Xylanase- and mannanase-aided ECF and TCF bleaching

ANNA SUURNÄKKI, THOMAS A. CLARK, ROBERT W. ALLISON, LIISA VIIKARI, AND JOHANNA BUCHERT

ABSTRACT

This work studies the effects of T. reesei *xylanase and mannanase treatments on the bleaching of radiata pine and northern pine sulfate pulps in three-stage peroxide- and chlorine dioxide-based DED bleaching sequences. The xylanase treatment was more effective in enhancing pulp bleaching than the mannanase treatment. Pulp origin, method of pulping, and the sequence used in bleaching influenced the effectiveness of enzymatic treatment. Enzymatic treatment affected bleaching of northern pine kraft pulp more than radiata pine kraft pulp with a similar kappa number. Radiata pine kraft pulp responded more readily to the xylanase and less readily to the mannanase treatment than the radiata pine modified continuous cooking pulp.*

Application:

Progress toward more environmentally friendly bleaching sequences has created a need for easily bleached kraft pulps. This work suggests that using a suitable combination of enzyme treatment and bleaching sequence can enhance the bleachability of low kappa number pulps produced by modified or new kraft pulping methods.

ENZYMATIC PRETREATMENT IS A common technique today in industrial chemical pulp bleaching processes. Since pulp hemicellulose—mainly xylan—undergoes limited hydrolysis in enzymatic treatment, the leaching of lignin in kraft pulp fibers increases in the subsequent bleaching sequence *(1–5)*. The increased bleachability of xylanase-treated pulp can be used to increase the final brightness value or to reduce the consumption of bleaching chemicals. A consequence of this is a reduction of the environmental effects from bleaching.

In conventional softwood kraft pulps, mannanase treatment alone has only a minor effect on bleaching compared to that of xylanase treatment *(6)*. New pulping methods such as modified continuous cooking (MCC) and superbatch cooking use an altered alkali profile *(7, 8)*. This should result in pulps with a modified composition of fiber surfaces when compared to that of conventional kraft fibers. Surprisingly, *T. reesei* mannanase treatment readily increases the hydrogen peroxide bleaching of softwood pulps produced by modified cooking methods *(9)*. In low kappa number pulps, the effect of mannanase even exceeded that of xylanase treatment.

Most xylanases can increase the bleaching of pulp regardless of the origin of the enzyme *(10)*. *T. reesei* mannanase is the only mannanase free from other enzyme activities reported to enhance considerably the bleaching of modified softwood pulps in peroxide or chlorine dioxide bleaching *(9)*. The *B. subtilis* mannanase studied by Clark *et al. (11)* increased the bleaching of softwood kraft pulps, however. Along with glucomannan, it also solubilized polymeric xylan from the pulps.

This work studies the effects of *T. reesei* xylanase and mannanase treatments on the bleaching of softwood kraft pulps bleached with totally chlorine-free (TCF) and elemental chlorine-free (ECF) sequences. It also examines the effects of pulp origin, pulping method, and bleaching sequence on enzyme-aided bleaching.

MATERIALS AND METHODS

Pulps

Four radiata pine *(Pinus radiata)* pulps came from conventional kraft and MCC cooking and oxygen delignification methods. A northern pine *(Pinus sylvestris)* pulp came from conventional kraft cooking. The radiata pine pulps were produced from the same lot of wood chips described previously *(12, 13)*. Buchert *et al.* have previously presented the cooking conditions of the northern pine kraft pulp *(14)*. Table I shows the properties and carbohydrate compositions of the pulps.

Enzymatic hydrolyses

The pulps underwent treatment with xylanase and mannanase purified from *Trichoderma reesei* RUT C-30 culture filtrate *(15, 16)*. Pulp hydrolyses used distilled water with an enzyme dosage of 500 nkat/g of o.d. pulp at 5% consistency at 45°C for 2 h. Adjustment of the pH of the pulps to pH 5 used H_2SO_4. The reference treatments were the same but had no enzyme addition. After the hydrolyses, the pulps underwent filtration. Filtrate boiling for 2 min inactivated the enzymes. High-pressure liquid chromatography analyzed the carbohydrates solubilized in the enzymatic treatments as monosaccharides after a secondary enzymatic hydrolysis *(17)*.

