A Case Study on Enzymatic Pretreatment of Agro-Based Pulps for Improved Bleaching

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Abstract

Bio-bleaching has been recognized as a potential pretreatment of pulp to reduce the environmental impact of bleaching operations due to reduced bleaching chemical consumption and to improve the properties of pulp and paper. A number of micorbial strains were isolated from different habitals such as soil, forest litter etc and screened after enrichment with xylan for the production of xylanse without any significant cellulase activity. The various parameters for the production of xylanase from selected microbial stains were optimized. The rude xylanase produced from these microbial were evaluated for enzyme activity over a wide range of pH, temperature and substrate concentration and under optimized reaction conditions, the agro-pulp was subjected to xylanase pretreatment to evaluate the effect fo enzyme on subsequent chlorine consumption and pulp quality. The performance of te crude enzymes from the isolated strains was also compared with the commercial enzyme preparation available in the market. It was observed that enzymes from isolated strains showed comparatively better adaptability towards extreme conditions of temperature and pH in the process. The further purification and modifications of crude xylanase are required to improve the performance of these enzymes. The results of the stuies carried out on the enzymatic prebleaching with commercial xylanase on agro -pulps from both alkaline pulping and alkaline-sulphite pulping have also been explained. The alkaline-sulphite agro-pulp was found to be more susceptible to xylanase prebleaching and chlorine consumption was reduced by 16-17%. It has been observed that reduced chlorine consumption alone could make enzymatic pretreatment ecomonically viable with improvement in brightness, stength properties and the reduced AOX content in the bleach plant effluent as additinal benefits.

INTRODUCTION

Toxic effluents produced during chlorine bleaching have strong impact on air, water micoorganisms and aquatic life and ultimately human health. Halides, the most reactive elements in the halogen family bind easily with organic substances. allowing quick entry into the halogen family bind easily with organic substances, allowing quick entry into the environment and food chain (1,2).

Keeping in view the above facts and other environmental concerns, paper mills have now started finding possible alternative bleaching agents to make the process total chlorine free (TCF). Technologies using chlorine dioxide, ozone and oxygen for pulp bleaching are available which can substantially reduce the release of chlorinated organic substances (AOX). However, these technologies though eco-friendly, are still not economically viable for small mills and hence find limited applications in industry and scientists are switching over to suitable bio-bleaching aids.

One of the alternative approches is the direct delignification of unbleached pulp using enzymes called ligninases or laccases, which act directly on the residual lignin in the pulp. Xylanolytic and lignolytic enzymes with specific advantages are also being preferred as another alternative for enhancing lignin removal. The microorganisms release extracellular enzymes called xylanases, which convert the xylan polymer to monomeric form (xylose) thereby, weakeing the bond between

cellulose and lignin. The initial hydrolysis of hemicellulose fractions of raw materials by enzymes before chemical bleaching results in the reduction of bleach chemicals in the bleaching process.

Enzymes used in paper industry should be devoid of cellulases and must be effective at high temperature and at alkaline pH in order to make maximum use of avaliable cellulose fibres and also to get better quality paper. Therefore, the use of available cellulose fibres and also to get better quality paper. Therefore, the use of cellulase free hemicellulose degrading enzymes (microbial enzymes) in pulp and paper industry provides a better alternative to chemical bleaching due to positive environment effects in terms of reduction in bleaching chemicals and economic feasibility as well. Under the prevalent conditions, enzymatic pre-bleaching of pulps employing xylanase enzymes could prove to be promising environment friendly technological option.

EXPERIMENTAL

Methods given by Reese and Mandels (3) for enzyme reaction and Miller (4) for reducing sugar estimation (5) were used.

Methods given by Reese and Mandels(3) for enzymes reaction and Miller (4) for reducing sugar estimation (6) were used.

Based on the results of primary screening, two bacterial strains XPB-21 and XPB-28 were selected for further studies based on their ability to produce maximum xylanase and minimum celluase activities. In order to determine the optimal conditions for xylanase production, the selected bacterial isolates were grown in the liquid medium under varied conditions of pH, temperature, time concentration of substrate (xylan) and enzyme activity was measured in each case.

