0273-2289/02/97/0091/$13.25

Fenton's Reagent–Mediated Degradation of Residual Kraft Black Liquor

Elisa Araujo,[1,2] Antonio J. Rodríguez-Malaver,*[,1] Aura M. González,[2] Orlando J. Rojas,[2] Nancy Peñaloza,[1] Johnny Bullón,[2] Mayra A. Lara,[1,2] and Natalia Dmitrieva[1]

[1]Laboratorio de Bioquímica Adaptativa, Departamento de Bioquímica, Facultad de Medicina, and [2]Laboratorio FIRP, Departamento de Ingeniería Química, Facultad de Ingeniería, Universidad de Los Andes, Mérida 5101, Venezuela, E-mail: antoniorod@cantv.net

Received June 29, 2001; Revised November 7, 2001; Accepted November 16, 2001

Abstract

In this work, the effect of Fenton's reagent on the degradation of residual Kraft black liquor was investigated. The effect of Fenton's reagent on the black liquor degradation was dependent on the concentration of H_2O_2. At low concentrations (5 and 15 m*M*) of H_2O_2, Fenton's reagent caused the degradation of phenolic groups (6.8 and 44.8%, respectively), the reduction of reaction medium pH (18.2%), and the polymerization of black liquor lignin. At a high concentration (60 m*M*) of H_2O_2, Fenton's reagent induced an extensive degradation of lignin (95–100%) and discoloration of the black liquor. In the presence of traces of iron, the addition of H_2O_2 alone induced mainly lignin fragmentation. In conclusion, Fenton's reagent and H_2O_2 alone can degrade residual Kraft black liquor under acidic conditions at room temperature.

Index Entries: Fenton's reagent; free radicals; hydrogen peroxide; hydroxyl radical; Kraft black liquor; lignin degradation; pulp mill effluent treatment.

Introduction

Black liquor, an intermediate product of the Kraft process for production of pulp, is one of the most important industrial fuels *(1)*. It is burned in recovery boilers with the objective of simultaneous recovery of heat and chemicals (sodium and sulfur) *(1)*. However, in most developing countries,

*Author to whom all correspondence and reprint requests should be addressed.

Kraft plants are not always fully equipped with recovery units. Therefore, the pulp industry in these countries produces high amounts of residual Kraft black liquor, which are rather difficult to dispose of and represent an environmental problem. On the other hand, Kraft black liquor has valuable chemical compounds that could be utilized such as lignin and some metals *(2)*.

Fenton's reagent has been widely used to treat a variety of industrial wastes containing a range of toxic organic compounds such as phenols, formaldehydes, pesticides, and wood preservatives *(3–10)*. This reagent has been applied to induce organic pollutant destruction, reduction of toxicity, improvement in biodegradability, removal of biologic oxygen demand/chemical oxygen demand, and removal of odor and color. Fenton's reagent *(11)*, a combination of hydrogen peroxide and a ferrous salt, is a potent oxidizing agent of organic compounds in acidic aqueous solution *(12–15)*. Fenton's reagent generates a highly reactive free radical, hydroxyl radical *(16,17)*, which reacts with various wood compounds, including lignin *(18–21)*. However, to date there is little knowledge about the effects of the Fenton's reaction on the industrial black liquors. Thus, the general aim of the present investigation was to study Kraft black liquor degradation by Fenton's reagent. The particular objective was to examine the effect of H_2O_2 concentration on the performance of Fenton's reagent under acidic pH in order to establish its optimum conditions for the degradation of Kraft black liquor.

Materials and Methods

Materials

All reagents were of the highest quality available. Hydrogen peroxide (30% [w/w] H_2O_2 in water) solution and mannitol were purchased from Sigma (St. Louis, MO), and $FeCl_2 \cdot 4H_2O$ (99%) was obtained from Fluka. All solutions were prepared with Millipore-quality water (Milli-Q plus, Ultrapure water system, 18 MΩ·cm).

Residual Kraft Black Liquor

Residual Kraft black liquor was kindly provided by Smurfit-Mocarpel pulp mill (Venezuela). This black liquor is derived from the digestion of Caribbean pine (*Pinus caribaea*) and was taken from the recovery plant at the process line, where the total solid content was 32.7%, density was 1.2 g/mL, and pH was 12.5. The Kraft lignin content was determined to be 17.8% according to Kim's method *(22)*.

Degradation of Kraft Black Liquor by Fenton's Reagent

Hydroxyl radical was generated by the Fenton's reagent ($Fe^{2+} + H_2O_2$) *(16)*. Fifteen microliters of black liquor were added to 5 mL of an aqueous solution of $FeCl_2$ (1 m*M*). With the solution stirring at room temperature,

570 µL of H_2O_2 (for final concentrations of 5, 15, 30, and 60 m*M*) were added over 1 min. The vessels were capped and the reaction mixture was under continuous agitation for 20 min.