Metal removal and three-stage peroxide bleaching

Before peroxide bleaching, the metal contents of the enzymatically and reference treated pulps underwent reduction by ethylenedi-

Pulp	Kappa number	Carbohydrates, cellulose	% of d.w. of pulp GM	Xylan	Ratio of ARA:Xylan	Ratio of GAL:GM
Radiata kraft	26.9	78.8	7.3	8.1	1:13	1:23
Radiata MCC	20.5	81.9	6.7	7.7	1:12	1:21
Radiata kraft + O_2	14.2	81.2	7.2	8.5	1:11	1:23
Radiata MCC + O_2	10.5	83:3	6.8	7.6	1:18	1:22
Northern kraft	25.9	79.6	7.4	9.4	1:7	ND

GM = Glucomannan
ARA = Arabinose
GAL = Galactose
Xylan = Xylanase
ND = Not determined

I. Carbohydrate compositions of the radiata pine and northern pine softwood pulps

aminetetraacetic acid (EDTA) treatment. The conditions were 0.2% EDTA, 5% consistency, 1 h at 45°C, and pH adjusted to 5.0 with H_2SO_4. After the EDTA treatment, the pulps underwent washing with distilled water. Peroxide bleaching used three identical stages after EDTA treatment. Each stage had 10% consistency at 80° for 2 h. The chemical dosages of each peroxide stage were 3% H_2O_2, 1.5% NaOH, 0.5% $MgSO_4$, and 0.2% diethylene triamine pentaacetic acid (DTPA). After each peroxide stage, the pulps underwent washing with distilled water. After the third bleaching stage, we measured the brightness (ISO 3688-1977), kappa number (TAPPI T 236), and viscosity (TAPPI T 230) values of the pulps.

DED Bleaching

After the enzymatic treatments, we fluffed the pulps and divided them into three lots for different subsequent treatments. The first chlorine dioxide stage (DO) was at 10% consistency at 50°C for 1 h. The initial DO stage used three active chlorine multiples (ACM) of 0.20, 0.25, and 0.30. Following each bleaching stage, we filtered the pulps to 20% consistency, diluted them to 1% consistency with 50°C water for 1 h, and filtered them again to 20% consistency. The conditions in the following extraction (E) stage were 10% consistency, 70°C, 1 h, and NaOH charge equivalent to half the active chlorine charge used in the DO stage. After the E stage, we analyzed the kappa numbers of the pulps. The second chlorine dioxide (D1) stage used 10% consistency at 70°C for 3 h with 0.8% ClO_2. Adjustment of the NaOH charge gave a final pH of approximately 3.8. We measured final brightness values of the pulps.

RESULTS AND DISCUSSION

Characteristics of the pulps

The study used five laboratory cooked softwood pulps. Radiata pine kraft, MCC, oxygen delignified kraft (kraft + O_2), and oxygen delignified MCC (MCC + O_2) pulps all underwent cooking and delignification to different kappa number levels from the same lot of wood chips in Table I. We included the northern pine kraft (northern kraft) pulp cooked to the same kappa number level as radiata pine kraft pulp in the pulp series to evaluate the influence of fiber origin on the effects of enzymes on pulp bleachablility.

Table I shows that the hemicellulose contents of the radiata pine pulps varied from 14.4–15.7% of dry weight (d.w.). The kraft pulps contained more xylan and glucomannan than the MCC pulps. The northern kraft pulp clearly contained more xylan than the radiata pine pulps. Table I also shows that the xylan of the northern kraft pulp also had more arabinose substitution than that of any radiata pine pulps. The degree of arabinose substitution was especially low in the oxygen delignified radiata pine MCC pulp.

