Effect of Xylanases from *Aspergillus niger* NKUC3-0.2 Mutant Strain on Prebleaching of Hardwood Mixed Pulps and its Impact on CE_PHH Bleaching Sequence

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ABSTRACT

Effects of *Aspergillus niger* NKUC3-0.2 pretreatment on total chlorine demand of pulp during conventional bleaching sequence and its impact on pulp brightness, viscosity and mechanical strength of mixed hardwood pulps were studied. Mixed hardwood pulps of *Populus deltoides* and *Eucalyptus tereticornis* in the ratio of 90:10 was cooked by kraft pulping process and then pre-bleached with *A. niger* NKUC3-0.2 to improve pulp brightness and pulp strength. *A. niger* NKUC3-0.2 reduces kappa number from 19.6 to 5.0. The enzyme treated pulp shows an improvement in brightness and viscosity by 1.8 and 1.6 per cent (^{0}PV) respectively at enzyme dose of 20 and 40 IU/g of pulp over untreated pulp and after enzyme pretreatment, the total chlorine demand reduces by 1.3 and 1.7 per cent respectively. The average pulp brightness and viscosity of enzyme treated pulp at two different enzyme doses i.e. 20 and 40 IU/g are 86.8 and 87.0 per cent (^{0}PV) and 8.07 and 8.06 cps respectively.

Keywords: *Aspergillus niger* NKUC3-0.2, *Populous deltoids*, *Eucalyptus tereticornis*, Kraft pulping, Bleaching, Brightness and Viscosity

INTRODUCTION

The marketing persons are demanding paper of high brightness. Efforts were made to improve brightness by increasing total chlorine demand during pulp bleaching but it adversely affects the pulp strength, brightness stability and pollution load. It was found that 'C' stage was generally the first point where 2,3,7,8-TCDD, 2,3,7,8-TCDF and 1,2,7,8-TCDF congeners were always present[1-3]. The E-stage filtrate was found to have the highest concentrations of dioxins[4]. They were found to change blood chemistry and cause liver damage, skin disorders, lungs lesions and tumor types at a number of sites within the body including the liver and thyroid[5-7]. Despite the commercial feasibility of the process, oxygen delignification remained non-commercialized in the most of the mills. The main reason was that under conditions necessary to achieve appreciable delignification cellulose was attacked and degraded, leading to sharp a decrease in pulp viscosity and strength. This obstacle was finally removed when Robert and *et al*[2] discovered that the addition of small amounts of magnesium salts sharply decreased the damage suffered by the polysaccharide components of the pulp during oxygen bleaching[8-9].The remaining obstacle of high mass transfer rates made necessary by the low solubility of oxygen in water for commercialization was soon overcome[10]. Even at very high oxygen pressure useful mass transfer of molecular oxygen at the reaction site in to the fiber walls is difficult to obtain in one stage pulping. Delignification after pulping takes place in the chlorination stage of bleaching process. A major portion of lignin can be removed by oxygen delignification before the start of actual bleaching operation. The dissolved lignin during oxygen delignification is taken to recovery section. In oxygen delignification stage, about 50 per cent lignin left after cooking stage can be removed[11-12]. Since the lignin remaining in the pulp after oxygen delignification is low and chlorination becomes less intensive. The level of AOX, TOCl, chlorinated dioxines, chlorophenols, chlorinated organo compounds and chloroform in pulp bleaching effluents can considerably be decreased by oxygen delignification stage than that of conventional bleaching sequence.

Another way to mitigate kappa number of pulp prior to bleaching is enzyme treatment. Ligno-carbohydrates are mainly decomposed by rot fungi belonging to Basidiomycetes. One type- the brown rot fungi- utilizes the carbohydrates and leaves the lignin only slightly affected[13]. White rot fungi, however, metabolize the lignin as well carbohydrates to lower molecular weight products, some of which are then further metabolized by facultative and obligate anaerobic soil bacteria and actinomycetes[14-17]. White rot fungi are usually fast degraders, which completely metabolize the complex polymer, exhibit the highest reported rates[18-19]. All of them have the enzymatic capacity to use the holocellulose components as a source of carbon and energy; hence, total biomass breakdown usually occurs and lignin removal is accompanied by removal of polysaccharides[17]. Under certain culture conditions white rot-fungi use lignin preferentially, producing soluble polymers, breaking up the cellulose-hemicellulose matrix, and making the solid more susceptible to further enzymatic action by other microorganisms e.g. rumen bacteria[10].

