Xylanase Pretreatment of Mechanically Destructured Chips of *Eucalyptus tereticornis* and its Effect on Kraft Pulping

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Mechanical pulping of raw wood material is a highly energy intensive and pollution generating step in the papermaking process. This study focused on combined mechanical and xylanase treatment prior to the kraft pulping of *E. tereticornis.* A screened pulp yield of 49.1% (on oven-dry wood basis) with a Kappa number of 24.9 was obtained at the optimum cooking temperature of 160 °C without any pretreatment of the wood chips. After mechanical treatment (destructuring), a slightly higher screened pulp yield (49.4%) was obtained with a Kappa number of 24.2 at the cooking temperature of 145 °C with the same active alkali charge (15%). The optimum cooking temperature was further reduced to 140 °C for the destructured xylanase-treated wood chips. The xylanase treatment resulted in a 2% reduction in screened pulp yield due to hydrolysis of xylan. However, the Kappa number was reduced to 18.2 after xylanase pretreatment of the mechanically destructured wood chips. The combined pretreatment (destructured and xylanase treatment) of wood chips resulted in a reduction in cooking temperature by 20°C compared to untreated wood chips. Such a reduction in cooking temperature can effectively reduce steam consumption. The combined pretreatment improved the pulp brightness by 2.0 (ISO points) and physical strength properties, which included the tensile index, tear index, and burst index by 11.06%, 21.72%, and 21.79%, respectively, compared to the control.

Keywords: Eucalyptus tereticornis; Xylanase pretreatment; Chemical pulping; Biopulping; Impressafiner; Kraft pulping

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INTRODUCTION

The pulp and paper industry is the 6th largest pollution-generating industry, and it produces solid, liquid, and gaseous wastes (Söderholm *et al.* 2019). In addition, it is the 4th most energy-intensive manufacturing industry. Pulping, bleaching, and papermaking are the three basic steps in the paper manufacturing process (Ezeudu *et al.* 2019; Sharma *et al.* 2020; Gautam *et al.* 2021). The pulping process involves the cleavage of lignocellulosic linkages in the woody structure to separate cellulosic fibers by dissolving (chemical pulping) or breaking (mechanical pulping) the middle lamella so that the individual fibers can be utilized for papermaking. During chemical pulping, raw materials are cooked with the appropriate chemicals in aqueous solution at high temperature and pressure. Chemical pulping consumes high amounts of energy and chemicals, and it is considered one of the main sources of pollution in the pulp and paper industry (Kumar *et al.* 2020).

Environmental regulations have pushed the industry to develop green alternatives for pulp and paper processing. The fungal pre-treatment of wood chips prior to mechanical or chemical pulping is known as biopulping. White-rot fungus has been shown to degrade the lignin selectively in wood after colonization, thereby leaving cellulose relatively intact. Biopulping is an energy-efficient and environmentally-friendly approach to minimize pollution generation and energy consumption during pulping. The biopulping process reduces chemical consumption, pulping time, lignin content, and Kappa numbers. Biological pretreatment decreases the energy requirement of pulping and improves the physical strength properties of paper (Bari *et al.* 2021). Fungal treatment also has several limitations. Fungal treatment can decrease the brightness of pulp after a long treatment time. Longer incubation times, which typically range from 2 weeks to 8 weeks, were effective in terms of chemical consumption, pulping time, and lignin content reduction, since it is understood that lignin content tends to be detrimental to industrial application of the fibers. Moreover, effective colonization by white-rot fungi requires the raw materials to be in a state of asepsis (Bajpai 2018; Kumar *et al.* 2020). If enzyme is used as a pretreatment agent, such limitations of biopulping can be avoided. Recently, several studies have reported the effect of xylanase pretreatment on chemical pulping (Varghese *et al.* 2020a,2020b; Akgül *et al.* 2021). Xylanase pretreatment facilitates the separation of fibrils in the initial stage of fibrillation *via* the removal of easily accessible xylan, which is mainly located among micrometer-sized cellulose fibrils (Zhou *et al.* 2019; Akgül *et al.* 2021; Kumar 2021). The pretreatment of sugarcane bagasse and wheat straw with xylano-pectinolytic enzyme reduced pulping chemical consumption by 12 to 15% during kraft pulping. In addition, the superior physical strength properties were observed due to enzymatic treatment of the substrate (Varghese *et al.* 2020a,b). Akgül *et al.* (2021) evaluated the effect of xylanase pretreatment on the kraft pulping of poplar and reported improved yield and pulp viscosity after xylanase pretreatment. Further, the xylanase pretreatment resulted in remarkable improvement in the tensile index, breaking length, and burst index. The mechanical pre-treatment with Impressafiner resulted in partial disintegration of chips into fragmented chips with cracks and reduced the electrical energy consumption of pre-treated Norway spruce chips to achieve a tensile index of 47 Nm/g by 120 kWh/bdt, *i.e.* 6% (Nelsson *et al.* 2018).

This study evaluated the effect of combined mechanical and xylanase treatment on the kraft pulping of *E. tereticornis* chips. Eucalyptus is an important type of hardwood that is grown in 18 million ha in 90 countries under temperate, tropical, and subtropical climates for industrial and commercial purposes, such as timber and papermaking. Different species of the genus *Eucalyptus* exhibit rapid growth and are cultivated in many countries. *E. tereticornis* is mainly grown in India and was selected for this study (Rockwood *et al.* 2008; Knapic *et al.* 2014; Neiva *et al.* 2015).

