Effects of enzymatic modification on radiata pine kraft fibre wall chemistry and physical properties

THOMAS A. CLARK*, ROBERT W. ALLISON† AND R. PAUL KIBBLEWHITE‡

SUMMARY

This paper examines the effects of treatments with six purified enzymes (two xylanases, one cellobiohydrolase and three endoglucanases from Trichoderma reesei) on a range of properties of kraft pulps from radiata pine. After initial dose response experiments, 20 individual treatments were performed involving combinations of the six enzymes and four pulp types, i.e. bleached and unbleached kraft pulps from radiata pine slabwood and thinnings.

Slabwood and thinnings pulps responded similarly to the treatments. Bleached pulps showed increased carbohydrate removal after treatments with some enzymes, but not others.

Cellulose depolymerization after endoglucanase treatments was strongly correlated with decreases in fibre strength, i.e. wet zero span tensile index. Decreases in fibre strength after endoglucanase treatments were, in turn, strongly correlated with losses of handsheet strength. This suggests that the weakening of handsheet webs by endoglucanase treatments was mainly due to weakening of individual fibres and not to effects on interfibre bonding potential or overall fibre length.

KEYWORDS

enzymes, kraft pulp, fibre modification, radiata pine, endo-glucanase, xylanase, cellulase, pulp properties, carbohydrates, pulp strength, cellobiohydrolase

* Senior scientist, Member Appita
Now with Carter Holt Harvey Pulp and Paper, Kinleith Mill, Private Bag, Tokoroa, New Zealand

† Group Leader, Member Appita

‡ Senior Scientist, Member Appita
PAPRO New Zealand, Pulp and Paper Division, New Zealand Forest Research Institute, Private Bag 3020, Rotorua, New Zealand

The application of enzymes in the pulp and paper industry has become of some interest following the successful introduction of xylanases as kraft pulp bleaching aids (*1*). Cellulases have also found application as drainage aids for low freeness pulps (*2*) and in deinking (*3*). Cellulases and hemicellulases are a growing segment of the world enzyme market, having found application in areas of plant tissue modification (in the brewing and food industries), the processing of textiles (e.g., stone washing of denim), and in laundry detergent applications. Being very selective in their mode of action and capable of modifying important commercial properties of cellulosic fibres, the potential for the use of such enzymes in papermaking has been recognized for some time (*4*). Thus the introduction of enzymatic treatments may offer a new technology for modifying fibres in ways that are impossible with chemical or mechanical processing and may offer new opportunities for integrating enzymatic steps into existing or modified pulp and paper processes.

There is a good understanding of how current processing technologies influence fibre properties, and how these in turn influence end use requirements. The application of enzyme technologies in papermaking requires a similar level of knowledge on the effects of these enzymatic agents on fibre properties. The literature is deficient in this knowledge and more basic research is required to address the information gap.

Despite the lack of studies on practical pulp substrates, considerable knowledge is available on the production, molecular biology, characteristics and modes of action of cellulases and hemicellulases (*5*). The enzyme systems from *Trichoderma reesei*, in particular, have been well studied as this fungus is widely used as a production micro organism for cellulases and hemicellulases. The cellulase system is composed of two exoglucanases and at least three endoglucanases. Endoglucanases are thought to attack the amorphous regions in native cellulose and artificial substrates, such as carboxymethyl cellulose, resulting in a rapid decrease in chain length. Cellobiohydrolases are thought to act by an endwise attack on the cellulose chain to cleave off cellobiose units and these enzymes work synergistically with the endoglucanases in degrading natural substrates. The xylanases of *Trichoderma reesei* have also been well studied and these enzymes act by randomly hydrolysing the xylan chain, releasing xylo-oligomers of various sizes (*6*).

Frequently, studies of the action of these enzymes have used culture filtrates containing a mixture of the various enzymes, such that the effects of individual component enzymes cannot be separated. It is essential that the effects of the individual enzymes are understood, so that advances can be made in their practical application. However work with purified mono-component enzymes has frequently been only on artificial cellulose substrates, which bear little relevance to commercial substrates such as those from the pulp and paper industry.

A recent study by Pere et al. (*7*) on the effects of purified endoglucanases and cellobiohydrolases on kraft pulp fibre properties showed that it was only the endoglucanases that cause rapid reductions in pulp viscosity and strength. This paper expands on the work of Pere et al. and describes the first stage of a two-part study into the effects of six purified enzymes (xylanases and cellulases from *T. reesei*) on the properties of selected radiata pine kraft pulps. It focuses on the enzyme characteristics and their effects on fibre chemistry and strength. The second part of this study focuses on fibre and handsheet property effects (*8*).

EXPERIMENTAL

Wood samples

Radiata pine slabwood and 12 year old thinnings wood samples were from the Kaingaroa Forest (Compartment 1029) in the central North Island of New Zealand. The basic densities for the slabwood and thinnings chips were 465 and 350 kg/m^3.

