Effect of enzyme pretreatment on ozone bleaching

Robert W. Allison and Thomas A. Clark

ABSTRACT: The effect of enzyme pretreatment on ozone and chlorine-based bleaching of kraft-oxygen pulp from radiata pine was studied in the laboratory. Pretreatment conditions with Pulpzyme HA, a xylanolytic preparation from Novo-Nordisk a/s, were optimized separately for both ZED and (DC)ED bleaching. The presence of cellulase activity in the preparation caused significant cellulose degradation under some pretreatment conditions, particularly low pH and temperature. At optimum pretreatment conditions, bleaching effectiveness for both sequences was improved about 25% while bleaching selectivity was unaffected.

KEYWORDS: Hemicellulase, kraft pulps, ozone bleaching, pinus radiata, pretreatment.

Kraft pulp bleaching with chlorine-based chemicals produces chlorinated organic material which is considered harmful to the environment. There is worldwide interest in developing chlorine-free bleach treatments which are cost-effective and preserve pulp quality. Ozone bleaching has great potential in this regard and has been extensively studied both in the laboratory and on the pilot plant scale *(1–3)*. Recently, a softwood kraft pulp producer in the United States announced plans to install ozone delignification in a 975-ton/day commercial bleach line *(1)*.

Questions still remain over the selectivity of ozone treatment and its ability to retain the important strength advantages of softwood kraft pulp, especially after oxygen delignification. Selectivity is normally defined as the ratio of a desirable bleaching effect (delignification or brightening) versus an undesirable bleaching effect (loss of pulp viscosity or strength). Many pretreatments and additives to improve ozone bleaching selectivity have been proposed though none to date has proved commercially viable *(2)*. More effective systems to increase the selectivity of ozone still need to be developed.

Recent work has shown that enzymatic pretreatments with hemicellulases prior to chlorine-based bleaching improve delignification effectiveness, i.e., increase lignin removal per unit of consumed active chlorine *(3–6)*. The exact mechanism of improvement is unknown. However, it may involve physical effects on the cell wall structure or the partial removal of redeposited xylan which may increase overall pore size and thus facilitate lignin removal *(3, 7)*. If this is the case, then hemicellulase pretreatment may act as a general enhancement for all delignification processes including those using oxygen, hydrogen peroxide, and ozone. Increases in the selectivity of ozone delignification may also accompany any increases in ozone effectiveness.

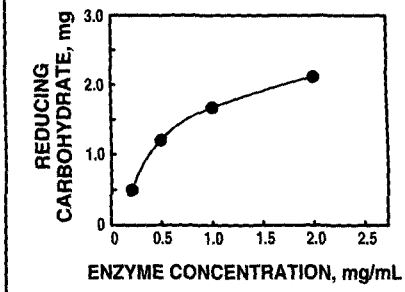
This study characterized the effects of pretreatment with a commercial hemicellulase product, Pulpzyme HA from Novo-Nordisk a/s, on ozone delignification and subsequent chlorine dioxide brightening of kraft-oxygen pulp from radiata pine. For comparison, pretreated pulps were also bleached with a conventional chlorine-based sequence. After initial pretreatment experiments at the manufacturer's recommended conditions, pretreatment conditions were optimized using statistically designed experiments. Filtrates from pretreatments were analyzed for residual enzyme activity and total carbohydrate dissolution. Pretreated pulps were bleached with standard ZED and (DC)ED sequences. Effects on pulp extracted kappa number and viscosity, as well as final brightness were then determined.

Allison is group leader, PAPRO-New Zealand, and Clark is scientist, Wood Technology Division, Forest Research Institute, Private Bag 3020, Rotorua, New Zealand.

I. Experimental variables and their levels in the factorial design.

	-1	0	+1
A. Temperature, °C	40	50	60
B. Enzyme charge, XU/g .o.d. pulp	3.3	6.6	9.9
C. Buffered pH	5.0	6.0	7.0
D. Time, h	3.0	5.0	7.0
E. Consistency, %	6.5	11.0	15.5

1. Effect of Pulpzyme HA concentration on reducing carbohydrate release in a standard xylanase assay



Experimental

Pretreatment

Pulpzyme HA is a xylanase preparation derived from a selected strain of *Trichoderma reesei*. According to the manufacturer it contains endo-1,4-beta D-xylanase as well as exo-1,4-beta-D-xylanase activities. A certain amount of cellulase activity is also present which is minimized at pH 7.0 and 40–50°C according to the manufacturer. Substantial xylanase activity is still reported under these conditions.

Pretreatments were performed using 100-g o.d. samples of commercial kraft-oxygen pulp (13.0 kappa number) from radiata pine. Pulps were hand mixed with enzyme in plastic bags then immersed in a water bath at the required temperature. Following pretreatment, spent filtrate was collected and pulp was washed with warm tap water.