UV/VIS Spectrophotometer Analysis

For UV/VIS spectrophotometric analysis, a Lambda 3B (Perkin-Elmer®) spectrophotometer was used. Samples were prepared as follows: to 200 µL of black liquor sample was added 1.8 mL of 0.1 *N* NaOH. Samples were scanned over the wavelength range from 200 to 400 nm using the PECSS program (Perkin-Elmer® Computerized Spectroscopy Software, version 3.2). In some experiments, the absorbance maximum of Kraft lignin was measured at 280 nm *(23)*.

Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) was employed to obtain information on the molecular weight distribution of residual black liquor samples. The high-performance SEC of black liquor was performed on a high-performance liquid chromatograph with a size-exclusion column (310 × 10 mm id, Superose® 12HR 10/30; Pharmacia Biotech), a binary pump (LC Pump 250; Perkin-Elmer) with a manual injector (Rheodyne), and a UV detector (UV/VIS Detector LC 295; Perkin-Elmer) at 280 nm connected to a PC (PE NELSON, model 1022; Perkin-Elmer). The optimum conditions for black liquor analysis were as follows: the mobile phase was 0.1 *N* NaOH, flow was 0.4 mL/min, injection volume was 20 µL, and black liquor dilution was 200 µL of sample and 200 µL of 0.1 *N* NaOH *(24)*. Prior to SEC, all samples were filtered through 0.45-µm Millipore membranes. A calibration curve was done to obtain the molecular weight distribution of black liquor samples using sodium polystyrene sulfonate standards (Polysciences) *(24–27)*.

Determination of Phenolic Groups

Phenolic groups were measured using the procedure of Markwell et al. *(28)* with a solution of known concentration of precipitated Kraft black liquor as standard.

Determination of Iron Ions

The determination of iron in the black liquor was performed by atomic absorption spectroscopy with an absorption/emission spectrophotometer 200-A (Buck Scientific).

Sample Color

Photographs of all preparations were taken to keep a record of the sample color. A commercial camera with Gold Kodak® film was employed.

Statistical Analyses

Results were expressed as the mean ± SE of three different preparations. Data were analyzed by student's *t*-test (GraphPad, Prism®, Version 2.01). The level of significance was accepted as $p < 0.05$.

Results and Discussion

Effect of Time and H_2O_2 Concentration on pH of Incubation Medium

Since the optimum pH for the completion of Fenton's reaction is found over the range of acidic pH *(12,16,29–34)*, the incubation medium's pH was adjusted to approx 5.5 with 250 m*M* H_2SO_4. Then, ferrous iron as $FeCl_2$ (final concentration: 1 m*M*) was added to the incubation medium. This addition caused a low reduction in medium pH, which could be owing to traces of H_2SO_4, contained in $FeCl_2$ *(16,30)*. When the H_2O_2 was added to the incubation medium, a further reduction in pH occurred. This change in pH happened within 2 min after adding the H_2O_2. This decrease in pH has been attributed to the fragmentation of organic material into organic acids *(12,16,30)*. After the incubation for 20 min, the pH value was unchanged (Fig. 1). Samples without $FeCl_2$ did not show any significant change in pH. The same pattern of pH changes was observed for all concentrations of H_2O_2 (Fig. 1).

Effect of H_2O_2 Concentration on ΔpH

Fenton's reagent caused a change in ΔpH, which was negative; for example, the final pH was lower than the initial one. As discussed, this change was explained by the fact that during the Fenton's reaction acidic compounds (organic acids) formed, as has been proposed by other investigators *(12,16,30)*. The highest concentration of H_2O_2 (60 m*M*) produced the largest change in ΔpH (Fig. 2). The samples of black liquor that were treated with increased concentrations of H_2O_2 alone did not show any change in ΔpH (Fig. 2).

Effect of H_2O_2 Concentration on Phenolic Groups of Kraft Black Liquor

Lignin is considered the main organic compound in the black liquor *(2,25)*, and it is well known that lignin has phenolic hydroxyl groups in its structure *(35)*. Therefore, it was decided to investigate the effect of Fenton's reagent on phenolic hydroxyl groups of black liquor lignin. Increased concentrations of H_2O_2 in the Fenton's reagent induced the degradation of black liquor phenolic groups (Fig. 3). From a chemical point of view, this is not surprising because these functional groups have a very high affinity for reactive oxygen species (ROS) *(18)*. This degradation reached its maximum at a concentration of 60 m*M* H_2O_2 (Fig. 3). On the other hand, H_2O_2 alone caused only a partial degradation of black liquor phenolic groups (Fig. 3).