Kraft pulping

Kraft pulps of Kappa number 27±2 were prepared under the following conditions: 4:1 liquor to wood ratio; 15% effective alkali (Na_2O); 30% sulfidity; 90 minutes to 170°C maximum temperature; H-factor 2100 for slabwood chips and 2600 for thinnings chips. Half of each pulp was then oxygen delignified (1.5% NaOH, 0.7 MPa O_2, 100°C, 60 minutes) and bleached to market brightness (>86% ISO) using a conventional DEoD sequence (*9*). A total of four kraft pulps were thus produced: bleached and unbleached slabwood, and bleached and unbleached thinnings.

Enzyme treatments

Six purified *Trichoderma reesei* enzymes were supplied as concentrated solutions by Genencor International (South San Francisco, CA). The six enzymes were three endoglucanases labelled EG-A, EG-B, and EG-C; two xylanases labelled D and E; and a cellobiohydrolase (CBH). They were purified by conventional techniques, but some contained minor amounts of contaminating side activities (Table 1).

Table 1
Enzyme properties

Enzyme	Concentration mg protein /mL	Purity %
EG-A	2.5	90
EG-B	6.5	90
EG-C	15.0	90
Xylanase D	1.2	85
Xylanase E	12.0	95
CBH	45.0	90

Enzyme treatments were performed on pulp at 10% pulp concentration sealed in plastic bags and immersed in a water bath for the prescribed time. Pulp pH was adjusted with sulfuric acid and then equilibrated overnight to achieve the desired pH level (xylanase D, pH 4; xylanase E, pH 6; and pH 5 for the endoglucanases and CBH). Enzyme was added initially to the dilution water which was, in turn, mixed with the pulp to achieve the target pulp concentration. Following treatment, pulps were thoroughly washed with hot tap water, thickened and stored at 4°C. Pulps were not post treated immediately to prevent ongoing enzyme activity due to adsorbed enzyme. This was performed after several months storage by treating pulps at pH 12 with sodium hydroxide for 15 minutes, then thoroughly washing to neutral pH.

Pulp and filtrate composition

The Klason lignin, acid soluble lignin, and individual carbohydrate compositions of pulp samples were determined as previously described (*9*). Samples of enzyme treatment filtrates were subjected to 4% sulfuric acid post hydrolysis and HPLC analysis of the individual carbohydrates as previously described (*9*).

Carbohydrate DP

Pulp viscosity was determined according to TAPPI Method T 230. Carbohydrate degree of polymerization (DP) was calculated from the pulp viscosity (V) according to the formula:

$$DP = [0.75(954 \times \log_{10}(V)) - 325)]^{1.105}$$

as indicated in SCAN Standard C15:62.

Pore Volume

The physical accessibility of the pulps (accessible pore volume) was determined using the solute exclusion technique of Stone and Scallan (*10*). The method measures the water which is accessible in pulp fibres to probes of various nominal diameters. The probes were dextrans T6 (3.9nm), T10 (5.1nm), T40 (9.0nm), and T2000 (56.0nm) (Pharma-

Table 2
Composition of the pulps

Pulp	Kappa No.	Viscosity mPa.s	Composition, % on pulp							
			Klason Lignin	Acid Sol. Lignin	Ara	Gal	Glu	Xyl	Man	Total
Unbl. SW	27.5	31.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	-
Bl. SW	n.d*	16.3	0.4	0.43	0.54	<0.25	77.1	7.1	5.2	91.0
Unbl. Thin.	25.5	22.9	4.0	0.45	0.53	0.27	72.6	7.6	5.5	91.0
Bl. Thin.	n.d.	16.1	1.3	0.41	0.56	<0.25	74.9	7.7	5.6	90.7

* Not determined

Table 3
Dimensions of kraft fibres reconstituted from handsheets (prepared from the unbleached pulps)

Wood	Chip basic density kg/m^3	Length mm	Coarseness mg/m	Width µm	Thickness µm	Width x thickness µm^2	Wall area µm^2	Wall thickness µm	Width / thickness
Slabwood	465	2.82	0.250	30.9	12.2	378	223	3.70	2.68
Thinnings	350	1.99	0.180	31.8	10.2	327	177	2.86	3.38
LSD*				1.7	0.7	30	15	0.18	0.24

* Least significant difference between means at 95% level of significance

Table 4
Untreated pulp fibre saturation points and accessible pore volumes for solutes of three different molecular sizes.

Pulp Type	FSP mL/g	Accessible volume, mL/g		
		90 Å	51 Å	39 Å
Unbleached SW	1.44	0.28	0.63	0.61
Bleached SW	1.36	0.32	0.63	0.67
Unbleached Thin.	1.45	0.31	0.63	0.69
Bleached Thin.	1.34	0.28	0.65	0.73

cia, Sweden). The water inaccessible to the T2000 dextran defines the Fibre Saturation Point (FSP) of a pulp.

