Effect of Xylanase Pre- and Post-Treatment on oxygen Bleaching of Oak Kraft Pulp

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SUMMARY

The use of genetically cloned xylanase acquired from *Bacillus strearthermophillus* improves bleachability for oak kraft pulps. Combination of xylanase(X), oxygen(O), ozone(Z), peroxide(P), alkaline extraction(Eo, Eop), and chlorination(C/D, D) have been tested in a variety of bleaching sequences. The effectiveness of xylanase pre-treatment(XO) and post-treatment(OX) in oxygen bleaching is mainly compared.

With xylanase treatment the brightness increase by 1.5-2.1% ISO in OZEP, OZEoP, OZEopP and OPZP sequences. There is only numerically difference of brightness gains between OX and XO sequences. With xylanase treatment, chemical requirements for bleaching decrease by 42.6-48.6% in OC/DEoD sequence and 47.9-54.7% as active chlorine in OC/DEopD sequence at the same brightness. The reduction of bleaching chemicals is higher in XO sequence than those in OX sequence.

Following xylanase treatment, the viscosity increases from 11.7-12.0 mPa· s to 12.4-13.5 mPa· s, and the brightness stability is considerably improved, however the difference of effectiveness between XO and OX sequence is not present. Compared to tensile index vs tear index, the physical properties are similar for TCF bleaching sequences with and without xylanase treatments. However, in OC/DEoD and OC/DEopD sequences, the physical properties decrease with xylanase treatment. There is no difference in the physical properties between XO and OX sequences. COD, BOD and color of bleaching effluents increase slightly with xylanase treatment, however the discharge of COD end-load into environmental impact decrease.

KEYWORDS : Bleaching, Kraft pulps, Xylanase, Oxygen, Brightness, Viscosity, Brightness reversion, Pulp strengthes, COD, BOD

INTRODUCTION

Bleaching of kraft pulps has been traditionally achieved using a combination of chlorination and alkaline extraction. However the bleaching sequences produces large amounts of chlorinated organic compounds which are harmful to the environment. Considering the growing public concerns over the environmental impact, it seems urgent to reduce or to eliminate the use of elemental chlorine in pulp bleaching. As a part of new bleaching technologies, elemental chlorine free(ECF) bleaching and totally chlorine free(TCF) bleaching have been developed and applied in Mills(1). However the former demands excessively the use of expensive chlorine dioxide, while the latter is difficult to achieve up to the target brightness of over 85% ISO(2). The use of xylanase enzyme for kraft pulp bleaching has been recognized one new technology to the problems of ECF and TCF bleaching process(3).

The concept of using xylanase to improve bleachability of kraft pulps was introduced by Viikari et al.(4) in Stockholm in 1986, and soon thereafter several studies have been published(5-8). The early studies have reported that xylanase treatment improves the delignification and brightness of pulps, and reduce the amount of chlorine used in the prebleaching stage. Also the possible mechanisms for xylanase action in kraft pulp bleaching have been suggested(7, 8). Thereafter the extensive studies have been performed, which elucidate numberous contributing factors affecting xylanase treatment efficiency including the bleaching agent, treatment conditions, enzyme preparation, wood species and pulping process etc. The excellent reviews in this subject have been published by some workers(9-12).

While oxygen bleaching reduce substantially the environmental impact by the replacement of elemental chlorine with chlorine dioxide. Also in TCF bleaching , oxygen bleaching is needed mostly. The use of xylanase in oxygen bleaching of kraft pulps bleaching has also produced very encouraging results. Xylanase treatment after oxygen bleaching results in the increase in brightness, the improvement of the viscosity, and little change of pulp properties(4, 5, 13, 14). However most reports lay emphasis on xylanase treatment after oxygen bleaching. There are a little reports on xylanase treatment before oxygen bleaching. Recently it has been also indicated that xylanase treatment can be applied successfully before oxygen bleaching(15, 16). Vidal et al.(17) compared the sequence ODPD with XODPD in the bleaching. According to the results, xylanase treatment increase the brightness by 2% ISO. At the some brightness, it is possible to reduce consumption of chemicals while retaining strength properties.

