Biobleaching Enzyme Production And Application

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ABSTRACT

A thermopilic fungus, THERMOMYCES LANUGINOSUS, isolated from stored bagasse could produce Xylanase enzyme of significant activity, after optimising the fermentation conditions, using cheap substrate available within the mill. Pretreatment of the unbleached hardwood pulp with the crude enzyme, under specified conditions, could improve the brightness of the bleached pulp by 3-4 points over the control, without significant effect on pulp properties. The preliminary studies on laboratory scale has given vide scope for improving the enzyme activity and its cost of production, to make it viable on plant scale. The enzyme pretreatment requires no major modification in the plant but a little amount of retrofitting.

INTRODUCTION

Growing consumer awareness and preference towards the environmentally friendly and safe products and processes, forced many industries to adopt environmentally friendly techniques in spite of high cost. One among such industries is the Pulp and paper industry. Biotechnology is emerging as a major substitute to traditional techniques because biotechnical processes put very little pressure on the environment when compared to others. In the recent past, the Pulp and paper industries are also exploiting this unique process, to introduce various stages of pulp and paper production, effluent treatment and waste management. Among the various applications, hemicellulase enzyme especially Xylanase aided bleaching showed its potential in reducing the chemical consumption and becoming commercially viable alternate and used in many industries in the Western countries.

CONCEPT

It is known that hemicellulose acts as a cementing material between lignin and cellulose in the kraft pulp. Partial and selective hydrolysis of hemicellulose by Xylanase enzyme would reduce the bonding between lignin and cellulose (1,2). In turn this would facilitate easy removal of lignin from pulp during the subsequent bleaching process. This results in lower chemical consumption or higher brightness (3).

COMMERCIAL XYLANASE ENZYME

Literature survey shows that different xylanase enzymes have been produced using different organism and substrate, under varying fermentation conditions, depending upon the nature of the strain being used for enzyme production (4,5,6). Considerable amount of work has been done on the development of xylanase enzyme, making it viable on plant scale application.

More than the application part of it, the production of Xylanase, free from contaminating enzymes like cellulase which affect the pulp properties significantly, on a commercial scale, with cheap substrate, has been a formidable challenge for the biotechnologists.

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TNPL XYLANASE PRODUCTION

In our earlier studies, we found that pretreatment of hardwood kraft pulp with our own Xylanase enzyme, (10XU/g of pulp for 2 hours) could give a brightness gain of around 3 to 4 points, with the CEH bleaching sequence, which was in agreement with the commercial xylanases (6). However, we were able to produce the enzyme with activity of only 112 Xu/ml, using the fungi Trichoderma sp. isolated from stored bagasse. This low enzyme activity, even after optimisation of the culture conditions, hindered the scaling up of the enzyme, as the volume of enzyme to be produced became enormous. Therefore, efforts were made to achieve enzyme of higher activity using new organism.

SCREENING OF FUNGI FOR XYLANASE ENZYME ACTIVITY

As a primary step towards identification of a new fungus for xylanase production, several fungal cultures were isolated from stored bagasse pile. Among the 17 species of thermophilic fungi, isolated using malt extract medium at 50 °C, nine species were selected for xylanase enzyme production based on the morphology and growth kinetics. These fungi were screened for enzyme production using Birchwoodxylan, as a carbon source. (Birchwoodxylan 20 g/lit, Peptone 6g/lit, K_2HPO_4 5g/lit pH 6.5 and temperature 60 °C). The results are given in **Table-1.** The fungal strain of "Thermomyces Lanuginosus", which produced xylanase enzyme of highest activity was selected for our studies.

TABLE-1

Xylanase enzyme production by various thermophilic fungi isolated from bagasse

Name of the fungi	Xylanase activity (XU/ml)
1. Thermomyces lanuginosus	79.8
2. Thermomyces lanuginosus	54.8
3. Thermomyces lanuginosus	15.7
4. Thermomyces lanuginosus	19.9
5. Thermomyces sp.	24.0
6. Aspergillus fumigatus	16.7
7. Aspergillus sp.	26.8
8. Penicillum sp.	13.8
9. Chetomium sp.	16.7

EXPERIMENTAL

Xylanase enzyme produced using Thermomyces Lanuginosus using Xylan, Unbleached chemical bagasse pulp and Corncob as carbon source, was used for pretreatment of Eucalyptus Hardwood Kraft pulp, Prior to bleaching, to study its effect on improvement of bleaching response. The hardwood pulp after enzyme pretreatment, was bleached using E-P and C-E-H bleaching sequences.