Both the bacterial isolates (XPB-21 and XPB-28) were separately grown in 50 ml of the liquid medium containing xylan as sole source of carbon at different pH ranging from 7.0 to 11.0 at 30°C in 250 ml. flask in an incubator shaker under continuous agitation (160 rpm.) Culture contents after 48 hrs were centrifuged at 10,000 rpm for 15 minutes at 4°C and the supernatant was stored at lower temperature in the refrigerator until further use.

To see the effect of temperature on the production of xylanase, Isolates XPB-21 and XPB-28 were separately grown at different temperatures (30°C, 37°C, 45°C and 55°C) at optimized pH and the xylanase activity was determined.

Effect of different substrate concentrations on the extracellular production of xylanase by XPD-21 and XPB-28 was studied by growing these organisms in John and Schmidt's liquid medium (pH 7.5, 160 rpm at 45°C) containing different concentrations of xylan (0.25%, 0.50%, 0.75%,1.0%, 1.25%, 1.50%, 1.75% and 2/0%) and the enzyme activity was assayed in the culture as described earlier. In order to determine the most suitable incubation period for optimum production of extracellular xylanase by the selected bacterial strains, there were separately grown in the liquid medium (pH 7.5, temperature 45°C and substrate concentration 1% for XPB-21 and 0.25% of XPB-28) and activity of enzyme was assayed at an interval of 3 hrs. until 36 hrs as described earlier.Optimization of reaction conditions for the extracellular production of xylanase by XPB-21 and XPB-28

Both the isolates were grown in John and Schmidt's liquid medium under optimized conditions (pH 7.5, temperature 45°C, time 27-30 hrs. and 1% xylan for XPB-21 and 0.25% xylan for XPB-28) and the activity of extracellular produced xylanases was studied under varied reaction conditions in order to determine the optimal conditions for enzyme activity.

In order to study the effect of pH on xylanase activity, 100µ of enzyme was added to the reaction mixture containing buffer at different pH i.e., 7.0, 7.5,8.0, 8.5, 9.0, 9.5, 10.0, 10.5 and 10.7 For the optimization of the pH, different buffers (citarte buffer, phosphate buffer, tris-HCl and sodium carbonate buffers) were used. The reaction mixture was incubated at 45°C for 15 minutes and absorbance was read at 540ηm.

In order to determine the best temperature for miximum activity of xylanase, the reaction mixture was incubated at different temperature ranging from 30°C to 80°C for 15 minutes and xylanase activity was performed at 540ηm as described earlier.

To study the effect of time of incubation on

xylanase activity the enzyme activity was checked after incubating the tubes containing the reaction mixture (pH 9.0 and temperature 55°C for XPB-21) and (pH 8.5 and temperature 50°C for XPB-28) for different time periods ranging from 5 minutes to 60 minutes.

The enzyme (100 ul) was added to the reaction mixture containting different concentrations of substrate (xylan) ranging from 0.25 to 2% in citrate buffer (0.05M, pH 5.0) and reaction mixture was incubated at 55°C for 30 minutes (for XPB-21) and at 50°C for 30 mintues (for XPB-28). The activity of enzyme was recorded in each case.

Sarkanda Grass (Saccharum munja) was used as a raw material for the studies.

Cooking Chemicals: Caustic Soda: Purity: 47.5%, GPL: 690-700, Na2CO3: 0.36%, Sodium Sulphite:75-78%, pH:11-11.75%, Moisture: 5-8%

Pulping process used in the study were alkaline pulping and alkaline sulphite pulping

Unbleaching Pulp: Consistency: 10-12%, pH; 8.5-9.5%, Kappa No. 29-31

Enzyme: Two Cellulase free xylanases obtained from a commercial source available in the market was used.

Bleaching Sequence:

Control: C-E-H

Enzyme : X-C-E-H

Tappi and other standard methods were followed for various testing & analysis in present study.

RESULTS AND DISCUSSION

Isolation of xylanolytic bacteria

In all, forty bacterial stains exhibiting activity of extacellulary produced xylanase were isolated from the samples collected form various habitats. Pure line cultures were established by streaking (Fig.1) repeatedly a single bacterial colony on John and Schmidt medium. These were maintained on nutrient agar (NA) slants (Fig. 2) and stored in the refrigerator (0-4°C) till further use with regular subculturing once in a month. Bacterial strains were selected for the production of xylanases because of their very fast growth rate in comparison to fungus, their increased activity at elevated pH and temperature and their ability to produce cellulase free xylanases so as to utilize these in commercial paper manufacturing process.

