

Xylanolytic enzyme consortia from *Bacillus* sp. NIORKP76 for improved biobleaching of kraft pulp

Pankaj Parab^{1,2}, Rakhee Khandeparker^{1*}

¹Biological Oceanography Division, CSIR-National Institute of Oceanography,
Dona Paula 403004, Goa, INDIA

²Department of Microbiology, Goa University, Taleigao Plateau, 403206, Goa, INDIA

*Corresponding author Dr. Rakhee Khandeparker

Phone No- 0832-2540540 Fax- 0832-2450602

E-mail: rakhee@nio.org, rakhee.khandeparker@gmail.com

Abstract

A cellulase free xylanolytic enzyme consortia consisting of a xylanase, arabinofuranosidase, and acetyl xylan esterase produced by *Bacillus* sp. NIORKP76 isolate under solid-state fermentation was assessed for its bio-bleaching ability on kraft pulp. In the biobleaching analysis, the xylanase dose of 5 U g⁻¹ dry pulp denoted the optimum bleaching of pulp at 40°C and pH 8.0 after 2 h of treatment. The reduction in kappa number of pre-treated hardwood pulp using xylanolytic enzyme consortium (XEC) was found to be ~55%, while solo xylanase could reduce the kappa number to 44-46%. In the case of chemical bagasse pulp, a reduction of ~27.5% and 19-20% was seen in kappa number using XEC and solo xylanase, respectively. Enzyme treated pulp (HW and CB) showed a 50% reduction in hypochlorite consumption during the chlorine treatment. The current study results reveal the significant potential of xylanolytic enzyme consortium from *Bacillus* sp. NIORKP76 on the environmentally friendly bio-bleaching process.

Keywords: *Bacillus* sp., bio-bleaching, xylanolytic enzymes, kappa number, brightness, viscosity

Introduction

The pulp and paper industry which is considered to be one of India's rapid emerging business sector, has shown remarkable development in the last few years. Government policies are building enormous pressure on paper industries so as to maintain a pollution-free and clean environment [1]. Pulp bleaching irrespective of their origin use large amounts of chlorinated compounds. The byproducts formed while chemical processing are mutagenic, toxic, and persistent and thus are the reason for numerous problems in the biological systems. In view of this pressure the pulp and paper mills/industries are trying to change chlorine-based chemicals and move towards environment friendly molecules [2]. Environment friendly bleaching enzymes mainly xylanases and laccases have great potential for bio-bleaching of agro-based pulps at an industrial scale, due to this, the interest in xylan degrading enzymes have developed extensively over the past few years.

Viikari et al., [3] first introduced the process where biological delignification of pulp was attempted using enzymes. Xylanase, which is a hydrolytic enzyme, is mainly used to breakdown the lignin-carbohydrate complex, which acts as a physical barrier during chemical bleaching. Pre-bleaching of kraft pulp using xylanase was reported to minimize the amount of chlorine required for bleaching and thus dipping chloro-organic discharges [3, 4]. There are several reports on the application of xylanases for pre-treatment of unbleached pulp, which proved to be efficient to reduce the use of chlorinated compounds in the subsequent bleaching process [5-10].

Due to the heterogeneity of hemicellulose, its hydrolysis requires the action of a complex enzyme system. This usually composed of β -1,4-endoxylanase, β -xylosidase, α -L-arabinofuranosidase, α -glucuronidase, acetyl xylan esterase, and phenolic acid esterases. All these enzymes act cooperatively to convert xylan to its constituent sugar. The presence of such multifunctional xylanolytic enzyme systems is quite widespread among bacteria and fungi [11-20]. The combined effect of hemicellulases on different pulp woods are published [21]. It was noticed that synergism does exist between the two types of endoxylanases reported from *Streptomyces* sp., which resulted in the enhancement of brightness in softwood [21].

However, the bioleaching process needs further development; different xylanolytic enzymes and their synergistic effect need to be studied. With this objective, the present study is mainly focused on the application of cellulase-free xylanolytic enzyme consortia (xylanase, arabinofuranosidase, and acetyl xylan esterase) isolated from *Bacillus* sp. NIORKP76 strain on pre-treatment of unbleached pulp samples (Hardwood pulp and Chemical bagasse pulp). As per our understanding, this is one among the few reports where the effect of sole xylanase and consortia of xylanolytic enzymes on

bio-bleaching of pulp is shown. This report is unique because the entire xylanolytic enzyme consortia is isolated from a single bacterial strain and used for bio-bleaching of different unbleached pulp samples.

Materials and methods

Microorganism, Growth conditions and enzymatic assays.

Xylanolytic enzymes used for this study were isolated from *Bacillus* sp. NIORKP76 strain (GenBank accession number: MH767158) [23]. The bacterial isolated was obtained from mangrove sediments of Goa. The xylanolytic enzyme consortium (*B*-XEC) production was carried out under solid-state fermentation (SSF) using modified basal salt solution (MBSS) [7] supplemented with 0.2% yeast extract and 0.03% peptone and wheat bran (substrate to moisture ratio 1:3) as a substrate. Xylanase enzyme (*B*-X) was produced under submerged fermentation (SmF) by replacing carbon source with 0.5% xylan.

The detailed composition of the MBSS medium (w/v) was as follows: NaCl, 30.00 g; MgSO₄, 7.00 g; NH₄Cl, 0.5 g; KCl, 0.75 g; KH₂PO₄ (10 %), 7.00 mL, K₂HPO₄ (10 %), 3.00 mL; trace metal solution, 1 mL; distilled water, 1 liter. The composition of trace metal solution is as follows: H₃BO₃, 2.85 g; FeSO₄.7H₂O, 2.49 g; MnCl₂.7H₂O, 1.80 g; CuCl₂, 0.03 g; Na-tartrate, 1.77 g; ZnCl₂, 0.02 g; Na₂MoO₄.2H₂O, 0.02 g; CoCl₂, 0.03 g; distilled water, 1 liter. Commercially available xylanase (CM-X) was purchased from Sigma-Aldrich, USA (*Trichoderma longibrachiatum*, X2629).

Xylanase, arabinofuranosidase (AFase), and acetyl xylan esterase (AXE) assays were performed using 1% xylan, 2.5 mM *p*-nitrophenyl- α -l-arabinofuranoside, [7] and 50 mM *p*-nitrophenyl acetate [24] respectively.

Optimization of xylanolytic enzyme pre-treatment conditions on kraft pulp

The enzymatic treatment studies were carried out on kraft pulp using a crude xylanolytic enzyme from *Bacillus* sp. NIORKP76 strain and commercial xylanase at Biological Oceanography Division of National Institute of Oceanography, Goa, India. Xylanase activity 2.5U, AFase activity 0.67U, and AXE activity 0.45U were measured in one milliliter of a crude xylanolytic enzyme mixture. The enzyme activities were determined before carrying out experimental work. Two different pulp samples, hardwood pulp (HW pulp) and chemical bagasse pulp (CB pulp) provided by Tamilnadu Newsprint and Papers Limited (TNPL), Tamilnadu, India, was used in this study. Unbleached pulp samples were washed thoroughly with tap water, oven-dried, and used for further studies.

