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Application of recombinant xylanase from *Orpinomyces* sp. in elemental chlorine-free bleaching of kraft pulps

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Abstract

A xylanase coding gene (xynA16) of Orpinomyces sp. was isolated and expressed in Escherichia coli. XynA16 encoded 291 amino acids to yield a protein of molecular mass 32.6 kDa. XynA16 had a catalytic domain of glycosyl hydrolase family 11 and a dockerin domain. The enzyme expressed in E. coli showed the highest activity at pH 6.0 and 50 °C. The enzyme retained 78% of its initial activity after 24 h preincubation at 50 °C. XynA16 was used in the pretreatment of wheat straw, eucalyptus and pine kraft pulps prior to oxygen delignification, alkali extraction and hydrogen peroxide stages. It has been demonstrated that the use of XynA16 for pulp bleaching could decrease kappa number and increase brightness, however the effects differed depending on the pulp type used. In terms of kappa number reduction, increase in brightness, release of chromophores and reducing sugars, eucalyptus kraft pulp was the most susceptible to XynA16 treatment.

Keywords: biobleaching, kraft pulp, *Orpinomyces* sp., xylanase

Introduction

Xylans are the major component of hemicelluloses in plant cell walls. Degradation of xylan by ruminant herbivores mainly occurs in the rumen (ORPIN & JOBLIN [1]) and anaerobic fungi play a significant role in degrading xylans of plant cell walls (LJUNGDAHL [2]). Xylanase (EC 3.2.1.8) is constantly produced at a basal level by anaerobic fungi while the media contained glucose or avicel (LOWE & al. [3], COMLEKCIOGLU & al. [4]). Due to their biotechnological potential many different xylanase genes have been cloned from anaerobic fungal isolates. Anaerobic fungal xylanase genes were expressed in *Escherichia coli*, lactic acid bacteria (OZKOSE & al. [5]), rumen bacteria (KRAUSE & al. [6]) and fungi (LI & al. [7]). Several applications of xylanase encoding genes of anaerobic fungi were tested for agricultural (OZKOSE & al. [5], KRAUSE & al. [6]) and industrial purposes (CLARKE & al. [8]). However more studies are needed that are focused on the agricultural and industrial application of anaerobic fungal xylanases.

In recent years there has been increasing interest in the use of xylanases, particularly in the bleaching process of pulp and paper industry (SANDRIM & al. [9]). Chlorine based bleaching in the pulp and paper industry has serious environmental effects such as the production of toxic and mutagenic chloroorganic compounds (SALEEM & al. [10]), therefore environmental demands have necessitated that the pulp and paper industry find various alternatives to chlorine-based chemical bleaching processes for the production of bleached kraft pulp (SUCHY & ARGYROPOULOS [11]). Xylanase treatment as an individual stage in

a bleaching sequence is a promising alternative to substitute for chlorine and chlorine derivatives (YANG & al. [12]). Enzymatic pretreatment of kraft pulp can be established as the most suitable stage to facilitate bleaching of pulp (BEG & al. [13]). It has been suggested that xylanases mainly hydrolyze relocated, reprecipitated xylan on the surface of the pulp microfibrils, allowing better chemical penetration (JIANG & al. [14]). Enzymatic bleaching also results from the cleavage of hemicellulose chains between lignin and carbohydrate complex (KULKARNI & al. [15]). In conclusion, xylanase pretreatment reduced the need for bleach to remove loosened lignin, therefore lowering bleaching chemical consumption (SALEEM & al. [16]).

In this study, the cloning of xylanase coding *xynA16* from *Orpinomyces* sp. and characteristics of XynA16 enzyme is reported. The bleaching effect of XynA16 was investigated on three different kraft pulps. For the bleaching of wheat straw, eucalyptus and pine kraft pulps, we have studied the combined effect of four chlorine free bleaching stages, xylanase, oxygen, alkali extraction and hydrogen peroxide.