Hardwood kraft pulp was collected from the plant. The pulp was washed thoroughly and shredded to uniform consistency. The characteristics of the unbleached pulp were

Kappa number : 17.0

Brightness % ISO : 25.6

Viscosity cPs : 13.1

The crude enzymes produced under the optimised conditions, using the three carbon sources as substrate, were used for further bleaching studies. The enzyme solution was filtered through sterilised cotton, to remove the mycelium and the filtrate was stored in amber bottles at 4 °C and was used for further studies.

Enzyme pretreatment

Hardwood kraft pulp was treated with the three xylanase enzymes

@ 10 XU/g under the following conditions

Consistency % : 10.0

Temperature °C : 60.0

Time mts : 120.0

pH : 6.5

A control was maintained without enzyme addition. After the pretreatment time, the pulp was dewatered and washed with water equivalent to 20 times the OD Weight of the pulp. The enzyme

filtrate was analyzed for reducing sugars. The kappa number and the brightness of the enzyme treated pulp were determined. The pH of the pulp was maintained at 6.5 throughout the pretreatment period.

Bleaching

The pulp after enzyme treatment, was subjected to E-P and C-E-H bleaching, to see the brightness improvement. The enzyme treated hardwood pulp was extracted using 2% NaOH followed by a Peroxide bleaching stage with 2.0% peroxide. The enzyme pretreated hardwood pulp was also bleached using C-E-H sequence. Kappa factor of 0.2 was used as chlorine charge in chlorination stages. The extraction and hypo stages were performed with 2% chemicals in the respective stages.

RESULTS AND DISCUSSION

OPTIMISATION OF FERMENTATION CONDITIONS

Improving the enzyme activity, to realise the maximum potential of the fungal strain, could be achieved through optimisation of fermentation conditions. Various conditions had to be optimised to enhance the enzyme activity viz nature of Carbon source and its concentration, nature of Nitrogen source and its concentration, pH, time, aeration.

TABLE-2

Effect of carbon sources on Xylanase enzyme production by thermophilic fungi Thermomyces lanuginosus

Carbon source	Sylanase activity (XU/ml)
Corn cobs (Coarse)	65.0
Corn cobs (fine)	22.5
Unbleached CBP	69.9
Bleached CBP	47.3
Unbleached HW pulp	41.9
Bleached HW pulp	39.2
Bagasse	17.4
Bagasse pith	17.4
Eucalyptus wood powder	2.4
Wheat bran	42.5
Birch wood Xylan	82.3
Lenzing Xylan	79.9
HW xylan	59.9
CBP xylan	65.0

TABLE-3

Effect of the initial medium pH and carbon source on xylanase production (XU/ml) by Thermomyces lanuginosus

	Carbon source		
pH	Xylan	Unbleached CBP	Corn Cobs
	(Xylanase Activity)		
5.5	124.7	64.9	65.0
6.0	224.5	137.2	99.2
6.5	174.0	124.7	78.3
7.0	109.8	64.9	45.0

Optimisation of the aforementioned parameters were carried out for Thermomyces Lanuginosus, to achieve maximum enzyme activity.

CARBON SOURCE

Various carbon sources **(Table-2)** were used to study the xylanase enzyme production by the thermophilic fungi T. Lanuginosus. Among the fourteen carbon sources tried, three carbon sources, namely Birchwood xylan, unbleached Chemical Bagasse Pulp and Corn Cobs, were selected for further optimisation. The basis of selection was purely based on the enzyme activity and cost factor. Birchwood Xylan was selected as the reference carbon source, as it is the best carcon source for Xylanase production.

pH

T. Lanuginosus was grown in various pH (Initial medium pH), to study the effect of the critical medium pH on xylanase enzyme production. It was found that the initial medium pH of 6.0 was highly favourable for the xylanase enzyme production **(Table-3).** In all the three carbon substrate, maximum enzyme activity was achieved only at pH 6.0.