The 2^{5-1} fractional factorial experimental design contained six center points to estimate pure error variance. Variable levels were as shown in **Table I.**

The half fraction of the full 2^5 was selected as a resolution V design with the defining relation I = ABCDE. The 32-run fractional factorial design was performed in two blocks for logistical reasons. Since the variables of time and consistency were thought to be the least significant, the two blocks were selected using the defining relation I = ± ABC. This blocking pattern causes the two-factor interaction variable (Time* consistency) to be confounded with any block effects which may be observed.

To obtain the pH range required (5.0–7.0), two buffers were used. Sodium citrate buffer (0.05 *M*) was used for pH 5.0 and 6.0, while sodium phosphate buffer (0.05 *M*) was used for pH 6.0 and 7.0. The replication of the center-point treatment conditions (which required pH 6.0) enabled several treatments to be performed with each of the two buffers. No differences in subsequent bleach response were observed between the two buffer systems.

Pretreatment experiments at optimized conditions were performed without buffer using sulfuric acid to adjust pH to the desired level. Some control pretreatments were also performed at optimal conditions, but without enzyme addition.

Bleaching

Pulp (30 g o.d.) at 10% consistency was acidified prior to ozone treatment. Pulp containing 0.4% DTPA was adjusted to pH 2.0 with sulfuric acid, then heated to 75°C for 60 min. After dewatering to 30% consistency, pulp was ozonated at room temperature in a rotary evaporator unit with 1.0% consumed ozone.

Pulp (25 g o.d.) at 1.5% consistency was also sequentially chlorinated in a modified pulp disintegrator with 2.6% active chlorine (50% ClO_2 substitution) at 35°C for 35 min.

Ozone treated and chlorinated pulps were washed with warm tap water then alkaline extracted in a plastic bag with 2.0% NaOH at 10% consistency and 70°C for 60 min. Extracted pulp was washed with warm tap water then analyzed for kappa number and viscosity according to TAPPI UM 246 and TAPPI T 230, respectively.

Extracted pulp (20 g o.d.) at 10% consistency was chlorine dioxide bleached in a plastic bag with 1.0% ClO_2 (0.4% NaOH) at 70°C, for 180 min. After treatment, pulp was washed with warm tap water then adjusted to pH 5 with sulfurous acid. ISO brightness was measured according to ISO 3688-1977.

Analyses

Pretreatment filtrates were analyzed for residual enzyme activity with a xylanase assay using beechwood 4-OMe-glucuronoxylan (1%) as sub-

II. Results after enzyme pretreatment at manufacturer's recommended standard conditions of 6.6 XU/g, 50°C, pH 6.0, 5.0 h and 11% consistency.

	Enzyme pretreatment				*ZED or (DC)ED Bleaching*			
	Enzyme survival, %	*Dissolved carbohydrate, %*	*Pulp yield, %*	*Kappa number*	*Extracted kappa number*	*Extracted pulp viscosity, mPa·s*	*Selectivity (Δ Kappa no. /Δ visc.)*	*Final brightness, % ISO*
ZED[a]								
Control	--	--	--	13.0	3.1	10.2	0.65	86.7
Pretreated	7.0	2.0	97.8	12.2	2.4	8.0	0.61	87.9
(DC)ED[a]								
Control	--	--	--	13.0	2.7	24.0	7.35	86.0
Pretreated	7.0	2.0	97.8	12.2	1.9	16.2	1.13	87.4

[a]Average of six or more replicates

strate in 0.05 *M* sodium acetate buffer at pH 5.0. One mL of substrate was mixed with 0.5 mL of suitably diluted enzyme. At least three enzyme concentrations were tested. The mixture was incubated at 46°C for 10.0 min, whereupon 1.5 mL DNS (3,5 dinitrosalicylic acid) reagent was added. After boiling for 5.0 min, the mixture was cooled; then 10 mL distilled water was added. Solution absorbance was measured at 530 nm against a buffer-only blank. Reducing carbohydrate released (mg) was determined by reference to a xylose standard curve. Readings were corrected for substrate and enzyme blanks. From each assay, a graph of milligrams reducing carbohydrate released versus enzyme concentration was prepared, and the xylanase activity was calculated for the enzyme concentration which released exactly 0.5 mg reducing carbohydrate (determined by interpolation). One xylanase unit (XU) of activity is the amount of enzyme which catalyzes the release of 1 micromole of reducing carbohydrate per minute.

Pretreatment filtrates were also analyzed for reducing carbohydrate and total carbohydrate content. The concentration of reducing carbohydrates was measured by the DNS procedure as already described for the xylanase assay. Total carbohydrate was determined by a modification of the orcinol/sulfuric acid method *(8)*. Xylose solutions (10 mg/L to 120 mg/L) were used to establish a standard curve. Sample or standard (1 mL) was dispensed into glass test-tubes immersed in an ice bath for 15 min. Two mL of freshly prepared orcinol reagent (0.2% w/v orcinol dissolved in conc. sulfuric acid) was rapidly added to each test tube which was vortexed and incubated in a boiling water bath for exactly 15 min. Tubes were then cooled in an ice bath and their absorbance at 540 nm determined. Samples were analyzed in triplicate.