Enzymatic treatments of the pulps

The pulps underwent treatment with a constant dosage of 500 nkat/g of *T. reesei* mannanase and xylanase before exposing them to the bleaching sequences. The production method of the pulp influenced the hydrolysis degree of pulp hemicellulose both in mannanase and xylanase treatments. In the conventionally cooked radiata pine kraft pulp, the mannanase solubilized 0.53% of the pulp d.w. Table II shows that in the MCC pulp the solubilization was only 0.29% of d.w. In the xylanase hydrolysis, the solubilization was 0.95% of the kraft and 0.65% of the MCC pulp. Oxygen delignification increased the accessibility of both glucomannan and xylan in radiata pine pulps. In the northern kraft pulp, the mannanase solubilized 0.84% of d.w. This was clearly more than the 0.53% of d.w. solubilization of radiata pine kraft pulp, although the two pulps initially had similar kappa numbers. In addition to glucomannan, xylan was

			SOLUBILIZATION OF CARBOHYDRATES, % of d.w. of pulp				
Pulp	**Treatment**	**Total**	**GLU**	**MAN**	**GAL**	**Xylan**	**ARA**
Radiata kraft	REF	0.03	0.01	0	0.01	0.01	0
	MAN	0.56	0.13	0.35	0.05	0.03	0
	Xylan	0.98	0.01	0	0.01	0.87	0.09
Radiata kraft + O_2	REF	0.04	0.01	0	0.01	0.02	0
	MAN	0.68	0.14	0.44	0.07	0.02	0.01
	Xylan	1.12	0.01	0	0.01	0.99	0.11
Radiata MCC	REF	0.04	0.01	0	0.01	0.02	0
	MAN	0.33	0.07	0.21	0.03	0.02	0
	Xylan	0.69	0.01	0.01	0.01	0.61	0.05
Radiata MCC + O_2	REF	0.04	0.02	0	0.01	0.01	0
	MAN	0.48	0.10	0.32	0.05	0.01	0
	Xylan	1.19	0.01	0	0.01	1.07	0.10
Northern kraft	REF	0.03	0.01	0	0	0.02	0
	MAN	0.87	0.13	0.64	0.08	0.02	0
	Xylan	1.92	0.01	0.01	0.01	1.70	0.19

GLU = Glucomannan
MAN = Mannanase
REF = Reference

The relative error of the analysis of glucose was 0.2% and that of the other monosaccharides was 2% at 95% confidence.

II. Solubilization of carbohydrates from the radiata pine pulps and northern pine kraft pulp by* T. reesei *xylanase and mannanase using 500 nkat/g for 2 h at 45°C

also more accessible to enzymatic attack in northern kraft than in radiata pine kraft pulp. The xylanase solubilized 1.89% of the northern kraft but only 0.95% of the radiata pine kraft pulp. Apparently pulp origin also had an effect on the accessibility of pulp hemicellulose to the enzyme.

Besides the amount, the composition of xylan solubilized from northern pine kraft pulp and radiata pine pulps by xylanase was also different. The ratios of arabinose:xylose were 1:8 and approximately 1:10 in the fractions solubilized from the northern kraft pulp and the radiata pine pulps, respectively. This indicates that especially in the radiata pine pulps the accessible xylan had more arabinose substitution than the total xylan in the pulps—average arabinose:xylose ratio 1:12. Earlier work indicates that the *T. reesei* xylanase (pI 9) prefers more substituted xylan as a substrate *(16)*. It is therefore possible that the difference observed in enzymatic solubilization of xylan between the pulps is partly due to the different degrees of substitution of xylan in the pulps.

Three-stage peroxide bleaching

The radiata pine pulps and the northern pine kraft pulp all underwent bleaching with a TCF three-stage peroxide sequence. In the radiata pine pulps, the final brightness values varied between 67.0–74.5%. Table III shows that the delignification obtained in bleaching ranged from 50–65%. It was easier to delignify the brownstock kraft and MCC pulps than the corresponding oxygen delignified pulps. The oxygen delignified pulps also consumed more peroxide during the bleaching sequence as Table III shows. Although the northern pine and radiata pine kraft pulps had similar final kappa numbers, the final brightness of the northern kraft pulp remained at a lower level than that of the radiata pine kraft pulp. Table III shows values of 61.8% vs. 67.0%, respectively. Obviously the bleachability of pulp depended on the wood species and on the method used in pulp production.