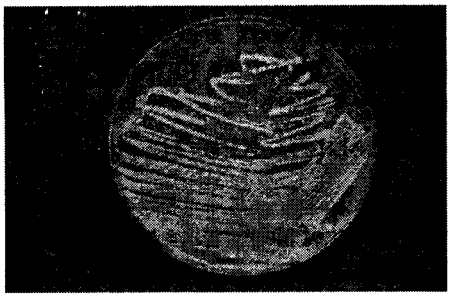

Fig.1 Streaking on agar-plate for establishing pure line culture

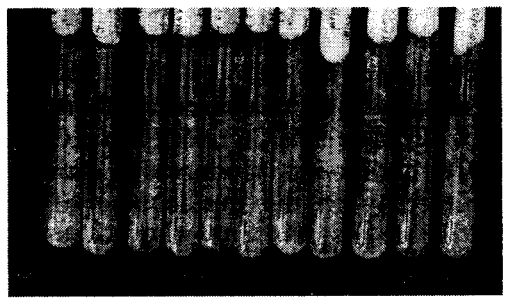

Fig.2 Isolated microbial strains on nutrient agar slants

Table 1: Quantitative determination of extracellular xylananse produced by bacterial isolates

Isolate	Sample/Habit	Xylanase Activity	Cellualase activity
XPB-8	Annadale, Shimla	1.235	0.045
XPB-9	Annadale, Shimla	3.124	0.034
XPB-21	Annadale, Shimla	5.157**	0.016
XPB-28	Sanjauli, Shimla	5.350**	0.010

* One International unit of enzyme activity represents μ moels of D-xylose released per minute, per ml. of culture supernatant under assay conditions.

* The values reported are average of three in each case.

** The isolates (XPB-21 and XPB-28) showed least celluase activity of all and hence were used for further experiments

Screening of xylanolytic bacterial isolates

Screening of bacterial isolates was initially done on the basis of their growth patterns in John and Schmidt's liquid medium containing xylan as a sloe carbon source and further confirmation was done by checking the enzyme activity in culture supernatants. Though, all the forty bacterial isolates exhibited xylanase activity but only four hyper xylanase producing strains (XPD-8, XPB-9, XPB-21, XPB-28) were selected on the basis of theri ability to produce increased extracellular xylanase in the liquid medium in comparison to the rest of the strains isolated by us. Among the four isolates, XPB-28 and XPB-8 exhibited maximum activity of extracellular xylanase in comparison to other two isolates (Table 1). Corresponding cellulase activity of these four isolates was minimum in isolates XPB-21 (0.0161U) and XPB-28 (0.010 IU). Based on the results of xylanase and cellulase activities, these two isolates were selected for further studies.

Charcterization of the hyper producing bacterial isolates

The observations were recorded regarding the colony and tell characteristics of the selected hyper xylanase producing bacterial strains XPB-21 and XPB-28. Based on these observations, it appears that these two isolates belong to the genus Bacillus. The detailed characterization of the isolates will be confiermed at Microbial Type Culture Collection (MTCC), IMTECG, Chandigarh and MGB (Microbial Germplasm Bank), DBT, H.P. University in the due course of time.

Optimization of the culture conditions for the increased production of extracellular xylanase by the isolates XPB-21 and XPB-28

Effort wer made to optimize the culture conditions for increased production of bacterial xylanase by growing the isolates XPB-21 and XPB-28 under varied conditions like pH, temperature, time and substrate concentration in the liquid medium and enzyme assay was done in each case.

XPB-21 and XPB-28 were grown in 50 ml of the liquid medium with different pH ranging from 7.0 to 11.0 (30°C for 48 hrs) in 250 ml flasks in incubator shaker under continuos agitation (160 rpm). Culture contents were centrifuged at 10,000 rpm for 15 minutes (4°C) and supernatant thus collected was assayed for xylanase activity.

Table 2: Effect of pH on the production of xylanase:

pH	Xylanase activity (IU)*	
	XPB-21	XP-28
7.0	4.785**	4.579
7.5	5.165	5.345
8.0	5.152	5.276
8.5	5.056	5.005
9.0	4.982	4.865
9.5	4.886	4.856
10.0	4.348	4.754
10.5	4.160	4.296
11.0	4.231	3.874

* One International Unit of enzyme activity represents μ moles of D-xylose released per minute, per ml. of culture supernatant under assay conditions.
** The values reported are average of three in each case.