In this paper, therefore we focused on the difference in effectiveness of xylanase between xylanase treatment before oxygen bleaching(XO) and xylanase treatment after oxygen bleaching(OX) of oak kraft pulp. Different bleaching sequences with chemicals such as oxygen, ozone, hydrogen peroxide, and chlorine dioxide were tested, and effects of the location of xylanase treatment on the brightness and chemical requirements, on pulp properties and bleach effluents were studied.

MATERIAL AND METHODS

Pulp sample

Oak(*Quercus mongolica*) kraft pulps were used for bleachings. At the initial Kappa numbers of 20.7, viscosity was 18.2 mPa s and the brightness value of the unbleached pulp were 27.8% ISO units.

Enzyme preparation and assay

The enzyme used in this study was the transgenic endo-xylanase acquired from *Bacillus stearothermophillus*. Genomic DNA of *Bacillus stearothermophillus*, was partially digested with *Hin*d III, cloned into pBR322, and subsequently transferred into the *Escherichia coli* HB101 cells. Among recombinated plasmids, pMG12 was isolated, which had the same 4 kb *Hind* III fragment originated from *B. stearothermophillus* which was responsible for endo-xylanse activity(18).

Endo-xylanase activity was routinely assayed by analysis of the reducing sugar from birchwood xylan(Sigma) using the modified dinitrosalicylic acid(DNS) method(19). Incubation conditions for the xylanase assay were 55℃ in 50 mM phosphate buffer,
pH 6.5 for 30 min. using 2.0% xylan. One unit of xylanase activity is the amount of enzyme that catalyses the release of 1μ mole reducing carbohydrate per minute.

Xylanase treatment of pulps

E. coli HB101/pMG12 was incubated on optimum conditions, and endo-xylanase was collected by centrifugation at 8000 rpm for 30 minutes. This xylanase had the xylanase activity of 0.48 Unit/mℓ and protein of 0.55 mg/mℓ. The xylanase was most effective at pH 7, but xylanase activity of 90% was kept up also to pH 11. The optimum temperature ranged from 50℃ to 60℃(20).

The pulps were hand mixed with xylanase in plastic bags, then immersed in a water bath. The xylanase reaction was carried out for 1 hour at 10% pulp consistency, at 50℃ and at pH 6.5. A xylanase charge of 2 Units on pulp was applied. After xylanase treatment, the pulps were washed at 1% consistency in water and were filterated using suction.

Pulp bleaching

Oxygen(O), oxygen extraction(Eo) and oxygen-peroxide extraction(Eop) stages were carried out with 3 L autoclave reactor with a rotary shaft . Ozone(Z) bleaching stages were performed in glass reactor equipped with a stirring mechanism. The residual ozone from the reaction vessel was analyzed by iodometric titration. Hydrogen peroxide(P), chlorination(C/D, D) and alkali extraction(E) stages were carried out in plastic bags immersed in a water bath. The bleaching conditions are as shown in Table 1.

Table 1. Bleaching conditions

Bleaching Stage	Pulp con. (%)	Temp. (℃)	Time (min.)	Chemical con. (%)
X	10	50	60	2 Unit
O	10	100	90	O_2 7kgf, NaOH 3, $MgSO_4$ 0.5
Z	40	30		0.8
E	10	70	60	NaOH 2.0
Eo	10	60	60	NaOH 2.0-3.0, O_2 1.5kgf
Eop	10	60	60	NaOH 2.0, O_2 1.5kgf, H_2O_2 0.5-0.6
P	10	70	70	H_2O_2 1.5-2.0, NaOH 0.2-0.5
C/D	3.5	40	30	TAC 0.73-.76, Cl_2:ClO_2=7:3
D	10	70	180	ClO_2 0.3-0.8

Analysis of chemical and physical properties of pulp

The chemical and physical properties of pulps were analyzed according to TAPPI Test Methods; Breaking length and Tensile index(T 494 om-88), Tear index(T 220 om-88) Brightness(T 452 om-83), Viscosity(T 254 om-85), Kappa number(T 236 om-85).