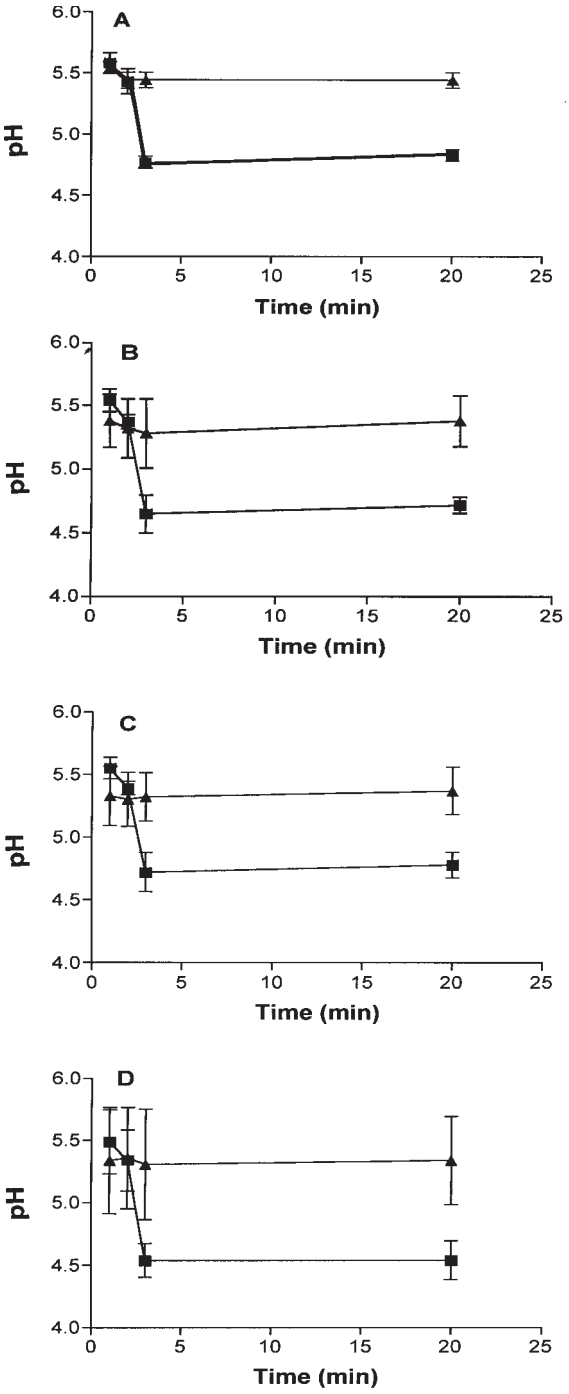

Fig. 1. Effect of time on pH of incubation medium. (■) Black liquor samples were treated with Fenton's reagent containing different concentrations of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature; (▲) black liquor samples were treated with different concentrations of H_2O_2 alone for 20 min at room temperature. **(A)** 5 m*M* H_2O_2; **(B)** 15 m*M* H_2O_2; **(C)** 30 m*M* H_2O_2; **(D)** 60 m*M* H_2O_2.

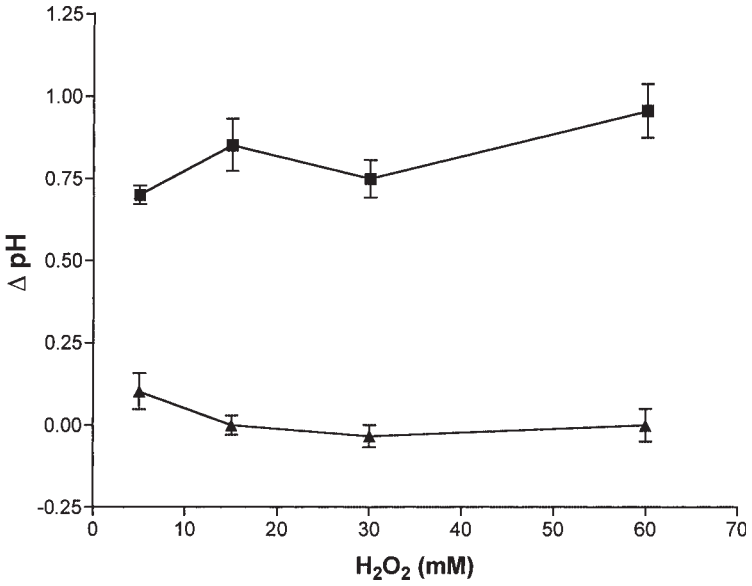

Fig. 2. Effect of H_2O_2 concentration on ΔpH. (■) Black liquor samples were treated with Fenton's reagent containing different concentrations (5, 15, 30, 60 m*M*) of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature; (▲) black liquor samples were treated with different concentrations of H_2O_2 alone for 20 min at room temperature.