Materials and Methods

Microorganisms and Culture Conditions: The *Orpinomyces* sp. GMLF16 was isolated from fresh cattle feces, and deposited in the culture collection of Biotechnology and Gene Engineering Laboratory of Kahramanmaras Sutcu Imam University, Kahramanmaras, Turkey. The fungus was maintained anaerobically and wheat straw was used as the sole energy source for the maintenance media. To obtain a relatively higher amount of mycelium for DNA extraction, glucose (0.5%, w/v) was used as the energy source. All incubations for the fungus used in this study were performed at 39 °C. *Escherichia coli* EC1000 was grown in Luria-Bertani (LB) broth at 37 °C in a gently shaking incubator (150 rpm).

Genomic DNA extraction and amplification: The fungus was grown for 2 days on glucose containing anaerobic medium. Fungal biomass was harvested by centrifugation at 1250 *g* for 10 min. Biomass was frozen using liquid nitrogen and ground to a fine powder by using Mixer Mill. Genomic DNA was isolated using DNA Extraction Kit (Favorgen Biotech. Corp., Taiwan) according to the manufacturer's protocol, and then stored at -20 °C for further analysis. The β-(1,4)-endoxylanase gene was amplified from the fungal genomic DNA using the OrpXF 5'-TGCCTCTGCTGGTCAAAGATTA-3' and OrpXR 5'-ACCATTCGTTGTTT-TCAACACC-3' primers.

Cloning, Transformation and Sequencing: The PCR product was cloned into pCT vector according to the manufacturer's protocol (Favorgen Biotech. Corp., Taiwan) and constructed plasmid named as pCTX16. The constructed plasmids were transformed into *E.coli* by $CaCl_2$ method to express the β-(1,4)-endoxylanase proteins. The resultant plasmid, pCTX16, was extracted using Plasmid DNA Extraction Kit (Favorgen Biotech Corp., Taiwan) according to the manufacturer's protocol and β-(1,4)-endoxylanase gene (*xynA16*) insert was sequenced on both strands by a commercial company Iontek (Istanbul, Turkey). Splits Tree 4 software (HUSON & BRYANT [17]) was employed to generate the phylogenetic tree, and the glycosyl hydrolase 11 family xylanase proteins were retrieved from the CAZY database (www.cazy.org). Multiple alignments of the xylanase proteins were performed using the ClustalX program.

Enzyme Assays: β-(1,4)-endoxylanase activity was determined by measuring the amount of reducing sugar liberated using dinitrosalicylic acid (DNS) (MILLER [18]). One unit of β-(1,4)-endoxylanase activity was defined as 1 μmol of reducing sugar released from beech wood xylan (Sigma) per minute under the standard assay conditions (50 °C, pH 6.0). The effect of pH was determined for different pH values ranging from 3.0 to 10.0 by using the

substrate in 50 mM sodium acetate buffer (for pH 3.5–5.5), sodium phosphate buffer (for pH 6.0–7.5) and Tris-HCl buffer (for pH 8.0–9.0) solutions at 50 °C. Optimum temperature was determined by assaying the enzyme activity at different temperatures ranging from 30 to 90 °C with 10 °C increments at pH 6.0. The pH stability was determined by incubating XynA16 with different buffers ranging from 3.0 to 10.0 at 50 °C for 30 min. For the thermal stability determination XynA16 was incubated at 40, 50, 60, 70, 80 and 90 °C for 1 h at pH 6.0. The effect of metal ions and some chemicals on enzyme activity was determined by pre-incubating XynA16 in the presence of substances with final concentrations of 1 and 10 mM for 30 min at 50 °C, and then performing the assay in the presence of the same substances at the optimum temperature (AYGAN & ARIKAN [19]). For the analysis of the hydrolysis products of xylan, 1.0% (w/v) beechwood xylan in 50 mM sodium phosphate buffer (pH 6.0) was incubated with XynA16 at 50 °C for 1 h and 2 h. Hydrolysis products were determined by thin layer chromatography (TLC) using TLC silica gel 60 plate (Merck).