Influence of xylanolytic enzyme dose

The effect of varying xylanolytic enzyme dosages was studied by treating oven-dried pulp samples (HW and CB) with different doses of xylanolytic enzymes. The xylanolytic enzyme dosages were varied by changing the volume of crude *B*-XEC. The enzyme doses ranging between 2.5 U to 25 U g⁻¹ pulp of xylanase, 0.67 U to 6.7 U g⁻¹ pulp of AFase, and 0.45 U to 4.5 U g⁻¹ pulp of AXE were used. The pulp samples were mixed with suitably diluted *B*-XEC and sodium phosphate buffer (pH 8.0) with 5% consistency [14] in zip lock polyethylene bags and incubated in a water bath maintained at a constant temperature of 40°C for 2h. Pulp sample was prepared and treated using the same physio-chemical parameters in the absence of any xylanolytic enzyme used as control. At the end of the incubation time, the pulp samples were washed with distilled water, filtered through a mesh sieve no. 200 [25] and filtrate was collected.

Influence of incubation period

To study the influence of the incubation period on bio-bleaching, the reaction mixture containing an optimized dose of *B*-XEC and pulp sample prepared in sodium phosphate buffer (pH 8.0) with 5% consistency was incubated in a water bath maintained at a constant incubation temperature of 40°C for varying time intervals (0-6h). At the end of a given time interval, pulp samples were washed with an equal volume of distilled water, filtered through a mesh sieve no. 200 [25] and filtrate was collected.

Influence of pulp consistency

The effect of pulp consistency on xylanolytic enzyme bleaching was studied by varying pulp concentration. Pulp samples with 2.5% to 10% consistency were prepared in sodium phosphate buffer (pH 8.0). Optimized *B*-XEC dose and the optimized incubation period were used to treat the enzymatic treatment of unbleached pulp samples under constant incubation temperature of 40°C. Following the incubation period, pulp samples were washed with an equal volume of distilled water, filtered through a mesh sieve no. 200 [25] and filtrate was collected.

Pulp properties

Kappa number is used to determine the lignin content in pulp. Kappa number of pulp was measured as per Technical Association of Pulp and Paper Industry (TAPPI) Test Methods T236 cm-85. Kappa number is defined as the volume of (mL) 0.1 N KMnO₄ solution consumed by 1 g moisture-free pulp under the conditions specified. The analysis of brightness and viscosity was carried out at Paper and Pulp Research Institute (PAPRI), Rayagada, Odisha-India. The brightness of pulp samples was determined as per the ISO 2470 method (L & W Elrepho, model-SE070, Kista-

Sweden). Viscosity was measured by standard TAPPI test method T230. The relative degree of delignification of enzyme pre-treated pulp samples and control pulp sample was determined using standard TAPPI protocols [7].

Analysis of pulp filtrates

The absorbance of the filtrates collected in the above experiments was measured spectrophotometrically at a wavelength ranging from 200 nm to 465 nm to determine the release of phenolic and hydrophobic compounds [7]. The reducing sugar released in the filtrate was measured using dinitrosalicylic acid method [26].

Efficacy of xylanolytic enzyme consortium

The efficiency of enzymatic pre-treatment for bio-bleaching of unbleached pulp samples (HW and CB) was examined by treating pulp samples with crude xylanolytic enzyme consortium, crude xylanase (free from other xylanolytic enzymes) isolated from *Bacillus* sp. NIORKP76 and commercially available xylanase (Sigma-Aldrich, USA). The optimized *B*-XEC dose containing 5 Ug⁻¹ pulp of xylanase, 1.34 Ug⁻¹ pulp of AFase, and 0.90 Ug⁻¹ pulp of AXE was used. Equivalent dose (5 Ug⁻¹ pulp) of commercial xylanase (CM-X) and *Bacillus* sp. NIORKP76 xylanase (*B*-X) was used for the treatment of pulp samples. The pulp samples were mixed thoroughly with optimized enzyme dose and sodium phosphate buffer (pH 8.0) at a 5% consistency in a polyethylene zip lock bag and plunged in a water bath, maintaining a constant temperature of 40°C for an incubation period of 2h. After enzyme pre-treatment, pulp samples were washed thoroughly with distilled water, and the filtrate was collected to carry out analysis as per the protocol mentioned above. Controls were prepared similarly, devoid of the enzyme. The pulp obtained was air-dried and stored for further analysis.

Chemical bleaching

An air-dried kraft pulp samples pre-treated with xylanolytic enzymes were used for hypochlorite and followed by hydrogen peroxide treatment. For hypochlorite treatment, the pulp samples were made to 5% consistency and divided into three equal parts. Hypochlorite solution of 8%, 4%, and 2% concentration was prepared, and each portion of the pulp sample was treated with the respective solution and incubated at 40°C for 1h [7]. At the end of the first chemical treatment step, pulp samples were washed and air-dried. All pulp samples obtained after hypochlorite treatment were treated with 1% hydrogen peroxide in a consecutive step. The pulp consistency, incubation period, and temperature were the same as used for the previous step. The pulp samples were washed with

distilled water and air-dried. Kappa number, brightness, and viscosity of bleached pulp samples obtained at the end of both chemical treatment stages were determined.

Result and Discussion

Optimization of bio-bleaching parameters

Optimization of bio-bleaching conditions is an essential step to maximize lignin removal and enhances pulp characteristics. The target properties were the kappa number, brightness and viscosity of the pulp.

The xylanolytic enzyme consortia extracted from *Bacillus* sp. NIORKP76 was optimized for pre-bleaching parameters such as enzyme dose, incubation period, and pulp consistency. The different parameters were optimized separately to improve the efficacy of enzyme treatment. Enzymatic biobleaching reported by Nair et al. [8], Khandeparker et al. [7] and Sridevi et al. [10] also provided importance of pertaining to use optimization parameters prior to enzymatic pre-treatment of pulp. The original kappa number of hardwood and chemical bagasse pulp was 21.51 and 14.13 and brightness was 41.3 and 53.1 (% ISO), respectively. As the kappa number is an estimation of the lignin content in pulp, it is evident that hardwood pulp has more lignin compared to chemical bagasse. The increase in delignification usually results in increased brightness as the brightness is directly linked to the residual lignin in the pulp [27]. The pulp brightness has been reported to have a linear relationship with the kappa number [28, 29]. Brightness of paper have a strong effect on the print quality, more the brightness better is the quality thus kappa number and brightness play important role in pulp properties [30].

Optimization enzyme dose

The xylanolytic enzymes consortia comprising 5 U g^{-1} of xylanase (1.34 U g^{-1} and 0.9 U g^{-1} of AFase and AXE, respectively) was most efficient for bio-bleaching pulp sample (HW and CB) when incubated for 2h at 40°C with 5% pulp consistency.

It was seen that the kappa number (κ) was reduced to 54.34%, and a 4-5-fold increase in the release of reducing sugar was observed for the HW pulp sample. Similarly, a 26.89% reduction in κ number and increase in the release of reducing sugar by 7-8-fold was measured for CB pulp sample at 5U of xylanase dose. The release of phenolic (237nm) and hydrophobic (465nm) compounds was also found to be elevated in both types of pulp samples with the reaction conditions above-mentioned (**Fig. 1a, b**). The decrease in κ number and the simultaneous increase in the release of reducing sugar, phenolic, and hydrophobic compounds reveal the delignification process by *B-XEC*. It was seen that *B-XEC* could delignify HW pulp better than CB pulp. We know that the original kappa

number of HW pulp was high, treatment with *B*-XEC brought the HW pulp at equivalent level to CB pulp in term of lignin content. The effect of xylanolytic enzyme dose on pulp bio-bleaching was highly significant, which is evident from a one-way analysis of variance (ANOVA) ($p < 0.05$).