EXPERIMENTAL

Collection of Raw Material and Chemical Composition

Eucalyptus tereticornis chips of thickness 4 to 6 mm were collected from ITC Bhadrachalam (Telangana, India). The chips were washed with water and manually chopped into small pieces of approximately 3.0 to 5.0 cm. The chopped chips were dried in sunlight until reaching the desired moisture content and stored in polythene bags for further use. Raw material was milled and the portion passed through –40 size mesh and

retained on +80 was used for chemical composition analysis. Extractives were removed using Soxhlet apparatus and mixture of ethanol-benzene (1:2 v/v) before compositional analysis as per TAPPI test method TAPPI T264 cm-97(1997). Extractive free samples were subjected to α-cellulose (TAPPI T203 cm-99 (1999)), holocellulose (TAPPI T249 cm-85(2009)), and lignin (TAPPI T222 om-02(2006)) determination.

Mechanical Treatment by Impressafiner

The wood chips were destructured by passing them through a wood destructuring machine **(**Impressafiner, Universal Engineering Corporation, Saharanpur, India) (Fig. 1) to reach the size of the spongy structure of about 25x25x4 mm. The wood chips were soaked in tap water overnight. After draining the water, the soaked chips were dewatered in a compression-cum dewatering unit (Universal Engineering Corporation, Saharanpur, India) at 10 RPM and 5800 psi. The unit completely compressed the chips and squeezed out soluble materials and water. The feeding capacity of the machine was 1 to 2 kg. The destructuring of 1kg of wood chips took 5 min and resulted in a spongy material without damaging the fibers. The destructured material was homogenized in a single lot to avoid compositional differences among aliquots and stored for further use.

The Impressafiner compressed and delaminated the chips to open the wood structure before refining, minimize variation in moisture content, maximize the removal of extractives, reduce variation in bulk density, and reduce the energy consumption during subsequent refining. The Impressafiner is designed to compress the chips to a uniform size distribution as they proceeded to the discharge of the compression screw press. The structural changes in the wood chips accelerated and improved the uniformity of heating, thus allowing a reduction in time required to soften the wood. The high compression squeezed out extractives and dissolved organic substances. Special attention had to be given to the retention time and pressure. High pressure and long retention time typically resulted in an increase in pulp strength but a decrease in pulp brightness (undesirable darkening reactions) (Fig. 2) (Sabourin *et al*. 2002).

Fig. 1. Wood destructuring machine (Impressafiner)

Xylanase Pretreatment

The commercial xylanase preparation (Xylanase ex) was purchased from SRL Pvt. Ltd. (Taloja, India). The xylanase activity of the enzyme was determined according to Kumar *et al.* (2016a), using birchwood xylan (SRL Pvt. Ltd., Taloja, India) (Kumar *et al.* 2016a). Pretreatment of the destructured *E. tereticornis* wood samples was performed in

separate conical flasks that contained 100 g (OD basis) of sample. Citrate buffer was added to the raw material (substrate) at a 3:1 ratio to maintain the pH at 5.0. The pretreatment of destructured wood chips was performed with an enzyme dose of 20 IU/g of OD samples and incubated at a temperature of 45 °C for 12h and as such the destructured wood was pretreated without deactivation of enzymes.

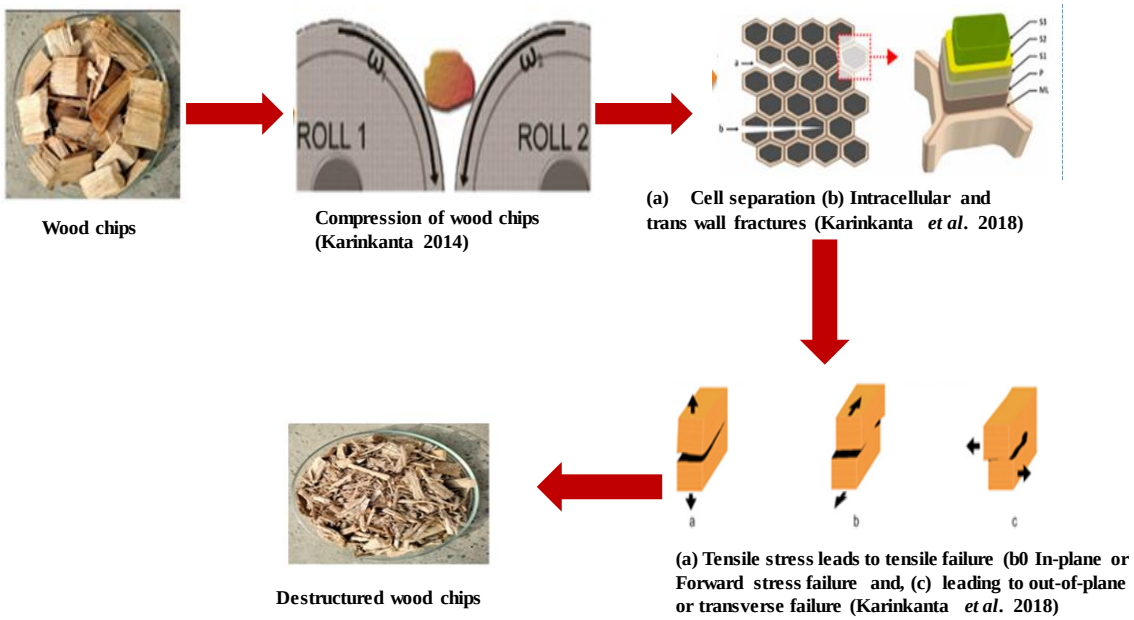

Fig. 2. Working principle of Impressafiner (Karinkanta 2014; Karinkanta *et al.* 2018)