Results and discussion

Characteristics of Pulpzyme HA

Pulpzyme HA is a liquid xylanase preparation with a stated xylanase activity of 500 XU/g of product. Its activity was determined using an in-house assay procedure as it is well known that different assay protocols will give different activity results. **Figure 1** shows the amount of reducing carbohydrate (as xylose equivalents) released in the assay versus the concentration of the enzyme preparation. The data indicate that this relationship is linear up to about 1.2 mg reducing carbohydrate released. The xylanase activity was calculated by this assay as 3300 XU/g product. The amount of cellulase activity was not determined.

Pretreatment effects under standard conditions

Initial enzyme pretreatment experiments were performed at standard conditions, as recommended by the enzyme manufacturer, i.e., 6.6 XU/g pulp, pH 6, 11% consistency, 50°C, 5 h. The bleach response of enzyme pretreated pulp was compared with that of untreated control pulps using similar ZED and (DC)ED bleach sequences. For ozone treatments, identical ozone charges were applied to both enzyme pretreated and control pulps. For (DC) treatments, a fixed ratio of active chlorine charge to kappa number was applied, i.e., 0.20 active chlorine multiple.

Pulp analysis after enzyme pretreatment but before bleaching showed that xylanase pretreatment at the standard conditions reduced kappa number by 0.8 units and incurred a 2.2% yield loss (**Table II**). This total yield loss coincided with the 2.0% total sugar yield measured in the pretreatment filtrate. The extent of delignification after both ZE and (DC)E prebleaching was increased by enzyme pretreatment

III. Predictive models for pretreatment and bleaching responses derived from statistical analysis of factorial design (95% confidence).

Factor	Enzyme pretreatment				(DC)ED Bleaching			ZED Bleaching		
	Kappa no.	Pulp yield, %	Dissolved carbohydrate, %	Enzyme survival, %	Ext. kappa No.	Pulp viscosity, mPa·s	Final brightness, % ISO	Ext. Kappa No.	Pulp viscosity mPa·s	Final brightness % ISO
Temp.	0.175	0.306		-15.2	0.095	0.810		0.156		
Enzyme charge			0.341		-0.080	-1.705				
pH		0.819	-0.562		0.126	3.875	-0.284	0.244		-0.662
Time			0.174							
Consistency				-5.31						
Temp* enz.						0.603	0.303		0.704	-0.575
Temp*pH		0.244	-0.251			1.195				
Temp*time										
Temp*cty				4.56						
Enz*pH			-0.181			0.710				
Enz*time		-0.256								
Enz*cty		-0.281						-0.069		
pH*time										
pH*cty								-0.231		
Time*cty			-0.210			0.475				0.576
Constant	12.4	97.9	1.53	16.9	1.92	18.1	87.4	2.57	8.81	87.4
Mean center point	12.2	97.8	1.96	7.0	1.86	16.2	87.4	2.38	7.98	87.9
R^2 % (adj. for dof)	18	76	91	85	82	98	26	62	19	66

(Table II). ZE and (DC)E kappa numbers were 0.7–0.8 units, respectively, lower than similarly treated control pulps. Thus final brightening after chlorine dioxide treatment was also increased by enzyme pretreatment, i.e., 1.2 and 1.4 units, respectively. Unfortunately, pulp viscosity after bleaching was substantially decreased by enzyme pretreatment, especially for the (DC)ED sequence (Table II).

Selective removal of xylan would be expected to increase pulp viscosity since xylan is relatively low in molecular weight. Apparently there was considerable cellulase activity at these standard pretreatment conditions. The selectivity of (DC)ED bleaching, as measured by the ratio of kappa number reduction to viscosity reduction, decreased dramatically from 7.4 to 1.2 after enzyme pretreatment. The selectivity ratio decrease for ZED bleaching was less pronounced, presumably due to the masking effect of the extensive attack by ozone itself on pulp carbohydrates *(1–3)*.

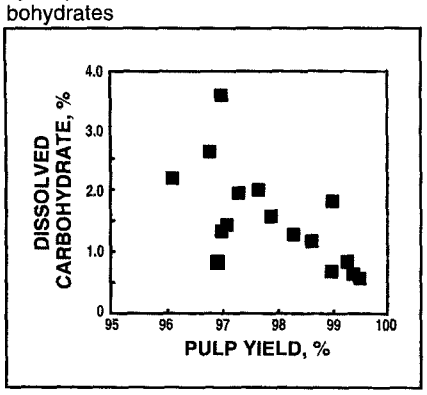
These results suggested that the standard pretreatment conditions were not optimal and that better conditions might be found which minimized cellulase activity while still promoting sufficient xylanase activity to result in improved delignification.

