fraction of relatively narrow molecular weight. Concentrations of stock solutions were determined by spectrometry; a standard calibration was made by using a solution of known solids content.

Hydroxyethyl cellulose was obtained from Aqualon Chemical Co., Wilmington, DE. Solutions were prepared by dissolving a preweighed amount of HEC into a known quantity of ultrapure water (usually 300 mL). Solutions were allowed to mix for one hour at room temperature and then overnight at 4°C to ensure complete swelling of the substituted cellulose.

Reactor and reaction sampling

All experiments were performed in a 300-mL Teflon™-lined, magnetically stirred batch reactor. The reactor was equipped with an air-tight lid, sampling line, pH probe, and gas buret. Samples were periodically removed and immediately placed on ice. Aliquots from this sample were removed immediately for chromatography and viscometry measurements; hydrogen peroxide was removed from these aliquots using sodium bisulfite.

High-performance size-exclusion chromatography

High-performance size-exclusion chromatography was performed using a Bio-Rad Bio-Sil SEC 125 size-exclusion column. A Varian 5020 liquid chromatograph, equipped with a Varian UV-50 variable-wavelength detector, and a Hewlett-Packard integrator were used. The mobile phase was 50-mM citric acid-disodium hydrogen phosphate buffer *(25)*, pH 3.0, and flowed at 0.8 mL/min. The column was calibrated using sodium polystyrene sulfonates.

Viscometry

An existing correlation between intrinsic viscosity and molecular weight (the Mark-Houwink equation) was used to obtain molecular weight values. Mark-Houwink parameters for dilute HEC solutions were obtained from Brown *et al. (26)*. Samples (1.0–5.0 mL) were introduced via pipet into a capillary viscometer, immersed in a 25°C water bath, and allowed to equilibrate for 5 min. Water was then added to obtain the minimum volume necessary for measurement. Dilutions were then made to obtain four different concentrations. Flow time was recorded for each of two passes. ■

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The authors would like to thank the Institute of Paper Science and Technology and its member companies for supporting this research. This work was used by Colleen Walker as partial fulfillment of the requirements for the Ph.D. degree at the Institute of Paper Science and Technology.

Received for review Sept. 16, 1994.
Accepted Oct. 22, 1994.

Presented at the TAPPI 1994 Biological Sciences Symposium.