Handsheet and fibre properties

Handsheets were prepared and pulp physical evaluations made in accordance with Appita standard procedures. Cross-section dimensions of pulp fibres were determined as described elsewhere (*8*).

RESULTS AND DISCUSSION

Pulp characteristics

The Kappa number, viscosity, and chemical composition of the four pulps are presented in Table 2. The dimensions of the unbleached kraft fibres, measured after reconstitution from dried handsheets are shown in Table 3. The slabwood fibres were longer and coarser than the thinnings fibres. Although fibre widths were similar for the two pulp types, fibre thickness, wall thickness, and wall area were substantially higher for the slabwood material. The unbleached pulps were slightly more swollen than the bleached pulps as indicated by the FSP data (1.44 compared with 1.35 mL/g.) (Table 4). Their physical accessibilities to enzyme-sized molecules (39–90 Å), however, appear very similar when expressed as accessible water in mL/g (*11*). When considering accessible water as a percentage of the FSP, the bleached pulps were slightly more accessible to enzyme-sized molecules.

Enzyme characteristics

Although all of the enzymes were highly purified, some did have measurable non-target activities (Table 1). For example, EG-B displayed substantial xylanase activity. EG-A and EG-C, on the other hand, contained only traces of contaminating xylanase activity.

Both xylanases were very pure and contained low or undetectable levels of endoglucanase impurities. None of the purified enzymes contained any detectable mannanase activity. All enzymes, being highly purified, were dosed on the basis of µg protein per gram oven dry pulp. This avoided interpretation difficulties implicit in the use of enzyme activity units as determined by different assay methods.

Screening experiments

The effects of increasing enzyme charge were initially investigated using the bleached slabwood (SW) pulp and two hours treatment time. Figure 1 shows total solubilized carbohydrate versus enzyme charge for each of the six purified enzymes. Treatment with xylanase E solubilized the greatest amount of carbohydrate, up to 2.5% on pulp dry mass at a charge of 1000 µg/g. Xylanase D removed about two-thirds this amount of carbohydrate at an equivalent charge level. The endoglucanases solubilized much less carbohydrate than the xylanases, especially at low enzyme charge (i.e. 100 µg/g). EG-A removed a little less carbohydrate than EG-B, with EG-C giving the lowest level of dissolved carbohydrate of the three endoglucanases. CBH caused relatively low extents of carbohydrate dissolution, with only 0.2% on pulp mass removed at 1000 µg/g charge.

Figure 2 shows the effect of increasing enzyme charge on carbohydrate degree of polymerization (DP), as estimated from pulp viscosity. The two xylanases and CBH had very little effect on DP as might be expected based on the substrate specificities of these enzymes (Table 4). The endoglucanases all caused substantial reductions in DP. EG-A had the greatest effect, while EG-B was the least aggressive in carbohydrate depolymerization.

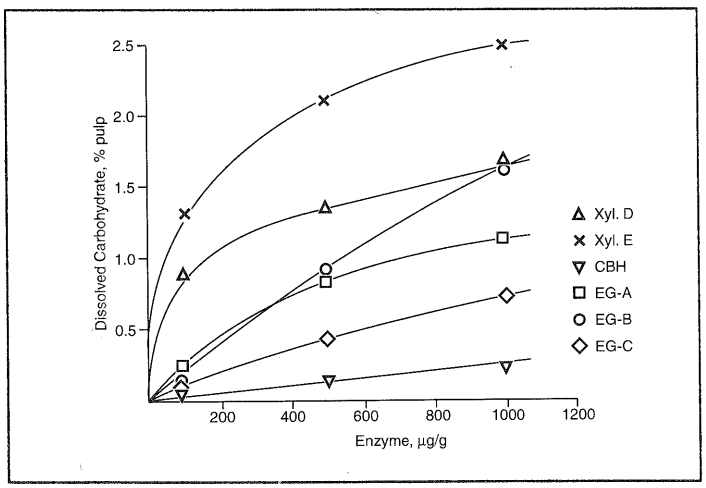

Fig. 1 Effect of enzyme charge on the amount of dissolved carbohydrate (bleached slabwood pulp).

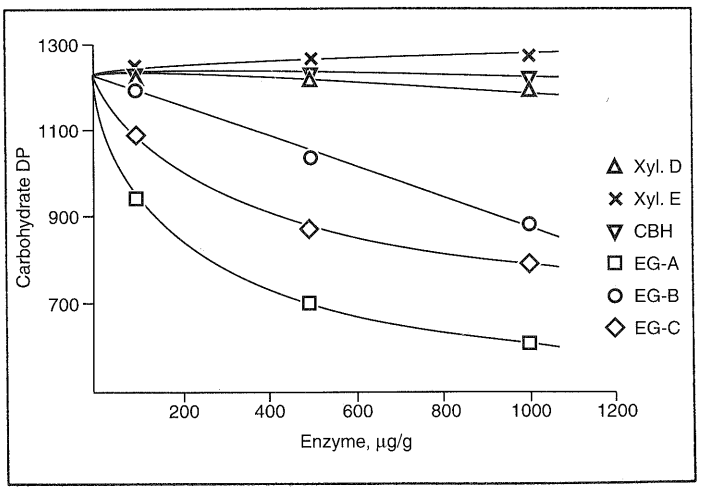

Fig. 2 Effect of enzyme charge on carbohydrate DP (bleached slabwood pulp).