Optimization of pretreatment conditions

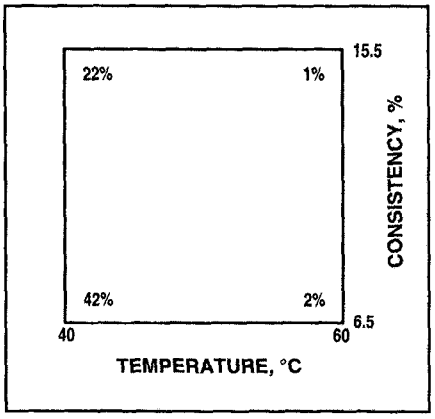
A fractional 2^{5-1} factorial design was constructed around the standard pretreatment conditions and the effects of variations in five process variables were determined: temperature, enzyme charge, pH, time, and consistency (Table I). With only two levels used for each variable factor, the factorial design was analyzed using linear response models. Center-point values for each factor were those used previously as standard pretreatment conditions. An estimate of pure error variance was obtained from center-point repeats, and this was used to confirm the statistical significance of the terms in each model. The predicted constants from these models were then compared to the actual responses at the center-point conditions to establish the degree of adequacy of the linear models. These models included all factors which showed statistically significant effects at greater than the 95% level.

The response models for enzymatic pretreatment factors are presented in **Table III**. Factor coefficients indicate the predicted change in the response when that

2. Relationship between pulp yield after enzyme pretreatment and total dissolved carbohydrates



3. Predicted effects of enzyme pretreatment conditions on enzyme survival



IV. Near-optimal enzyme pretreatment conditions for (DC)ED and ZED bleaching.

	(DC)ED	*ZED*
Temperature, °C	60	40
Enzyme charge, XU/g o.d. pulp	6.6	3.3
pH	7.0	5.0
Time, h	3.0	3.0
Consistency, %	11.0	6.5

factor is increased from its 0 to +1 level in Table I. The original pulp kappa number of 13.0 was decreased somewhat by pretreatment, though only temperature was shown to have a significant effect at the 95% level. There was a high level of unexplained variability in the data with a R^2 of only 18%.

Pulp yield was also affected by pretreatment conditions, with several factors showing significance at the 95% level. However, a general relationship existed between pretreatment pulp yield and dissolved carbohydrate in the filtrate since carbohydrates are the major pulp components removed by enzyme action (**Fig. 2**). Statistical analysis showed that the measurement of dissolved carbohydrate was more precise, and therefore, a better indicator of the yield effects of enzyme pretreatment.

Dissolved carbohydrate varied between 0.5 and 3.6% of the initial pulp dry weight, averaging 2.0% at the center-point conditions (Table II). Enzyme charge, pH, temperature, and time all affected the amount of carbohydrates dissolved from the pulp. Increasing enzyme charge and increasing time, both increased dissolved carbohydrate yield. The yield at pH 5 was also greater than that at pH 7, indicating that the xylanase (and also possibly the cellulase) was more active at pH 5. The rather high yields of dissolved carbohydrate under some conditions (at pH 5 in particular) suggested there was substantial attack on the cellulose component.

Significant factors affecting the survival of the enzyme were pretreatment temperature, consistency and their interaction (Table III). These effects are graphically illustrated by the two-way table in **Fig. 3**. Temperature had the greatest effect on enzyme survival with very little residual enzyme activity predicted at 60°C. This was likely due to excessive thermal inactivation of xylanase at this temperature. The signficiant decrease in enzyme survival at high consistency, especially at 40°C, was unexplained. Conditions which promote enzyme survival are desirable since there may be potential to recover and reuse residual enzymes and, therefore, reduce overall enzyme requirements.