The present study aims at prebleaching of mixed hardwood kraft pulp of *P. deltoides and E. tereticornis* in the ratio of 90:10 with different doses of *Aspergillus niger* NKUC3-0.2 in order to reduce kappa number and the total chlorine demand of the pulp.

Experimental Methodology

Raw Material Collection and Chips Classification-The logs of *P. deltoides and E. tereticornis* were chipped in Veco-plan chipper and screened. The screened chips were mixed in the ratio of 90:10 as per mill furnish. The chips

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Table1— Chips classification of *P. deltoides* and *E. tereticornis* mixed in the ratio of 90:10

Sl no	Fractions	units	Values
1	+30mm	Per cent	3.50
2	-30+19mm	Per cent	41.40
3	-19+10mm	Per cent	42.80
4	-10+3mm	Per cent	12.10
5	-3mm	Per cent	0.20

Table 2— Cooking conditions and results of mixed pulping of *P. deltoides* and *E. tereticornis* blended in the ratio of 90:10

Sl no	Parameters	Results
1	Unbleached pulp yield, per cent	49.75
2	Screened pulp yield, per cent	47.90
3	Screen Rejects, per cent	1.85
4	Residual alkali, g/L	0.23
5	Kappa Number	19.6
6	Unbleached pulp brightness. ^{0}PV	29.4
7	Viscosity, cps	11.5

Cooking conditions: Active alkali = 16 per cent (as Na_2O), sulphidity = 20 per cent, liquor to wood ratio = 2.8:1, digester pressure = 6.5 kg/cm^2, time taken to raise temp from ambient to 162 ^{0}C = 120 min and cooking at 162 ^{0}C = 75 min.

Table 3—Effect of temperature on the xylanase production under solid state fermentation using cotton hull as solid support

Sl. no.	Temperature, (^{0}C)	Xylanase activity, (IU g^{-1})
1	30	1618
2	32	1657
3	34	1679
4	36	1664
5	38	1634
6	40	1612

Condition: wheat bran =2%, Nutriment Salt Solution=40ml, pH=7.0 and incubation period (in days) =03

Table 4— Effect of pH on the xylanase production under solid state fermentation using cotton hull as solid support

Sl. no.	pH	Xylanase activity, (IU g^{-1})
1	5	1494
2	6	1570
3	7	1692
4	8	1603
5	9	1573

Condition: wheat bran =2%, Nutriment Salt Solution=40ml, Tamp=34° and incubation period (in days) =03

Table 5— Effect of incubation time on the xylanase production under solid state fermentation using cotton hull as solid support

Sl no	Time, (days)	Xylanase activity, (IU g^{-1})
1	1	1643
2	2	1658
3	3	1685
4	4	1673
5	5	1638
6	6	1598
7	7	1528

Condition: wheat bran =2%, Nutriment Salt Solution=40ml, pH=7.0 and Tamp=34°C

Table 6" Effect of enzyme dosage on kappa number of mixed hard wood kraft pulp.

Sl. no	Enzyme dosage IU g^{-1} of pulp	Kappa number
1	-	19.6
2	10	18.6
3	20	14.2
4	30	15.8
5	40	15.0
6	50	14.7

Conditions: Temperature 65 ^{0}C, pH 7.0, reaction time 2 hours and consistency 10 per cent.

were classified in Bauer McNett chips classifier and results are reported in Table1.

Pulping Studies-The chips of *P. deltoides and E. tereticornis* mixed in the ratio of 90:10 were cooked in electrically heated WEVERK rotary digester of capacity 0.02 m^3. The chips were digested at active alkali dose of 16 per cent as Na_2O, sulphidity 20.4 per cent, and liquor to wood ratio of 2.8:1. The total time taken to raise the temperature from ambient to 162^{o}C was 120 min and temperature keeping time at 162 ^{o}C was 75 min. At the end of cooking, the pulp was washed and screened on laboratory vibratory screen having slot size 150 mesh. The unbleached pulp produced was evaluated as per TAPPI standard test methods for kappa number (T 230 on-99), viscosity (T214 S-71) and brightness (T 452 on -98). The results are reported in Table 2.