Biobleaching of kraft pulp: The pulp samples were treated with XynA16 ranging from 1-4 U g^{-1} of pulp in 50 mM sodium phosphate buffer (pH 6.0) at 5% consistency. The incubation of the samples was carried out in an orbital shaker at 50 °C for 3 h. After incubation, pulp samples were washed with distilled water. Filtrate samples were used to determine absorbance at 237 nm to estimate the release of phenolic compounds. The removal of hydrophobic compounds was estimated by absorbance at 465 nm (SANDRIM & al. [9]). Reducing sugars in pulp filtrates were measured at 540 nm by DNS method. The control samples without enzyme were treated under the same conditions.

Chemical bleaching: Xylanase-treated pulp samples were chemically bleached using the oxygen (O)-alkaline extraction (E)-hydrogen peroxide (P) treatment sequence. Oxygen delignification was performed at three different pressures (3, 5, 7 bar) by using O_2 gas (99% purity). Concentrations of NaOH and $MgSO_4$ were 3% and 0.5%, respectively. Reaction temperature, reaction time and pulp consistency were kept at the levels of 100 °C, 60 min and 10%, respectively. In alkaline extraction, oxygen treated pulps (12% consistency) were subjected with appropriate amount of NaOH at 70 °C for 60 min. Then the alkali-treated pulps (12% consistency) were mixed with different amounts of H_2O_2 (3, 5, 7%), 0.5% EDTA, 0.5% $MgSO_4$ and 3% Na_2SiO_3 at 75 °C for 60 min.

Analysis of pulp properties: The treated pulp was washed and the handsheets were prepared by the standard TAPPI methods (T 402 om-88). Kappa numbers were determined by the reaction of pulp samples with potassium permanganate (T 236 cm-8). ISO Standard Methods were used to determine brightness (ISO/DIS, 2470) opacity (ISO/DIS, 2471) and whiteness (ISO/DIS, 11475) of pulp samples. ASTM E 313 method was used to measure yellowness of pulp samples. Breaking length (T 494 om-88) and burst index (T 403 om-91) were determined according to standard protocols of TAPPI. The viscosity of the pulp was measured according to the Scan Test Methods (SCAN cm-15).

Results and Discussions

Characterization of xynA16

The length of xylanase insert (*xynA16*) was 950 bp, and the predicted peptide consisted of 291 amino acids with the theoretical molecular weight of 32.6 kDa. Poly (A) tail was not found at its 5'end. A catalytic domain of glycosyl hydrolase family 11 (GH11) was found at *xynA16*. One dockerin domain was also observed at the C-terminal of *xynA16* and the dockerin domain was separated from the catalytic domain by the reiterated sequence RTTT. GC content of the coding region was found to be 44% which was similar to that of other rumen fungal xylanase genes. The codon usage of coding region was biased to T at the third

position. The percentages of A, G, C and T in the wobble position were 25.1, 10.7, 29.8 and 34.4, respectively. It was observed that 15 codons were not utilized in XynA16. XynA16 was aligned with 26 GH11 xylanases from bacteria, archaea and fungi, and phylogenetic relationship was analyzed (Figure 1). According to deduced amino acid analysis, *xynA16* exhibited a high level of similarity (97%) to that of *Orpinomyces* sp. PC2 *xynA* (LI & al. [20]) and *Orpinomyces* sp. LT3 β-(1,4)-endoxylanase (Accession no. AEO51791). XynA16 and other rumen fungal xylanases were found to be related with rumen bacteria and anaerobic bacteria according to the phylogenetic analysis. Although anaerobic fungi are true eukaryotic microorganisms, previous reports suggested that some rumen fungal enzyme genes were originated from bacteria (CHEN & al. [21]).

