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Biobleaching of Nonwoody Pulps Using Xylanase of *Bacillus brevis* BISR-062

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Abstract

The efficiency of xylanase of *Bacillus brevis* BISR-062 as a prebleaching agent was evaluated on three nonwoody pulps at two different pH values (7.0 and 8.5). Crude xylanase was found to have an optimum temperature and pH of 65–70°C and 7.0, respectively. The stability of the enzyme was determined at two pH values (7.0 and 8.0), and it lost approx 50% of its activity at both values within 2 h at 50°C. However, the enzyme was found to be effective as a prebleaching agent only with rice straw pulp. A maximum brightness gain of 6 points was obtained with this pulp at pH 7.0. The strength properties of the rice straw pulp at pH 7.0 also improved as the result of enzyme treatment.

Index Entries: Biobleaching; *Bacillus brevis*; xylanase; nonwoody pulps.

Introduction

Bleach plant effluents from the pulp and paper industry are becoming a matter of concern to environmentalists. Stringent regulations related to bleach plant effluents resulted in the development of alternative technology that can reduce the pollution load. One such method is the use of xylanase in the existing method of the bleaching sequence. This can result in either a reduction in bleaching chemicals or attainment of higher brightness for the enzyme-treated pulp at the same chemical dosage. Viikari et al. *(1)*, for the first time, demonstrated the usefulness of xylanase in reducing the consumption of bleach chemicals. Today, the beneficial effects of xylanase have also been commercially demonstrated in the pulp and paper industry *(2,3)*. Most of the reported studies on xylanase have concentrated

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on hardwood and softwood pulps, whereas only a few studies have also reported the effect of xylanase on nonwoody pulps *(4–9)*. Different world organizations have recommended using nonwoody pulps in the pulp and paper industry owing to the changes in agricultural policies, wood supply issues, and environmental concerns *(10,11)*.

In the present study, we assessed the efficiency of xylanase as a function of pulp raw material and pH of the pulp in a total chlorine-free bleaching sequence. A crude xylanase of a new isolate, *Bacillus brevis* BISR-062, was tested as a prebleaching agent on nonwoody pulps (wheat straw, rice straw, and jute pulps). Uses of wheat straw and jute pulps have been reported in the literature, but rice straw has rarely been used in biobleaching applications *(4–9)*. The effects of xylanase of *B. brevis* BISR-062 were evaluated on three nonwoody pulps at an optimum pH of the enzyme and at a higher pH of 8.5.

Materials and Methods

Medium and Culture Conditions

Xylanase was used as a prebleaching agent and the enzyme was obtained from *B. brevis* BISR-062. The culture was isolated from a saline lake of Rajasthan, India, and submitted to the Microbial Type Culture Collection, Chandigarh, India, having accession no. MTCC 7054. For *B. brevis* BISR-062 culture, medium with the following composition was used for the subculturing and enzyme production: 6 g/L of Na_2HPO_4, 3 g/L of KH_2PO_4, 1 g/L of NH_4Cl, 0.5 g/L of NaCl, 10 g/L of birchwood xylan or wheat straw powder, 1 mL/L of $MgSO_4 \cdot 7H_2O$, 1 mL/L of thiamine-HCl, 1 mL/L of $CaCl_2 \cdot 2H_2O$, and stock solution (246.5 g/L of $MgSO_4 \cdot 7H_2O$, 10 mg/L of thiamine-HCl, 14.7 g/L of $CaCl_2 \cdot 2H_2O$, pH 8.0). The production of xylanase was carried out with *B. brevis* BISR-062 in a fermentor under optimum conditions (45°C, pH 8.0, 200–400 rpm, aeration of 1 vvm) using 1% (w/v) wheat straw powder. The cell-free broth was used for determining enzyme activity and pulp treatment.

Determination of Enzyme Activity

The activity of the xylanase was determined with 1% birchwood xylan (Sigma, St. Louis, MO) in a 50 m*M* phosphate buffer, pH 7.0, at 70°C using the method described by Bailey et al. *(12)*. The enzymatic reaction was carried out for 5 min and the reducing sugar determined using the dinitrosalicylic (DNS) method *(13)*. Filter paper activity of the crude enzyme was measured using the method recommended by the International Union of Pure and Applied Chemistry using filter paper as a substrate *(14)*. The reaction was carried out at 50°C for 60 min.