Isolation of Fungus and Effect of Incubation Period, pH and Temperature on Xylanase Activities and Assays of Enzymes-The fungus *Aspergillus niger* isolated from decaying wood collected from the Department of Biotechnology, I.I.T. Roorkee and was confirmed as hyper-xylanase producer when cultured on xylan rich culture medium. The fungus was sub-cultured on medium having 2% cotton hull and 2% agar (w/v) without any nutrient salt at 40 ^{o}C for 5-8 days and maintained at 4 ^{o}C until used. Streptopenicilline (185 g / ml) was aseptically added to check any bacterial growth. The xylanase was produced under solid state fermentation in a medium having 0.8 g cotton hull and 40 ml of solution having 1.5 g/l KH_2PO_4, 4 g/l NH_4Cl, 0.5 g/l $MgSO_4$, 0.5 g/l KCl, and 1 g/l yeast extract in distilled water with 0.04 ml/l trace elements solution having 200 mg/l $FeSO_4$. $7H_2O$, 180 mgl $ZnSO_4$. $7H_2O$, and 20 mg/l $MnSO_4$ $7H_2O$. Desired pH of the solution was adjusted with NaOH /H_2SO_4. Two discs of 8 mm diameter from 5-d old culture of *Aspergillus niger* were aseptically inoculated in each of the flasks. These flasks were incubated at 100 rpm at optimum temperature and harvested as and when required. After desired growth under test conditions, the contents were centrifuged at 15000 x g at 4 ^{0}C (Sigma centrifuge - model 2K15). The pellets were boiled in 5 ml of 1.0 M NaOH for 10 minutes. The total volume was made up to 5 ml with 1.0 M NaOH. The activity was expressed as equivalent of reducing sugars produced, assayed by Miller's DNS method[13]. The enzyme activity is expressed as µ moles D-xylose equivalents released g^{-1} at 34 ^{0}C (IU). The results of xylanase activities at different incubation periods, pH and temperatures are reported in Tables 3-5.

Enzyme Pretreatment-In order to see the effect of xylanase, the wheat straw and mixed hardwood pulps were treated with different enzymes dosages at different pH and reaction time. After enzyme treatment the pulp was washed. The results of enzyme treatment and conditions are reported in Tables 6-8.

Bleaching Studies-The unbleached

Table 7— Effect of enzyme dosage time on kappa number of mixed hard wood kraft pulp.

Sl. no	Enzyme dosage time, min	Kappa number
1	30	19.2
2	60	18.3
3	90	17.5
4	120	16.4
5	150	15.9
6	180	15.5

Conditions: Enzyme dosage 40 IU/g, pH 7.0, temperature 65^{0}C and consistency 10 per cent.

Table 8— Effect of pH on kappa number of mixed hard wood kraft pulp.

Sl. no	pH	Kappa number
1	5.0	17.8
2	6.0	17.3
3	7.0	16.2
4	8.0	15.3
5	9.0	15.0
6	10.0	14.7

Enzyme dosage 40 IU/g, Temperature 65^{0}C, reaction time 2 hours and consistency 10 per cent.

pulp was treated with *Aspergillus niger* NKUC3-0.2 in order to reduce kappa number of the pulp. Both the pulps i.e. with *Aspergillus niger* NKUC3-0.2 treated or untreated were bleached by CE_pHH bleaching sequence and results are reported in Table 9. The pulp brightness and viscosity of XCE_pHH and CE_pHH bleaching sequences are compared and reported in Table 10.

RESULTS AND DISCUSSION

Table 1 reveals the chips classification of *P. deltoides* and *E. tereticornis* mixed in the ratio of 90:10. The middle fraction of chips i.e. -30+19mm and -19+10mm is about 84.20 per cent and the lower chips size i.e. -3 mm is only 0.20 per cent. The wood packing density of mixed chips was found to be 195 kg/cm^3.