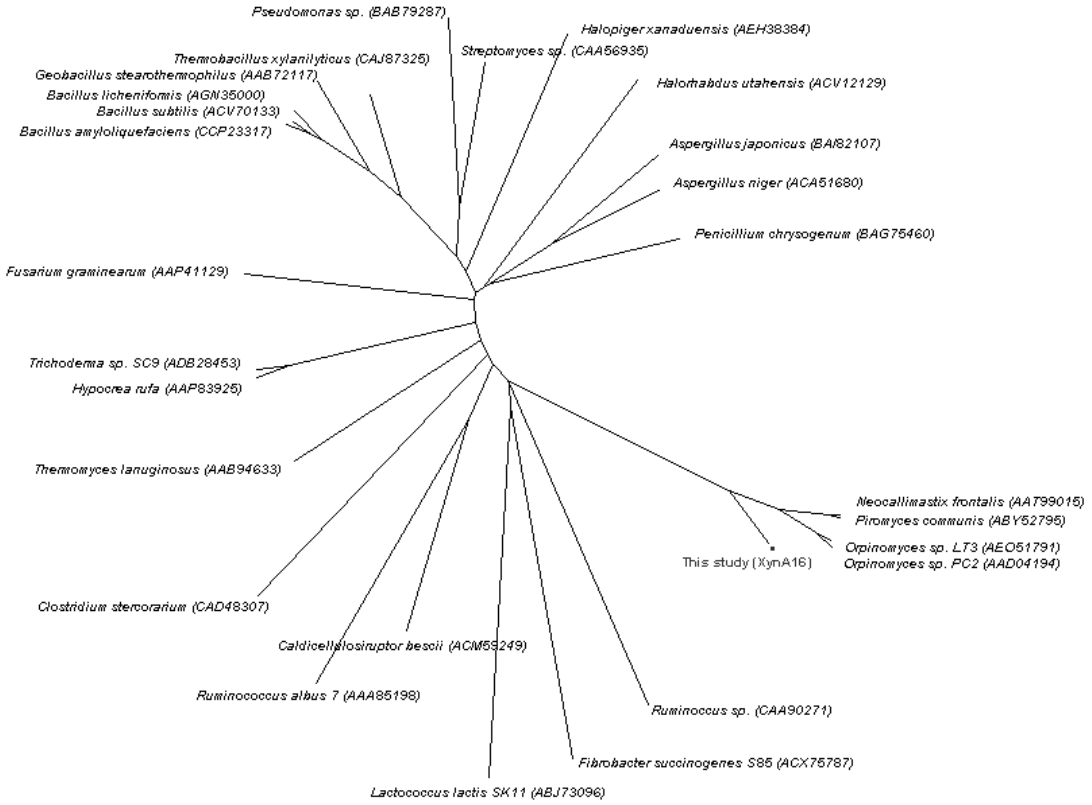


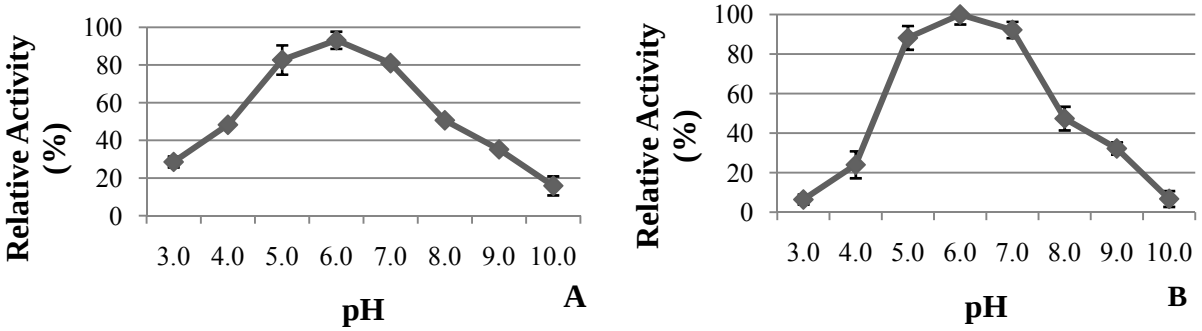
Figure 1. Phylogenetic tree of XynA16 with glycoside hydrolase 11 family proteins using the neighbor-joining method. GenBank accession numbers are indicated in parentheses.