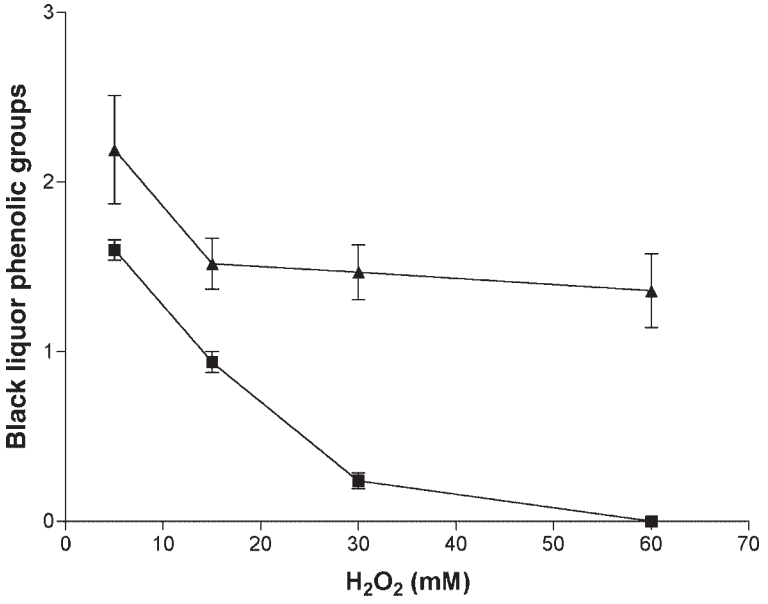

Fig. 3. Effect of H_2O_2 concentration on black liquor phenolic groups (g/L). (■) Black liquor samples were treated with Fenton's reagent containing different concentrations (5, 15, 30, 60 m*M*) of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature; (▲) black liquor samples were treated with different concentrations of H_2O_2 alone for 20 min at room temperature.

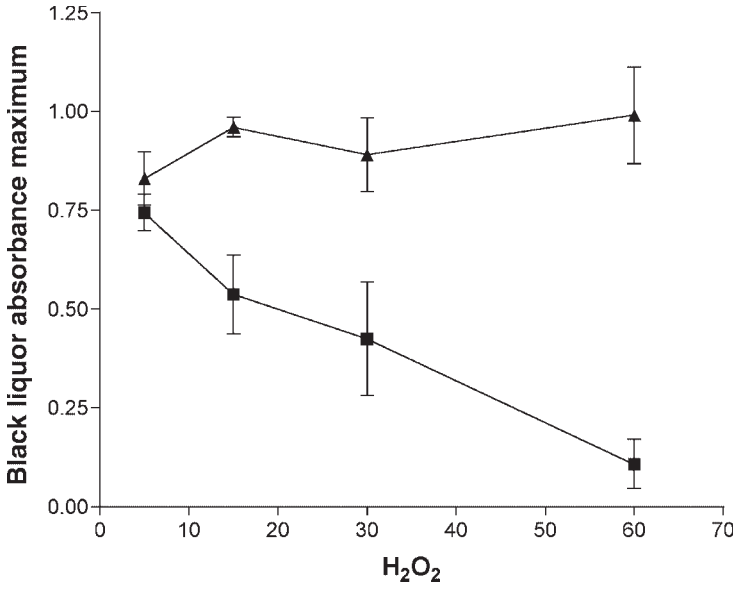

Fig. 4. Effect of H_2O_2 concentration (in m*M*) on black liquor absorbance maximum (280 nm). (■) Black liquor samples were treated with Fenton's reagent containing different concentrations (5, 15, 30, 60 m*M*) of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature; (▲) black liquor samples were treated with different concentrations of H_2O_2 alone for 20 min at room temperature.

Effect of H_2O_2 Concentration on UV Absorbance of Kraft Black Liquor Lignin

The molecule of Kraft lignin absorbs over the UV range and has an absorbance maximum at 280 nm *(23)*. When the pH of the incubation medium was adjusted to approx 5.5, the increase in H_2O_2 concentration caused a decrease in the maximum of lignin absorbance of black liquor samples treated with Fenton's reagent (Fig. 4). This indicated that the hydroxyl radical generated by the Fenton's reagent was very effective in degrading the black liquor lignin. It has been demonstrated by other investigators that ROS, particularly hydroxyl radical and singlet oxygen, are able to react with lignin, causing its chemical degradation *(18,36–41)*. On the other hand, absorbance did not change when samples were treated with H_2O_2 alone (Fig. 4). It is noteworthy to underline that those changes observed in samples treated with Fenton's reagent were not owing to a shift in lignin spectra as shown in Fig. 5.

Effect of H_2O_2 Concentration on Molecular Weight Distribution of Black Liquor Lignin

The increase in H_2O_2 concentration induced a reduction in both weight average molecular weight (M_w) and number average molecular weight (M_w) of black liquor samples treated with Fenton's reagent (Table 1). Interestingly, at low concentrations of H_2O_2, the values of M_w and M_n of samples