Optimization of the incubation period

The effect of time concerning enzyme treatment on pulp was investigated using an optimized enzyme dose. After 1h of the incubation period, HW and CB pulp samples showed 30.8% and 3.9% reduction in κ number, respectively. A significant decrease in κ number was seen after 2h of the incubation period. A steep reduction in κ number of 54.43% and 27.10% in HW and CB pulp, respectively, was observed. Similarly, the maximum release of reducing sugar, phenolic, and hydrophobic compounds were observed at a 2h incubation period for both types of pulp samples (**Fig. 2a, b**). It was also observed that the incubation time did not show further reduction in κ number with further increase in time. The effect of incubation time on bio-bleaching of both types of pulp was significant and evident from one way ANOVA ($p < 0.05$).

Optimization of pulp consistency

Pulp consistency optimization is a must to attain proper and effective spread of the enzyme so as to improve the enzyme treatment efficiency. During enzymatic prebleaching experiment, pulp consistency was varied from 2.5 to 10 %. Pulp consistency of 2.5% with 5U enzyme dose and at 2h incubation time could deliver up to 33.5% and 8.28% reduction in κ number for HW and CB, respectively. A maximum reduction in κ number was observed when the pulp consistency of 5% was subjected to the pre bleaching process by keeping treatment conditions at an optimized level. The reduction in κ number was 54.67% for hardwood pulp, whereas it was 27.24% for chemical bagasse pulp. Pulp consistency higher than 5% did not contribute to much reduction in κ number (**Fig. 3a, 3b**). The $p < 0.05$ from one-way ANOVA reveals the significant effect of pulp consistency on pulp properties such as κ number, the release of phenolic, hydrophobic compounds, and release of reducing sugars.

Color removal from the kraft pulp

The release of color from the pulp treated with xylanase (*B*-X and *CM*-X) as well as xylanolytic enzyme consortia (*B*-XEC) was studied using UV spectrum analysis. Enzymatically pre-treated pulp filtrate displayed an increase in absorbance and peak at 280 nm, which confirms the release of lignin from the pulp [31]. The correlation between the nature of chromophore released and enzyme is described in the literature [7, 32, 33]. Xylanolytic consortia from *Bacillus* sp. NIORKP76 showed the absorption of $\cong 0.5$ when treated with 5 Ug^{-1} of oven-dried hardwood and softwood pulp (**Fig. 4a, b**).

While commercial xylanase as well as single xylanase *Bacillus* sp. NIORKP76 from showed absorption of $\cong 0.3$. High absorbance at 280 when XEC was used compared to XE gives a clear indication of the effectiveness of XEC during pre-treatment. The xylanase enzyme from *Arthrobacter* sp. showed absorption of 1.5 when treated with 20 Ug^{-1} of oven-dried pulp [7], the absorption of 0.8 was recorded by Kulkarni and Rao, [32] using 20 Ug^{-1} of oven-dried pulp, while the absorption of 0.4-0.5 was noted when Dwivedi et al., [34] used 16 Ug^{-1} of oven-dried pulp.

Bio-bleaching of kraft pulp

The present work demonstrated that an improved delignification could be achieved by using xylanolytic enzyme consortia when compared to xylanase alone. In enzymatic prebleaching of hardwood and chemical bagasse pulp, *B-XEC* delivered excellent results in terms of κ number reduction compared to *B-X*, which was partially purified xylanase from *Bacillus* sp. NIORKP76 and *CM-X*, which is commercial xylanase. In the case of HW pulp samples, *B-XEC* treatment could reduce κ number by 55%, while 44.6% and 45.8% reduction in κ number were seen by *B-X* and *CM-X* treatment, respectively (**Fig. 5a**). In the case of CB pulp, enzyme pre-treatment showed a reduction in κ number as 27.53%, 20.6%, and 19.0% by *B-XEC*, *B-X*, and *CM-X*, respectively (**Fig. 5b**) (**Table 1**). Along with a reduction in κ number, the release of phenolic compounds, hydrophobic compounds and reducing sugars were highest in *B-XEC* treatment (**Fig. 5a, b**). The enzymatic biobleaching showed the highest reduction in κ number with an increase in brightness index, and without much alteration in viscosity by using xylanolytic consortia compared to xylanase alone, observed differences were highly significant as evident from one-way ANOVA analysis ($p < 0.05$). Kappa number was negatively correlated to brightness for HW as well as CB pulp ($r = -0.9803$; $p < 0.01$, and $r = -0.9870$; $p < 0.01$) respectively (Supplementary Fig. 1)

Biobleaching of eucalyptus pulp have been reported to reduce the kappa number by 3.0 and 3.3 units by using xylanase doses of 2 and 4 Ug^{-1} dried pulp after 1h treatment respectively, thus suggesting that *P. janczewskii* xylanases can be a promising candidate for biobleaching of kraft pulp [35] which is around 20 % reduction in kappa number. Enzymatic prebleaching of kraft pulp reported by Khandeparker and Bhosle, [7] also showed a 20% decrease in kappa number of the kraft pulp without compromising with viscosity. As per their claim, 29% reduction in chlorine requirement can be reduced by enzymatic treatment without any decrease in brightness. The biobleaching efficiency of xylanase obtained from *Streptomyces* sp. QG-11-3 was maximum after 2 h of incubation using a xylanase dose of 3.5 Ug^{-1} , at 50°C and at 6% pulp consistency. The reduction in kappa number was found to be 23% [2]. Sridevi et al., [36] reported reduction in kappa number by 4.2 units, and increase in brightness by 4 units as compared to untreated pulp samples when xylanase

produced by *Trichoderma asperellum* with enzyme dose of 100 U g^{-1} and 1h incubation period was used for pre-treatment. Xylanase from *Aspergillus niger* with optimum dose of 60 U g^{-1} , 1h incubation period and pulp consistency 3% enabled reduction in kappa number by 3.5 units and increase in brightness by 3.1 units as compared to untreated pulp sample [10].

There are reports in literature which describes about use of enzymes other than xylanolytic enzymes for bio bleaching purpose. Bhoria et al., [37] reported production and application of mannanase for enzymatic pre-treatment of wheat straw-rich soda pulp samples. In this report with optimum enzyme dose of 5 U g^{-1} and time interval of 4h showed increase in brightness of 8.7% ISO, and reduction in kappa number by 16% with 6% overall reduction in chlorine consumption.

Report by Gupta et al., [38] describes use of enzyme consortia (xylanase and laccase) obtained from two different bacterial species grown with co-culturing technique. Using eucalyptus kraft pulp mixture of enzyme pre-treatment raised brightness and reduced kappa number by 5% and 9.5% respectively which after final chemical bleaching stage improved to 13% and 15%.

Recently, Angural et al., [39] used a mixture of xylanase, laccase, and mannanase enzymes for pulp biobleaching on mixed wood pulp and got a 46.32/40.25% reduction in kappa number while 13.21/10.01% improvement in brightness. Xylanolytic consortia reported in the present study could reduce the kappa number of hardwood pulp by 55%, making it a suitable candidate for its application in pulp and paper mill.