XynA16 activity was mainly cell-associated and approximately 10% of the activity was found in the culture medium. XynA16 was optimally active at pH 6.0 (Figure 2). There was substantial activity between pH values 5.0 (88%) and 7.0 (92%). The pH stability of XynA16 was evaluated at 50 °C by measuring the residual activity of XynA16 after 30 min at different pH values. The enzyme retained more than 80% activity at pH values ranging from 5.0 to 7.0. Enzyme activity was also measured at different temperatures and the optimal temperature for the XynA16 was found to be 50 °C at pH 6.0. The enzyme retained more than 60% of the maximum activity when assayed between 25-80 °C. The optimum pH and temperature values of our study were in parallel to the findings of earlier reports focused on rumen fungal enzyme characteristics. In general, anaerobic fungal xylanases had pH and temperature optima of 5.5-6.5 and 40-50 °C, respectively. The specific activity of XynA16 was found to be 15.58 U mg^{-1} protein at the optimum pH and temperature conditions. XynA16 retained 100% activity after incubation for 1 h at 50 °C and pH 6.0. After incubation at 50 °C for 24 h, the enzyme retained 78% of its initial activity. Loss of 53% activity was observed after 1 h of

incubation at 60 °C. *xynA16* gene product showed good thermal stability at 50 °C. Even after 24 hours at 50 °C, XynA16 retained more than 75% of its activity. Xylanase activity from *Neocallimastix* sp. R1 was reduced by 58% after 7 h at 50 °C (LOWE & al. [3]), and *N. frontalis* xylanase lost its 50% of activity at 50 °C after 4 h (HEBRAUD & FEVRE [22]). An increment in thermal stability of a xylanase from *N. patriciarum* at 60 °C with a loss of 50% of its maximum activity after 78 min resulted from use of directed evolution (CHEN & al. [23]). The effects of metal ions and some chemicals on the enzyme activity were evaluated. Positive modulatory effect on enzyme activity was obtained with Mn^{2+} and Co^{2+} at 1 mM concentration. The presence of Pb^{2+}, Zn^{2+}, Ni^{2+}, Cu^{2+} and EDTA partially inhibited the enzyme. Strong inhibition was observed by Hg^{2+} and SDS (Table 1). Stimulation with Mn^{2+} and Co^{2+} was reported for several xylanases (KIDDINAMOORTHY & al. [24]). *Bacillus circulans* xylanases also showed strong inhibition at 1mM concentration of Hg^{2+} (DHILLON & al. [25]). XynA16 yielded mainly xylobiose, xylotriose, and higher xylooligosaccharides, and no xylose was observed (Figure 3). The hydrolysis products of XynA16 demonstrated a typical endoxylanase pattern. The principal hydrolysis products of rumen fungal xylanase enzyme XynR8 were xylobiose and xylotriose (LIU & al. [26]).

Table 1. Activity (%) of XynA16 after incubation for 30 min at 50 °C with metal chlorides, EDTA and SDS.

	Final Concentration	
Chemicals	1 mM	10 mM
None	100	100
$MnCl_2$	132	98
$CoCl_2$	127	85
$MgCl_2$	100	77
$PbCl_2$	87	68
$HgCl_2$	5	2
$ZnCl_2$	93	66
$NiCl_2$	95	69
$CuCl_2$	90	76
$BaCl_2$	101	64
EDTA	93	75
SDS	29	17