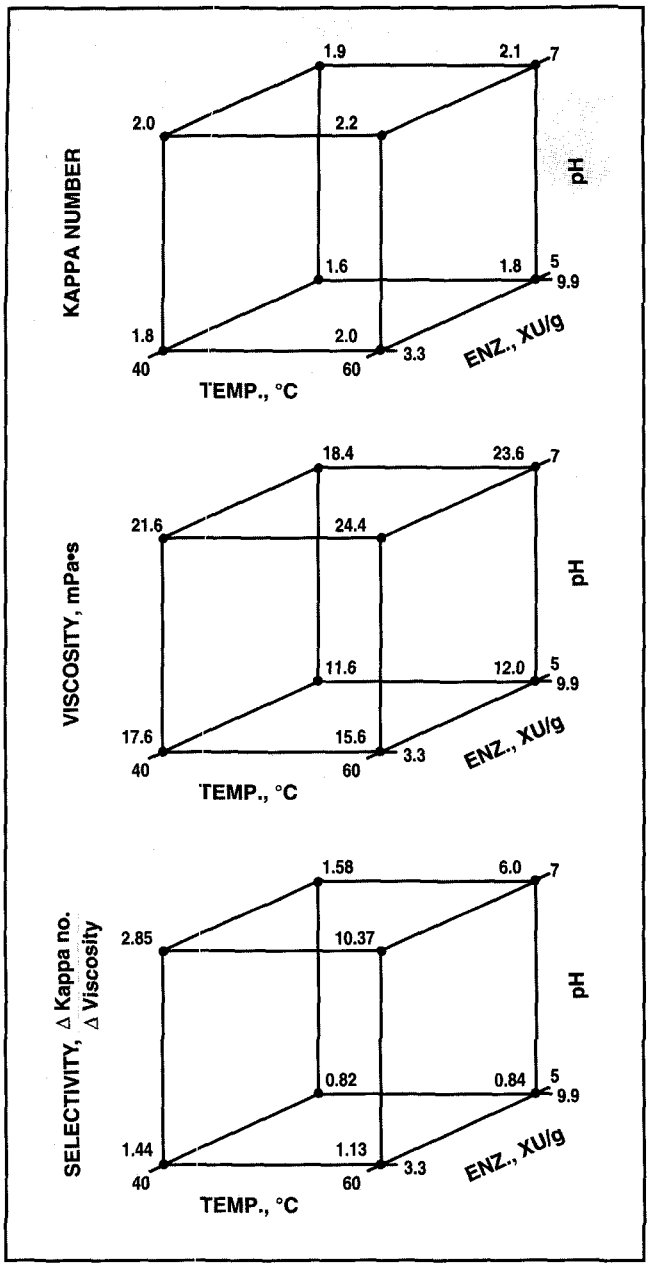
The effects of pretreatment conditions on subsequent chlorine-based bleaching are summarized in the predictive models in Table III. Extracted pulp kappa number and viscosity after (DC)E bleaching were significantly affected by enzyme charge, temperature, and particularly pH. There were also significant interactions between these variables. A graphical resolution of these complex effects is presented in **Fig. 4** as three-way tables for kappa number, viscosity, and the calculated selectivity ratio.

The response of kappa number at constant active chlorine charge is a measure of bleaching effectiveness. As shown in Fig. 4, the highest effectiveness, hence lowest kappa number, was obtained at pretreatment conditions of high enzyme (9.9 XU/g), low temperature (40°C), and low pH (5). Unfortunately, these conditions also resulted in the lowest pulp viscosity, indicating a positive correlation between the effects of enzyme pretreatment on overall removal of lignin and attack on cellulose after chlorine-based bleaching. The existence of substantial cellulase activity at these conditions was supported by the response of dissolved carbohydrate yield in the pretreatment filtrates (Table III).

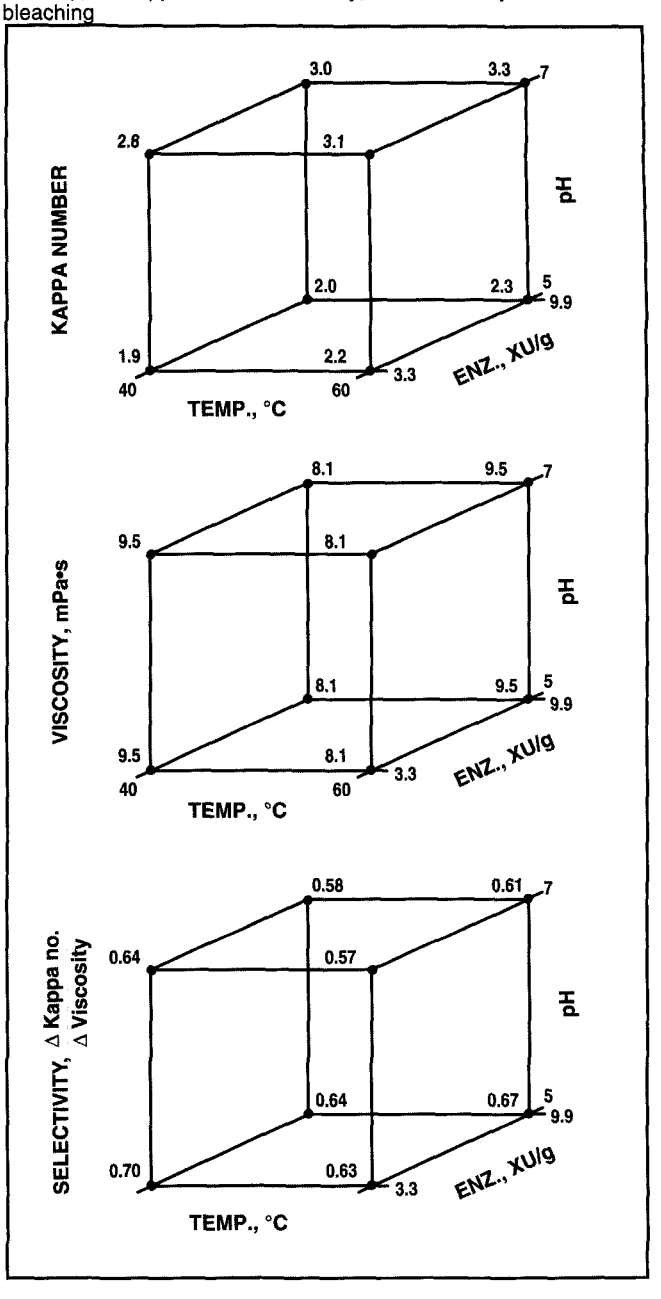
Calculated selectivity ratios based on predicted kappa number and viscosity responses showed that pretreatment at low enzyme (3.3 XU/g), high temperature (60°C), and high pH (7) resulted in the highest overall selectivity (Fig. 4). Extracted pulp kappa number at these conditions was predicted to be 2.2 at 24.4 mPa·s viscosity which was still a substantial improvement over control bleaching which resulted in 2.7 kappa number at 24.0 mPa·s viscosity (Table II). Interestingly, these conditions were opposite to those resulting in the greatest bleaching effectiveness, indicating that sufficient xylanase activity was maintained to promote delignification while at the same time cellulase activity was effectively suppressed.