Pulping Studies

Three different forms of *E. tereticornis* wood samples, which included non-destructured, destructured (mechanically treated), and destructured xylanase-treated (DXT) wood samples were cooked *via* the kraft pulping process. They were cooked at an alkali dose of 15% Na_2O in a WEVERK electrically heated rotary digester (Universal Engineering Corporation, Saharanpur, India) of 0.02 m^3 capacity that had four bombs (metal container of 1-L capacity), each at a maximum temperature that ranged from 150 °C to 170 °C. Cooking time ranged from 1.5 to 2 h, and the liquor to raw material (OD basis) ratio was 3:1. Further, the pulps were washed on a laboratory flat stationary screen with a 300-mesh wire bottom to remove the black liquor. Then, the pulp was disintegrated and screened through a WEVERK vibratory flat screen (Universal Engineering Corporation, Saharanpur, India) with a 0.15-mm slot size, and the screened pulp was washed with water, pressed, and crumbled. The pulp samples were analyzed for Kappa number as per TAPPI T236 om-99(1999), screened pulp yield, and screening rejects.

Laboratory Handsheets Preparation and Analysis of Pulp and Paper Properties

After pulping, the unbleached pulp samples were beaten in a PFI mill (Universal Engineering Corporation, Saharanpur, India) at a fixed beating level of 35 °SR. Laboratory handsheets of 60 g/m^2 were prepared using British sheet former (Universal Engineering Corporation, Saharanpur, India) as per TAPPI T205 sp-02(2002). Before determination of the physical strength properties, the handsheets were pre-conditioned at 23±2 °C and

relative humidity of 50±2% as per TAPPI T402 sp-03, 2003). The physical strength properties, which included burst index (TAPPI T403 om-97, 1997), tear index (TAPPI T414 om-04, 2004), tensile index (TAPPI T494 om-01, 2001), and double fold numbers (TAPPI T423 cm-98, 1998)) were analyzed. Thick pads of 4±0.2 g were prepared as per TAPPI T218 sp-02, 2002 and brightness determination according to TAPPI T452 om-02, 2002.

Field Emission-scanning Electron Microscopy (FE-SEM) and Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Pulp Samples

Morphological analyses of the pulp samples were performed by FE-SEM (TESCAN, MIRA 3 LMH, Libusina TR.21, Brno, Czech Republic). Pulp samples were dried before analysis and gold coated *via* a standard sputtering technique (Quorum, Leica EM ACE600, Libusina TR.21, Brno, Czech Republic) for 30 s. Samples were analyzed for morphological changes and images were captured at 15.0 kV at desired magnifications. Fourier transform infrared spectroscopy is a method of calculating structural deviations in the samples due to the chemical treatments. Fourier transform infrared analysis of the non-destructured, destructured (mechanical treatment), and destructured-xylanse pretreated pulp samples was performed using a FTIR (Perkin-Elmer, Singapore Pvt. Ltd., L1600400 Spectrum TWO DTGS, Ayer Rajah, Singapore) spectrophotometer. The samples were oven dried at 105 °C for 4 h to 5 h and mixed with KBr to prepare the pellets. The sample spectra were recorded in transmittance mode at the wavenumber range 4000 to 500 cm^{-1}.

Statistical Analysis

All experiments were carried out in triplicate, and the experimental results were analyzed by Origin Pro 8.5 (OriginLab Corporation, Northampton, MA, USA) software. All the results were recorded as the mean ± standard deviation of values.

RESULTS AND DISCUSSION

Pulping of Non-destructured Wood Chips of *E. tereticornis*

The proximate analysis of *E. tereticornis* showed that it contained 43.2%, 73.2%, and 27.1% of α-cellulose, holocellulose, and lignin content, respectively. According to the rating system designated by Nieschlag *et al*. (1960), cellulosic plants having more than 34% α-cellulose were characterized as promising for pulp and paper making for the chemical composition point of view because α-cellulose had undegraded and high molecular weight cellulose contents in the pulp. On the other hand, β-cellulose was degraded cellulose and γ-cellulose consisted of low molecular weight hemicelluloses. Kraft pulping of non-destructed wood chips was performed at cooking temperatures that ranged from 150 to 170 °C using 15% active alkali (Na_2O), and the other conditions, such as pulping time and bath ratio, remained constant (Table 1). When cooking temperature was increased from 150 to 160 °C and H-factor 290-691, the pulp yield and Kappa number decreased. Beyond a cooking temperature of 160 °C and H-factor 691, Kappa number was decreased slightly, and the screened pulp yield decreased sharply. In addition, the screened pulp yield decreased sharply, which may have been because of the degradation of cellulose due to the peeling reaction mechanism (Pandey *et al.* 2012). Higher temperature results in faster delignification and additional degradation (secondary peeling) of carbohydrates, which decreases the screened pulp yield (Lal *et al*. 2010). The optimum screened pulp yield

of 49.1% with a Kappa number of 24.9 was obtained at a maximum temperature of 160 °C, and other conditions were kept constant. Therefore, 160 °C was judged to be the optimum temperature for the pulping of the non-destructed wood chips of *E. tereticornis*.