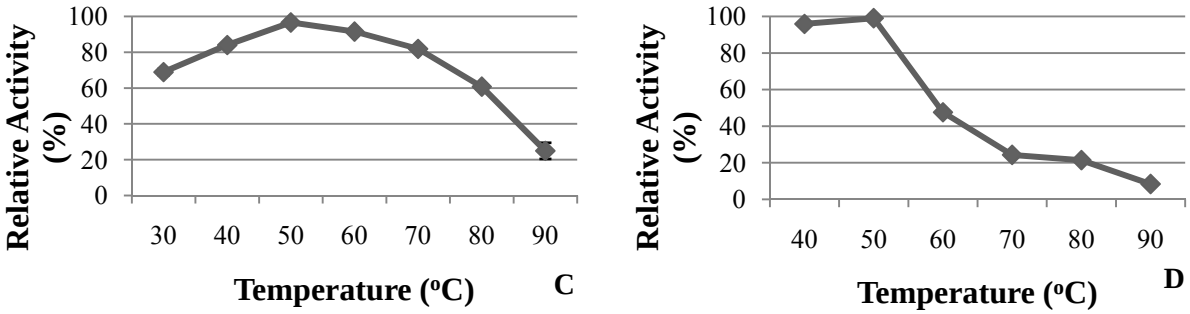


Figure 2. Characteristics of XynA16. (A) Optimum pH at 50 °C. (B) Stability at pH after incubation for 30 min at 50 °C. (C) Optimum temperature at pH 6.0. (D) Thermal stability after incubation for 1 hour at different temperatures and pH 6.0.

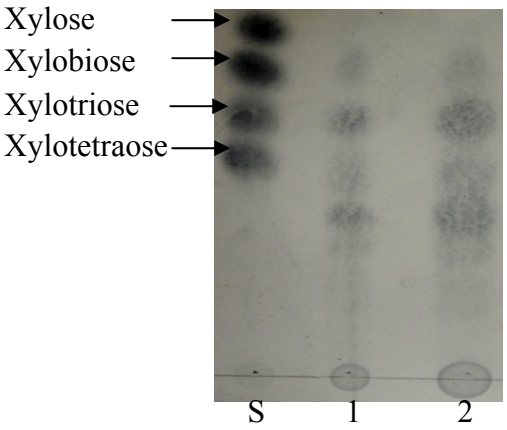


Figure 3. Thin layer chromatography of xylan hydrolysation products by XynA16. Lane S: Xylose standards, Lane 1: products of 1 h enzymatic reaction, Lane 2: products of 2 h enzymatic reaction

Biobleaching of kraft pulp

Results of spectrophotometric analysis of wheat straw, eucalyptus and pine kraft pulps treated with XynA16 are presented in Table 2. XynA16 was effective in releasing chromophoric material and reducing sugars from the kraft pulps. The pulps were treated with increasing enzyme concentration for 3h at pH 6.0 and 50 °C. Analysis of the treatment filtrates indicated that chromophore (measured at 237 and 465 nm) and reducing sugar release increased depending on the enzyme dose. XynA16 released maximum chromophore and reducing sugars at an enzyme dose of 4U g^{-1} in 3 h from kraft pulps. XynA16 was found to be more effective on wheat and eucalyptus pulps than pine pulp. The release of reducing sugars only determines xylanase activity, whereas the release of chromophores reflects the pulp bleachability (KIDDINAMOORTHY & al. [24]). Enzyme dose and incubation time are significant factors that enhance the release of chromophores and reducing sugars, however, higher enzyme dose or longer periods of incubation did not increase the biobleaching benefits significantly (BEG & al. [13]). BAJPAI [27] summarized that the optimum enzyme dose ranged between 2-5 international units of enzyme per gram of dry pulp, and most of the beneficial effect can be obtained after only 1-2 h of enzymatic treatment.

Table 2. Effect of XynA16 treatment on the release of chromophoric material and reducing sugars of kraft pulps

		237 nm			465 nm			Reducing Sugar (mg gr^{-1} pulp)		
		Wheat	Eucalyptus	Pine	Wheat	Eucalyptus	Pine	Wheat	Eucalyptus	Pine
Enzyme dose (U g^{-1} pulp)	0	0.282	0.109	0.155	0.043	0.035	0.035	1.651	1.684	1.076
	1	0.525	0.637	0.425	0.089	0.104	0.065	2.31	2.702	1.715
	2	0.915	1.197	0.714	0.123	0.129	0.101	2.938	4.048	2.741
	3	1.062	1.364	0.752	0.132	0.17	0.116	3.509	4.653	2.929
	4	1.363	1.531	0.762	0.15	0.192	0.12	4.676	4.611	3.212