The results of enzyme assay done after 48 hrs. have been reported in Table 2.

Both the isolates XPB-21 and XPB-28 showed maximum xylanase activity at pH 7.5. However, in both cases, good activity of xylanase was observed in a wide pH range from 7.5-10.0. This shows that both bacterial srains can grow in wider pH range 7.5 to 10.0 and this property can be effectively utilized in industrial processes where pH fluctuations are expected due to large samples and improper mixing during initial processes.

Both the isolates, XPB-21 and XPB-28 were

Table 3 : Effect of temperature on xylanase production

Temperature	Xlanase activity (IU)*	
(°C)	XPB-21	XPB-28
25°C	3.150**	3.125
30°C	3.697	3.954
37°C	4.929	4.981
45°C	5.165	5.345
55°C	4.676	4.940
65°C	3.285	3.294

* One International Unit of enzyme activity represents μ moles of D-xylose released per minute, per ml. of culture supernatant under assay conditions.
** The values reported are average of three in each case.

grown in 50 ml. of the liquid medium with ph 7.5 at different temperatures ranging from 25-60°C (160 rpm) and the results of enzymes activity after 48 hrs have been reported in Table-3. Although the bacterium exibited good growth at the temperature ranging from 30°C to 55°C but maximum enzyme production for XPB-21 and XPB-28 was observed at 45°C and least enzyme production was observed at 25°C.

Table 4 : Effect of substrate concentration on xylanase production by the isolates XPB-21 and XPB-28

Substrate conc. (%)	Xylanase activity (IU)*	
	XPB-21	XP-28
0.25	4.322**	4.420
0.50	4.365	4.983
0.75	5.075	5.542
1.0	5.165	5.345
1.25	4.995	5.231
1.50	4.699	4.943
1.75	4.378	4.356
2.0	4.155	4.163

* One International Unit of enzyme activity represents μ moles of D-xylose released per minute, per ml. of culture supernatant under assay conditions.

** The values reported are average of three in each case.

Effect of substrate concentration on the extracellular production of xylanase by XPB-21 and XPB-28 was studied by growing the organisms in John and Schmidt liquid medium (pH 7.5) containing varied concentrations of xylan ranging from 0.25%, 0.50%, 0.75%,1.0%,1.25%,1.50%,1.57% to 2.0%. 50 ml of broth with different concentration of xylan (10%) was incubtion at 45°C at 160 rpm and enzyme assay was done in each case agter 48 hrs of incubation (Table-4). In presence of 1% xylan in the medium, isolate XPM-21 exhibited maximum enzyme production. However XPB-28 showed maximum enzyme production with 0.75% xylan in the medium. Least enzyme was recorded with 0.25% xylan case of XPB-21 whereas; XPB-28 could utilize least substrate when 0.75% concentration of xylan was used in the medium.

The seed culture was prepared by growing the bacterial strains in 50 ml. of medium (pH 7.5 at 45°C for 36 hrs) in an incubator shaker under continuors of 0.1 O.D of both the isolates an incubated at 45°C at 160 rpm. The samples were drawn at an interval of 3 hrs until 36 hrs and the absorbance (600ηm.) and enzyme activity was recorded in each case (Table-5).

The results reported in Table5 indicated maximum

Table 5 : Growth profile of isolates XPB-21 and XPB-28

Time (hrs.)	XPB-21	XP-28
	Absorbance (600mm)	Absorbance (600mm)
0	0.070	0.179
3	0.101	0.28
6	0.193	0.303
9	0.283	0.345
12	0.373	0.493
15	0.621	0.686
18	0.883	0.938
21	1.286	1.243
24	1.369	1.363
27	1.487	1.320
30	1.410	1.310
33	1.402	1.303
36	1.350	1.200

enzyme activity after 27 hrs of incubation in both bacterial strains XPB-21 and XPB-28. However maximum absorbance was recorded after 24 to 27 hrs. of icubation. Thereafter, a decline was observed which shows that log phase ended at 24 to 27 hrs and stationary phase started after 30 hrs. A sharp decline in both O.D. and enzyme activity was recorded after 33 hrs.