Brightness reversions were determined according to Yang et al.(21). Airdry sheets of bleached pulps were kept in an oven at 105℃ for 4 hr. The difference in the brightnesses before or after the aging was corrected according to TAPPI Test Method T 260 om-91.

Analysis of bleaching effluents

COD, BOD and Color of bleaching effluents were analyzed according to APHA-AWWA-WPCF Standard Methods; COD(5220), BOD(5210) and Color(2120).

RESULT AND DISCUSSION

Effect of xylanase treatment on the brightness, Kappa number and chemical requirements.

Oak kraft pulps were bleached in a variety of bleaching sequences using xylanase treatment(X) in combination with oxygen(O), ozone(Z), hydrogen peroxide(P), and chlorine/chlorine dioxide(C/D).

One and two stages bleaching: Effect of xylanase treatment on the brightness and Kappa numbers in the first and the second stage bleaching is presented in Table 2.

Table 2. Effect of xylanase treatment on 1st and 2nd stage bleaching

Bleaching sequence	Stage	Kappa (no)	Brightness (% ISO)
Unbleached pulp		20.7	27.8
XO	X	18.7	33.1
	O	9.0	61.7
OX	O	9.1	59.1
	X	9.1	60.8
O C/D	C/D	2.3	67.2
XO C/D	C/D	2.0	70.8
OX C/D	C/D	1.8	70.5
OZ	Z	3.1	79.5
XOZ	Z	3.1	81.5
OXZ	Z	3.1	81.3
OP	P	7.0	70.5
XOP	P	6.5	72.0
OXP	P	6.4	71.8

Multistage bleaching: The brightness of pulps after multistage bleaching to the target brightness, 87 – 90% ISO, is presented in Table 3.

Table 3. Brightness of pulps after multistage bleaching

Bleaching Sequences	Brightness (% ISO)	Bleaching Sequences	Brightness (% ISO)	Bleaching Sequences	Brightness (% ISO)
$OZEP_{2.0}$	87.5	$XOZEP_{2.0}$	89.1	$OXZEP_{2.0}$	88.8
$OZEoP_{2.0}$	88.3	$XOZEoP_{2.0}$	89.8	$OXZEoP_{2.0}$	89.7
$OZEop_{0.5}P_{2.0}$	88.5	$XOZEop_{0.5}P_{2.0}$	90.5	$OXZEop_{0.5}P_{2.0}$	90.3
$OP_{2.0}ZP_{1.5}$	88.0	$XOP_{2.0}ZP_{1.5}$	89.5	$OXP_{2.0}ZP_{1.5}$	89.3
$OC/D_{1.76}EoD_{0.8}$	90.3	$XOC/D_{0.73}EoD_{0.6}$	90.2	$OXC/D_{0.88}EoD_{0.6}$	90.0
$OC/D_{1.76}Eop_{0.6}D_{0.5}$	90.5	$XOC/D_{0.73}Eop_{0.6}D_{0.3}$	90.2	$OXC/D_{0.88}Eop_{0.6}D_{0.3}$	90.4

* Chemical charges of O, X, Z and E stage(See Table 1).
Charges of in Eo stage is 3.0% without xylanase treatment and 2.0% with xylanase treatment, respectively.

Chemical requirements: The effect of xylanase treatment on the reduction of active chlorine in OC/DEoD and OC/DEopD sequences was investigated. The results are summarized in figure 1.The ratio of chemical reduction was calculated by the basis on the chemical consumption in a bleaching sequence without xylanase treatment. All pulps were bleached to a brightness level of 90% ISO.