The chemical dosages used in peroxide bleaching did not allow achievement of full brightness values. The sequence was suitable for observing the effects of enzymatic treatments on the bleaching of pulps in multi-stage peroxide bleaching, however.

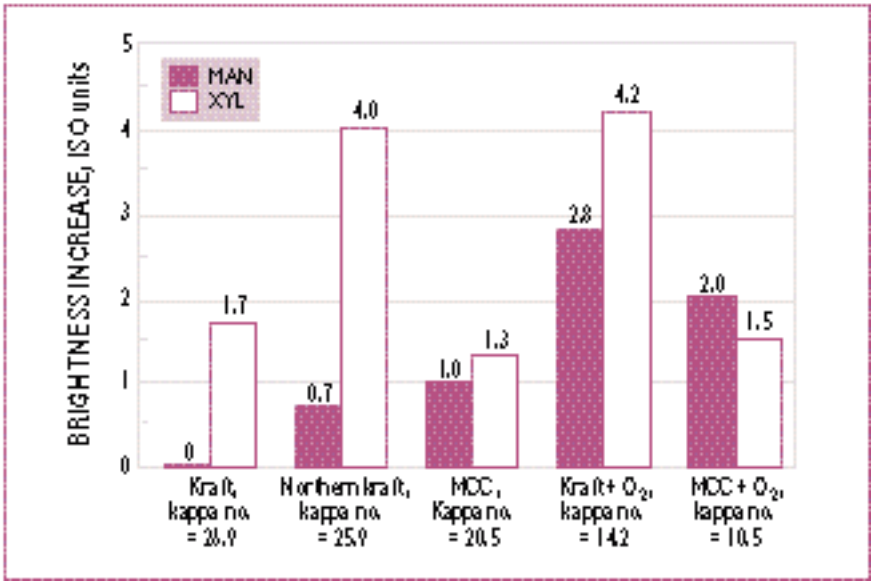
We studied the effects of *T. reesei* mannanase and xylanase treatments on pulp bleachability after bleaching. Oxygen delignification increased the effect of mannanase on the bleaching of the radiata pine pulps. Figure 1 shows that without oxygen delignification mannanase treatment did not affect the brightness of the kraft pulp. In the oxygen delignified kraft pulp, the final brightness increased by 2.8 units,

however: to 71.4% with mannanase treatment compared to the brightness of the reference treated pulp. Table III presents the brightness values of the reference treated pulps in Fig. 1. There was also a less pronounced effect of oxygen delignification on mannanase-aided bleaching with the MCC pulp as Fig. 1 shows. Earlier work reported a positive influence of oxygen delignification on the effectiveness of mannanase pretreatment on bleaching in industrial softwood kraft pulps produced from mixed Scandinavian softwood *(9)*. Despite pulp origin, oxygen delignification apparently modifies the structure of pulp fibers in a way that enhances the effect of mannanase treatment on pulp bleaching.

Xylanase treatment was generally more effective in increasing the bleachability of the radiata pine pulps than mannanase treatment. Figure 1 shows that only for oxygen delignified MCC pulp did the mannanase treatment result in a higher brightness and lower kappa number than the xylanase treatment after peroxide bleaching. The most pronounced effect of xylanase treatment occurred with oxygen delignified kraft pulp. Here the brightness increased by 4.2 units to 72.8% and the kappa number decreased by 1.4 units to 5.8 compared with the corresponding values of the reference treated pulp after bleaching. See Table III and Fig. 1. Interestingly, the increase in the effect of xylanase treatment by oxygen delignification only occurred in the final brightness values of the pulps. The mannanase treatment also affected the final kappa numbers of the pulps. Table III shows that the consumption of peroxide in bleaching was similar in reference and enzymatically treated radiata pines. With kraft pulps, however, some benefit of mannanase treatment could have been from the increased consumption of hydrogen peroxide in bleaching.