Apart from microbial origin there are reports in which metagenomic approach for novel xylanase is used. Chawannapak et al., [9], reported metagenomic endoxylanase, with optimum enzyme dose of 50 U g^{-1} used for bio bleaching which was able to fetch 4.5-5.1% ISO increase in brightness.

Chemical bleaching of pre-treated pulp

To evaluate the potential of *B-XEC*, *B-X*, and *CM-X* in reducing chlorine consumption, enzyme pre-treated pulp was treated with sodium hypochlorite followed by H₂O₂. Results showed *B-XEC* significantly reduced hypochlorite consumption without compromising the brightness and viscosity compared to control after processing the pulp sample.

Pulp samples (HW and CB) pre-treated with *B-XEC* resulted in a 50% reduction in hypochlorite consumption, by keeping the brightness level same as that of control (**Fig. 6a, b, 7a, b**). Pulp samples pre-treated with xylanolytic enzyme consortia utilized 2% hypochlorite and proffered brightness and reduction in kappa number results superior to that of control pulp sample bleached with 4% hypochlorite solution (**Table 2**). The xylanolytic enzyme pre-treatment was more prominently effective against HW pulp as compared to CB pulp samples. When compared with kappa number of untreated kraft pulp, solo xylanase (*B-X* and *CM-X*) pre-treatment followed by 2%

hypochlorite treatment lead to 84-85.5% reduction in kappa number. The reduction in kappa number was ~87% when same procedure was followed using *B-XEC*. The control pulp sample treated with 4% hypochlorite displayed only 81.77% reduction in kappa number. In case of CB pulp sample highest reduction in kappa number was recorded for pulp samples pre-treated using *B-XEC* followed bleaching using 2% hypochlorite which was ~89%, whereas, control pulp sample bleached using 4% hypochlorite harnessed only 86%. Further, hypochlorite treated pulp was subjected to H₂O₂, which increased the brightness index with a mild reduction in κ number of pulp samples without affecting viscosity values (**Fig. 8a, b, 9a, b**).

Beg et al., [2] reported pulp treated with xylanase when exposed to 4.5% chlorine; it reduced kappa number by 25% and also the brightness was enhanced (% ISO) by 20%. Ninawe and Kuhad, [40] reported xylanase isolated from *Streptomyces cyaneus* SN32, which was used for biobleaching of pulp. The resulted bleached pulp on treating with a 10% less hypochlorite (5.4%) the brightness was similar to fully bleached pulp (6% hypochlorite). Xylanase enzyme from *Arthrobacter* sp. MTCC 521 could reduce the chlorine use by 29% without much change in the brightness of kraft pulp [7]. Xylanase and laccases mediator system reported by Kapoor et al., [41] facilitated 15% reduction in hypochlorite consumption as compared to controls. With the use of optimum conditions such as xylanase dose of 25 U_g⁻¹, 5h incubation period, and 10% pulp consistency, Nair et al., [8], reported elemental chlorine reduction of 14.3%. Chawannapak et al., [9] reported 20% decrease in hypochlorite consumption by using 50 U_g⁻¹ of endoxylanase for biobleaching of pulp samples. 6% overall reduction in chlorine consumption was reported by Bhorla et al., [37] by using 50 U_g⁻¹ of endoxylanase for biobleaching of pulp samples. Demand of chlorine-based chemicals was reported to be reduced up to 15% with use of xylanase for pre-treatment of kraft pulp [42]. Nagar et al., [5] applied xylanase for pretreatment of pulp which achieved 29.16% reduction in chlorine consumption while maintaining brightness equivalent to control.

Conclusion

The current study gave us the clear view, that if the pulp is treated with xylanolytic enzyme consortia, the amount of chlorine required during bleaching of pulp can be reduced to a great extent and also it gives better results when compared to single xylanase. The reduction in chlorine requirement due to prebleaching of pulp with xylanase enzyme and xylanolytic enzyme consortia has made the bio-bleaching process not only economical but also eco-friendly.

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Tables:

Table 1. Effect of xylanolytic enzyme pre-treatment on kappa number, brightness and viscosity of different kraft pulp

Control: Treatment only with buffer; CM-X: Treatment with commercial xylanase; B-X: Treatment with *Bacillus* sp. NIORKP76 xylanase; B-XEC: Treatment with *Bacillus* sp. NIORKP76 xylanolytic enzyme consortia

Bleaching state		Hardwood pulp			Chemical bagasse		
		Kappa number	Brightness (%)	Viscosity (cP)	Kappa number	Brightness (%)	Viscosity (cP)
Untreated		21.51±0.749	41.30±0.94	6.30±0.021	14.13±0.645	53.10±0.82	7.70±0.041
Pre-treated	Control	20.37±0.652	45.70±0.75	6.20±0.024	13.36±0.583	56.30±0.65	7.50±0.021
	CM-X	11.64±0.559	61.00±0.45	6.00±0.031	11.22±0.562	64.70±0.52	7.30±0.023
	B-X	11.92±0.575	60.80±0.63	6.00±0.012	11.44±0.550	63.40±0.63	7.30±0.021
	B-XEC	9.70±0.485	71.00±0.23	5.80±0.026	10.24±0.515	71.70±0.24	6.90±0.035
2% NaOCl	Control	7.56±0.175	70.00±0.23	3.90±0.045	2.70±0.090	64.70±0.35	5.80±0.036
	CM-X	3.11±0.160	77.65±0.15	3.70±0.031	2.16±0.060	75.20±0.12	5.40±0.031
	B-X	3.38±0.170	76.95±0.76	3.70±0.033	2.43±0.036	74.90±0.19	5.50±0.029
	B-XEC	2.84±0.150	82.15±0.36	3.50±0.028	1.49±0.070	76.00±0.24	4.70±0.041
1% H ₂ O ₂	Control	4.05±0.125	72.45±0.51	3.70±0.027	2.53±0.080	67.20±0.58	4.60±0.048
	CM-X	2.23±0.168	78.40±0.62	3.60±0.035	1.95±0.060	76.80±0.51	4.80±0.026
	B-X	2.73±0.132	78.15±0.31	3.60±0.043	2.34±0.070	76.20±0.37	4.20±0.036
	B-XEC	1.75±0.149	83.70±0.85	3.40±0.021	1.36±0.050	78.40±0.54	4.40±0.019
4% NaOCl	Control	3.92±0.149	73.50±0.62	3.70±0.035	1.98±0.075	75.50±0.32	5.30±0.021
	CM-X	2.84±0.140	79.30±0.90	3.50±0.044	1.49±0.045	76.70±0.31	4.80±0.025
	B-X	2.90±0.180	78.95±0.30	3.65±0.019	1.62±0.029	75.90±0.61	5.10±0.028
	B-XEC	2.30±0.170	84.40±0.21	3.45±0.022	1.22±0.035	77.70±0.37	4.30±0.027
1% H ₂ O ₂	Control	3.04±0.142	77.65±0.25	3.60±0.021	1.75±0.050	76.00±0.24	4.10±0.036
	CM-X	1.82±0.154	80.25±0.23	3.45±0.031	1.36±0.040	77.70±0.31	3.90±0.016
	B-X	1.95±0.186	79.15±0.26	3.50±0.048	1.56±0.080	77.50±0.19	3.95±0.031
	B-XEC	1.56±0.157	85.60±0.34	3.35±0.046	1.17±0.070	79.20±0.53	3.90±0.034
8% NaOCl	Control	3.11±0.190	78.20±0.41	3.70±0.032	1.62±0.050	76.90±0.41	4.60±0.038
	CM-X	2.57±0.180	80.15±0.38	3.50±0.014	1.22±0.080	78.40±0.32	3.80±0.051