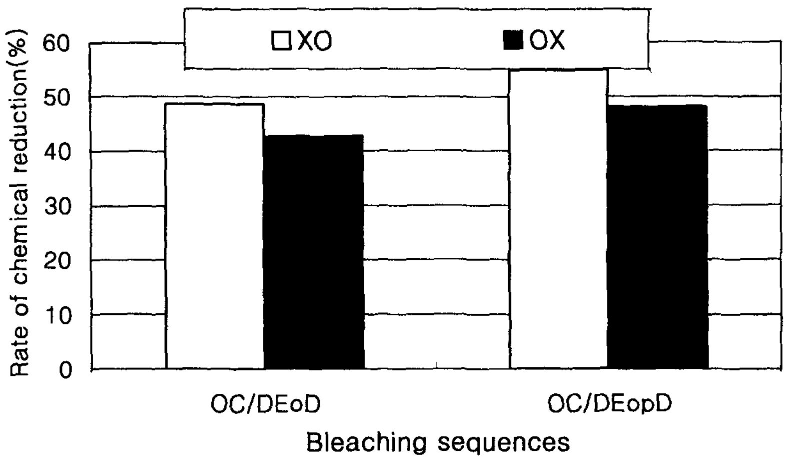

Fig.1. Rate of chemical reduction by xylanase treatment.

Effect of xylanase treatment on pulp properties

Viscosity: Figure 2 summarizes the effect of xylanase treatment on viscosity of bleached pulps. As a result of xylanase treatment, the viscosity of pulps increased.

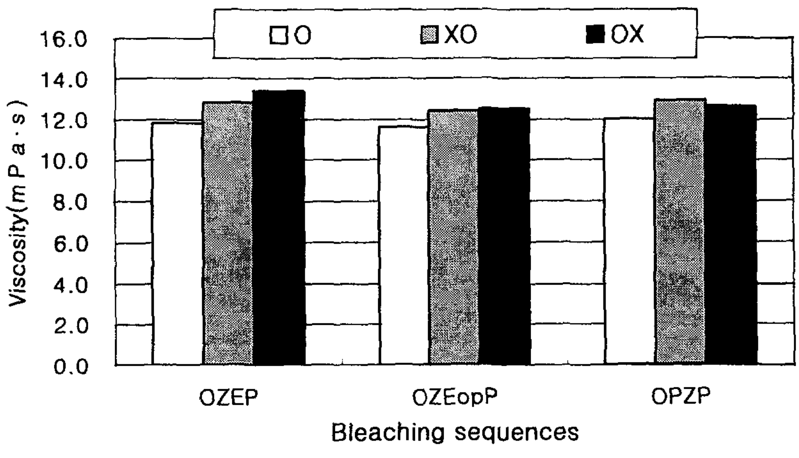

Fig. 2. Effect of xyalanase on viscosity of fully bleached pulps.

Brightness reversion: The effect of xylanase treatment on brightness reversion for aging is presented in Table 4.

Table 4. Comparison of P.C. no. in fully bleached pulp

Bleaching sequences	Brightness(% ISO)		P. C. no.
	Before aging	After aging	
CEDED	90.1	87.2	0.40
C/DEoDED	91.0	88.8	0.26
OC/DeopD	90.5	88.6	0.23
OZPD	90.4	87.2	0.43
OZEP	87.5	83.8	0.67
OPZP	88.0	83.5	0.81
OZEopP	88.5	86.1	0.38
OXZEP	88.8	87.4	0.20
XOZEP	89.1	87.9	0.17
OXPZP	90.3	88.4	0.24
XOPZP	89.5	88.3	0.16
OXZEoP	89.7	88.5	0.16
XOZEoP	89.8	88.7	0.14
OXZEopP	90.3	88.5	0.23
XOZEopP	90.5	88.7	0.22
OXC/DeoD	90.0	87.2	0.38
XOC/DeoD	90.2	87.8	0.32
OXC/DEopD	90.4	88.6	0.22
XOC/DEopD	90.2	88.6	0.22

Reversion time: 4hr. at 105℃

Physical properties: A Figure 3 shows breaking lengths of bleached pulps following beating with PFI Mill. With xylanase treatment, breaking lengths decreased slightly at same charge of bleaching chemicals. Breaking lengths of pulps with xylanase treatment ranged from 6.8 km to 7.4 km, while those without xylanase treatment ranged form 7.5 km to 7.6 km in TCF bleaching. However in OC/D sequence, breaking lengths decreased more than in TCF bleaching sequences by xylanase treatment. While breaking lengths in XO sequences were slightly higher compared to those in OX sequence. As shown at a figure 4, with xylanase treatment tear index slightly increased or halted at the same range compared to pulps without xylanase treatment.