Table 1. Pulping of the Non-destructured Wood Chips of *E. tereticornis*

Parameter	H-factor	Pulp Yield (%)	Screen Rejects (%)	Screened Pulp Yield (%)	Kappa No.	Pulping Conditions
Temperature (°C) Variation						
150 ± 2	290	51.1 ± 0.52	2.70 ± 0.11	48.4	33.8 ± 0.32	Time from ambient temperature to 105 °C = 45 min, time from 105 °C to maximum temperature = 60 min, time at maximum temperature = 90 min, bath ratio =1:3, active alkali 15% (Na_2O), sulphidity=25%
155 ± 2	450	50.5 ± 0.31	1.90 ± 0.05	48.6	26.3 ± 0.41	
160 ± 2	**691**	**49.8 ± 0.48**	**0.57 ± 0.08**	**49.1**	**24.9 ± 0.27**	
165 ± 2	1051	48.1 ± 0.40	0.35 ± 0.04	47.5	23.7 ± 0.43	
170± 2	1585	47.3 ± 0.24	0.23 ± 0.03	47.3	21.6 ± 0.21	
Active Alkali (%) Variation						
12		50.9 ± 0.32	2.50 ± 0.10	47.8	30.4 ± 0.32	Time from ambient temperature to 105 °C = 45 min, time from 105 °C to 160 °C = 60 min, time at 160 °C =90 min, bath ratio =1:3, sulphidity=25%, active alkali doses= varied from 12% to 20% (Na_2O)
14		50.1 ± 0.15	1.70 ± 0.12	48.4	26.8 ± 0.28	
15		**49.8 ± 0.48**	**0.57 ± 0.08**	**49.1**	**24.9 ± 0.27**	
16		48.0 ± 0.33	0.25 ± 0.11	47.7	24.6 ± 0.10	
18		46.5 ± 0.37	0.15 ± 0.13	46.4	23.1 ± 0.45	
20		45.1± 0.16	0.10 ± 0.10	45.0	22.4 ± 0.12	

Note: Figures following "±" are standard deviations

Active alkali influences effective delignification during kraft pulping. In another set of experiments, the active alkali doses were varied from 12% to 20% (Na_2O), and the other conditions remained constant. Table 2 showed that the screened pulp yield of *E. tereticornis* wood chips improved as the active alkali increased to 15% (Na_2O), and beyond a 15% active alkali dose, the screened pulp yield started to decline. The pulp Kappa number decreased sharply up to a 15% alkali dose, and beyond 15% the decrease in Kappa number was insignificant. Therefore, an optimum active alkali of 15% gave a reasonable screened pulp yield with the minimum Kappa number and was selected as the optimum dose for the pulping of non-destructured wood chips of *E. tereticornis.* The screened pulp yield and Kappa number of the resultant pulp were controlled by the active alkali dose during kraft pulping (Gautam *et al.* 2016). The kraft pulping process is an alkaline process that utilizes NaOH and Na_2S as active chemicals in the white liquor for lignin degradation and the liberation of fibers. The hydroxide ion (HO^-), hydrogen sulphide ions (HS^-), and carbanions were generated during kraft pulping, and they competed to react with lignin. The delignification rate is substantially higher in kraft pulping than in soda pulping because HS^- ion improves the rate of lignin degradation by specifically promoting the rate of cleavage of β-aryl-ether linkages in the phenolic units of lignin (Ghazy 2016; Brännvall 2017). Santos *et al.* (2015) studied the effect of acid pretreatment of *E. grandis* and *E. urograndis* wood chips prior to kraft pulping and reported several benefits, such as reduction in white liquor charge, increased pulp yield, and increased pulp viscosity and black liquor heating value. The carbohydrate contents were not adversely affected due to the acidic pretreatment (Santos *et al.* 2015).

Pulping of Destructured Wood Chips of *E. tereticornis*

The effect of mechanical treatment by the Impressafiner was studied during the kraft pulping of *E. tereticornis* wood chips. The effect of temperature and active alkali variations were also studied for the destructured wood chips. The screened pulp yield increased as cooking temperature increased from 135 to 145 °C and H-factor from 73 to 185, whereas the Kappa number decreased sharply each time the temperature was measured. Beyond a temperature of 145 °C and H-factor 185, the screened pulp yield decreased significantly (Table 2). Therefore, 145 °C (and H-factor 185) was selected as the optimum temperature to optimize the alkali dose in the next set of experiments. The effect of active alkali doses was studied from 12% to 20% at an interval of 2% alkali dose. The screened pulp yield increased as alkali dose increased until reaching 15%, after which there was a sharp decrease in screened pulp yield. However, the Kappa number decreased sharply as active alkali dose increased from 12% to 20%. Therefore, an active alkali dose of 15% was determined to be optimal for the delignification of destructured wood chips of *E. tereticornis*. The fiber length and width of *E. tereticornis* were 0.82 and 15 µm respectively.