Table 5
Viscometric DP and yield of individual carbohydrates dissolved by treatments with each of the six purified enzymes (xylanases and CBH = 400 µg/g, 4 hours; EG's = 100 µg/g, 2 hours).

Enzyme	Bleached	Carbohyd. DP	Dissolved carbohydrates % on pulp					
			Ara	Gal	Glu	Xyl	Man	Total
Slabwood								
Control	No	1670	-	-	0.01	0.01	-	0.02
	Yes	1225	0.01	-	-	0.09	-	0.10
Xyl. D	No	1611	0.11		-	1.24	-	1.35
Xyl. E	No	1651	0.13	-	-	1.41	-	1.54
	Yes	1203	0.17	-	0.02	1.98	-	2.17
CBH	No	1602	-	-	0.11	-	-	0.11
	Yes	1207	0.01	-	0.19	0.11	-	0.32
EG-A	No	1333	-	-	0.06	0.10	-	0.16
	Yes	927	0.01	-	0.16	0.10	-	0.27
EG-B	No	1511	0.02	-	0.06	0.25	-	0.33
	Yes	1126	0.02	-	0.07	0.19	-	0.27
EG-C	No	1288	0.01	-	0.07	0.07	-	0.15
	Yes	1020	0.01	-	0.06	0.06	-	0.13
Thinnings								
Control	No	1455	-	-	-	0.01	-	0.01
	Yes	1218	0.01	-	-	0.07	-	0.08
EG-A	No	1057	0.01	-	0.09	0.13	-	0.23
	Yes	930	0.01	-	0.15	0.10	-	0.26
EG-B	No	1278	0.03	0.01	0.09	0.30	0.02	0.44
	Yes	1152	0.02	-	0.08	0.23	-	0.34
EG-C	No	1051	0.01	-	0.12	0.07	-	0.20
	Yes	1052	0.01	-	0.06	0.08	-	0.15

Treatment of four pulp types

Based on the screening results, 20 individual treatments were performed involving combinations of the six enzymes and the four pulp types (*i.e.*, bleached and unbleached slabwood, and bleached and unbleached thinnings). The unbleached slabwood pulp was treated with all enzymes, some at two levels. For the xylanases and CBH, a relatively high enzyme charge of 400 µg/g and a treatment time of four hours were chosen to ensure high levels of enzyme attack so that any consequent effects on pulp properties might be accentuated. Endoglucanase treatments of unbleached slabwood pulp were at 100 and 400 µg/g for two hours to achieve low and high extents of cellulose depolymerization.

Bleached slabwood pulp was treated with xylanase E and CBH (400 µg/g) as well as the three endoglucanases at the low dose of 100 µg/g. The effects of bleaching on these enzyme treatments could be determined by direct comparison with unbleached slabwood pulp responses. Bleached and unbleached thinnings pulps were only treated with the three endoglucanases at the low dosage rate (100 µg/g).

A wide range of analytical procedures were applied to these 20 pulps (plus the four original pulps which received control treatments without enzyme). The full evaluation of fibre and handsheet properties is described in Part 2 of this work (*8*). The only handsheet data discussed here, reflect effects on fibre wall chemistry.

Dissolved carbohydrates

Table 5 shows the yields of dissolved carbohydrates released by the six enzymes, comparing the unbleached and bleached pulps. The two xylanases were very selective in their action, attacking only arabinoxylan as evidenced by the release of xylose and arabinose in approximately the same ratios as in the original pulp (Table 2). For xylanase E,

Table 6
Comparisons of cellulose DP reduction and chain scissions per gram of cellulose with percentage glucan removal after endoglucanase treatments at 100 µg/g.

Pulp Type	EG-A			EG-B			EG-C		
	ΔDP	Chain Scis.*	Glucan %	ΔDP	Chain Scis.*	Glucan	ΔDP %	Chain Scis.*	Glucan %
Unbl SW	-337	5.8	0.06	-159	2.3	0.06	-382	6.6	0.07
Bl SW	-298	9.8	0.16	-99	2.7	0.07	-205	6.1	0.06
Unbl Thin	-398	9.6	0.09	-177	3.5	0.09	-404	9.8	0.12
Bl Thin	-288	9.5	0.15	-66	1.8	0.08	-166	4.8	0.06

* 10^{17}/g cellulose = $[(DP_o/DP) - 1] \times [6.023 \times 10^{23}] \div [162 \times DP_o]$

carbohydrate solubilized from the bleached slabwood pulp was substantially greater than that solubilized from the unbleached pulp, suggesting that bleaching had enhanced the accessibility of the xylan component to this enzyme. This trend was in contrast with that measured by the solute exclusion technique where bleaching had little effect on the accessibility of enzyme-sized dextran probes (Table 4).