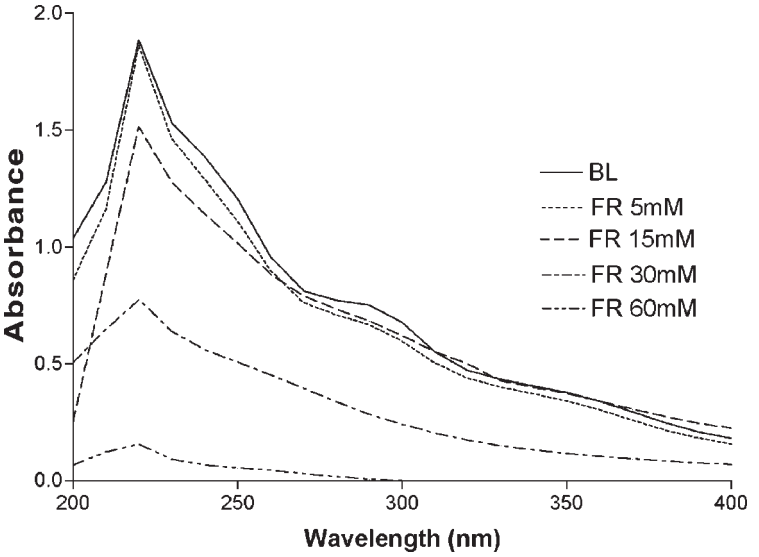

Fig. 5. Effect of H_2O_2 concentration on UV spectrum of black liquor. BL, black liquor alone; FR, black liquor samples treated with Fenton's reagent containing different concentrations (5, 15, 30, 60 m*M*) of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature.

Table 1
Effect of H_2O_2 Concentration on Weight Average Molecular Weight (M_w), Number Average Molecular Weight (M_n), and Dispersity (D) of Black Liquor

Treatment $[H_2O_2]$[a]	M_w	M_n	D (M_w/M_n)
Black liquor alone	1561.1 ± 62.3	720.0 ± 5.8	2.2 ± 0.1
5 m*M* FR	2961.2 ± 321.0	1200.0 ± 57.8	2.5 ± 0.4
15 m*M* FR	2408.7 ± 76.6	1133.3 ± 33.4	2.1 ± 0.03
30 m*M* FR	1906.1 ± 9.7	1233.3 ± 33.4	1.6 ± 0.03
60 m*M* FR	867.6 ± 45.6	820.0 ± 61.2	1.1 ± 0.03
5 m*M* H_2O_2	1847.9 ± 33.4	793.3 ± 12.0	2.4 ± 0.03
15 m*M* H_2O_2	1628.5 ± 140.3	643.3 ± 78.9	2.6 ± 0.1
30 m*M* H_2O_2	1309.7 ± 89.0	503.3 ± 23.4	2.6 ± 0.03
60 m*M* H_2O_2	1152.6 ± 73.1	456.7 ± 16.7	2.5 ± 0.03

[a]FR, samples treated with Fenton's reagent; H_2O_2, samples treated with H_2O_2 alone.

treated with Fenton's reagent were higher than those of black liquor without any treatment (Table 1). This result could indicate that the molecule of lignin was fragmented by the Fenton's reagent and then these fragments copolymerized, creating compounds with higher molecular weight, as shown by SEC (Fig. 6). It has been proposed that unlike other natural polymers, lignin cannot be degraded to give structurally intact precursors. This is owing to the presence of many reactive sites in the molecule; therefore, hydrolysis reactions are often coupled to condensation reactions *(42)*.