The response of final brightness to changing process conditions was less precisely modeled (Table III). Only pretreatment pH was shown to have a significant main effect while

4. Predicted effects of enzyme pretreatment conditions on kappa number, viscosity, and selectivity ratio after (DC)E bleaching



5. Predicted effects of enzyme pretreatment conditions (6.5% consistency) on kappa number, viscosity, and selectivity ratio after ZE bleaching



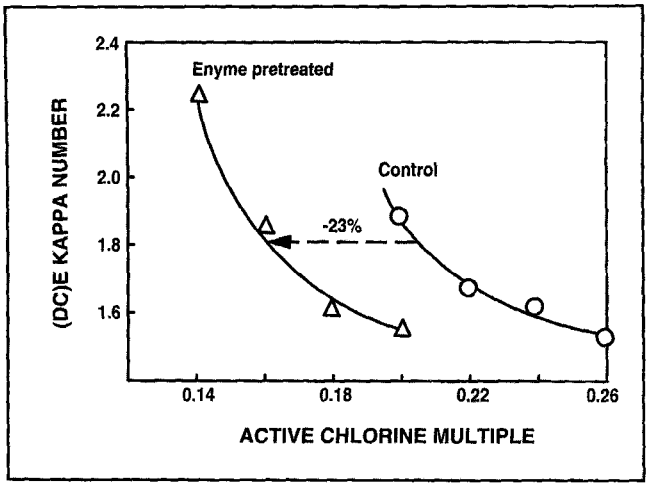
the model explained only 26% of the experimental variation. The predicted brightness at the optimum conditions for selectivity ratio resulted in 87.4% ISO brightness compared with 86.0% for control bleaching (Table II).

Table IV presents near-optimal enzyme pretreatment conditions chosen for conventional (DC)ED bleaching. They were based primarily on maintaining adequate selectivity. Pretreatment at 60°C and pH 7 were clearly required to preserve pulp viscosity. Since time and consistency are not significant, the shorter time of 3 h and 11% consistency were chosen. Enzyme charge was selected at 6.6 XU/g.

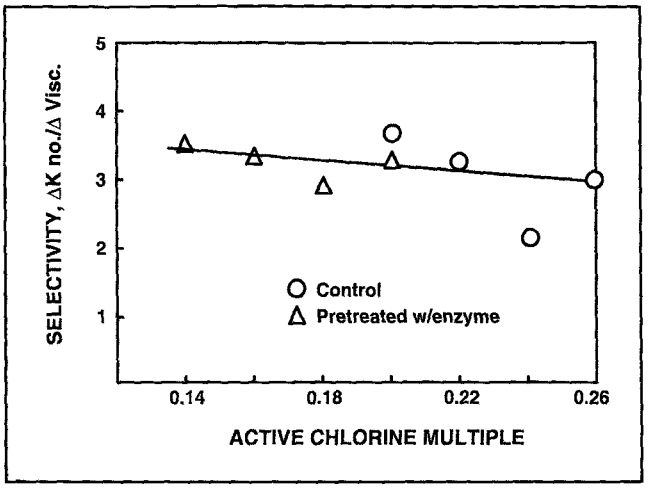
The effects of pretreatment conditions on subsequent ozone bleaching are summarized in the predictive models in Table III. There were much higher levels of random error variance apparent in the data (compared with the (DC)ED data), with the fitted models explaining only 20–70% of the overall experimental variation.