After considering the carbohydrate release of the controls, CBH was shown to solubilize only glucan from both unbleached and bleached slabwood pulps (Table 5). Again, a greater dissolution was observed for bleached pulp which indicates increased accessibility after bleaching for this enzyme, too. The low level of glucan dissolved by CBH is consistent with the findings of Pere et al. (*7*) which showed very low carbohydrate dissolution and viscosity drop after CBH treatment of a northern pine kraft pulp. Cellobiohydrolases are very different in their mode of action and effects on pulp fibres, compared with the more aggressive endoglucanases.

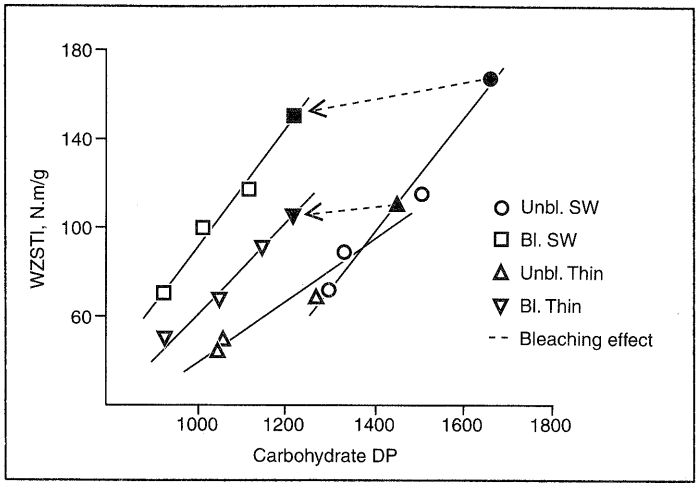

Fig. 3 Wet zero span tensile index (500 PFI revolutions) versus carbohydrate DP after treatment with endoglucanases at 100 µg/g (filled symbol = untreated pulp; open symbol = EG treated pulp)

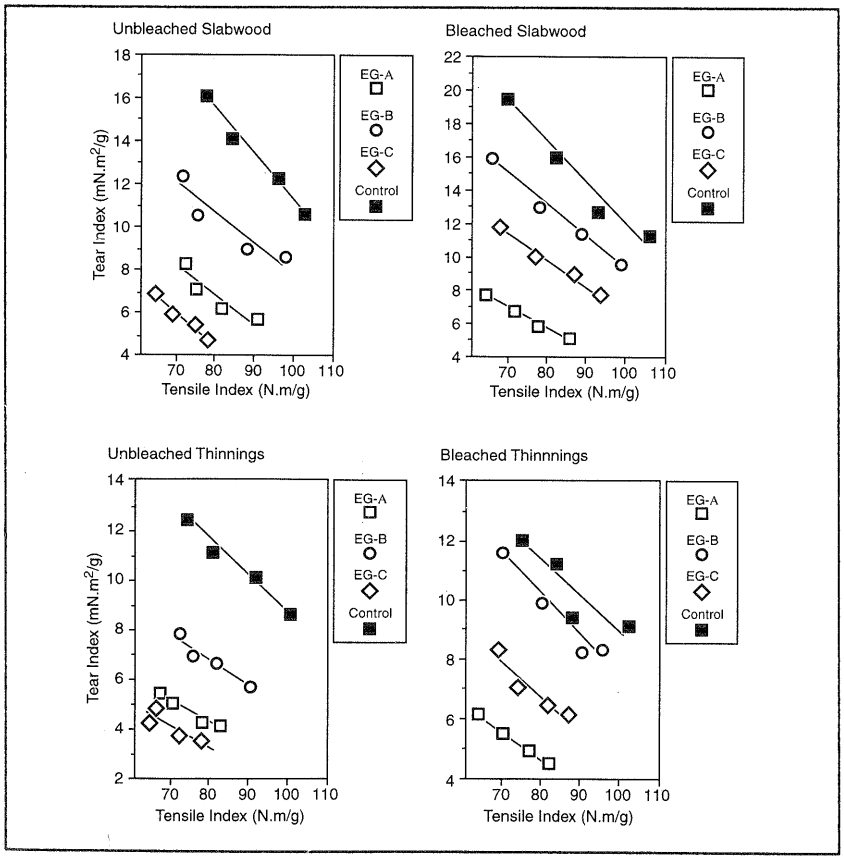

Fig. 4 Tear index versus tensile index for untreated and EG treated pulps at 100 µg/g.