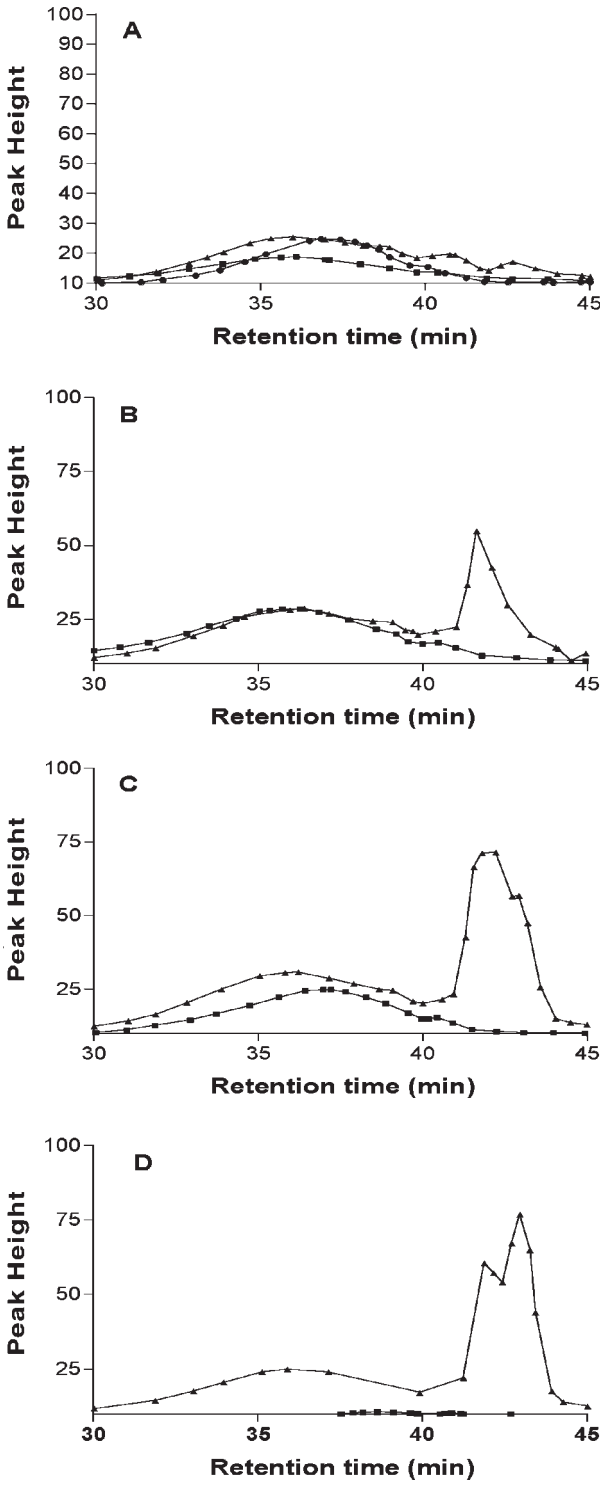

Fig. 6. Effect of H_2O_2 concentration on molecular weight distribution of black liquor. (■) Black liquor samples were treated with Fenton's reagent containing different concentrations of H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature; (▲) black liquor samples were treated with different concentrations of H_2O_2 alone for 20 min at room temperature; (●) black liquor alone incubated for 20 min at room temperature. **(A)** 5 m*M* H_2O_2; **(B)** 15 m*M* H_2O_2; **(C)** 30 m*M* H_2O_2; **(D)** 60 m*M* H_2O_2.

In the samples treated with H_2O_2 alone, although there was a small reduction in M_w and M_n values, SEC clearly showed that there was a fragmentation of black liquor lignin molecules, and the appearance of a peak over the range of lower molecular weights (Table 1 and Fig. 6). It seems that H_2O_2 alone only caused the fragmentation of lignin molecule, whereas the Fenton's reagent induced an initial fragmentation, which was rapidly followed by the polymerization of lignin fragments at low concentrations of H_2O_2. At the highest concentration of H_2O_2, Fenton's reagent could readily degrade lignin molecule (Fig. 6) and the decrease in M_w and M_n was approx 50% (Table 1).

Since black liquor sample treated with H_2O_2 alone showed a fragmentation of lignin molecules (Fig. 6), it was decided to measure iron ions in the black liquor stock solution by atomic absorption spectrometry. The concentration of iron ions was 0.842 ppm in the black liquor stock solution. It is possible that these traces of iron were sufficient to reduce H_2O_2 and produced hydroxyl radical, which then reacted with lignin and caused its fragmentation into molecules with lower molecular weight. An alternative explanation for the fragmentation of lignin is the action of cationoid species. Cationoid species are formed by the protonation of H_2O_2 under acidic conditions, and they are able to react with the π electrons of the benzene ring, resulting in ring cleavage reactions of lignin molecules *(43,44)*.

As shown in Table 1, Fenton's reagent also produced a reduction in dispersity (*D*) of approx 50%, whereas H_2O_2 alone did not modify this parameter. The value of *D* of black liquor lignin was similar to those values reported by other investigators *(25)* and was close to what would be expected from a polymer degraded through a random process.

Effect of H_2O_2 Concentration on Black Liquor Color

Black liquor solution darkened on the addition of H_2O_2 and cleared up as the Fenton's reaction reached completion. Samples with H_2O_2 alone also darkened on the addition of H_2O_2, but their clearance was not completed (results not shown). It has been reported that in certain conditions H_2O_2 induces the formation of chromophoric species such as quinones, cinnamaldehyde, and ring-conjugated ketones *(44,45)*.

Effect of Mannitol (100 mM) on Degradation of Kraft Black Liquor

Mannitol is widely used as a hydroxyl radical scavenger to demonstrate the formation of this radical under certain experimental conditions *(36,46)*. Therefore, to ensure that hydroxyl radical was formed in our conditions, the effect of mannitol on Fenton's reagent–mediated black liquor degradation was studied. Mannitol was able to prevent the degradation of black liquor by Fenton's reagent (about 30–45% of protection) as shown by the inhibition of reduction in area under the curve and absorbance maximum loss (Fig. 7). This result confirmed that hydroxyl radical was formed