NITROGEN SOURCES

The effect of various Nitrogen sources such as Soya peptone, meat peptone and yeast extract were studied for xylanase enzyme production by T. Lanuginosus. Among the Organic Nitrogen sources, Yeast extract gave the highest sylanase activity **(Table-4)**, and was selected for further studies.

TABLE-4

Effect of nitrogen and carbon source on xylanase production (XU/ml) by Thermomyces lanuginosus

Nitrogen source	Carbon source		
	Xylan	Unbleached CBP	Corn Cobs
	(Xylanase Activity)		
Soya peptone	69.6	78.3	20.2
Meat peptone	212.0	109.8	78.3
Yeast extract	311.8	137.2	99.2

CONCENTRATION OF CARBON AND NITROGEN SOURCES

Out of the three carbon sources ie Birch wood Xylan, Unbleached Chemical Bagasse Pulp and Corn cobs, unbleached Chemical Bagasse Pulp was selected for further optimisation based on the cost and its availability within our mill. The results of the effect of various concentration of nitrogen source (yeast extract) and carbon source (unbleached Chemical Bagasse Pulp) on xylanase enzyme production by T. Lanuginosus, is presented in **Table-5** which suggests that 30 gpl of Yeast extract and 35 gpl of unbleached Chemical Bagasse Pulp is ideal for xylanase enzyme production.

BUFFER pH AND XYLANASE ENZYME ASSAY

To find out the optimum treatment pH for the xylanase enzyme produced by the T. Lanuginosus, Enzyme assay experiments were carried out at different pH, using 50 mM Sodium citrate buffer. The results are presented in **Table-6.** Assay medium pH 6.5 gave the highest enzyme activity of 624 XU/ml, 398 XU/ml and 374 XU/ml for

TABLE-5

Optimization of carbon (unbleached CBP) and nitrogen (yeast extract) sources for xylanase production by Thermomyces lanuginosus

	Unbleached CBP			
	20g/lit.	25g/lit.	30g/lit.	35g/lit.
Yeast extract		(Xylanase Activity)		
15g/lit.	137.2	137.2	212.0	149.7
20g/lit.	156.6	199.6	212.0	212.0
25g/lit.	198.4	187.1	------	224.5
30g/lit.	212.0	212.0	249.0	311.8

TABLE-6

Effect of assay medium pH on xylanase enzyme activity (XU/ml) of Thermomyces lanuginosus

Carbon source	Medium pH				
	5.5	6.0	6.5	7.0	7.5
	(Xylanase Activity)				
Xylan	474.3	574.8	623.6	474.3	311.8
Unbleached CBP	311.8	383.0	398.9	311.8	224.5
Corn Cobs	286.9	335.0	374.2	255.0	149.7

Birchwood Xylan, Unbleached chemical Bagasse Pulp and Corn cobs respectively. Hence pulp pH was maintained at 6.5 throughout our enzyme pretreatment stage, in all our bleaching experiments.

Thermomyces Lanuginosus, could produce Xylanase enzyme of significant activity, under specific optimised conditions given in **Table-7.** The substrate chosen were very cheap and easily available within the mill. Being a thermophilic strain, the temperature of incubation was 50 °C, which is very much favourable for the mill bleaching conditions. This temperature tolerance aspect of the T. Lanuginosus, widens the temperature optimum range between 45-60 °C, during pretreatment, which is easy to maintain on plant scale. So also, the enzyme produced can be stored at room temperature, requiring no refrigerated storage conditions.

The activity of crude enzyme with unbleached chemical bagasse pulp and Corn cobs, (400 and 350 XU/ml), gives us a good start for commercial production of the enzyme, on large scale. The pulp pretreatment conditions as given in **Table-8,** show that enzyme pretreatment does not require any

TABLE-7

Optimised conditions for Xylanase enzyme production by Thermomyces lanuginosus

Parameter	Condition
Intial medium pH	6.0
Temperature °C	50
Carbon source	Unbleached CBP 35 gpl
Nitrogen source	Yeast extract 30 gpl
Enzyme assay conditions	50mM Sodium Citrate 6.5 pH, 60 °C

TABLE-8

Enzyme pretreatment followed by E-P Bleaching of hardwood

XYLANASE FROM T.LANUGINOSUS

Hardwood decker :