Pulp-Making Process

The pulps were produced by the typical method of "open hot digestion" in which raw materials were cooked with 8% NaOH for a period of 3 h at boiling temperature.

Enzyme Prebleaching

The pulps were subjected to enzyme treatment in polyethylene bags. For this, two lots of each pulp were taken. One lot was added to the enzyme and the other lot as a control was subjected to similar conditions without adding enzyme. Pulp consistency was 6% and the pulp was enzymatically treated at two pH values (7.0 and 8.5) and 50°C. To maintain the initial pH of the pulp at the desired value, 4 *N* NaOH and 4 *N* H_2SO_4 were used. An enzyme dose of 20 IU/g of oven-dried pulp was used for all studies and incubated for 3 h. Pulps were then squeezed to collect the water extracts and washed with hot water. The enzyme filtrates were collected and analyzed for color and reducing sugar (as xylose) to determine the efficacy of the enzyme. The color of the filtrate was analyzed by measuring the absorbance at 465 nm, and the κ number of the treated pulps was evaluated to assess the effect of enzyme treatment on lignin using TAPPI test method T236cm-85 *(15)*. The reducing sugar was determined by the DNS method using xylose as the standard *(13)*.

Bleaching Methods

Both the treated and control pulps were subjected to peroxide bleaching after enzyme treatment. All the pulps were given an additional EDTA treatment (0.2% EDTA, pH 4.0–5.0, ambient temperature, 45 min, 5% consistency) before peroxide bleaching so as to increase the effect of peroxide, because EDTA is a chelating agent, which traps the hindering metal ions in the pulps. Peroxide bleaching was carried out at a consistency of 8%, 1% NaOH, 2% H_2O_2, 70°C, 2 h, with a final pH >9.0. In the case of wheat straw and rice straw pulps, an additional step of peroxide bleaching was carried out with 1 and 2% H_2O_2, respectively, to improve the final brightness of the pulp.

Evaluation of Optical and Strength Properties

The bleached pulps were used to make hand sheets (T-205om-88 *[16]*) to evaluate their optical (brightness: ISO-2471 *[17]*) and strength properties (tensile strength: ISO-1924; tear strength: ISO-1974 *[17]*) using standard procedures. From each pulp sample, nine sheets were made and three sheets of each pulp were used to determine the individual parameters. Brightness, tensile, and tear index values presented in the following section are the average of three independent analyses.

Results and Discussion

We have described the effects of crude xylanase of *B. brevis* BISR-062 on three nonwoody pulps (wheat straw, rice straw, and jute) at two different pH values in a total chlorine-free bleaching sequence. It has been mentioned in the literature that pulp raw material influences the bleaching efficiency for a particular enzyme *(18)*. Thus, in this context, the efficiency

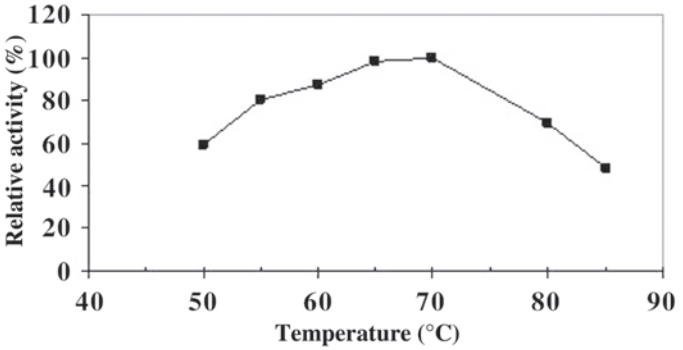

Fig. 1. Effect of temperature on activity of crude xylanase of *B. brevis* BISR-062 at pH 7.0. Values presented here are the average of three analyses having a standard deviation within the range of 4–6%.

of xylanase of *B. brevis* BISR-062 was assessed as a function of pulp raw material and initial pH of pulp.

Optimization of enzyme activity as a function of pH and temperature was carried out with cell-free broth. The enzyme was produced in a 7-L fermentor using *B. brevis* BISR-062 under optimum conditions. A maximum xylanase activity of 12.8 IU/mL was obtained with 1% wheat straw powder.