Table 2 shows the results of kraft pulping of mixed chips of *P. deltoides* and *E. tereticornis.* The mixed cooking of *P. deltoides* and *E. tereticornis* produces a screened yield of 47.9 per cent at kappa number of 19.6 at cooking conditions mentioned in table 2. On the other hand, the brightness and viscosity of unbleached pulp are found 29.4 ^{o}PV and 11.5 cps respectively.

Table 3 reveals the effect of temperature on xylanase production by *A. niger* NKUC3-0.2. At 30 ^{0}C, *A. niger* NKUC3-0.2 yields the lowest enzyme activity, which increases with increasing temperature up to 34 ^{0}C and thereafter, it decreases. It may possible due to lower transport of substrate across the cells at lower temperature causing lower yield of the product. At higher temperature, the maintenance energy requirement of cellular growth is high due to thermal denaturation of enzymes of the metabolic pathway resulting in lower production of the metabolites[14]. The optimum activity of *A. niger* NKUC3-0.2 was 1679 IU g^{-1} at 34^{0}C. It suggests that the fungus is slightly thermophilic and for the optimum production of enzyme incubation temperature of 34^{0}C should be used.

Table 4 reveals the effect of pH on xylanase production by *A. niger* NKUC3-0.2 using liquid medium at various pH levels ranging from 5.0 to 9.0 at a difference of 1.0. It is observed that initial pH of the medium shows remarkable effects on the enzyme production. It is evident from Table 2 that increase in pH from 5.0 towards neutrality enhances the production of enzyme and the optimum enzyme activity of 1692 IU g^{-1} is observed at pH 7.0.

Table 5 reveals the effect of incubation period on xylanase production by *A. niger* NKUC3-0.2. On cotton hull the production of xylanase by solid state fermentation by *A. niger* NKUC3-0.2 increases with increasing incubation period and the highest activity i.e. 1685 IU g^{-1} is observed after 3 days of incubation. Xylanase production declines after 3 days of incubation. It suggests that the enzyme production is dependent on biomass but only during exponential phase of growth of fungi. On onset of death phase, the enzyme activity decreases[21]. It may be concluded that the best results were obtained on 3rd day of incubation under solid-state fermentation condition using cotton hull as the solid matrix. Temperature optima and pH was found to be 34^{o}C and 7.0 respectively.

Table 6 reveals the effect of enzyme dosage on kappa number of mixed wood pulps. An enzyme dosage of 40 IU g^{-1} decreases the kappa number of mixed kraft pulp from 19.6 to 15.0 at the same enzyme dosage at temperature 65 ^{0}C, pH 7.0, reaction time 2 hours and consistency 10%.

Table 7 shows the effect of enzyme dosage time on kappa number of mixed hard wood pulps. On increasing retention time of enzyme prebleaching, the kappa number of mixed hardwood pulps reduces to 15.9 after 150 minutes. It means *A. niger* NKUC3-0.2 reduces kappa number by 18.9 % for mixed hard wood pulps respectively at enzyme dosage of 40 IU/g, pH 7.0, temperature 65^{0}C and consistency 10%.

Table 8 shows the effect of pH on kappa number of mixed hard wood pulps. The kappa number reduces sharply up to pH 7 and beyond that the magnitude of decrease in kappa number is insignificant. If we go for more alkaline pH, the requirement of alkali will be increasing for maintaining the pH of prebleaching stage and the cost of prebleaching stage will be increasing accordingly.

Table 9 shows the results of CE_pHH bleaching of mixed pulp of *P. deltoides* and *E. tereticornis* in the ratio of 90:10 with or without pre-bleaching with A niger NKUC3-0.2 The kappa number reduces by 5.4 and 6.9 units at an enzyme dose of 20 and 40 IU/g of pulp respectively. On the other hand, the unbleached pulp brightness improves by 2.6 and 4.1 per cent respectively at the given two different enzyme doses. The decrease in kappa number after enzyme treatment can be explained based on the mechanism of enzymes. Enzymes cause hydrolysis of hemicelluloses. The lignin fragments, which are linked with hemicelluloses, are separated with hemicelluloses. After enzyme pretreatment, the total chlorine demand reduces 1.3 and 1.7 per cent respectively whereas, both pulp brightness and viscosity improves marginally. Both the pulps i.e. treated and untreated when bleached by CE_pHH bleaching sequence, the brightness of untreated pulp after hypochlorite 2nd stage is observed 85.2 per cent and viscosity is 7.93 cps. Whereas, the average pulp brightness