1% H ₂ O ₂	<i>B-X</i>	2.63±0.150	79.65±0.54	3.50±0.019	1.22±0.055	78.60±0.16	4.20±0.057
	<i>B-XEC</i>	1.76±0.140	85.35±0.36	3.45±0.024	1.08±0.035	79.10±0.72	3.70±0.036
	Control	1.82±0.156	79.80±0.21	3.45±0.023	1.56±0.090	77.00±0.63	3.90±0.022
	<i>CM-X</i>	1.42±0.135	81.20±0.32	3.40±0.035	1.17±0.080	78.65±0.34	3.55±0.015
	<i>B-X</i>	1.56±0.175	81.15±0.38	3.50±0.016	1.17±0.050	79.30±0.26	3.75±0.011
	<i>B-XEC</i>	1.36±0.137	86.60±0.60	3.25±0.011	1.07±0.040	80.80±0.12	3.45±0.019

Table 2. Effect of xylanolytic enzyme pre-treatment on kappa number, brightness and viscosity of different kraft pulp after chlorine reduction

Control: Treatment only with buffer; CM-X: Treatment with commercial xylanase; *B-X*: Treatment with *Bacillus* sp. NIORKP76 xylanase; *B-XEC*: Treatment with *Bacillus* sp. NIORKP76 xylanolytic enzyme consortia; 4C: 4% hypochlorite treatment on pre-treated pulp; 2C: 2% hypochlorite treatment on pre-treated pulp; H: 1% hydrogen peroxide treatment on pre-bleached pulp

Bleaching state	Hardwood pulp			Chemical bagasse			
	Kappa number	Brightness (%)	Viscosity (cP)	Kappa number	Brightness (%)	Viscosity (cP)	
Untreated	21.51±0.749	41.30±0.94	6.30±0.021	14.13±0.645	53.10±0.82	7.70±0.041	
Pre-treated	Control	20.37±0.652	45.70±0.75	6.20±0.024	13.36±0.583	56.30±0.65	7.50±0.021
	CM-X	11.64±0.559	61.00±0.45	6.00±0.031	11.22±0.562	64.70±0.52	7.30±0.023
	<i>B-X</i>	11.92±0.575	60.80±0.63	6.00±0.012	11.44±0.550	63.40±0.63	7.30±0.021
	<i>B-XEC</i>	9.70±0.485	71.00±0.23	5.80±0.026	10.24±0.515	71.70±0.24	6.90±0.035
NaOCl	Control-4C	3.92±0.149	73.50±0.62	3.70±0.035	1.98±0.075	75.50±0.32	5.30±0.021
	CM-X-2C	3.11±0.160	77.65±0.15	3.70±0.031	2.16±0.060	75.20±0.12	5.40±0.031
	<i>B-X</i> -2C	3.38±0.170	76.95±0.76	3.70±0.033	2.43±0.036	74.90±0.19	5.50±0.029
	<i>B-XEC</i> -2C	2.84±0.150	82.15±0.36	3.50±0.028	1.49±0.070	76.00±0.24	4.70±0.041
1% H ₂ O ₂	Control-4CH	3.04±0.142	77.65±0.25	3.60±0.021	1.75±0.050	76.00±0.24	4.10±0.036
	CM-X-2CH	2.23±0.168	78.40±0.62	3.60±0.035	1.95±0.060	76.80±0.51	4.80±0.026
	<i>B-X</i> -2CH	2.73±0.132	78.15±0.31	3.60±0.043	2.34±0.070	76.20±0.37	4.20±0.036
	<i>B-XEC</i> -2CH	1.75±0.149	83.70±0.85	3.40±0.021	1.36±0.050	78.40±0.54	4.40±0.019

Figure:

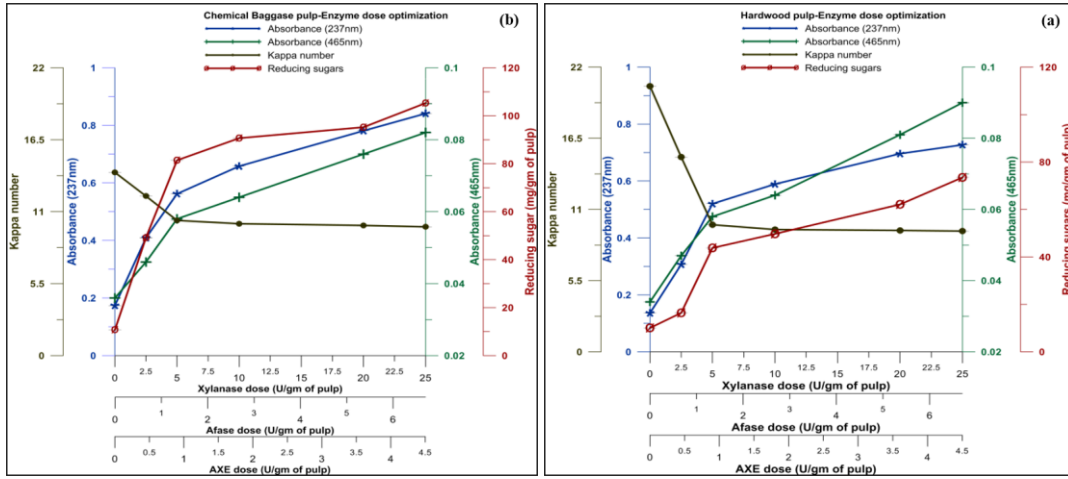


Fig.1 Xylanolytic enzyme dose optimization (Ug^{-1} pulp) for pre-treatment of pulp. a) Hardwood pulp, b) Chemical bagasse pulp

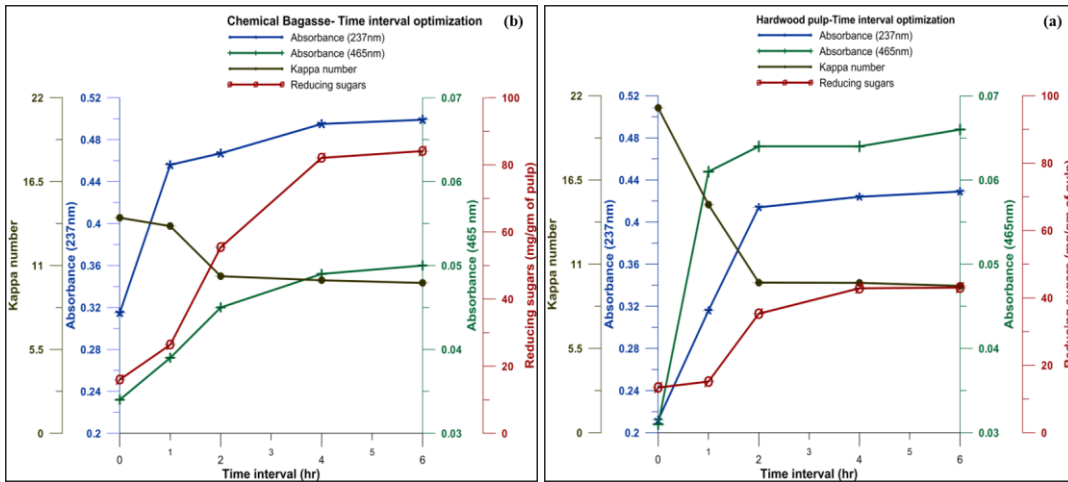


Fig.2 Time interval optimization (h) for pre-treatment of pulp. a) Hardwood pulp, b) Chemical bagasse pulp