Based on the results of the growth of both bacterial isolates under varied culture conditions, it was observed that both isolates. can be used in varied levels of pH(7-9) and good enzyme could be produced even upto 50°C in 24-27 hrs. These properties make these isolates good candidature to be used in plant scale where large volumes result in pH and temperture fluctation specially during initial stages. The pH (8.5-10) and temperature (45-50°C) optima observed for both the bacterial isolates are the ideal conditions in paper manufacturing processes. These isolates can be effectively utilized in paper industries provided the enzyme production activity can be increased further.

Optimization of reaction conditions for xylanase

production by isolates XPB-21 and XPB-28

In order to see the effect of different reaction conditions on the activity of extracellular xylanase production by both the isolates XPB-21 and XPB-28, these were grown under optimized conditions (pH 7.5, temperature 45°C, 1% xylan for XPB-21 and pH 7.5, temperature 45°C, 0.25% xylan for XPB-28) for 30 hrs. under continuous agitation (160 rpm) and enzyme activity was measured by varying pH, temperature, time of incubation and substrate concentration of the reaction mixture.

Table 6 : Effect of pH on the xylanase activity

Assay pH	Xylanase activity (IU)*	
	XPB-21	XP-28
7.0	5.162**	5.305
7.5	5.202	5.327
8.0	5.273	5.392
8.5	5.316	5.439
9.0	5.415	5.419
9.5	5.409	5.385
10.0	4.655	4.961
10.5	4.573	4.818
11.0	4.184	4.310

* One International Unit of enzyme activity represents μ moles of D-xylose released per minute, per ml. of culture supernatant under assay conditions.

** The values reported are average of three in each case.

The activity of extrcelllulary produced xylanase was assayed at different pH ranging from 7.0 to 11.0 for both the isolates (XPB-21 and XPB-28). The results obtained indicated great influence of pH on the relative xylanase activity for both the isolates (Table-6) Maximum activity was recorded at pH 9.0 for XPB-21 and at pH 8.5 for XPB-28. Interestingly, though the optimum pH for enzyme production by these orgnisms was 7.5, but activity at this pH was comparatively low. A sharp decline in the xylanase activity was recorded by increasing pH from 9.5 to 11.0 in both the cases.

To see the effect of varying temperature on activity and stability of enzyme, the enzyme activity was assayed at different temperatures ranging from 30°C to 80°C and the results have been reported in Table 7. Althouh enzyme from both the isolates were active at temperature ranging from 30°C to 70°C but maximum enzyme activity was observed at 55°C for XPB-21 and at 50°C for XPB-28, Enzyme activity upto 70°C was quite good but above 70°C there was an abrupt decline. The xylanase from both bacterial strains showed good activity in wider temperature range (40-60°C) as reported in Table7.

Table 7 : Effect of Temperature on xylanase activity

Temperature	Xylanase activity (IU)*	
(°C)	XPB-21	XP-28
30	4.178**	4.154
35	4.622	4.752
40	5.225	5.251
45	5.410	5.442
50	5.476	5.469
55	5.487	5.401
60	5.105	5.162
65	4.266	4.489
70	3.254	3.162

* One International Unit of enzyme activity represents μ moles of D-xylose released per minute, per ml. of culture supernatant under assay conditions.

** The values reported are average of three in each case.

These observatios points towards the thermostability of the xylanase from the isolates and the suitability of the enzyme for industrial applications especially in paper industry after pilot scale trials and after improvements in enzyme activities. Further studies are needed to confirm the stability of these enzymes at elevated temperatures for different lengths of time.

The enzyme was assayed by incubating the reaction mixture at different time intervals from 5 minutes upto 60 minutes and it was observed that maximum activity of the xylanase in the raction was after 30 minuts of incubation. The enzyme activity gradually increased with time from 5 minutes till 30 minutes. The enzyme activity decresed slightly with observed upto 60 minutes, but no significant decline in enzyme activity was observed upto 1 hr. which is a good prospect considering the possible industrial application of these enzymes.

In order to see the effect of varying concentrations of substrate (xylan) on the activity of enzyme, the raction mixture with different

concentrations of xylan ranging from 0.25% to 2% was incubated with enzyme and the enzyme activity was recorded in each case. For XPB-28 the reaction mixture with different substrate concentration ranging from 0.25% to 2% showed continuous increase in enzyme activity while in case of XPB-21, enzyme activity increased with increasing substrate concentration from 0.25% to 0.75%. No significant decline in enzyme activiry was recorded even upto substrate concentration of 1.25%. The enzymatic conversion of xylan to monomer can be effectively experimented by using the low cost substrate like straw, bran etc. to check its effectiveness in the commercial processes where maximum deradation of hemicellulose present in raw material by using least/less possible enzyme is desirable.