Kappa number	:	17.0
Brightness %ISO	:	25.6
Viscosity cPs	:	13.1

Particulars	Control	XYLAN SUBSTRATE	CB PULP SUBSTRATE	CORN SUBSTRATE
Enzyme activity XU/ml	---	600	400	350
Enzyme charged XU/g	---	10	10	10
Brightness % ISO	25.9	26.8	26.7	26.7
Kappa number	16.2	15.4	15.7	15 5
Reducing sugars released % on pulp	0.13	1.13	1.14	1.05
EXTRACTION				
Alkali as NaOH %				
applied	2.00	2.00	2.00	2.00
consumed	0.53	0.56	0.56	0.60
pH Initial/Final	11.8/11.4	11.7/11.4	11.7/11.4	11.7/11.4
Brightness % ISO	27.5	29.1	29.0	29.1
Kappa number	15.8	13.4	13.9	13.3
PEROXIDE STAGE				
H_2O_2 %				
applied	2.00	2.00	2.00	2.00
consumed	2.00	2.00	2.00	2.00
pH Initial/Final	11.0/10.9	11.0/10.8	11.0/10.8	11.0/10.8
Final Brightness % ISO	40.2	44.8	44.2	43.3
Brightness gain	---	**4.6**	**4.0**	**3.1**
Viscosity cPs	10.4	9.7	9.5	9.6

Enzyme pretreatment	: 10.0 % cy, 60 °C, 120 mts, 6.5 pH
E stage	: 8.0 % cy, 60 °C, 60 mts
Peroxide stage	: 8.0 % cy, 90 °C, 90 mts

TABLE-9

Enzyme pretreatment followed by CEH Bleaching of hardwood

XYLANASE FROM T.LANUGINOSUS

Hardwood decker :

Kappa number	:	17.0
Brightness %ISO	:	25.6
Viscosity cPs	:	13.1

Particulars	Control	XYLAN SUBSTRATE	CB PULP SUBSTRATE	CORN SUBSTRATE
Enzyme activity XU/ml	---	600	400	350
Enzyme charged XU/g	---	10	10	10
Brightness % ISO	25.9	26.8	26.7	26.7
Kappa number	16.2	15.4	15.7	15.5
Reducing sugars released % on pulp	0.135	1.072	1.072	1.031
CHLORINATION				
Chlorine as Cl_2 %				
applied	3.40	3.40	3.40	3.40
consumed	3.29	3.16	3.16	3.26
Final pH	2.5	2.4	2.4	2.4
EXTRACTION				
Alkali as NaOH %				
applied	2.00	2.00	2.00	2.00
consumed	1.39	1.28	1.26	1.26
pH Initial/Final	11.2/11.1	11.3/11.2	11.4/11.3	11.4/11.3
Kappa number	4.5	3.3	3.5	3.6
Brightness % ISO	44.5	48.5	47.3	47.6
Yellowness %	17.8	17.4	17.5	17.9
HYPO I STAGE				
Hypo as Cl_2 %				
applied	2.00	2.00	2.00	2.00
consumed	1.72	1.68	1.69	1.68
pH Initial/Final	10.0/8.4	9.9/8.2	9.6/8.3	9.8/8.2
Final Brightness % ISO	77.9	80.9	80.8	81.3
Brightness gain	---	**3.0**	**2.9**	**3.4**
Viscosity cPs	5.2	4.4	4.3	4.4

Enzyme pretreatment	: 10.0 % cy, 60 °C, 120 mts, 6.5 pH
C stage	: 3.0 % cy, Ambient, 30 mts
E stage	: 8.0 % cy, 60 °C, 60 mts
H stage	: 8.0 % cy, 40 °C, 120 mts