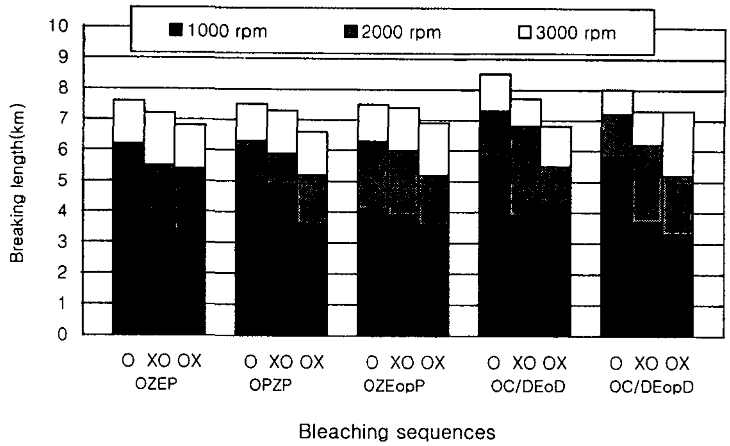

Fig. 3.Breaking length of fully bleached pulps with according to PFI revolutions

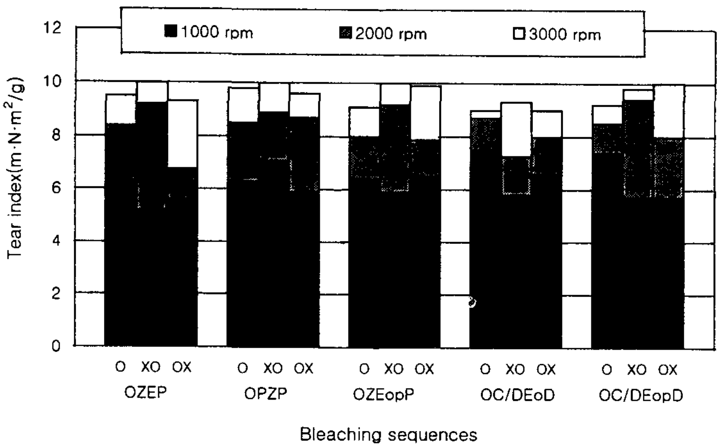

Fig. 4.Tear index of fully bleached pulps with according to PFI revolutions.

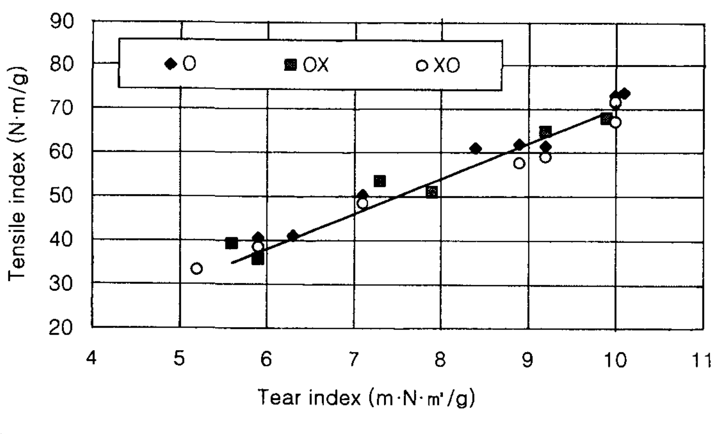

Fig. 5. Tensile vs tear index of fully bleached pulps in TCF bleaching sequences.