Optimum Temperature of Crude Xylanase

The activity of the enzyme was determined in the temperature range of 50–85°C at pH 7.0. From the results in Fig. 1, it can be seen that the enzyme had an optimum temperature of 65–70°C and that it had activity even at a higher temperature of 85°C. It retained 48% of its maximum activity at 85°C. Values reported in the literature for the optimum temperature of enzyme activity were within the range of 50–100°C, and for *Bacillus* species the highest enzyme activity was obtained at 75°C *(2,3)*. Thus, for biobleaching applications, this enzyme was found to be suitable at a higher temperature. However, note that most of the Indian pulp and paper industries operate in batch mode in which enzyme with an optimum of approx 50°C is more suitable.

Optimum pH of Crude Xylanase

The activity profile of the enzyme was evaluated in the pH range of 6.0–12.0 by using 50 m*M* various buffers at 70°C. Maximum activity of the enzyme was obtained at pH 7.0, but the enzyme was active even at pH 11.0–12.0 (Fig. 2). At pH 9.0, it retained 50% of its maximum activity. Thus, this enzyme was suitable for biobleaching applications in which enzyme activity at higher alkaline pH is desirable. Most of the reported xylanase of *Bacillus* sp. has optimum activity in the pH range of 5.5–7.0, with the exception of xylanase of *Bacillus stearothermophilus* T-6, which has optimum activity at pH 9.0 *(2,3)*.

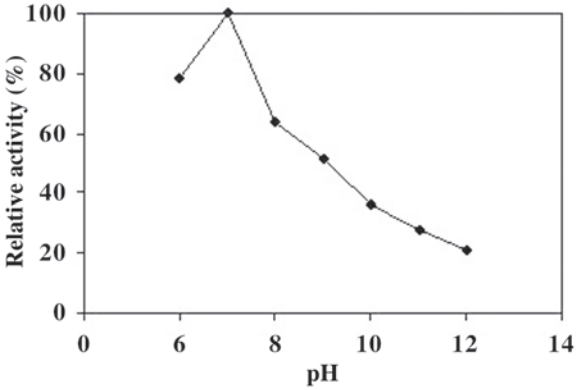

Fig. 2. Effect of pH on activity of crude xylanase of *B. brevis* BISR-062 at 70°C. Various pH buffers of 50 m*M* were used to determine the activity at different pH values. Values presented here are the average of three analyses having a standard deviation within the range of 4–7%.

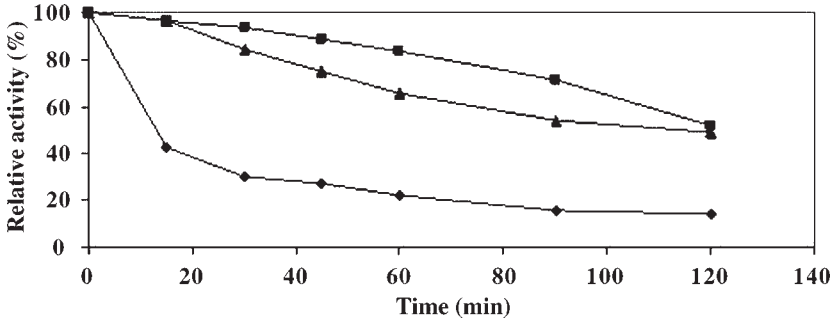

Fig. 3. Stability of crude xylanase of *B. brevis* BISR-062 at 50°C (pH 7.0 [■] and 8.0 [▲]) and 70°C (pH 7.0 [◆]). Values presented here are the average of three analyses having a standard deviation within the range of 5–8%.

Stability of Enzyme

The previous section stated that the enzyme had a maximum activity at pH 7.0 and 70°C. However, the suitability of the enzyme depends on its stability at optimum temperature and pH. Thus, in our study, the stability of the enzyme was determined at pH 7.0 and 70°C. From the results in Fig. 3, it can be seen that the enzyme lost 85% of its activity within 2 h. We therefore studied the stability of the enzyme at pH 7.0 and 50°C; this temperature was chosen considering the needs of Indian pulp and paper industries. The stability of the enzyme at a higher pH of 8.0–9.0 is desirable, because the pulp's pH lies within this range. Thus, we also studied the stability of the enzyme at pH 8.0 and 50°C. It was observed that the enzyme lost only 50% of its activity within 2 h at pH 7.0 and 50°C, compared with 85% loss within the same time period at 70°C (Fig. 3). However, the stability of the enzyme at pH 8.0 was similar to that at pH 7.0 at the same temperature of

50°C (Fig. 3). Thus, from the point of enzyme stability, this enzyme was found to be suitable for biobleaching application at higher pH.