TABLE-10

PULP EVALUATION

PFI Beating Load : 17.7 N/10 mm bar length

S. No.	Particulars	PFI Rev	Freeness ml CSF	Sub g/m²	Cal mic	Bulk cc/g	Tensile Index Nm/g	Tear Index mN.m²/g	Burst Index kPa.m²/g	Bri %ISO	Opacity %(ptg)%	Scat. coefft. m²/kg	Yel %
6	Control-E-P	0	385	60.0	127	2.12	49.59	5.82	2.78	39.2	99.1	51.8	34.0
		1000	280	60.3	112	1.86	65.91	6.61	4.32	38.7	98.6	46.2	34.4
		2000	210	59.3	101	1.70	77.49	7.30	5.23	36.6	98.4	42.3	35.7
		3000	175	60.1	99	1.65	82.12	7.24	5.44	36.4	98.5	42.3	36.0
7	X_{xyl}-E-P	0	420	61.5	132	2.15	44.47	5.11	2.49	42.8	98.9	54.5	31.4
		1000	310	59.3	105	1.77	66.30	7.08	4.23	41.3	98.0	45.9	32.5
		2000	260	60.0	101	1.68	75.49	7.27	5.17	40.3	98.0	44.2	33.3
		3000	235	59.9	98	1.64	79.02	7.94	5.68	39.5	97.9	40.6	33.9
8	X_{cbp}-E-P	0	440	60.1	127	2.11	50.10	4.98	2.50	42.4	99.0	57.2	31.6
		1000	330	59.4	108	1.82	72.87	6.67	4.35	41.4	98.3	47.8	32.3
		2000	265	59.4	100	1.68	81.06	7.32	5.16	39.9	98.0	44.4	33.5
		3000	240	59.6	96	1.61	81.80	7.37	5.72	39.3	97.7	43.8	34.0
9	X_{corn}-E-P	0	405	60.0	127	2.12	46.94	5.02	2.55	42.8	98.8	54.5	31.3
		1000	325	60.1	108	1.80	70.50	6.84	4.40	41.0	98.5	48.5	32.7
		2000	285	59.9	99	1.65	71.20	7.28	5.19	40.0	97.8	42.7	33.5
		3000	245	60.1	98	1.63	80.49	6.99	5.63	39.7	97.7	41.8	33.8

X indicates Enzyme pretreatment stage

major modification in the plant, except a heater mixer at the inlet to the screened pulp tower, for mixing the enzyme and buffer (for desired pH) with the pulp and for preheating the pulp to 50 °C. Hence, other than the enzyme production aspect, the pretreatment of the pulp requires no major modification but a little amount of retrofitting.

BLEACHING

The E-P bleaching results as evident from the **Table-8,** shows that the xylanase enzyme pretreatment gives a brightness gain of 3.0 to 4.5 points over the control. Kappa number of the unbleached pulp reduces by 0.5-0.8 over the control, upon enzyme pretreatment. The pulp evaluation results are given in **Table-10,** and the strength properties computed at 300 ml CSF are given in Table-12 and in **Fig.1.** The results show that there is no significant change in the strength properties except a slight improvement in bonding properties, in comparison to the control (1).

The C-E-H- results show that there is about 3.0-3.5 points gain in brightness over the control, in case of the enzyme pretreated pulp samples. The pulp evaluation results given in **Table-11** and the strength properties computed at 300 ml CSF are given in **Table-12** and presented graphically in **Fig.2,** which shows no significant change in strength properties due to enzyme pretreatment, except a slight reduction in Tear.

The bleaching and pulp evaluation results of the enzyme treated and the control pulps show that the T. lanuginosus Xylanase enzyme can improve the final brightness of the bleached pulp by 3-4 points, without affecting the pulp properties. This further throws light on the fact that the enzyme produced is Cellulase free, the contaminating