Table 2. Pulping of De-structured Wood Chips of *E. tereticornis*

Parameters	H-factor	Pulp Yield (%)	Screen Rejects (%)	Screened Pulp Yield (%)	Kappa No.	Pulping Conditions
Temperature (°C) Variation						
135 ± 2	73	48.9 ± 0.56	2.10 ± 0.13	46.8	31.7 ± 0.22	Time from ambient temperature to 105 °C = 45 min, time from 105 °C to maximum temperature = 60 min, time at maximum temperature = 90 min, bath ratio =1:3, active alkali 15% (as Na_2O), sulphidity = 25%
140 ± 2	117	50.2 ± 0.46	1.25 ± 0.03	49.0	25.6 ± 0.51	
145 ± 2	**185**	**49.7 ± 0.48**	**0.35 ± 0.06**	**49.4**	**24.4 ± 0.37**	
150 ± 2	290	48.5 ± 0.39	0.25± 0.04	48.2	23.8 ± 0.53	
155± 2	450	47.0 ± 0.27	0.20 ± 0.05	46.8	21.0 ± 0.44	
Active Alkali (%) Variation						
12		49.6 ± 0.46	1.25 ± 0.14	48.4	29.2 ± 0.23	Time from ambient temperature to 105 °C = 45 min, time from 105 °C to 145 °C = 60 min, time at 145 °C = 90 min, bath ratio =1:3, sulphidity = 25%, active alkali doses = varied from 12% to 20% (as Na_2O)
14		49.2 ± 0.50	0.64 ± 0.11	48.7	26.1 ± 0.22	
15		49.4 ± 0.27	0.25 ± 0.17	49.2	24.2 ± 0.38	
16		47.4 ± 0.68	0.15 ± 0.28	47.3	23.2 ± 0.16	
18		46.2 ± 0.55	0.10 ± 0.15	46.10	22.2 ± 0.33	
20		45.8± 0.32	0.10 ± 0.17	45.7	19.2 ± 0.13	

A screened pulp yield of 49.1% with a Kappa number of 24.9 was observed at a cooking temperature of 160 °C during the kraft pulping of non-destructured wood chips. In addition, a similar screened pulp yield (49.4%) and Kappa number (24.4) were observed at a cooking temperature of 145 °C during the kraft pulping of mechanically destructured wood chips. These results indicated saving in electrical energy in terms of cooking temperature reduction of 15 °C for mechanically destructured wood chips. The mechanical treatment broke the compact structure of the wood chips and converted the wood chips into a spongy material. Further, the mechanical treatment opened the compact fibers to improve the accessibility of pulping chemicals to the interior of the fibers (Gupta *et al.* 2013). The improvement in the penetration of white liquor resulted in improved delignification of destructured wood chips at lower temperature.

Pulping of Destructured-xylanase-treated (DXT) Wood Chips of *E. tereticornis*

The effect of the combined mechanical and xylanase treatment of wood chips was studied at different cooking temperatures and active alkali doses. Figure 3 shows an overview of pulping of DXT wood chips of *E. tereticornis*.