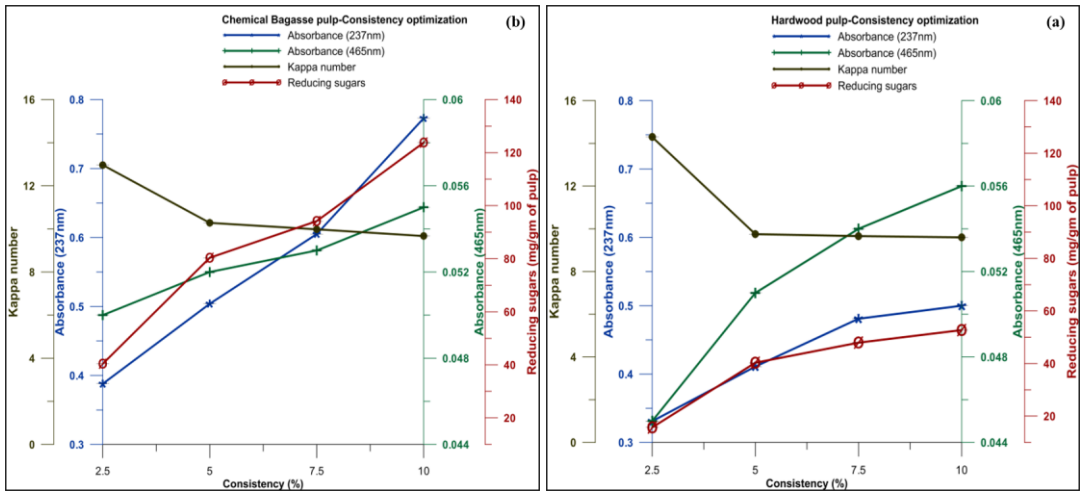


Fig.3 Pulp consistency optimization (%) for pre-treatment of pulp. a) Hardwood pulp, b) Chemical bagasse pulp

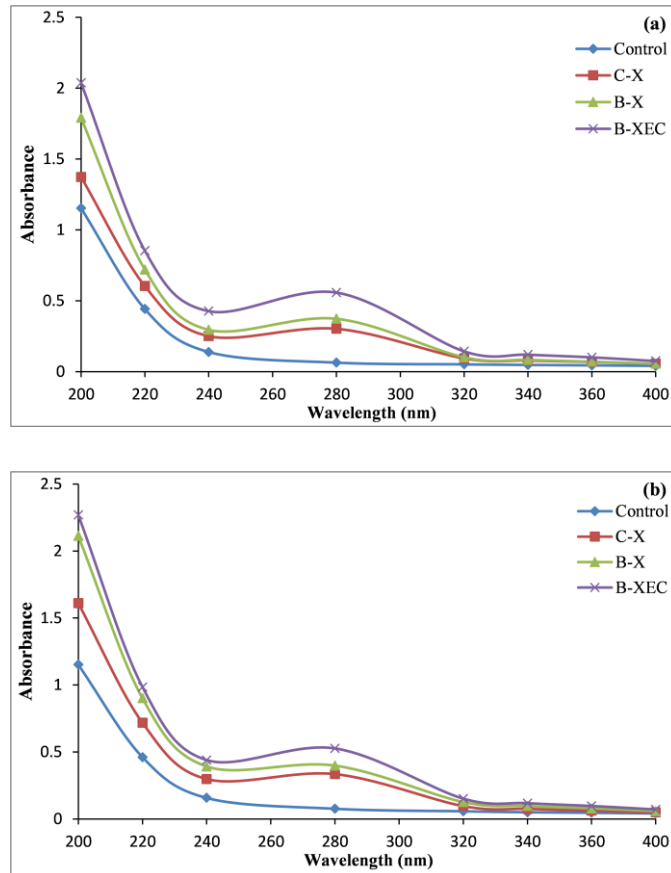


Fig.4 UV spectrum of colored compounds released during xyloanalytic enzyme treatment, a) Hardwood pulp, b) Chemical bagasse pulp

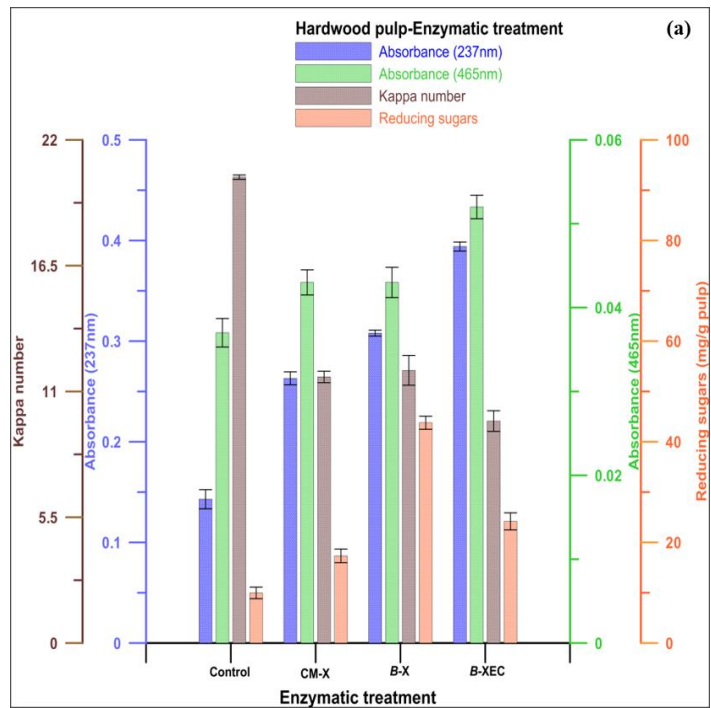
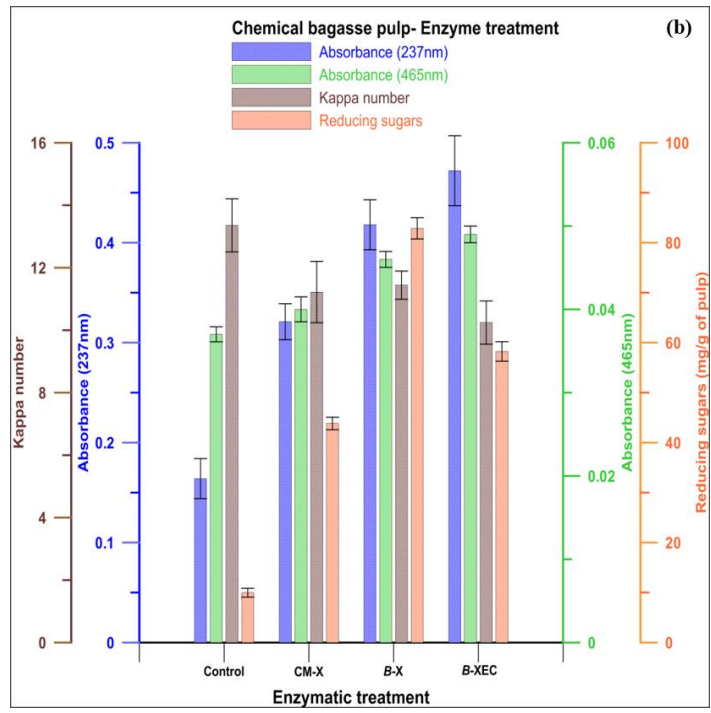