The effects of the three endoglucanases on glucan dissolution also varied with pulp type. For the unbleached pulps, dissolved glucan levels were generally similar for the three endoglucanases, though that for EG-C with thinnings pulp was somewhat elevated (Table 5). After bleaching, only EG-A treatment showed a marked increase in glucan removal (i.e. from about 0.08 to 0.16% on pulp), while the dissolved glucan levels for EG-B and EG-C were generally unchanged. Thus, of all six enzymes studied, only the endoglucanases EG-B and EG-C failed to show any improvement in carbohydrate removal on bleached pulp. These relative effects indicate that there are strong interactions between pulp treatment history (e.g. bleached or unbleached) and the activities of these *T. reesei* enzymes.

Again it should be noted that EG-B has a substantial reactivity with xylan as evidenced here by its high levels of xylose dissolution (Table 5). The other endoglucanases also showed some xylan removal above the background levels of the control pulps, indicating trace xylanase contamination. In contrast to the results reported by Pere et al. (*7*), endoglucanases in this study did not show any mannanase activity as no dissolution of mannose-containing oligomers was observed (Table 5).

Table 6 shows the effects of the endoglucanase treatments on carbohydrate depolymerization for the bleached and unbleached pulps, as estimated from pulp viscosity. Pulp viscosity is predominantly affected by the DP of the backbone cellulose in fibres, but also reflects a lesser influence of lower DP hemicelluloses. Changes in viscometric DP after endoglucanase treatments with minimal side reactivities can be assumed to be due to depolymerization of cellulose, whereas the effects of bleaching on viscometric DP may be due to this as well as changes in overall carbohydrate composition. It is interesting to note that the xylanase treatments in this study removed substantial amounts of arabinoxylan from the pulps (up to 30% of the original content), yet very little effect on viscometric DP was observed (Fig. 1,2).

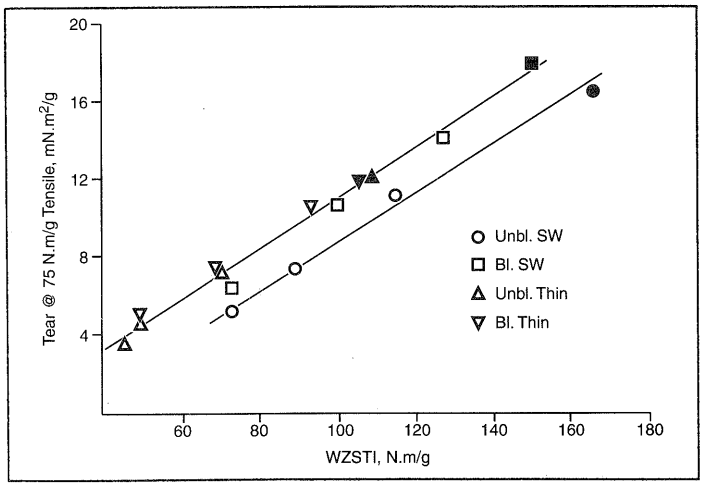

Fig. 5 Tear index at 75 N.m/g tensile index versus wet zero span tensile index (500 PFI revolutions) after treatment with endoglucanases at 100 µg/g (filled symbol = untreated pulp; open symbol = EG treated pulp).

Carbohydrate depolymerization (DP) after treatment with EG-A, EG-B and EG-C was substantially less for bleached versus unbleached slabwood and thinnings pulps (Table 6). This might suggest that bleaching decreased the extent of endoglucanase attack on the cellulose fraction in pulp. However, to account for the lower initial DP of bleached pulps, endoglucanase attack was normalized by calculation of the number of chain scissions per gram of cellulose (Table 6). These data show a less uniform trend with only EG-B and EG-C consistently displaying decreased attack after bleaching, especially for thinnings pulps.

Results in Table 6 also show that two separate measures of endoglucanase attack (i.e., depolymerization and glucan removal) did not correlate well. As an extreme example, EG-A treatment data for bleached and unbleached thinnings pulp indicated – that glucan dissolution was *greater* for bleached pulps (0.15 versus 0.09% on pulp); decreases in cellulose DP were *less* after bleaching (DP -288 versus -398); and number of chain scissions per gram of cellulose was *unaffected* by bleaching (9.5 versus 9.7 x 10^{17}).

There is some disagreement in the literature concerning the extent of pulp material loss, and its correlation with changes in cellulose DP (*7*). This is perhaps explainable in terms of a topochemical action on the fibre wall. For endoglucanases, localized attack at specific sites could result in substantial cellulose dissolution without much overall reduction in measured DP, while more even attack over the bulk of the fibre wall may give much greater extents of DP reduction at a given level of cellulose dissolution. The effect of bleaching on the action of specific enzymes might then be to somehow promote the former effect while diminishing the latter.