Fig.1. X - E - P Bleached Pulp Evaluation

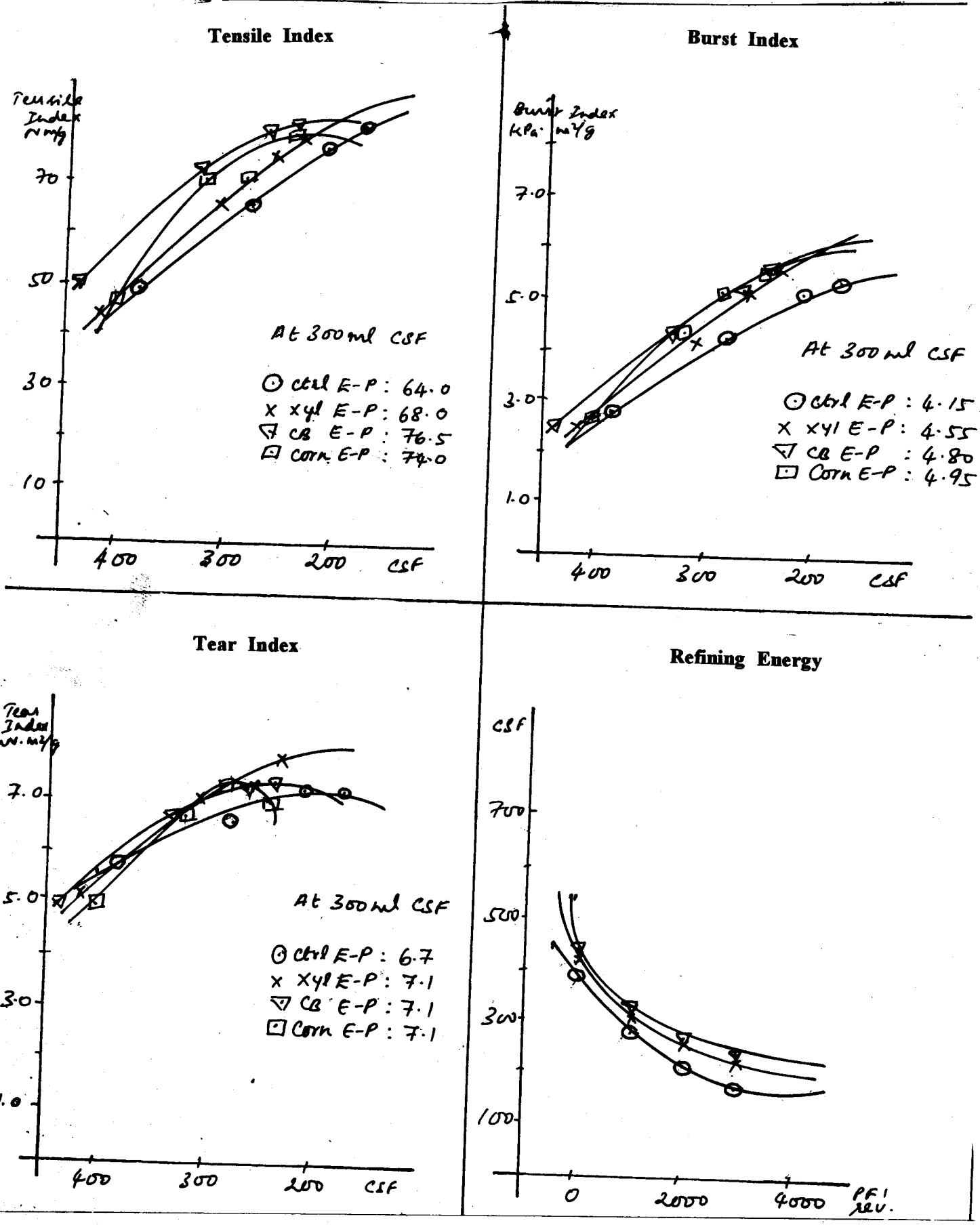

Fig.2. X - C - E - H Bleached Pulp Evaluation

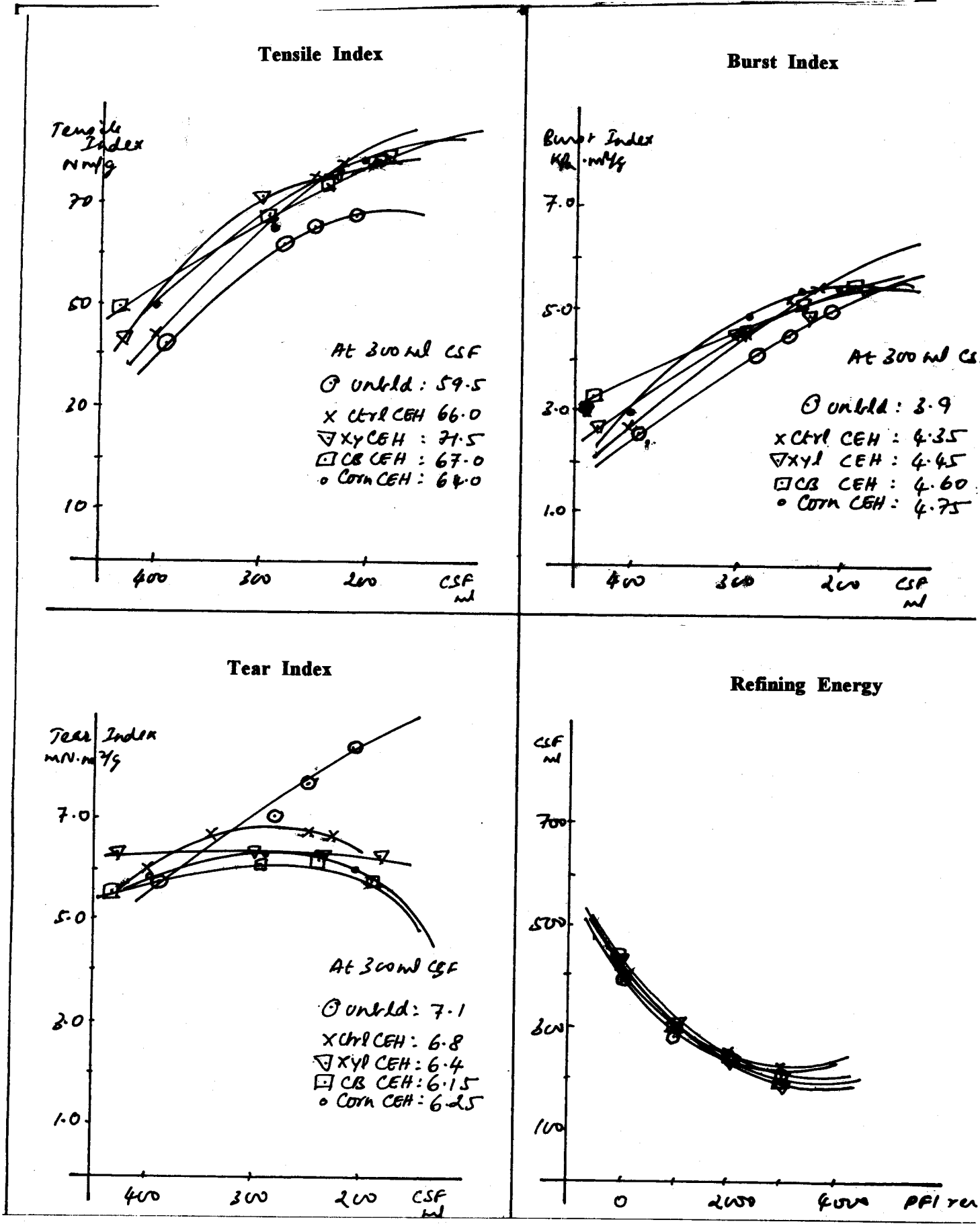

TABLE-11

PULP EVALUATION

PFI Beating Load : 17.7 N/10 mm bar length

S. No.	Particulars	PFI Rev	Freeness ml CSF	Sub g/m²	Cal mic	Bulk cc/g	Tensile Index Nm/g	Tear Index mN.m²/g	Burst Index kPa.m²/g	Bri %ISO	Opacity (ptg)%	Scat. coefft. m²/kg	Yel %
1	Hardwood Decker	0	390	63.2	136	2.15	42.04	5.73	2.60	26.3	99.8	37.7	36.4
		1000	280	64.4	121	1.88	62.10	7.05	4.08	25.1	99.4	37.2	36.1
		2000	250	62.9	113	1.80	65.40	7.68	4.46	25.5	99.3	29.8	37.0
		3000	210	62.1	111	1.79	67.70	8.33	5.01	25.4	99.0	28.1	37.1
2	Control-C-E-H	0	400	62.6	126	2.01	44.51	6.01	2.72	77.0	88.0	57.4	13.8
		1000	290	61.7	105	1.70	66.19	6.70	4.46	76.2	85.2	48.6	14.1
		2000	250	61.7	96	1.56	75.53	6.74	5.21	75.4	85.1	47.3	14.5
		3000	225	61.4	94	1.53	77.56	6.71	5.42	75.0	84.2	44.9	14.5
3	X_{xyl}-C-E-H	0	430	62.0	124	2.00	43.62	6.30	2.67	79.4	87.3	57.5	11.6
		1000	300	61.4	103	1.68	71.50	6.35	4.46	78.0	85.6	50.9	12.3
		2000	230	61.6	96	1.56	75.45	6.28	4.90	77.5	83.9	45.8	12.4
		3000	180	60.0	90	1.50	79.37	6.27	5.39	76.4	83.2	43.3	12.8
4	X_{cbp}-C-E-H	0	435	61.4	113	1.84	49.78	5.54	3.29	79.3	86.8	56.2	11.7
		1000	295	62.7	106	1.69	67.38	6.10	4.50	78.5	85.8	50.7	11.7