Biobleacing of Xylanase on agro pulps

The process conditions provided during pre-bleaching trials have been shown Table 8. The various results of bio-bleaching trials carried out have been summarized in Tables 9-12. During optimization of enzyme dosage in pre-bleaching stage on agro pulps from alkaline pulping and alkalie-sulphite pulping, it was observed that excess dosages of xylanase enzyme for a longer time or temperature below 45°C has resulted in decreased pulp yield, strength and brightness of pulp. This could probably be due to loss of hemicelluoses by the action of enzyme. However limited hydrolysis of hemicelluloses in the pulp by the enzymes, especially xylanases, results in a higher final brightness or saving in bleaching chemical to reach a particular brightness level. The enzyme, therefore, used for the treatment must be free from cellulose, which attacks the cellose in the fibers and reduces the strength properties of the pulp.

Effects of xylanase in bleah chemical demand

Alkaline agro pulp and alkaline Sulphite agro pulp

Table 8 : Process conditions during Pulp Bleaching

Particulars	Chlorination Stage	Alkali Extarc-tion Stage	Hypo Stage
Temperature, °C	Ambient	60	40
Pulp Consistency,%	3.0	8.5	8.5
Retention time, min.	30	60	120
Final pH	1.8-2.0	10.5±2	8.8-9.2

shows different trend while bleaching with conventional C E H bleach sequence. Chlorine demand substantially reduced in both the agro pulp treated with alkaline and alkaline Sulphite process. Elemental chlorine consumption reduced to 17.8% while brigtness increaed by 2 to 2.5 poit against control pulp sample.

Improved pulp brightness and reduced chlorine consumption reflected from the reduction in Kappa No. of the unbleached pulp after enzyme treatment. The reduction in Kappa No. by 4%-9% after enzyme treatment and more than 23% after alkali extraction with indigenously manufactured enzyme treated pulp.

Effect fo Enzyme on pulp yield in Alkaline Agro Pulp and Alkaline Sulphite Agro Pulp

It is evident from the results that chlorine saving is achieved nearly 17.8% in chlorination stage when enzyme treated pulp processed under optimized condition. Hypo consumption reduced by 18.7 to 25% in Enzyme I,II & xylanase from isolated strain XPB-28 treated pulp. Burst index, Tensile index and Tear index have also been observed to be improved substantially in enzyme treated Alkaline Sulphite Agro Pulp. Yellowness decreased by 40%-42%. Literature reviewed on bleach effluent generated form chemical pulping of lignocellulosic raw materials before and after enzyme treatment indicated the reduction of AOX (Adsorbable Organic Halides) through the use of xylanase which helps in release of lignin and other chloro-phenolic compounds and allowing the use of lesser chlorine in chlorination stage. A reduction of 26%-29% in AOX level when pulp is treated with. An indirect saving of power consumption to treat same amount of untreated bleach effluent in ETP have also been noticed. The economics of the enzyematic treatment fo agro-pulp has been explained in the Table 11. inal teatment effluent

CONCLUSION

The enzyme treatment of agro-pulps resulted in approximately 17% and 18-25% reduction in terms of element chlorine and hypo consumption respectively under optimixed reaction conditions. The enzyme treatment has alo shown a positive effect on strength properties of the pulp. It has been observed that Alkaline Sulphite Agro Pulp respond better than Alkaline Agro Pulp when

Table 9 : Bleach Chemical consumption of enzyme pre-treated Pulp using various xylanase preparation on Agro Pulp