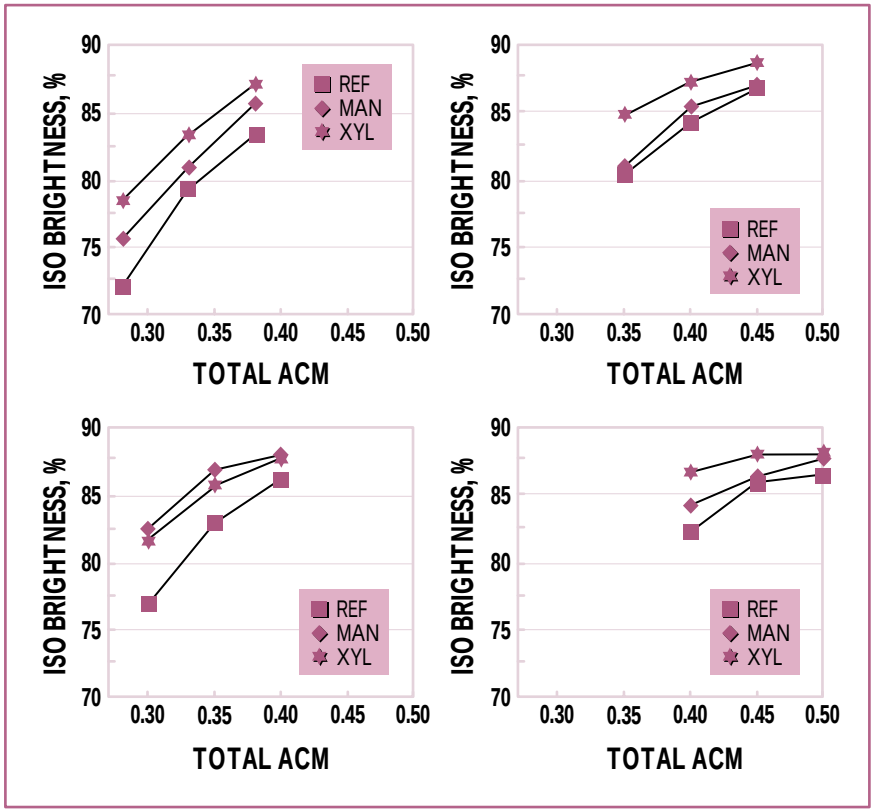
Pulp	Treatment	Brightness, % (ISO)	Kappa number	H_2O_2 consumption, % of total
Radiata kraft,	MAN	66.3	9.4	37.6
kappa no. 26.9	Xylan	68.7	8.2	27.6
	REF	67.0	9.5	27.8
Northern kraft,	MAN	62.5	8.9	ND
kappa no. 25.9	Xylan	65.8	7.2	ND
	REF	61.8	9.4	ND
Radiata kraft + O_2,	MAN	71.4	6.6	43.0
kappa no. 14.2	Xylan	72.8	5.8	36.0
	REF	68.6	7.2	33.3
Radiata	MAN	69.4	7.3	24.5
kappa no. 20.5	Xylan	69.7	6.8	26.8
	REF	68.4	7.8	17.4
Radiata	MAN	76.5	4.5	30.2
MCC + O_2,	Xylan	76.0	5.1	28.8
Kappa no. 10.5	REF	74.5	5.3	25.9

III. Properties of the* T. reesei *mannanase and xylanase treated pulps measured after three-stage peroxide bleaching for three 2-h periods at 80°C



1. Effects of* T. reesei *mannanase and xylanase treatments on the brightness of softwood pulps after three-stage peroxide bleaching

Figure 1 and Table III show that the effects of both mannanase and xylanase treatments on bleaching were more pronounced in the northern pine kraft than in the radiata pine kraft pulp. Table II shows that an improved response in enzyme-aided bleaching also reflected the higher accessibility of hemicellulose of northern kraft pulp to enzymatic attack. In all the pulps studied, there was stronger correlation between the enzymatic solubilization and the effect of enzyme treatment on bleaching in xylanase- than in mannanase-aided peroxide bleaching. The effect of xylanase treatment on pulp bleaching increased with



2. Effects of **T. reesei** *mannanase and xylanase treatments on the final brightness of radiata pine pulps in DED bleaching*