Most of the reported studies on xylanase stability have considered the effect of either pH or temperature, and very few have presented the variation in activity profile with time *(2,3)*. In the present study, we considered the effects of both temperature and pH for stability and treated the pulp at pH 7.0 and 50°C. The efficiency of the enzyme as a prebleaching agent was also evaluated at a higher pH of 8.5 after giving due consideration to its stability at higher pH.

Enzyme Treatment of Pulp

Cell-free crude enzyme obtained from fermentation run was further concentrated in a 10-kDa ultrafiltration system, and the final xylanase activity was 94 IU/mL with a corresponding filter paper activity of 0.21 IU/mL. Three different pulps produced from wheat straw, rice straw, and jute were treated with the concentrated enzyme at a dose of 20 IU/(g of oven-dried pulp) at 50°C and two different initial pH values of pulp. The water extracts or pulp filtrates collected from the treated and control pulps were than analyzed for color and reducing sugars (as xylose) release. The κ number of enzymatically treated and control pulps was evaluated to determine the effect of enzyme treatment on the lignin content of pulp.

From Table 1 it can be observed that the effects of enzyme treatment on the release of reducing sugar were different on the three pulps. A minimum amount of reducing sugar was released from jute pulp at both pH values. The effect of the initial pH of the pulp was more prominent in the case of wheat straw pulp. In the case of rice straw pulp, there was a marginal increase in the release of reducing sugar at higher pH compared with pH 7.0.

The color of the filtrate was analyzed by measuring the absorbance at 465 nm. Table 1 shows that maximum color was released from rice straw and wheat straw pulps at a higher initial pH of the pulp. The effects of enzyme treatment were more prominent in the case of rice straw and wheat straw pulps.

κ number is one of the most important parameters in analyzing the effectiveness of any xylanase. In the case of xylanase of *B. brevis* BISR-062, a maximum reduction in κ number of 2.6 was observed with rice straw pulp at an initial pH of 8.5. At pH 7.0, the reduction in κ was 50% of the maximum value obtained at pH 8.5 for rice straw pulp. In other pulps, the reductions in κ number were minimal compared with that of the control pulp. Thus, it can be concluded that the effect of enzyme was more significant in the case of rice straw pulp.

After the enzyme treatment of pulps, subsequent EDTA treatment and bleaching with 2% H_2O_2 was carried out. The bleached and unbleached pulps were used to make sheets for analyzing optical and strength properties. Brightness gain values of pulps after enzyme treatment and bleaching were compared to evaluate the bleach-boosting effects of crude enzyme on three different pulps.

Table 1
Release of Reducing Sugar and Color From Nonwoody Pulps and κ Number of Pulps at Two Initial pH Values

	Wheat straw		Rice straw		Jute	
	pH 7.0	pH 8.5	pH 7.0	pH 8.5	pH 7.0	pH 8.5
Reducing sugar as xylose (g/L)						
Control	2.02 ± 0.16	4.01 ± 0.27	0.031 ± 0.005	0.03 ± 0.009	4.13 ± 0.31	6.08 ± 0.38
Enzyme treated	4.64 ± 0.24	5.39 ± 0.31	2.93 ± 0.11	3.57 ± 0.17	4.8 ± 0.41	6.88 ± 0.42
Color of pulp filtrate in PCU[a]						
Control	326.8 ± 19	406 ± 38	565.8 ± 33	591.5 ± 49	135.4 ± 16	274 ± 27
Enzyme treated	620.7 ± 31	928 ± 49	660.9 ± 46	1152.4 ± 62	294 ± 28	507.3 ± 43
κ Number						
Control	33.4 ± 0.42	33.6 ± 0.38	29.7 ± 0.28	29.6 ± 0.21	34.9 ± 0.43	35.4 ± 0.48
Enzyme treated	33.2 ± 0.48	33.2 ± 0.43	28.4 ± 0.23	27 ± 0.24	34.7 ± 0.49	35.9 ± 0.41

[a]Platinum-cobalt color units.