Overall, the data from this study show that the different endoglucanases do have distinct differences in their relative substrate affinities and modes of action, as the findings of Pere et al. (*7*) have suggested when they compared the actions of several endoglucanases on a single pulp sample. Therefore, each endoglucanase has some unique characteristics in terms of its effect on kraft pulps and all such enzymes should not be considered as being the same.

Fibre strength

Just as the strength of the cellulose component of kraft fibres can be estimated from viscometric DP measurements, so can the mean strength of kraft fibres be estimated from the wet zero span tensile index (WZSTI) of handsheets formed from untreated and enzyme treated pulps (*14*). In theory, tensile tests on wetted samples with zero span between the instrument jaws measures the work required to rupture individual fibres, without any effects of interfibre bonding (*15*).

Correlations of cellulose strength (DP) with fibre strength (WZSTI) are presented in Figure 3 for the four untreated pulps and those treated with the three endoglucanases at 100 µg/g. As DP was reduced by endoglucanase treatments, so did WZSTI decrease. The unbleached slabwood and thinnings pulps formed similar relationships in this regard. Bleaching, however, reduced DP significantly with relatively little change in fibre strength, and greater DP reductions were observed for the slabwood pulps. After bleaching, endoglucanase treatment then decreased DP with a similar relationship to WZSTI as that observed for EG-treated unbleached pulps.

Figure 3 illustrates the distinct difference between cellulose depolymerization induced by chemical processing and that due to cellulase treatment. In the former case, the bleaching process substantially reduced overall cellulose DP, but without significant effect on fibre strength. In contrast, the enzymatic reduction in DP was accompanied by dramatic fibre strength reduction. This phenomenon has been discussed by Gurnagul et al. (*13*) who examined the relationship between fibre strength loss and cellulose degree of polymerization. They found that treatments which induce homogeneous and random degradation of cellulose, such as those due to chemical processing (e.g. bleaching), cause relatively little fibre strength loss. In agreement with the current data, they also showed that agents such as cellulase enzymes can induce quite dramatic reductions in WZSTI, yet only moderate reductions in cellulose DP. They ascribed this to the heterogeneous nature of cellulase hydrolysis of fibre wall components due to the limited accessibility of these enzymes to all regions of the fibre wall. The localized cellulose attack may occur at fibre wall defects of high accessibility, resulting in the creation of weak points in the fibre which reduce measured fibre strength after little measured overall DP loss.

Handsheet strength

The handsheet strength of a pulp is a useful indicator of its potential to impart wet web strength to papermaking furnishes and dry strength to the final paper product. Handsheet strength is normally determined from the tear-tensile relationship, with pulps achieving a higher tearing resistance at a given tensile strength being considered stronger (*16*).

Plots of tear index versus tensile index for each of the four pulps treated with the endoglucanases at 100 µg/g are shown in Figure 4. All endoglucanase treatments caused substantial handsheet strength reductions. For unbleached

pulps, the order of strength reduction was consistently EG-C > EG-A > EG-B. For bleached pulps, however, the order was altered with EG-A > EG-C > EG-B. Closer inspection reveals that the relative impact of both EG-B and EG-C on handsheet strength reduction was affected by bleaching, with the effect on EG-C being greater. For example, reductions in tear-tensile relations after EG-C treatment of both slabwood and thinnings pulp were 70% for unbleached *versus* 40% for bleached pulp. EG-A treatment effects on handsheet strength reduction, while substantial, remained relatively constant for bleached and unbleached slabwood and thinnings pulps (i.e. ≈ 60%). These trends are in keeping with the cellulose depolymerization effects observed after endoglucanase treatment as presented in Table 6. Bleaching reduced the extent of depolymerization for EG-B and EG-C treatments while having a lesser effect on EG-A treatments.

Handsheet strength can be expressed as a single value by estimating the tear index at a given tensile index. If this value is plotted against the WZSTI, then the relationship of fibre strength to handsheet strength can be examined. Fibre strength has a large influence on the web strength of dry paper, which is also affected by other factors such as interfibre bonding and fibre length. Figure 5 shows that the rate of pulp strength loss with decreases in fibre strength after endoglucanase treatment was similar for all pulps. There was very close to a single relationship between fibre strength losses and handsheet strength losses, with only the unbleached slabwood pulp showing a somewhat different correlation. Overall this suggests that endoglucanase treatments were affecting pulp strength mainly through fibre weakening, and not through changes to interfibre bonding or decreases in fibre length.

CONCLUSIONS

This study of kraft pulp treatments with six purified enzymes from *T. reesei* showed that, at equivalent mass charges of enzyme protein on pulp, xylanases hydrolysed the greatest amount of carbohydrate (up to 2.5% on pulp at 1000 µg/g charge) while cellobiohydrolase removed the least (< 0.2% on pulp at 1000 µg/g). The three endoglucanases dissolved intermediate levels of carbohydrate. The xylanases and CBH were relatively pure in their activity, while endoglucanases displayed some activity toward xylans.