Parameters	CHLORINATION STAGE							
	Control		Pulp treated with enzyme					
			Xylanase-I		Xylanase-II		Xylanase-XPB-28	
	AAP	ASAP	AAP	ASAP	AAP	ASAP	AAP	ASAP
Chlorine applied, %	7.3	7.3	6.2	6.2	6.0	6.0	6.0	6.0
Chlorine consumed,%	-	-	85	87	83	87	84	87.5
Chlorine saving,%	-	-	15.06	15.06	17.8	17.8	17.8	17.8
Final pH	1.8	1.9	2.0	1.8	1.9	1.8	1.8	1.9
	ALKALI EXTRACTION STAGE							
% NaOH applied	3.0	3.0	2.8	2.8	2.8	2.8	2.8	2.8
% NaOH consumed	75	76.5	74.5	75	75.5	76	74.5	75.5
Final pH	10.6	10.3	10.7	10.4	10.5	10.5	10.3	10.4
Kappa No. of Pulp	5.9	5.6	5.0	4.6	4.5	4.3	4.4	4.3
	HYPO STAGE							
% Hypo applied	3.2	3.2	2.6	2.6	2.4	2.4	2.4	2.4
% Hypo cosmed	82	83	81	81.5	80	80	79.5	80.5
Final brightness of PUlp % ISO	82	81.5	84	83	84	84.5	83.5	84
	STRENGTH & OPTICAL PROPERTIES							
Freeness, °SR	38	36	38	37	38	36	36	36
Apparent densigy, g/m³	0.88	0.86	0.92	0.88	0.90	0.88	0.94	0.87
Burst index Kpa M²/g	1.90	2.1	1.90	2.35	2.0	2.3	1.90	2.4
Tensile index Kpa M²/g	35.5	33.0	36.5	34.0	36.5	33.5	38.0	36.0
Tear index Mn. M²/g	5.05	5.2	5.1	5.4	5.3	6.0	5.2	6.2
	OPTICAL PROPERTIES							
Brightness of Pulp, % ISO	82	81.5	84	83.5	84.5	84	84	83.5
Yellowness	15	14	8.8	8.2	8.6	8.3	86	8.3

Table 10 : Bleaching Chemical consumption of enzyme pre-treated pulp using various xylanase preparation

Parameters	Control		Pulp treated with enzyme					
			Xylanase-I		Xylanase-II		Xylanase-XPB-28	
	AAP	ASAP	AAP	ASAP	AAP	ASAP	AAP	ASAP
Saving in elemental Chlorine,%	-	-	15.06	15.06	17.8	17.8	17.8	17.8
% reduction in Kappa No. after final unbleached washer	-	-	4.0	5.7	6.0	9.4	6.0	9.4
% reduction in Kaapa No. E stage in	-	-	15.2	17.8	23.7	19.6	25.4	23.2
Final bright ness of Pulp, % ISO	82	81.5	84.0	83.5	84.5	84.0	84.0	83.5

Table 11 : Yield and other parameters of Alkaline and Alkaline-Sulphite agro-pulp with control and after enzyme treatment

Parameters	Control		Pulp treated with enzyme					
			Xylanase-I		Xylanase-II		Xylanase-XPB-28	
	AAP	ASAP	AAP	ASAP	AAP	ASAP	AAP	ASAP
Kappa No.	25	26.5	24	25	23.5	24	23.5	24
CED Viscosity, CM^3/g	10	590	620	615	625	630	620	630
Brightness %, ISO	28	27.5	29	29	29.5	29	29.5	29.5
Pulp Yield, %	99.1	98.8	99.0	98.02	99.0	98.0	99.06	98.8

AAP- Alkaline Agro Pulp
ASAP- Alkaline Sulphite Agro Pulp

Table 11 : Yield and other parameters of Alkaline and Alkaline-Sulphite agro-pulpwith control and after enzyme treatment

Parameters	Control		Pulp treated with enzyme	
	AAP	ASAP	AAP	ASAP
Cl2 applied, %	7.3	7.3	6.0	6.0
Hypo applied, %	3.2	3.2	2.4	2.4
Total Cl2 applied, %	10.5	10.5	8.4	8.4
Cost of Cl2 per tonne pulp (Rs/T)	976.50	976.50	781.20	781.20
Enzyme applied, %	-	-	0.03	0.03
NaOH cost per tonne pulp (Rs/T)	-	-	180	180
Net saving/tonne pulp (Rs/T)	375.00	375.00	350.00	350.00
	-	-	40.30	40.30

treated with enzyme. The process condition namely pH, temperature and retention timings are three critical & liniting parameters for optimum application of enzymes towards pre-bleaching to achieve the targete results. The organisms isolated from the forest soils have been found to have good protential for use in enzymatic prebleaching of the agro-pulps. However, further studies are in progress to purify the xylanase from the siolated bacterial strain to enhance its

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