From the results in Table 2, it can be seen that after enzyme treatment (prior to the bleaching stage) the brightness of the pulp improved only for rice straw pulp at pH 7.0 and that there was an insignificant brightness gain at pH 8.5. A similar phenomenon has been reported for enzyme-treated kraft pulp, with a brightness gain of 2–4 points prior to the bleaching stage. After the bleaching stage, the maximum brightness gain improved to 7.9 points with the same kraft pulp *(17)*. In the present study, after the first stage of bleaching, the brightness of wheat straw and rice straw pulps was less, so an additional stage of bleaching was carried out for wheat straw and rice straw pulps with 1 and 2% H_2O_2, respectively. Table 2 gives the brightness values of pulps after the second stage of bleaching. In the case of bleached pulps, maximum brightness gains of 5.5 and 5 were observed with rice straw pulp at pH 7.0 and 8.5, respectively. In the case of wheat straw pulp at pH 7.0, a brightness gain of 0.6 points was observed, and there was no gain in brightness value at pH 8.5. Similarly, jute pulp was found to be ineffective with xylanase of *B. brevis* BISR-062. In the case of rice straw pulp, further bleaching with 1% H_2O_2 was carried out, and at pH 7.0 brightness gain was improved to 6 points, whereas at pH 8.5 brightness gain decreased to 1.6 points. However, the beneficial effect of higher pH in improving brightness gain has been reported for wheat straw pulp treated with xylanase of *Thermomyces lanuginosus* CBS 288.54 *(7)*. In that study, the effect was investigated up to a pH of 11.0, and a maximum brightness gain was observed at pH 9.0. Roncero et al. *(4)* studied the effect of xylanase treatment on wheat straw pulp in a total chlorine-free bleaching sequence and obtained a final brightness value above 80%. Scanning electron microscopy of the enzyme-treated wheat straw pulp showed changes in the surface of the fiber resulting in a more open surface. Herpoel et al. *(6)* used a laccase mediator system along with xylanase for treatment of wheat straw pulp in a total ethylchloroformate sequence that resulted in 69% ISO brightness, but there was a reduction in the degree of cellulose polymerization. Jiménez et al. *(8)* optimized the experimental conditions for enzyme treatment of wheat straw pulp with Cartazyme enzyme followed by H_2O_2 bleaching. Although enzyme treatment reduced the pulp yield, there was an increase in the final brightness value of the pulp. Roncero et al. *(5)* reported the kinetic study of ozone treatment of wheat straw pulp, and it has been compared with eucalyptus pulp. As far as studies on enzyme treatment on nonwoody pulps are concerned, there is rarely any report on rice straw pulp. In the present study, the brightness gain achieved with wheat straw pulp was less compared with that of other reported studies *(4–8)*.

Sheets made from bleached pulp were also used to determine the strength properties of enzyme-treated bleached and control pulps. In the case of tensile index, there was a marginal decrease in value for jute pulp at both pH 7.0 and 8.5 and for rice straw pulp at the higher pH value of 8.5 (Table 3). Marginal effects on tear index were observed in the case of jute pulp at both pH values and for wheat straw pulp at pH 7.0 (Table 3). Thus, the enzyme was found to be suitable for rice straw pulp, for which along

Table 2
Brightness Values of Bleached and Unbleached Pulps of Three Different Raw Materials at Two Initial pH Values

	Control at pH 7.0 (brightness [%ISO])	Enzyme treated at pH 7.0 (brightness [%ISO])	Control at pH 8.5 (brightness [%ISO])	Enzyme treated at pH 8.5 (brightness [%ISO])
		Wheat straw pulp		
Unbleached	21.7 ± 1.21	21.6 ± 1.33	22.6 ± 1.27	21.6 ± 1.22
After second stage of bleaching	31.3 ± 1.37	31.9 ± 1.39	31.6 ± 1.31	30.9 ± 1.37
		Rice straw pulp		
Unbleached	20.7 ± 1.08	21.7 ± 1.14	21.1 ± 1.03	21.4 ± 1.11
After second stage bleaching	24 ± 1.17	29.5 ± 1.27	26.8 ± 1.28	31.8 ± 1.32
After third stage of bleaching	28.5 ± 1.37	34.5 ± 1.31	32.2 ± 1.51	33.8 ± 1.59
		Jute pulp		
Unbleached	18.1 ± 1.16	17.9 ± 1.21	17.8 ± 1.08	17.8 ± 1.16
After first stage of bleaching	26.1 ± 1.31	24 ± 1.37	27.9 ± 1.27	26.5 ± 1.31