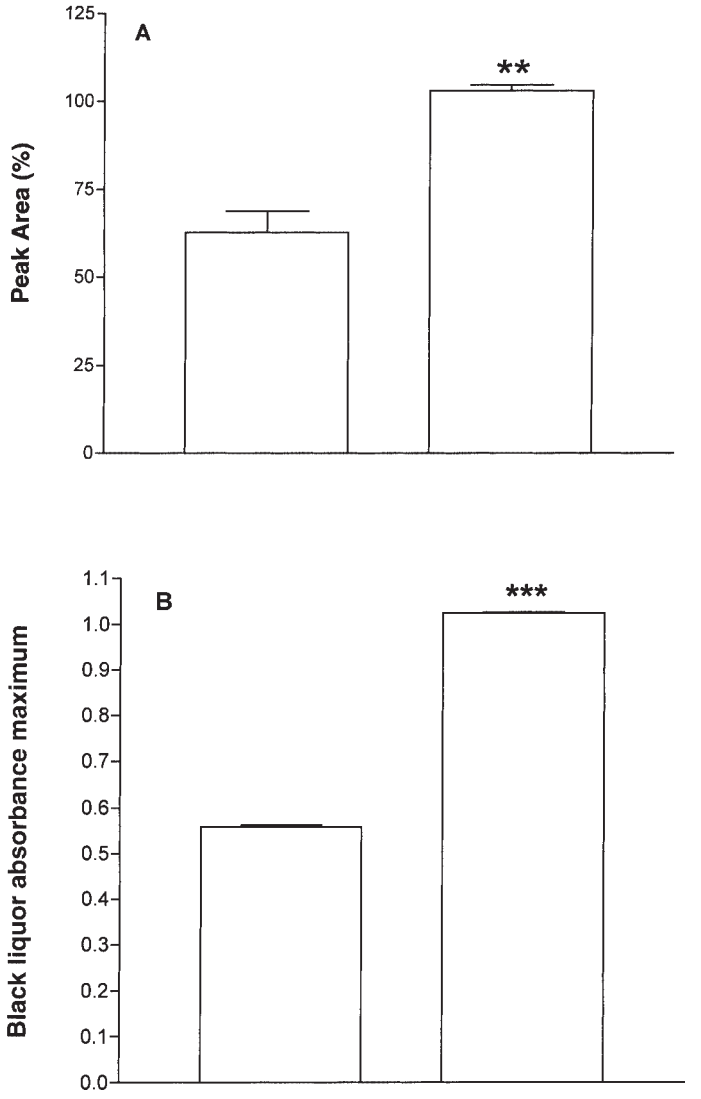

Fig. 7. Effect of mannitol (100 m*M*) on black liquor degradation. Samples were treated with Fenton's reagent containing 30 m*M* H_2O_2 and 1 m*M* $FeCl_2$ for 20 min at room temperature. **(A)** Area under the curve from sample size-exclusion chromatograms (**$p < 0.001$, in relation to samples without mannitol); **(B)** absorbance maximum of samples (***$p < 0.0001$, in relation to samples without mannitol).

during the Fenton's reaction under the conditions of this investigation. However, the partial protection brought about by mannitol might indicate that the formation of hydroxyl radical occurred very close to the lignin molecule and mannitol was unable to interact with this radical. Another explanation was that other ROS were involved in black liquor degradation; however, they were not quenched by mannitol.

Conclusion

The degradation of residual Kraft black liquor by Fenton's reagent was investigated at room temperature under acidic conditions and was found to be easily degraded. In the presence of Fenton's reagent and at a low concentration of H_2O_2, the main degradation products seemed to polymerize and form molecules with higher molecular weight, whereas at a high concentration of H_2O_2, black liquor lignin appeared to be converted into compounds with lower molecular weight. By contrast, the use of less reactive reagent (H_2O_2 and traces of iron) could fragment lignin molecule and produce intermediate products of lower molecular weight.

The problem of environmental pollution has led to a growing interest in the potential use of different treatments for the degradation of lignin containing industrial waste. This investigation showed that Fenton's reagent could be an efficient treatment for the degradation of industrial black liquors.

Acknowledgments

We gratefully acknowledge the financial support provided by FONACIT-Venezuela (grant no. S1-2000000816) and by Universidad de Los Andes (CDCHT-ULA, grant no. I710-01-08-F). We also thank the Petroleum Laboratory from Engineering School for the atomic absorption spectrometry determinations.

References

1. Macek, A. (1999), *Prog. Energy Combust. Sci.* **25,** 275–304.
2. Passinen, K. (1968), *Recovery of Pulping Chemicals Symposium Notes*, Kauppakirjapaino OY, Helsinki.
3. Arslan, I., Balcioghi, I. A., and Tuhkanen, T. (1999), *Chemosphere* **39,** 2767–2783.
4. Balanosky, E., Fernandez, J., Kiwi, J., and Lopez, A. (1999), *Water Sci. Technol.* **40,** 417–424.
5. Dercova, K., Vrana, B., Tandlich, R., and Subova, L. (1999), *Chemosphere* **39,** 2621–2628.
6. Fallmann, H., Krutzler, T., Bauer, R., Malato, S., and Blanco, J. (1999), *Catalysis Today* **54,** 309–319.
7. Kang, S.-F., Liao, C.-H., and Hung, H.-P. (1999), *J. Hazard. Mater.* **65,** 317–333.
8. Rodriguez, J., Contreras, D., Parra, C., Freer, J., Baeza, J., and Duran, N. (1999), *Water Sci. Technol.* **40,** 351–355.
9. Wu, K., Xie, Y., Zhao, J., and Hidaka, H. (1999), *J. Mol. Catalysis A: Chem.* **144,** 77–84.
10. del Río, J. C., Romero, J., and Gutierrez, A. J. (2000), *J. Chromatogr. A* **874,** 235–245.
11. Fenton, H. J. H. (1894), *J. Chem. Soc.* **65,** 899–910.
12. Oliveros, E., Legrini, O., Hohl, M., Müller, T., and Braun, A. M. (1997), *Chem. Eng. Proc.* **36,** 397–405.
13. Shah, M. M., Barr, D. P., Chung, N., and Aust, S. D. (1992), *Toxicol. Lett.* **64–65,** 493–501.
14. Nada, A.-A. M. A., Yousef, M. A., Shaffei, K. A., and Salah, A. M. (1998), *Polym. Degrad. Stabil.* **62,** 157–163.
15. Kang, Y. W. and Hwang, K.-Y. (2000), *Water Res.* **34,** 2786–2790.
16. Walling, C. (1975), *Acc. Chem. Res.* **8,** 125–131.
17. Sheldon, R. A. and Kochi, J. K. (1981), *Metal-Catalyzed Oxidations of Organic Compounds*, Academic, New York.