XynA16 was further tested for its ability to improve bleachability of kraft pulps, therefore XynA16 was used in wheat, eucalyptus and pine pulp bleaching sequence (Table 3). XynA16 treatment (X) as the first stage reduced the kappa number of wheat straw, eucalyptus and pine kraft pulps by 2.96, 7.27 and 4.99 units and increased the brightness by 0.9%, 1.07% and 0.82% ISO, respectively. Kappa number reduction showed the treatment of kraft pulps with XynA16 improves lignin extractability and brightness characterizes the route of the pulp delignification process (DANIELEWICZ & SURMA-SLUSARSKA [28]). In terms of kappa number reduction, the largest impact by XynA16 occurred in eucalyptus kraft pulp (7.27 units). *Thermomyces lanuginosus* xylanase decreased the kappa number of kraft pulp 0.5 and 0.7 points at an enzyme dose of 5 U g^{-1} and 10 U g^{-1}, respectively (MADLALA & al. [29]). Also an increase in whiteness value and a decrease in yellowness value of the XynA16 treated pulps were observed. XynA16 pretreated wheat straw pulp resulted in remarkable increase of 3.47% in whiteness. GARG & al. [30] reported that wheat straw pulp pretreated with crude xylanase isolated from *Bacillus stearothermophilus* SDX increased the whiteness by 3.63%. Breaking length, burst index and viscosity of pulp handsheets decreased slightly following xylanase pretreatment. However, there were insignificant changes in the physical properties of XynA16 treated samples. Decrease in breaking length (KIM & PAIK [31]), burst index (SALEEM & al. [10]) and viscosity (BISSOON & al. [32]) were reported with several xylanase treatments.

Table 3. Physical and optical properties of bleached kraft pulps

	Treatments*	Kappa number	Breaking length (km)	Burst index (kPa.m^2/g)	Viscosity (cm^3/g)	Yellowness (%)	ISO Brightness (%)	ISO Opacity (%)	ISO Whiteness (%)
Wheat Straw	Control	40.89	5.94	2.75	992.8	36.63	32.27	99.41	40.79
	X	37.93	5.71	2.69	927.7	36.11	33.17	99.12	44.26
	O1 (3 bar)	26.96	5.27	2.68	885.1	30.4	46.32	98.58	59.16
	O2 (5 bar)	10.75	5.03	2.57	874.9	25.55	53.7	97.46	66.07
	O3 (7 bar)	9.92	4.73	2.55	820.6	20.14	60.2	95.96	70.65
	E	9.08	4.6	2.5	808.8	20.45	60.05	96.04	70.65
	P1 (3%)	8.67	4.35	2.42	773.7	17.1	63.53	95.37	72.75
	P2 (5%)	7.42	4.17	2.25	733	16.25	64.97	95.01	73.89
	P3 (7%)	5.77	4.1	2.1	719.9	15.2	66.9	95.91	75.45

Eucalyptus	Control	52.72	5.98	3.75	1020.4	46.26	22.09	99.6	31.3
	X	45.45	5.74	3.6	980.4	44.48	23.16	99.12	33.6
	O1 (3 bar)	35.53	5.48	3.41	872.6	33.57	36.29	98.77	52.31
	O2 (5 bar)	30.39	5.3	3.3	851.7	31.76	52.19	96.65	68.06
	O3 (7 bar)	24.8	5.25	3.18	811.5	21.85	62.06	94.36	74.3
	E	22.24	5.18	3.05	779	21.5	62.61	94.57	74.74
	P1 (3%)	20.51	5.06	2.95	761.2	17.31	66.44	94.59	76.52
	P2 (5%)	18.34	4.91	2.81	745.7	14.81	69.02	93.91	77.86
	P3 (7%)	14.55	4.82	2.68	722.2	12.24	71.47	93.5	78.94
Pine	Control	55.24	6.75	4.82	1041.4	57.2	18.23	98.88	29.58
	X	50.25	6.53	4.31	1024.7	56.29	19.05	98.82	31.38
	O1 (3 bar)	35.6	5.98	4.05	989.2	54.03	23.65	97.97	38.83
	O2 (5 bar)	30.47	5.74	3.9	960	50.56	32.8	95.06	51.1
	O3 (7 bar)	28.81	5.5	3.75	878.6	39.51	43.69	92.19	61.56
	E	26.1	5.42	3.67	815.3	38.72	44.79	92.79	62.6
	P1 (3%)	25.22	5.35	3.5	776.4	30.9	52.16	91.59	67.89
	P2 (5%)	22.48	5.25	3.35	767.7	28.42	54.77	92.5	70.03
	P3 (7%)	18.36	5.17	3.2	757.3	25.4	57.19	90.57	70.88