Table 3
Effects of Xylanase Treatment on Tensile and Tear Indexes of Three Different Nonwoody Pulps at Two Different Initial pH Values

	Tensile index (Nm/g)		Tear index (mNm^2/g)	
Pulp raw material	Control pulp	Enzyme-treated pulp	Control pulp	Enzyme-treated pulp
Wheat straw at pH 7.0	39 ± 1.43	40.4 ± 1.51	7.6 ± 0.21	7.1 ± 0.28
Wheat straw at pH 8.5	40.7 ± 1.28	41.1 ± 1.33	7.14 ± 0.19	7.6 ± 0.23
Rice straw at pH 7.0	42.5 ± 1.31	44.2 ± 1.28	5.46 ± 0.13	5.6 ± 0.17
Rice straw at pH 8.5	43.1 ± 1.67	42.6 ± 1.73	4.9 ± 0.12	5.7 ± 0.19
Jute at pH 7.0	45.2 ± 1.18	44.6 ± 1.23	11.4 ± 0.31	10.8 ± 0.37
Jute at pH 8.5	44.4 ± 1.27	44.3 ± 1.32	11.7 ± 0.35	11 ± 0.39

with brightness gain there was an improvement in the strength properties of the pulp at pH 7.0. However, note that the differences in values of either tensile or tear index of pulp samples were statistically insignificant.

The differences in enzyme efficiency for different pulps can probably be explained in terms of variation in chemical structure and composition of pulps. Xylanase of *B. brevis* BISR-062 was found to be suitable for biobleaching of rice straw pulp at an initial pH of 7.0, and ours is probably the first report on enzyme treatment of rice straw pulp.

Acknowledgments

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References

1. Viikari, L., Pauna, M., Kantelinen, A., Sundquist, J., and Linko, M. (1986), in *Proceedings of the Third International Conference on Biotechnology in the Pulp and Paper Industry*, pp. 67–69.
2. Kulkarni, N., Shendye, A., and Rao, M. (1999), *FEMS Microbiol. Rev.* **23,** 411–456.
3. Techapun, C., Poosaran, N., Watanabe, M., and Sasaki, K. (2003), *Process Biochem.* **38,** 1327–1340.
4. Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2003), *Bioresour. Technol.* **87,** 305–314.
5. Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2003), *Bioresour. Technol.* **87,** 315–323.
6. Herpoel, I., Jeller, H., Fang, G., et al. (2002), *J. Pulp Paper Sci.* **28,** 67–71.
7. Li, X. T., Jiang, Z. Q., Li, L. T., et al. (2005), *Bioresour. Technol.* **96,** 1370–1379.
8. Jiménez, L., Navarro, E., Ferrer, J. L., Lopéz, F., and Ariza, J. (1999), *Process Biochem.* **35,** 149–157.
9. Kundu, A. B., Ghosh, B. S., and Chakrabarti, S. K. (1993) *Textile Res. J.* **63(8),** 451–454.
10. Atchinson, J. E. (1998), in *Proceedings of the North American Nonwood Fiber Symposium*, pp. 1–3.
11. Moore, G. (1996), *Nonwood Fiber Applications in Papermaking*, Pira International Leatherhead, Surrey, UK.
12. Bailey, M. J., Biely, P., and Poutanen, K. (1992), *J. Biotechnol*. **23,** 257–270.
13. Miller, G. L. (1959), *Anal. Chem.* **31,** 426–428.
14. IUPAC (International Union of Pure and Applied Chemistry). (1987), *Pure. Appl. Chem*. **59,** 257–268.
15. TAPPI Test Methods (1992), Kappa no. of pulp, T236 cm-85, TAPPI Press, Atlanta, GA.
16. TAPPI Test Methods (1992), Forming handsheets for physical tests of pulp, T205 om-88, TAPPI Press, Atlanta, GA.
17. *ISO Standard Handbooks, Paper, Board and Pulps* (2003), vol. 1, 3rd ed.
18. Suurnakki, A., Clark, T. A., Allison, R. W., Viikari, L., and Buchert, J. (1996), *Tappi J.* **79,** 111–117.