		2000	240	61.3	96	1.57	73.89	6.17	5.08	78.6	83.9	46.9	11.5
		3000	190	61.5	91	1.48	79.02	5.82	5.53	77.8	82.9	43.6	11.7
5	X_{corn}-C-E-H	0	400	61.5	117	1.90	49.96	5.84	2.97	80.0	86.7	56.5	10.9
		1000	290	60.9	105	1.72	64.93	6.19	4.89	77.9	85.4	51.1	13.0
		2000	240	60.6	96	1.58	74.08	6.16	5.38	77.2	84.0	47.2	13.4
		3000	205	61.1	93	1.52	78.33	5.97	5.42	76.5	83.0	44.0	13.8

X indicates Enzyme pretreatment stage

TABLE-12

Strength properties computed at 300 ml CSF

S.No.	Sample	Tensile Index Nm/g	Tear Index mN.m²/g	Burst Index kPa.m²/g
1	Hardwood Decker	59.5	7.10	3.90
2	Control-E-P	64.0	6.70	4.15
3	X_{xyl}-E-P	68.0	7.10	4.55
4	X_{cbp}-E-P	76.5	7.10	4.80
5	X_{corn}-E-P	74.0	7.10	4.95
6	Control-C-E-H	66.0	6.80	4.35
7	X_{xyl}-C-E-H	71.5	6.40	4.45
8	X_{cbp}-C-E-H	67.0	6.15	4.60
9	X_{corn}-C-E-H	64.0	6.25	4.75

enzyme which degrades cellulose even in very little quantity (7). Also, obtaining higher brightness without affecting pulp properties is a significant aspect. The xylanase enzyme production using cheap substrate, has given us lot of scope for developing the same, to make it still more cheaper. The substitution of the Yeast extract Nitrogen source, with some other cheaper Organic nitrogen source will pave a long way for reducing the cost of enzyme production. But the substitution should not affect the activity of the enzyme produced significantly.