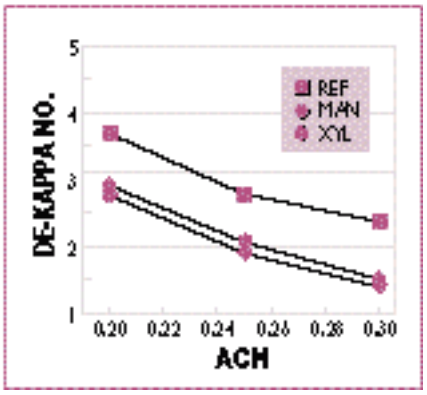
greater solubilization of xylan, but mannanase treatment was independent of the amount of glucomannan solubilized. Another study has also shown similar observations with industrial softwood pulps after one-stage peroxide bleaching *(9)*.

Chlorine dioxide bleaching

The radiata pine pulps underwent bleaching with a DED sequence with three different ACM in the initial chlorine dioxide stage. After a fixed chlorine dioxide charge in the final D stage, the total ACM values were 0.28–0.38 in the kraft, 0.35–0.45 in the oxygen delignified kraft, 0.30–0.40 in the MCC, and 0.40–0.50 in the oxygen delignified MCC pulp. Figure 2 shows that the final brightness values of the reference treated pulps ranged from 72–83% in the kraft, 80–87% in the oxygen delignified kraft, 77–86% in the MCC, and 82–87% in the oxygen delignified MCC pulps, respectively. In this figure, the error limit of the DED bleaching was ± 0.5 brightness units at 95% confidence. The final brightness was higher in the MCC than in the kraft pulp at all ACM values. This indicates that the MCC pulp was easier to bleach with chlorine dioxide. Oxygen delignification further increased the bleaching of both kraft and MCC pulps. The delignification obtained after the DE stages with the highest ACM value was approximately 89% in the kraft, approximately 85% in the kraft + O_2, approximately 88% in the MCC, and approximately 83% in the MCC + O_2 pulp.

The radiata pine pulps treated with *T. reesei* mannanase and xylanase also underwent bleaching with a DED sequence. The effects of enzymatic treatments on the final brightness and DE-kappa number values of the pulps varied depending on the enzyme, pulp, and bleaching chemical charge. Figure 2 shows that the xylanase treatment was generally more effective in enhancing the bleaching of the pulps than mannanase treatment. Figure 2 also shows that the xylanase treatment increased the final brightness values of the pulps by 4.4–6.4 brightness units with the lowest chlorine multiple and by 1.5–3.8 brightness units using the highest chlorine multiple compared to the brightness values of the reference treated pulps. The effect of xylanase treatment on final pulp brightness was greatest in the kraft pulp that was at a lower brightness level than the other pulps after bleaching. Figure 2 shows that in all the pulps the effect of xylanase on final brightness decreased by increasing the active chemical charge. Table IV indicates that the approximate chemical savings achieved by xylanase treatment were more pronounced in the oxygen delignified than brown-stock kraft and MCC pulps. For example, in the xylanase treated MCC and MCC + O_2 pulps a brightness level of 86% was obtained with 9% and 20% lower total ACM, respectively, compared to the corresponding reference treated pulps. As earlier reported *(1)*, xylanase pretreatment can either reduce chlorine chemical consumption in bleaching to a target brightness value or increase the final brightness value obtainable with the same chemical charge.

Figure 2 shows that the effect of *T. reesei* mannanase treatment on the bleaching of the kraft, oxygen delignified kraft, and oxygen delignified MCC pulps was approximately half that of the xylanase treatment. Surprisingly, however, the *T. reesei* mannanase treatment was more efficient than the xylanase treatment in increasing the bleachability of the MCC pulp in chlorine dioxide bleaching. The brightness increase of MCC pulp ranged from 1.8 to 5.5



3. Effects of* T. reesei *mannanase and xylanase treatments on the DE delignification of radiata pine MCC pulp in DED bleaching

Pulp	Treatment	Chemical savings, % of total ACM
Kraft, kappa no. 26.9	MAN	7
	Xylan	15
Kraft + O_2, kappa no. 14.2	MAN	4
	Xylan	14
MCC, kappa no. 20.5	MAN	14
	Xylan	9
MCC + O_2, kappa no. 10.5	MAN	10
	Xylan	20

Calculations for the chemical savings obtained in DED bleaching of kraft pulp by enzymatic treatments use a target brightness value of 83%.