Fig.5 Enzymatic pre-treatment of pulp, a) Hardwood pulp, b) Chemical bagasse pulp

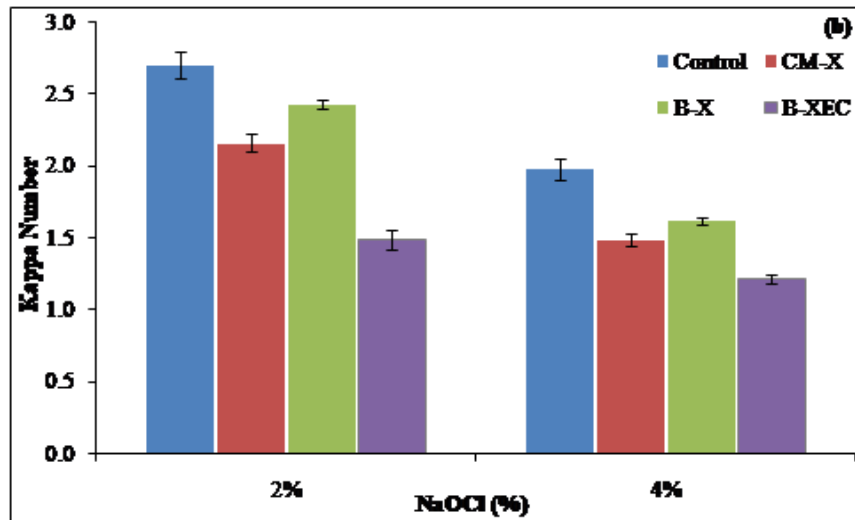
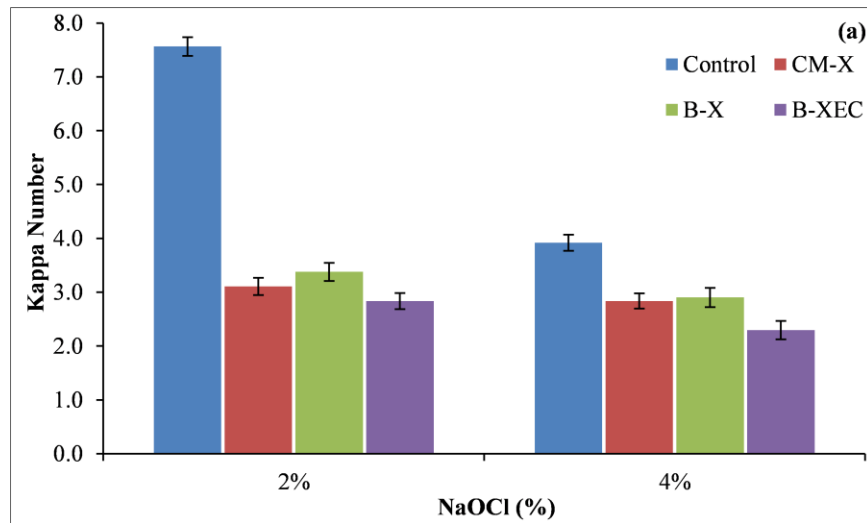


Fig.6 Effect of hypochlorite treatment on kappa number of enzymatically pre-bleached pulp: (a) Hardwood, (b) Chemical bagasse

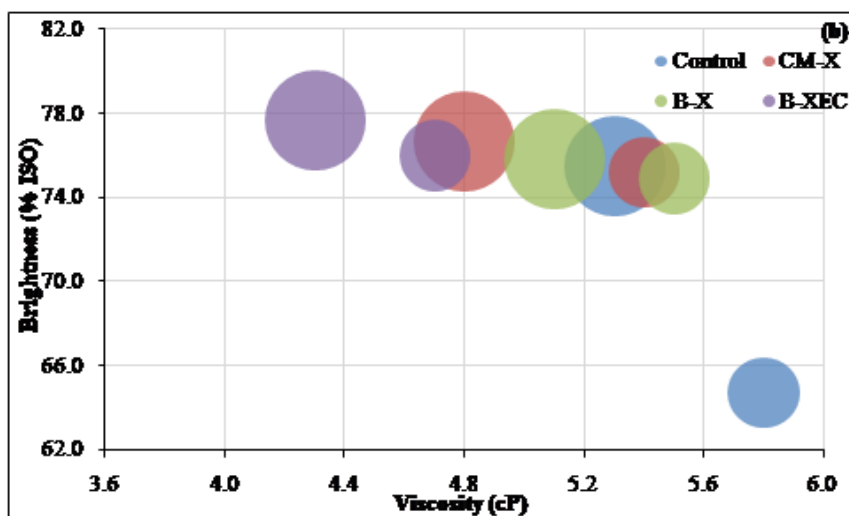
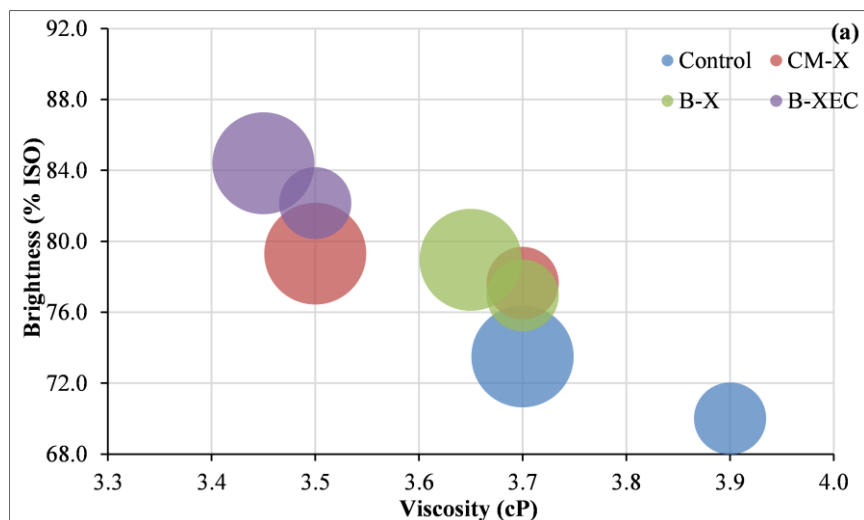


Fig.7 Effect of hypochlorite treatment on brightness and viscosity of enzymatically pre-bleached pulp: (a) Hardwood, (b) Chemical bagasse
 (Note: Size of the bubble represents the concentration of hypochlorite used in the bleaching of enzymatically pre-treated pulp samples)