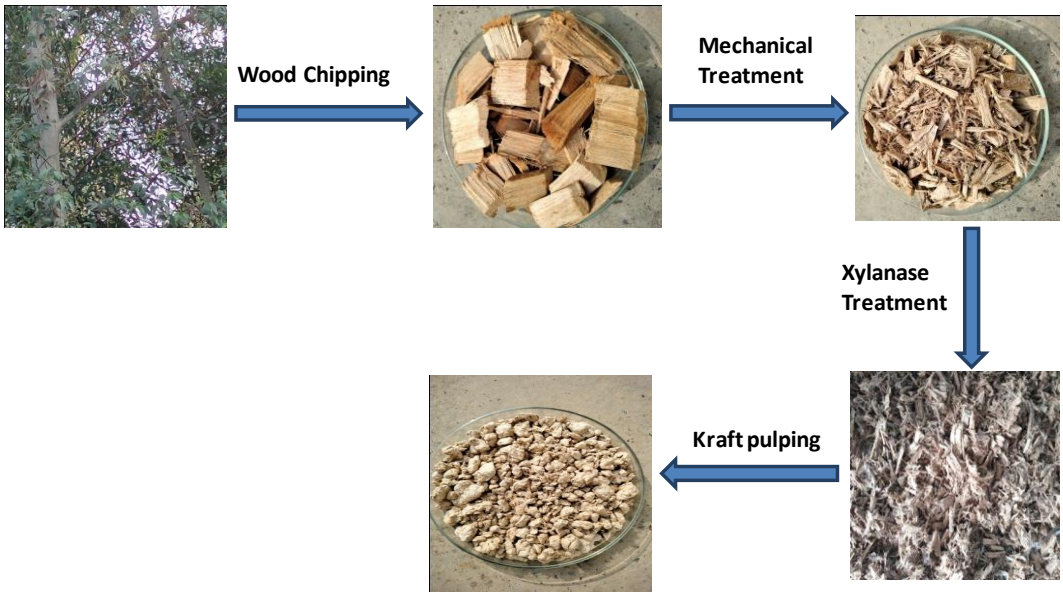

Fig. 3. Process overview for pulping of mechanically DXT wood chips

Table 3. Pulping of DXT Wood Chips of *E. tereticornis*

Parameter	H-factor	Pulp Yield (%)	Screen Rejects (%)	Screened Pulp Yield (%)	Kappa No.	Pulping Conditions
Temperature (°C) Variation						
135 ± 2	73	48.6 ± 0.46	1.88 ± 0.09	46.5	18.8 ± 0.32	Time from ambient temperature to 105 °C = 45 min, time from 105 °C to maximum temperature = 90 min, bath ratio = 1:3, active alkali = 15% (Na_2O), sulphidity = 25%
140 ± 2	117	48.1 ± 0.57	0.69 ± 0.02	47.4	18.2 ± 0.41	
145 ± 2	185	47.2 ± 0.44	0.50 ± 0.05	46.7	16.4 ± 0.57	
150 ± 2	290	46.2 ± 0.39	0.30± 0.03	45.9	15.9 ± 0.23	
155± 2	450	45.1 ± 0.22	0.10 ± 0.06	45.0	13.2 ± 0.64	
Active Alkali (%) Variation (140 °C)						
12		48.8 ± 0.23	1.90 ± 0.12	46.9	24.7 ± 0.33	Time from ambient temperature to 105 °C = 45min, time from 105 °C to 140 °C = 90 min, bath ratio =1:3, temperature = 140 °C, sulphidity=25%,
14		48.5 ± 0.54	1.20 ± 0.10	47.3	21.4 ± 0.32	
15		48.1 ± 0.57	0.69 ± 0.02	47.4	18.2 ± 0.41	
16		47.1 ± 0.55	0.40 ± 0.19	46.7	16.9 ± 0.20	
18		45.5 ± 0.48	0.32 ± 0.13	45.2	14.7 ± 0.19	
20		43.8 ± 0.37	0.10 ± 0.20	43.7	13.4 ± 0.11	

The wood chips were first mechanically destructured and then pretreated with xylanase (20 IU/g of oven-dried substrate) before pulping. The cooking temperature was varied from 135to 145 °C and H-factor from 73 to 185 at an interval of 5 °C. The screened pulp yield increased to 47.4% with a sharp reduction in screening rejects. Further increases in temperature resulted in a significant decrease in both screened pulp yield and Kappa

number (Table 3). Therefore, 140 °C was determined to be the optimum cooking temperature for DXT wood chips of *E. tereticornis.* An active alkali dose of 15% was found most suitable for improving screened pulp yield (47.4%) and reducing the Kappa number (18.2). Active alkali doses beyond 15% reduced the Kappa number and screened pulp yield sharply.

The objective of mechanical and xylanase pretreatment was to make the raw material more suitable for pulping and papermaking. The comparative analysis of the pulping of destructured and DXT wood chips showed that xylanase pretreatment decreased the screened pulp yield from 49.4% to 47.4%. In addition, the xylanase pretreatment resulted in a Kappa number reduction by 26.9% relative to the control (without any pretreatment). The cooking temperature was also lowered by 5 °C after xylanase pretreatment. If the cooking temperature were lowered by 5 °C, it would reduce the energy requirement during pulping. The compact structure of wood chips was broken during mechanical treatment, and the wood chips were converted into spongy material. The mechanical treatment loosened the compact structure of the fibers and improved the accessibility of xylanase to the fibers (Gupta *et al.* 2013). The decreased screened pulp yield and reduced Kappa number indicated that there was better xylanase penetration, and it acted on the xylan. The xylanase treatment hydrolyzed the xylan, broke down the lignin-carbohydrate bonds, and opened the polymer to facilitate the removal of lignin by white liquor during pulping and decreased the Kappa number of resultant pulp (Akgül *et al.* 2021; Gautam *et al.* 2021). Varghese *et al.* (2020b) performed a xylano-pectinolytic enzyme pretreatment of sugarcane bagasse before soda-anthraquinone pulping. The maximum effect of the enzyme treatment was reported with an enzyme dose of xylanase (175 IU/g bagasse) and pectinase (75 IU/g bagasse) and treatment duration of 180 min. The enzyme pretreatment reduced the Kappa number 49.1% compared to the control. Similarly, the enzyme treatment also reduced the requirement of active alkali by 15% to attain a Kappa number similar to that obtained with 100% active alkali (Varghese *et al.*2020b). In another study, the pretreatment of wheat straw was performed with a crude xylano-pectinolytic concoction with a xylanase dose of 400 IU/g wheat straw and a pectinase dose of 120 IU/g wheat straw at 55 °C for 3 h of treatment (Varghese *et al.* 2020a). The enzymatic pretreatment resulted in a 15.67%reduction in Kappa number, and the pulp brightness was improved 16.04%. Moreover, the pulping chemical requirement decreased 12% relative to the control (without enzyme treatment) (Varghese *et al.* 2020a).