Table 9— CE_pHH bleaching of mixed pulp of *P. deltoides* and *E. tereticornis* in the ratio of 90:10 with or without pre-bleaching with A. Niger NKUC3-0.2

Sl no	Particulars	Blank (with out enzyme)	Enzyme dosage, IU/g Set I	Enzyme dosage, IU/g Set II	Enzyme dosage, IU/g Set I	Enzyme dosage, IU/g Set II
1	Prebleaching with enzyme					
	Enzyme dosage, IU/g	Nil	20	20	40	40
	Enzyme dilution, times	Nil	200	200	200	200
	Kappa number	19.6	14.2	14.1	15.0	15.0
	Brightness, per cent (^{o}PV)	29.4	31.9	32.0	33.5	33.4
2	Chlorination (C) stage					
	Molecular Cl_2 added as available Cl_2 on o d pulp basis	2.3	1.8	1.8	1.6	1.6
	Residual Cl_2, ppm	10.7	7.1	8.9	7.8	7.5
	Brightness, ^{o}PV	39.2	39.6	40.4	41.0	41.7
3	Peroxide reinforced extraction (Ep) stage					
	NaOH added on o d pulp per cent basis	1.16	1.06	1.06	0.86	0.86
	H_2O_2 added on o d pulp per cent basis	1.25	1.25	1.25	1.25	1.25
	Brightness, per cent (^{o}PV)	63.1	64.1	65.0	66.2	66.5
4	Hypochlorite (H_1) stage					
	$Ca(OCl)_2$ added on o d pulp per cent basis	1.61	1.26	1.26	1.12	1.12
	Residual Cl_2, g/l	0.135	0.121	0.156	0.604	0.730
	Brightness, per cent (^{o}PV)	85.0	85.5	85.4	85.8	86.0
5	Hypochlorite (H_2) stage					
	$Ca(OCl)_2$ added on o d pulp per cent basis	0.69	0.54	0.54	0.48	0.48
	Residual Cl_2, g/l	0.227	0.228	0.140	0.213	0.316
	Brightness, per cent (^{o}PV)	85.2	86.9	86.7	86.8	87.4
	Viscosity, cps	7.93	8.10	8.04	8.14	7.98

Bleaching conditions	X	C	E_p	H_1	H_2
Retention time, min	120	30	90	120	150
Temperature, ^{0}C	65	ambient	73±2	40	40
Consistency, per cent	10	3	10	10	10
pH	7.0	2.6	10.5	11.2	11.2

Table 10— Effect of *P. chrysosporium* on pulp brightness and viscosity

Sl no	Particulars	Enzyme dosage, IU/g 0	20	40
1	Brightness, per cent (^{0}PV)	85.2	86.8	87.0
2	Pulp viscosity, cps	7.93	8.07	8.06

and viscosity of enzyme treated pulp at two different enzyme doses i.e. 20 and 40 IU/g are 86.8 and 87.0 per cent (^{0}PV) and 8.07 and 8.06 cps respectively.

Table 10 shows the comparison of XCE_pHH and CE_pHH bleaching results of mixed pulp of *P. deltoides* and *E. tereticornis*. The XCE_pHH bleached pulp at two different doses of enzymes shows improvement in brightness by 1.6 and 1.8 per cent (^{0}PV) over CE_pHH bleached pulp. The viscosity of pulps at two different enzyme doses improves marginally to 0.14 cps only.

CONCLUSIONS

Aspergillus niger NKUC3-0.2 reduces kappa number from 19.6 to 12.7. The enzyme treated pulp shows an improvement in brightness and viscosity by 1.8 and 1.6 per cent (^{0}PV) respectively at enzyme dose of 20 and 40 IU/g of pulp over untreated pulp and after enzyme pretreatment, the total chlorine demand reduces 1.3 and 1.7 per cent respectively. The average pulp brightness and viscosity of enzyme treated pulp at two different enzyme doses i.e. 20 and 40 IU/g are 86.8 and 87.0 per cent (^{0}PV) and 8.07 and 8.06 cps respectively.

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