IV. Approximate reduction obtained by enzymatic pretreatment in the total active chlorine charge needed for reaching a brightness value of 86% in DED bleaching of radiata pine pulps

KEYWORDS
Bleachability, chlorine dioxide, chlorine free bleaching, enzymes, enzymolysis, kraft pulps, multistage process, peroxide bleaching, pinus, softwood pulps, xylanase.

brightness units with mannanase treatment compared to 1.5–4.6 brightness units with xylanase treatment depending on the chemical load. Figure 3 shows that the reduction of DE-kappa number was also higher in the mannanase than xylanase treated MCC pulp. The pronounced effect of the *T. reesei* mannanase treatment on the bleaching of MCC pulp in chlorine dioxide bleaching did not correlate with the enzymatic solubilization. See Table II and Fig. 2. Apparently the action of mannanase in the MCC pulp enhanced the leaching of lignin in the subsequent chlorine dioxide bleaching stage despite limited solubilization of pulp glucomannan. It is possible that the composition and structure of accessible fiber surfaces change in the MCC process in such a way that the pulp is especially suitable for mannanase aided chlorine dioxide bleaching. Unlike peroxide bleaching, Table IV and Fig. 3 show that oxygen delignification reduced the positive effect of mannanase treatment on the bleachability of MCC and kraft pulps in the chlorine dioxide sequence. Besides the method used in pulp production, the effectiveness of mannanase treatment in bleaching also apparently depends on the type of sequence used in bleaching.

CONCLUSIONS

This work compared the effects of *T. reesei* mannanase and xylanase treatments on bleaching of radiata and northern pine pulps in three-stage peroxide and DED bleaching sequences. Xylanase treatment was more effective than mannanase treatment in enhancing the bleachablity of pulps in both bleaching sequences. The correlation between solubilization of pulp xylan and the effect of xylanase treatment on pulp bleachability was also better than that between solubilization of glucomannan and the effect of mannanase treatment. Earlier work has reported similar observations concerning xylanase- and mannanase-aided bleaching with other softwood pulps *(6, 9, 11)*.

Pulp origin influenced the action of enzymes in bleaching. The northern pine kraft pulp was more accessible to enzymatic attack and responded more readily to both xylanase- and mannanase-aided peroxide bleaching than did the radiata pine kraft pulp cooked to the same kappa number level. Besides the pulp origin, the sequence used in bleaching also affected the response to enzyme-aided bleaching. In three-stage peroxide bleaching, the effect of both xylanase and mannanase treatment on bleachability was less pronounced in the brownstock radiata pine pulps than in the oxygen delignified pulps. An earlier study reported a similar effect of oxygen delignification on enzyme-aided peroxide bleaching in northern softwood pulps *(9)*. In chlorine dioxide bleaching, however, oxygen delignification decreased the effect of mannanase on the bleachability of the radiata pine pulps. The role of oxygen delignification in mannanase-aided bleaching needs further study in more detail.

Surprisingly, the effect of mannanase treatment on bleaching clearly exceeded that of xylanase

treatment in the MCC pulp with chlorine dioxide bleaching. This pronounced effect of mannanase treatment did not occur with any other combination of pulp and bleaching sequence tested. Apparently, the positive effect of mannanase is especially dependent on the pulp production method and on the sequence used in pulp bleaching. With a suitable combination of pulp and bleaching sequence, mannanase may compete with xylanase in the effectiveness of enzyme-aided bleaching. TJ

Suurnäkki is research scientist, Viikari is head of biotechnology research, and Buchert is leader of enzyme application group at VTT Biotechnology and Food Research, P.O. Box 1500, FIN-02044 VTT, Espoo, Finland. Clark is scientist and Allison is group leader at Forest Research Institue-Papro, Private Bag 3020, Rotorua, New Zealand.

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