Extracted pulp kappa number gave the best fit to the data with pretreatment temperature and pH having significant main effects. Surprisingly, consistency was also a significant variable in two first-order interaction terms (Table III). The model pre-

6. Effect of optimized enzyme pretreatment on (DC)E bleaching effectiveness



7. Effect of optimized enzyme pretreatment on (DC)E bleaching selectivity ratio



dicted that lower kappa number is obtained at pH 5 when the pretreatment is performed at low consistency (6.5%); however, at high consistency (15.5%), pH 7 and pH 5 conditions result in similar kappa number. Overall the lowest predicted kappa number was at low consistency (6.5%), low temperature (40°C), low enzyme charge (3.3 XU/g) and low pH (5) (**Fig. 5**). Treatment time had no significant influence.

The model for final brightness after ZED bleaching showed that pretreatment pH had the only signficiant main effect (Table III). Enzyme pretreatment at pH 5 resulted in greater final brightness which was similar to the result for (DC)ED bleaching.

In contrast to chlorine-based bleaching, the effect of pretreatment conditions on extracted pulp viscosity was barely observable after ozone bleaching (Fig. 5). Only one first-order interaction term was significant in the viscosity model. Ozone treatment itself caused substantial cellulose degradation as evidenced by the low viscosity after control bleaching (Table II). The effect of cellulase activity during enzyme pretreatment was apparently masked by the much greater effect of ozone on pulp cellulose.

Optimum selectivity ratio was, therefore, not constrained by the adverse effect of cellulase activity on viscosity and was achieved at conditions resulting in superior delignification (Fig. 5). As shown in Table IV, the chosen optimum pretreatment conditions were quite different for ozone and chlorine-based bleaching due primarily to the need to preserve the inherently higher selectivity of chlorine-based bleaching through suppression of cellulase activity.

Pretreatment effects under optimized conditions

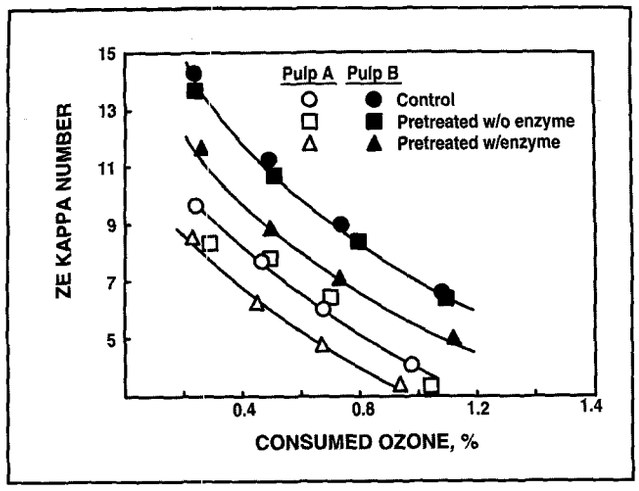
Kraft-oxygen pulp after enzyme pretreatment at near-optimal conditions (Table IV) was ZE and (DC)E bleached over a range of bleach chemical charges and compared with untreated control pulps. To better simulate industrial conditions, the use of citrate and phosphate buffers during pretreatment was abandoned and pulps were adjusted to target pH with sulfuric acid.

Active chlorine multiple for the control (DC)E pulp was varied from 0.20 to 0.26 while active chlorine multiple for pretreated pulp (based on the kappa number after pretreatment) was varied from 0.14 to 0.20. As expected, enzyme pretreatment improved the effectiveness of chlorine-based bleaching. Pretreated pulps were bleached to lower extracted kappa number at constant active chlorine multiple, or conversely, bleached with less active chlorine at constant extracted kappa number (**Fig. 6**). For example at 1.8 extracted kappa number, enzyme pretreatment reduced active chlorine multiple by 23%. This level of chemical savings for chlorine-based prebleaching of pine kraft pulps was similar to that reported in other enzyme studies using this, as well as other, commercial xylanase products *(3–6)*.

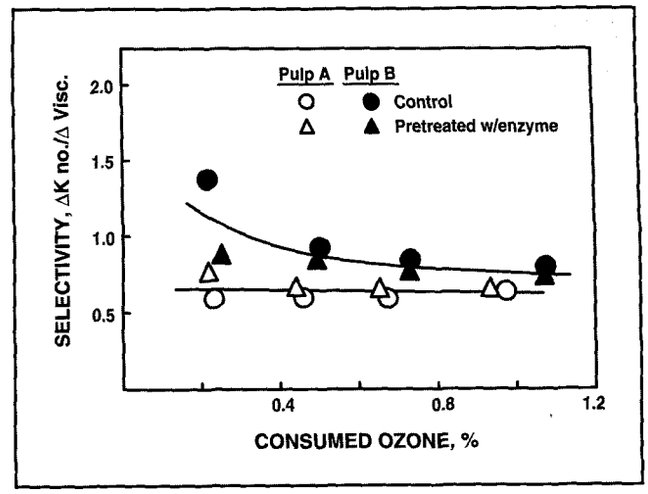
(DC)E bleaching both with and without enzyme pretreatment at optimized conditions had very little effect on selectivity ratio (**Fig. 7**). This confirmed that optimization of the enzyme pretreatment conditions had minimized the detrimental effects of cellulase activity on pulp viscosity after (DC)E bleaching. There was, however, a small drop in selectivity as active chlorine multiple increased from 0.14 to 0.26.