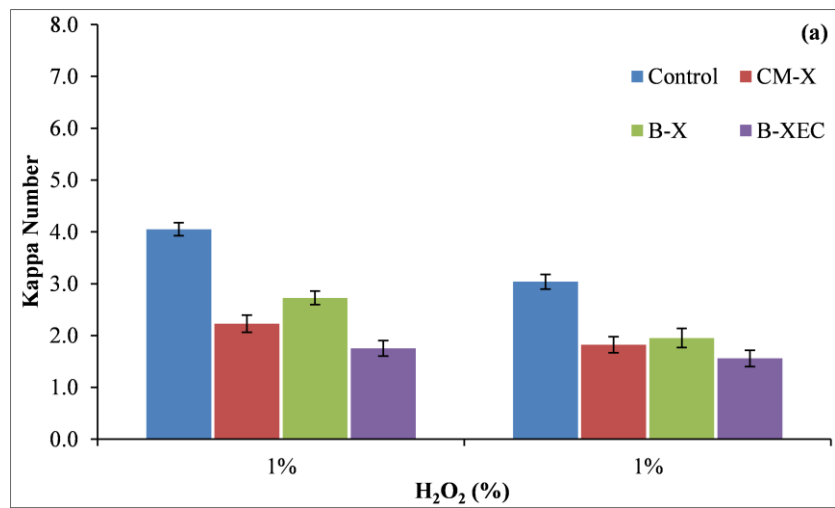
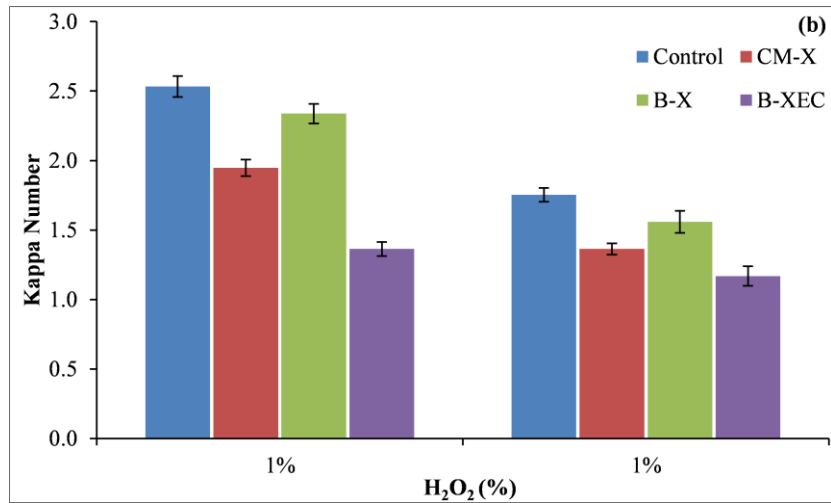


Fig.8 Effect of hydrogen peroxide treatment on kappa number of enzymatically pre-bleached pulp: (a) Hardwood, (b) Chemical bagasse

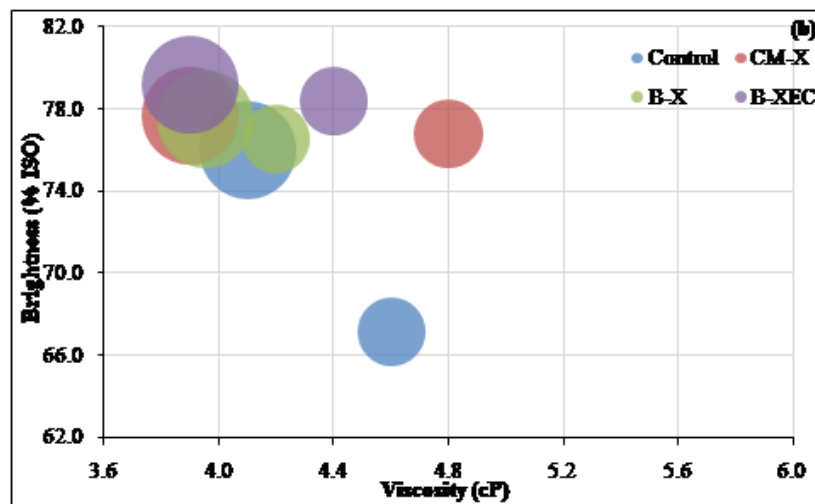
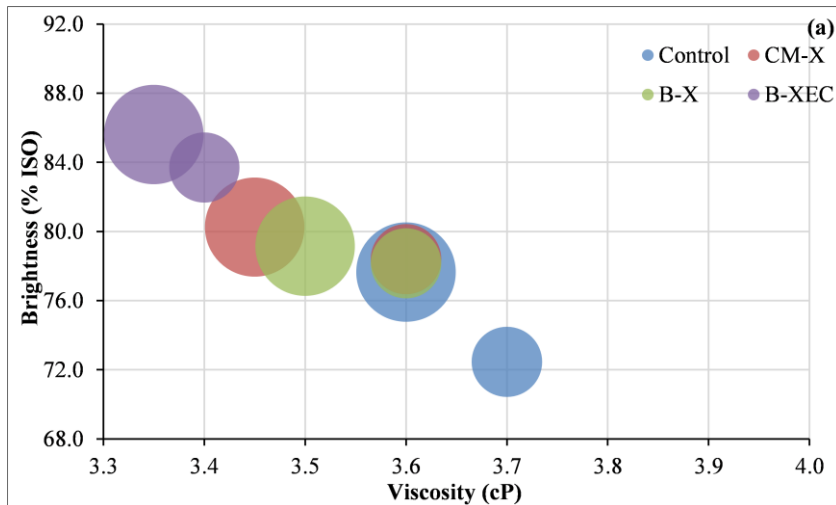
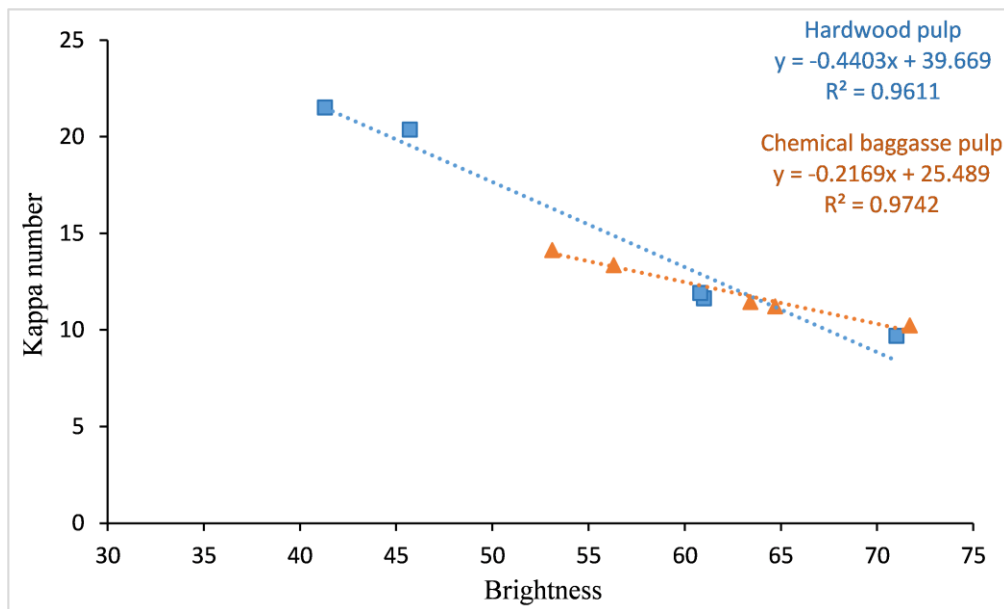


Fig.9 Effect of hydrogen peroxide treatment on brightness and viscosity of enzymatically pre-bleached pulp: (a) Hardwood, (b) Chemical bagasse

(Note: Size of the bubble represents the concentration of hypochlorite used in the bleaching of enzymatically pre-treated pulp samples)



Supplementary Fig. 1 Correlation between brightness development and lignin content of kraft pulp samples