18. Hall, P. L. (1980), *Enzyme Microbiol. Technol.* **2,** 170–176.
19. Berlin, A. A. and Kislenko, V. N. (1996), *Eur. Polym. J.* **32,** 1023–1029.
20. Henriksson, G., Zhang, L., Li, J., Ljungquist, P., Reitberger, T., Pettersson, G., and Johansson, G. (2000), *Biochim. Biophys. Acta* **1480,** 83–91.
21. Lindsey, M. E. and Tarr, M. A. (2000), *Chemosphere* **41,** 409–417.
22. Kim, H., Hill, M. K., and Fricke, A. L. (1987), *TAPPI J.* **12,** 112–116.
23. Janshekar, H., Brown, C., and Fiechter, A. (1981), *Anal. Chim. Acta* **130,** 81–91.
24. González, A. M. (1998), MSc thesis, Universidad de Los Andes, Mérida, Venezuela.
25. Sarkanen, K. and Ludwing, C. (1971), *Lignin Occurrence, Formation, Structure and Reaction*, John Wiley & Sons, New York.
26. Wagner, B. A., To, T., Teller, D. E., and McCarthy, J. L. (1986), *Holzforschung* **40,** 67–73.
27. Himmel, M. E., Tatsumoto, K., Grohmann, K., Johnson, D., and Chum, H. (1990), *J. Chromatogr.* **498,** 93–104.
28. Markwell, M. A., Haas, S. M., Bieber, L. L., and Tobert, N. E. (1978), *Anal. Biochem.* **87,** 106–210.
29. Eisenhauer, H. R. (1964), *J. WPCF* **36,** 1116–1128.
30. Bishop, D. F., Stern, G., Fleischman, M., and Marshall, L. S. (1968), *I&EC Proc. Design Dev.* **7,** 110–117.
31. Sedlak, D. L. and Andren, A. W. (1991), *Environ. Sci. Technol.* **25,** 777–782.
32. Lipczynska-Kochany, E. (1991), *Chemosphere* **22,** 529–536.
33. Pignatello, J. J. (1992), *Environ. Sci. Technol.* **26,** 944–951.
34. Zepp, G. R., Faust, B. C., and Hoigné, J. (1992), *Environ. Sci. Technol.* **26,** 313–319.
35. Higuchi, T. (1980), in *Lignin Biodegradation: Microbiology, Chemistry, and Potential Applications*, vol. I, Kirk, T. K., Higuchi, T., and Chang, H.-M. eds., CRC Press, Boca Raton, FL, pp. 1–20.
36. Bes, B., Ranjeva, R., and Boudet, A. M. (1983), *Biochimie* **65,** 283–289.
37. Nakatsubo, F., Reid, I. D., and Kirk, T. K. (1981), *Biochem. Biophys. Res. Commun.* **102,** 484–491.
38. Forney, L. J., Reddy, C. A., Tien, M., and Aust, S. D. (1982), *J. Biol. Chem.* **257,** 11,455–11,462.
39. Kelley, R. L. and Reddy, C. A. (1982), *Biochem. J.* **206,** 423–425.
40. Kutsuki, H. and Gold, M. H. (1982), *Biochem. Biophys. Res. Commun.* **109,** 320–327.
41. Bentivenga, G., Bonini, C., D'Auria, M., De Bona, A., and Mauriello, G. (1999), *Chemosphere* **39,** 2409–2417.
42. Himmel, M. E., Oh, K. K., Sopher, D. W., and Chum, H. L. (1983), *J. Chromatogr.* **267,** 249–265.
43. Levitt, L. S. (1955), *J. Org. Chem.* **20,** 1297.
44. Xiang, Q. and Lee, Y. Y. (2000), *Appl. Biochem. Biotechnol.* **84–86,** 153–162.
45. Sun, R., Tomkinson, J., Zhu, W., and Wang, S. Q. (2000), *J. Agric. Food Chem.* **48,** 1253–1262.
46. Cohen, G. and Cerderbaum, A. I. (1979), *Science* **204,** 66–67.