Effect of Mechanical and Xylanase Pretreatment on Strength Properties

The maximum pulp brightness (34% ISO) was observed for DXT wood chips rather than non-destructured or destructured wood chips. The higher brightness of pulp from DXT was attributed to increased removal of lignin-carbohydrate complexes due to xylanase pretreatment. The kraft pulp produced by the 3 different treatments was analyzed for physical strength properties at a fixed beating level of 35±2 °SR. The xylanase pretreatment improved the tensile index of the pulp by 11.06% and 6.74% compared to non-destructured and destructured wood chips, respectively. Similarly, destructured-xylanase treatment caused slight improvements in the tear and burst indexes compared to non-destructured and destructured wood chips (Table 4). The xylanase hydrolyzed the xylan, caused the separation of the secondary wall, and increased the surface area by fibrillation, which improved the fiber-to-fiber bonding. The improved fiber-to-fiber bonding may have improved the physical strength properties (Akgül *et al.* 2021; Kumar *et al.* 2021). The FE-SEM analysis of the pulp samples also indicated that there was improved fiber bonding for

the destructured-xylanase treated pulp samples (Fig. 3). Akgül *et al.* (2021) performed xylanase pretreatment before the Kraft pulping of poplar and reported 28.18% and 25.27% improvements in tensile index and burst index, respectively, compared to the control (without xylanase pretreatment). Varghese *et al.* (2020b) pretreated sugarcane bagasse with xylano-pectinolytic enzyme before chemical pulping and found 13.55%, 40.21%, 19.04%, and 42.5% improvements in breaking length, burst index, tear index, and double fold numbers, respectively.

Table 4. Comparison of Strength and Optical Properties of Pulp from Non-destructured, Destructured, and DXT Wood Chips

Type of Treatment	Tensile Index (Nm/g)	Tear Index ($mN*m^2/g$)	Burst Index ($kPa\ m^2/g$)	ISO Brightness (%)	Bulk (cm^3/g)	Double Fold (Numbers)
Non-destructured	55.42 ± 2.3	5.57 ± 0.21	3.12 ± 0.11	32± 0.3	1.66 ±0.03	340 ± 4
Destructured	57.66 ± 2.8	6.22± 0.35	3.74 ± 0.20	32± 0.4	1.65 ± 0.05	360 ± 7
DXT	61.55± 3.1	6.78± 0.32	3.80 ± .13	34± 0.3	1.76 ± 0.02	370 ± 6

Morphological Analysis of Pulp

Analysis of the FE-SEM images showed that the separation of fibers was improved for pulp obtained from mechanically destructured wood chips compared to non-destuctured wood chips (Fig.4a-b).

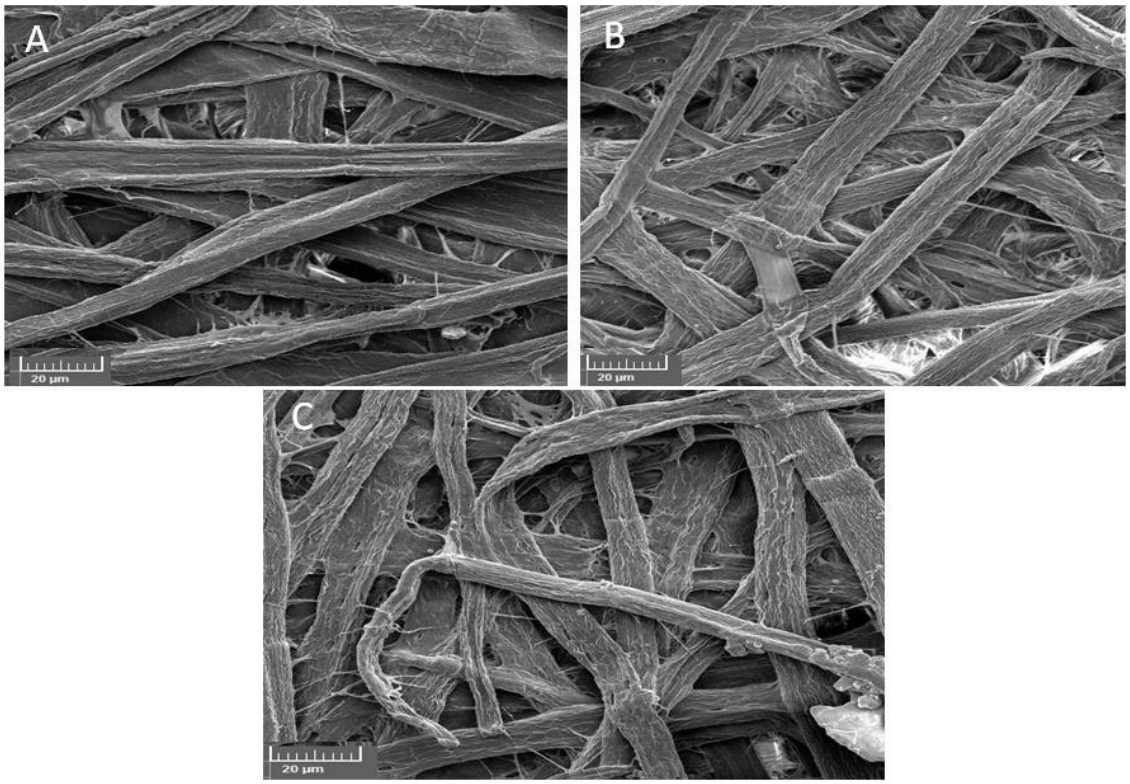

Fig. 4. Morphological analysis of pulp: (A) non-destructured pulp fiber (1.0 kx); (B) destructured (mechanical) pulp fiber (1.0 kx); (C) DXT pulp fiber (1.0 kx)

The DXT samples exhibited increased swelling, peeling, and loosening of pulp fibers. The fiber roughness increased for the xylanase treated pulp samples. The FE-SEM analysis showed prominent external fibrillation in xylanase pretreated pulp samples, which was supported by the improved physical strength properties (Fig. 3c). The changes in surface structure indicated that xylan hydrolysis occurred (Nagar *et al.* 2013).