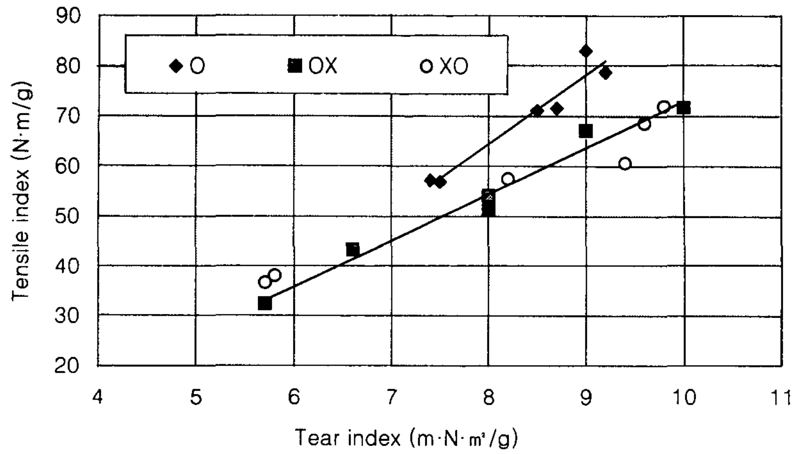

Fig. 6. Tensile vs tear index of fully bleached pulps in OC/DEoD and OC/DEopD bleaching sequences.

Effect of xylanase treatment on bleach effluents

COD, BOD and color of bleaching effluents are presented in Table 6.

Table 6. COD, BOD and Color of bleaching effluents

Bleaching sequences	COD (kg/ton)	BOD (kg/ton)	Color (Pt· Co unit)
CEDED	53.4(53.4)	17.3	1558
OZE_OP	20.5(4.3)	5.1	1360
$XOZE_OP$	23.4(3.6)	6.1	1487
$OXZE_OP$	23.4(3.6)	5.5	1499
OPZP	18.8	5.9	1249
XOPZP	23.7	6.1	1462
OXPZP	24.0	7.4	1492
OC/DEoD	18.8(10.4)	5.0	1337
XOC/DeoD	19.2(7.3)	5.2	1348
OXC/DeoD	18.8(6.3)	5.3	1460

( ) means environmental end-load.

COD of E and Eo stages is calculated as environmental impact.

The brightness of bleached pulps range form 88.3 to 90.5% ISO.

CONCLUSIONS

This work shows that xylanase treatment (transgenic endo-xylanase acquired from *Bacillus stearothermophillus*) improves the bleachability in oxygen bleaching of oak kraft pulps. With xylanase treatment the brightness of pulp increase. The brightness gains are similar for OX and XO sequences in TCF bleaching. While in OC/D EoD and OC/DEopD sequences, chemical requirement for bleaching reduce, and the rates of a reduction are higher in XO sequence than those in OX sequence. The results suggest that the order of xylanase treatment is important in ECF bleaching.

Using xylanase treatment, we also show that the viscosities and the brightness stabilility of fully bleached pulps are also improved, however no significant differences is observed in OX and XO bleaching sequences. The physical properties of the bleached pulps with xylanase treatment are comparable to those of the pulps without xylanase in TCF bleaching, but in bleaching sequences with the chlorination stages, the physical properties of the xylanase treated pulps are inferior to those of the non-xylanase treated pulps. While there is no difference in the physical properties between OX and XO bleaching sequences.

Results reveal that COD, BOD and color of bleaching effluents increase slightly with xylanase treatment, however the discharge of COD end-load into environmental impact reduces with xylanase treatment.