For ozone bleaching, the response of pulp pretreated both with and without enzyme present was compared to that of untreated control pulp. Again, optimized pretreatment conditions were used (Table IV). Two kraft-oxygen pulps were assessed; the original Pulp A at low kappa number (13.0) and Pulp B at high kappa number (19.8). Ozone applications were varied from about 0.2 to 1.0% consumed O_3.

8. Effect of optimized pretreatment with and without enzyme on ZE bleaching effectiveness



9. Effect of optimized enzyme pretreatment on ZE bleaching selectivity ratio



Pretreatment with no enzyme present functioned effectively as a further washing stage and reduced kappa number from 13.0 to 12.4 for Pulp A and 19.8 to 17.8 for Pulp B. Similar pretreatment with enzyme present (3.3 XU/g) resulted in kappa numbers of 12.7 and 17.1, respectively, demonstrating that the enzyme had limited direct interaction with residual lignin. However, the effect of the enzyme was apparent on subsequent ZE bleaching. Extracted kappa number was about 25% lower for both Pulp A and Pulp B after enzyme pretreatment (**Fig. 8**). Thus the extent of improved bleaching effectiveness after enzyme pretreatment was similar for both ozone and chlorine-based bleaching.

The main aim for pretreatments prior to oxygen-based bleaching is not so much to increase bleaching effectiveness but to improve bleaching selectivity. This will allow these treatments to be safely extended to greater extents of delignification without concomitant pulp strength degradation. As more bleaching is performed by oxygen-based treatments, less of the total bleaching will remain for chlorine-based treatments.

For ozone bleaching, enzyme pretreatment had little effect on overall selectivity (**Fig. 9**). While there were differences in the selectivity ratios for high kappa number pulp versus low kappa number pulp, the effect of enzyme pretreatment in both cases was negligible.

Conclusions

Pretreatment with Pulpzyme HA was shown to increase the effectiveness of both conventional chlorine-based bleaching and ozone bleaching to similar extents. However, the selection of pretreatment conditions was very important due to the presence of cellulase activity in the preparation. This was particularly critical for (DC)ED bleaching where substantial reductions in pulp viscosity occurred when the enzyme pretreatment was performed at low pH and temperature. At optimized pretreatment conditions (6.6 XU/g, 60°C, pH 7.0, 3 h, 11% consistency), active chlorine charge could be reduced 23% compared with bleaching untreated pulp, while maintaining pulp viscosity.

For ZED bleaching, the adverse effects of cellulase activity were less discernible during optimization studies since ozone itself caused a substantial reduction in pulp viscosity. Selection of optimal pretreatment conditions was, therefore, less constrained by the need to control cellulase activity. The optimal pretreatment conditions for ozone bleaching (3.3 XU/g, 40°C, pH 5.0, 3 h, 6.5% consistency) were markedly different from those for chlorine-based bleaching. Optimized enzyme pretreatment improved only ozone bleaching effectiveness (≈ 25%) but not selectivity over that for untreated control bleaching.

Literature cited

1. Allison, R., *Appita J.* 44(6): 405(1991).
2. Liebergott, N., Van Lierop, B., and Skothos, A., *Tappi J.* 75(1): 145(1992); *Tappi J.* 75(2): 117(1992).
3. Clark, T., Steward, D., Bruce, M., *et al.*, *Appita* 44(6): 389(1991).
4. Pedersen, L. and Elm, D., *Proceedings of the 1991 International Pulp Bleaching Conference,* SPCI, Stockholm, Vol. 2, p. 107.
5. Pedersen, L. "Pulpzyme HA for bleach boosting of oxygen delignified softwoods," Novo Nordisk a/s, Report No. EM-900634/LSPe (1990).
6. Tolan, J. and Canovas, V., *Proceedings of the 1991 Spring Conference of the CPPA* (Technical Section), CPPA, Montreal, p. 1.
7. Kantelinen, A., Sundquist, J., Linko, M., *et al., Proceedings of the 1991 International Symposium on Wood and Pulping Chemistry,* APPITA, Melbourne, Vol. 1, p. 493.
8. White, C. A. and Kennedy, J. F., "Oligosaccharides," in *Carbohydrate analysis: A practical approach* (M. F. Caplin and J. F. Kennedy, Eds.), Oxford: IRL, 1986, p. 37.

The skillful technical assistance of D. Steward, N. Chandler, and S. Wrathall is gratefully acknowledged.

Received for review June 29, 1992.

Accepted Feb. 20, 1994.

Presented at the 1992 Pan Pacific Conference.