OBSERVATIONS AND CONCLUSIONS

Xylanase enzyme produced using Thermomyces Lanuginosus with Xylan, Unbleached chemical bagasse pulp, and Corn cobs had an enzyme activity

of 600, 400, 350 XU/ml respectively under optimised conditions (Table-7).

The bleached pulp brightness increases by 3-4 points over the control, without significant influence on pulp properties.

There is scope for improvement of the enzyme with respect to its activity and cost of production, with cheaper nitrogen source.

The results obtained are in line with Commercial Xylanases (8).

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REFERENCES

1. Clark.T.A., McDonald .A.G., Senior.D.J., Mayers.P.R. Biotechnology in Pulp and Paper manufacture 1989, P162-164.
2. Vikari, 1., Kantelinen, A., Sundquist, J., and Linko, M., FEMS Microbioi, Rew, 1995, 13, P335-350.
3. Yang.J.L., Gu Lou, Eriksson. K.L., Tappi Journal Dec 1992, P95.
4. Jager.A., Sinner.M., Purkarthofer.H., Esterbaur.H., Ditzelmuller.G., Voest Alpine Industrieanlagenbau GmbH, Austria.
5. Skerker.P.S. Farell. R.L., Chang. H.m. Repligen Sandoz Research Corporation USA, P93-105.
6. Pedersen.L.S., Elm.D.D., Choma.P.P., NovoNordisk, P107-121.
7. Paice.M.G., Gurnagul.N., Page D.H., Jurasek.L., 78th Annual Meeting. Technical session, CPPA, P A45.