LITERATURE CITED

1. McDonough, T. J., Recent advance in bleached chemical pulp manufacturing technology. Part 1: Extended delignification, oxygen delignification, enzyme applications, and ECF and TCF bleaching. *Tappi J.*, 78(3): 55(1995).
2. Kukkonen, K. and Reilama, I., Experiences and conclusions with TCF bleaching at Metsa-Botnia. *Proc. Non-chlorine Bleach. Conf.*(Mar. 14-18, Hamilton Head, USA), Paper No.20(1993).
3. Forber, C. L., Twenty-first century bleaching will be a challenging lottery. *Pulp Pap.*, 66(11): 90(1992).
4. Viikari, L., Ranua, M., Kantelinene, A., Sundquist, J. and Linko, M., Bleaching with enzymes. *Proc.3rd Int: Conf. Biotechnol. Pulp Pap.Ind.*(Jun. 16-19, Stockholm, Sweden), p. 67(1986).
5. Kantelinen, A., Ratto, M., Sundquist, J., Ranua, M., Viikari, L. and Linko, M., Hemicelluloses and their potential role in bleaching. Tappi Proc. Int. Pulp. Bleach. Conf.(Jun. 5-9, Orlando, USA), p. 1(1988).
6. Paice, M., Bernierm M. and Jurasek, L., Viscosity enhancing bleaching of hardwood kraft pulp with xylanase from a cloned gene. *Biotechnol. Bioeng.*, 32, pp. 235-239(1998).
7. Kantelinen, A., Hortling, B., Sundquist, J., Linko, M. and Viikari, L.(1993) Proposed mechanism of

the enzymatic bleaching of kraft pulp with xylanase. *Holzforschung*. 47,. 318-324(1993).

8. Paice, M. G., Gurnagul, N., Page, D. H. and Jurasek, L., Mechanism of hemicellulose-directed prebleaching of kraft pulps. *Enzyme Microb. Technol.*, 14: 272-276(1992).
9. Daneault, C., Leduc, C. and Valade, J. L., The use of xylanase in kraft pulp bleaching: a review, *Tappi J.*, 77(6): 125(1994).
10. Viikari, L., Kantelinen, A., Sundquist, J. and Linko, M., Xylanase in bleaching: From an idea to the industry. FEMS Microbiology reviews, 13: 335-350(1994).
11. Wong, Ken. K. Y., de Jong, E., Saddler, J. N. and Allison, R. W., Mechanisms of xylanase aided bleaching of kraft pulp Part 1: Process parameter, *Appita J.*, 50(5): 415(1997).
12. Wong, Ken. K. Y., de Jong, E., Saddler, J. N. and Allison, R. W., Mechanisms of xylanase aided bleaching of kraft pulp. Part 2: Target aubstrates. *Appita J.*,50(6): 509(1997).
13. Allison, R. W., Clark, T. A. and Wrathall, S. H., Preteatments in radiata pine kraft pulp with a thermophilic enzyme, Part 1: Effect of oxygen on conventional bleaching. *Appita J.*, 46(4): 269(1993).
14. Allison, R. W., Clark, T. A., and Wrathall, S. H., Preteatments in radiata pine kraft pulp with a thermophilic enzyme, Part 2: Effect of oxygen, ozone and chlorine dioxide bleaching. *Appita J.*,46(5): 349(1993).
15. Hemilton, J., Senior, D.J., Rodriguez, A., Santiago, D., Szwec, J. and Ragauskas, A. J., Improvements in ECF bleaching. Use of activated oxygen species and xylanase. *Tappi J.*, 79(4): 231(1996).
16. Wong, Ken. K. Y., Nelson, S. L., Saddler, J. N., Xylanase treatment for the peroxide bleaching of oxygen delignified kraft pulps derived from three softwood species. *J. Biotechnol.*,48: 137(1996).
17. Vidal, T., Torres, A. L., Colom, J. F. and Siles. J., Xylanase bleaching of eucalyptus kraft pulp- an economical ECF process. *Appita J.*,50(2): 144(1997).
18. Cho, S. G., Park S. S., Park, Y.I. and Choi, Y. J., Molecular cloning and expression of an Endo-xylanase Gene from *Bacillus strearothermophillis* into Escherichia coli. Kor. *J. Appl. Microbiol. Biotechnol.*, Vol. 20, No. 3, p. 271(1992).
19. Miller, G.L. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31: 426(1959).
20. Kim, D. H., Paik, K. H., Variation of Oak Kraft pulp properties by xylanase treatment in C/D, P and Z stage, *Kor. J. Wood Scie and Tenol.* Vol. 25, No. 2, 100(1997).
21. Yang, J. L., Lou, Gu. and Eriksson, K. E. L., The impact of xylanase on bleaching of kraft pulps. *Tappi J.*, 75(11): 95(1992).