Endoglucanase treatments were shown to cause substantial cellulose depolymerization in treated pulps, while CBH and xylanase treatments had little effect. The extent of cellulose depolymerization varied among the three endoglucanases, with EG-A being the most aggressive. Wood source (i.e. slabwood versus thinnings) had little effect on DP loss after endoglucanase treatment.

For the three endoglucanase treatments, there was no common correlation between the extent of glucan removal from the fibre and the extent of depolymerization of the cellulose remaining in the fibre. This indicates that each endoglucanase had a unique relationship between extent of hydrolytic chain scission and dissolution of degraded cellulose fragments. Furthermore, pulp bleaching altered this relationship in different ways for each endoglucanase. Bleached pulps lost twice as much glucan after EG-A treatment, but with less cellulose depolymerization. For EG-B and EG-C treatments, the extent of depolymerization was also decreased by bleaching whereas glucan removal was relatively unaffected. Overall, the different endoglucanases displayed distinct differences in their relative substrate affinities and modes of action, in agreement with earlier studies of *T. reesei* enzymes.

Endoglucanase treatments substantially reduced intrinsic fibre strength, as estimated by the wet zero span tensile index. This reduction was strongly correlated with cellulose DP loss, suggesting that the degradative enzyme attack was focused on accessible areas of the fibre and created fibre weak points. In contrast, the more homogeneous chemical reactions during pulp bleaching caused minimal fibre strength loss despite substantial, but dispersed, cellulose depolymerization.

The aggressive attack of endoglucanase treatments also diminished pulp handsheet strength as measured by the tear-tensile relationship. After treatment with two of the three endoglucanases, the extent of handsheet strength loss differed between bleached and unbleached pulps, indicating that bleaching altered fibre properties affecting the action of some endoglucanases. Handsheet strength loss was well correlated with decreases in wet zero span tensile index, suggesting that endoglucanase treatments were affecting pulp strength mainly through fibre weakening, and not through changes to interfibre bonding or decreases in fibre length.

ACKNOWLEDGEMENTS

The excellent technical work of Sylke Campion and Frances Signal is gratefully acknowledged. For the preparation and supply of the enzymes and the many helpful discussions of results, thanks are extended to Genencor International, especially Anne Kantelinen, Bette Bodie, Philippe Lavielle, Oli Jokinen, and Ed Larenas.

REFERENCES

(1) Viikari, L., Sundqvist, J., and Kettunen, J. – *Paperi Puu* **73** (5): 384 (1991).
(2) Pommier, J-C., Goma, G., Fuentes, J-L. and Rousset, C. – *Tappi J.* **73** (12): 197 (1990).
(3) Jeffries, T. W., Sykes, M. S., Rutledge-Cropsey, K., Klungness, J. H. and Abubakr, S. M. – Proc. 6th Int. Conf. Biotechnol. Pulp and Paper Industry, Vienna, p.23 (1995).
(4) Daniels, M. J. – *Paper Technol.* **33** (6): 14 (1992).
(5) Kubicek, C. P., *et al.* – *Trichoderma reesei* cellulases: Biochemistry, Genetics, Physiology, and Applications. The Royal Society of Chemistry, Cambridge, (1990).
(6) Poutanen, K. and Puls, J. – *App. Microbio. Biotech.* **28**: 425 (1988).
(7) Pere, J., Siika-aho, M., Buchert, J. and Viikari, L. – *Tappi J.* **78** (6): 71 (1995).
(8) Kibblewhite, R. P. and Clark, T. A. – Proc. 50th Appita An. Gen. Conf., Auckland, (1996).
(9) Allison, R., Clark,T. and Ellis, M. – *Appita J.* **48** (3): 201 (1995).
(10) Stone, J. and Scallan, A. – *Pulp Paper Mag. Can.* **69** (6): T288 (1968).
(11) Cowling, E. B. and Brown, W. – Structural features of cellulosic materials in relation to enzymatic hydrolysis. In: **Cellulases and Their Applications, Advances in Chemistry Series 95,** G. Hajny and E. Reese, eds. American Chemical Society, Washington (1969).
(12) Biely, P. – Artificial substrates for cellulolytic glycanases and their use for differentiation of Trichoderma enzymes. In: **Trichoderma reesei Cellulases: Biochemistry, Genetics, Physiology, and Applications.** (Kubicek, C. P. et al, eds.) The Royal Society of Chemistry, Cambridge (1990).
(13) Gurnagul, N., Page, D. H. and Paice, M. G. – *Nordic Pulp Paper Res. J.* **3**: 152 (1992).
(14) Boucai, E. – *Pulp Paper Mag. Can.* **72** (10): T313 (1991).
(15) Cowan, W. – Proc. CPPA An. Meeting, Tech. Sec., Montreal, p.A149 (1988).
(16) MacLeod, M. – *Pulp Paper Can.* **88** (9): T330 (1987).

Revised manuscript received for publication 10.9.96