*X: xylanase treatment, O1, O2 and O3: Oxygen treatments, E: Alkaline extraction, P1, P2 and P3: Hydrogen peroxide treatments

Chemical bleaching of kraft pulp

It has been proven from numerous reports that biobleaching with xylanase is an environmentally friendly bleaching technology (BISSOON & al. [32]) and xylanase treatment as a bleaching step improves bleaching in subsequent stages. Besides xylanase, various applications and bleaching materials have been suggested to meet environmental demands for totally chlorine free bleached kraft pulps (VAN LIEROP & al. [33]). Oxygen and peroxides are expected to be the leading candidates for chlorine replacement in bleach plants (SUCHY & ARGYROPOULOS [11]). In this study, chemical treatments further decreased kappa number and improved brightness of enzyme treated pulps. The kraft pulps of high kappa number were delignified in three-stage oxygen treatment and 75.73, 52.95 and 47.85% kappa number reduction was observed in wheat straw, eucalyptus and pine, respectively, at the end of oxygen delignification. A very significant increase in brightness is achieved after oxygen was applied to kraft pulps. Oxygen treated wheat straw, eucalyptus and pine kraft pulps exhibited 46.40, 64.40 and 58.27% increment in brightness, respectively. It is demonstrated that xylanase treatment can be used either before or after the oxygen delignification stage (YANG & al. [12]); however, KIM & PAIK [31] reported that the xylanase pretreatment was slightly more effective rather than posttreatment in the oxygen bleaching sequence. Better results were also obtained from *Streptomyces* sp. QG11-3 and *Bacillus pumilus* from xylanase pretreatments, therefore xylanase treatment at the first step can be established as the most suitable stage to facilitate bleach boosting of pulp (BEG & al. [13], BATTAN & al. [34]).

The alkaline extraction stage was introduced as a part of the chemical bleaching processes. Kappa number of wheat straw pulp handsheets decreased slightly following alkaline extraction, while kappa numbers of eucalyptus and pine pulps decreased by 2.56 and 2.71 units, respectively. However, the largest increase on brightness and whiteness by alkaline extraction was observed only in pine pulp. Alkaline extraction removes soluble lignin which has been oxidized in previous bleaching stages and reactivates residual lignin for subsequent oxidative stages (SHACKFORD & al. [35]). Three-stage hydrogen peroxide delignification makes it possible to lower kappa numbers of wheat straw, eucalyptus and pine kraft pulps finally to 5.77, 14.55 and 18.36, respectively. Total brightness increments were observed as

51.76, 69.09 and 68.12%, respectively, at the end of the bleaching process. It is possible to improve the effectiveness of oxygen delignification and the reinforcement of the delignification effect by the addition of hydrogen peroxide (DANIELEWICZ & SURMA-SLUSARSKA [28]), therefore hydrogen peroxide was used as the final bleaching stage in this study, and remarkable effects on kappa number and optical properties were observed after the peroxide stage.

Conclusions

A xylanase coding gene of *Orpinomyces* sp. expressed in *E. coli*, and used in a totally chlorine-free bleaching sequence. The thermostability of XynA16 was found to be better than previously reported anaerobic fungal xylanases. The bleaching effect of XynA16 varied with the type of pulp material. XynA16 was able to remove reducing sugars and chromophoric material from wheat straw, eucalyptus and pine kraft pulps. Kappa number reduction and increase in brightness after enzyme pretreatment suggested that XynA16 can be suitable as a bleaching agent for pulp and paper industry in an environmental friendly process.

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