FTIR Analysis

Figure 5 shows the FTIR spectra of the pulp samples from the three treatments. The band at 2893 to 2900 cm^{-1} was attributed to the C-H stretching of the methyl/methylene groups of cellulose and hemicelluloses for pulp from non-destructured wood chips. The band shifted to 2912 cm^{-1} for mechanically destructured wood chips. The shifting effect was more pronounced after the xylanase treatment of destructured wood chips and reached approximately 2926 cm^{-1}. This may have been due to the change in the environment around the C-H bond due to removal of hemicelluloses and lignin during treatments (Chen *et al.* 2015; Dowarah *et al.* 2020). The band at 1512 cm^{-1} was assigned to the vibration of the aromatic ring in lignin. This band disappeared in the pulp obtained from the mechanical and xylanase treatments due to removal of lignin (Kumar *et al.* 2016b).

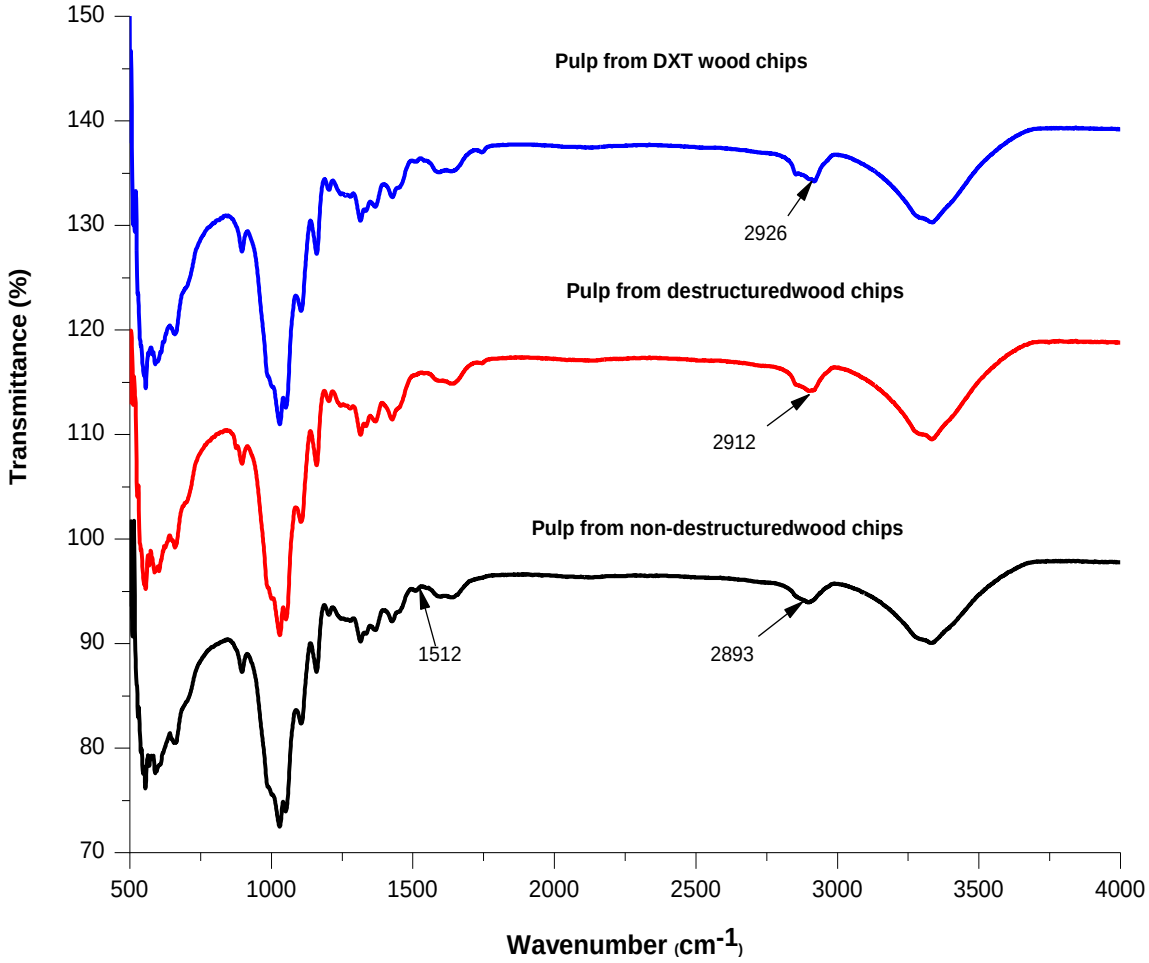

Fig. 5. The FTIR spectra of non-destructured, destructured, and DXT pulp samples

CONCLUSIONS

1. The combined mechanical and xylanase pretreatment effectively reduced energy consumption during the kraft pulping of *E. tereticornis*.
2. The combined pretreatment reduced the cooking temperature 20 °C and H-factor 546 compared to control (without any pretreatment) and reduced the Kappa number of the resultant pulp 26.9%.
3. The reduction in temperature could save steam during kraft pulping.
4. The combined pretreatment improved the physical strength properties of the resultant pulp compared to the control.

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