Applicability of Microbial Xylanases in Paper Pulp Bleaching: A Review

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The pulp and paper industries are attempting to bring changes to the bleaching process to minimize the use of chlorine to satisfy regulatory and market demands. Xylanases offer a cost-effective way for mills to realize a variety of benefits in bleaching. One main benefit is reducing Adsorbable Organic Halides (AOX) discharge. This is achieved primarily by decreasing chlorine gas usage. Other benefits include eliminating chlorine gas usage in mills with high chlorine dioxide substitution levels and increasing the brightness ceiling (particularly for mills contemplating Elemental Chlorine Free (ECF) and Totally Chlorine Free (TCF) bleaching sequences and in mills using large amounts of peroxide or chlorine dioxide). These benefits are achieved in the long term when the enzymes are properly selected and integrated into the process. This review summarizes the application of xylanases in the bleaching of pulp, with emphasis on the mechanism and effects of xylanase treatment on pulp and paper and the factors affecting the bleaching process and its efficiency. Brightness gains of up to 1.4 to 2.1 units have been achieved with xylanase treatment with the reduction of chlorine consumption by 15.0%. Xylanase treatment can lower the AOX amount in filtrate by 25.0% as compared to references. The Chemical Oxygen Demand (COD) can be reduced by 85%.

Keywords: Adsorbable organic halides; Bleaching effluents; Chlorine compounds; Enzymatic bleaching; Kraft pulps; Pulp properties; Xylanases

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INTRODUCTION

Biotechnology is an area with a high potential to improve various aspects of pulp and papermaking processes through cost reduction, quality improvement, and reduction of the environmental impact of an industry that has historically been considered a polluting industry (Valls *et al.* 2010a; Senior *et al.* 1999). Increased environmental awareness has forced paper manufacturers to consider new bleaching strategies, as chlorine-based bleaching leads to the formation of dangerous adsorbable organic halides (AOX). In this regard, the use of biotechnology in the paper industry has provided some fascinating and eco-friendly results. Spence *et al.* (2009) observed that enzymatic pretreatment of hardwood kraft pulp reduced the overall cost of bleaching to 89% ISO brightness by about US $ 2.32 per oven-dry ton of pulp.

According to mill-scale experiments, xylanase treatment can substantially improve the final brightness of bleached pulps while simultaneously decreasing bleaching costs, when it is used with hydrogen peroxide and ozone (non-chlorine bleaching chemicals) within totally chlorine-free (TCF) bleaching sequences (Allison *et al.* 1994).

It has also been demonstrated that xylanase pretreatment usually results in up to a 20 to 25% reduction in total elemental chlorine for hardwoods and 10 to 15% for softwoods, all while decreasing AOX by 12 to 25% (Shatalov and Pereira 2008; Tolan *et al.* 1996).

Organochlorine compounds are very important to the pulp and paper industry. These compounds are generated by the reactions between the residual lignin present in wood fibers and the chlorine used in some mills for bleaching. According to Bajpai *et al.* (2006), some of these compounds are toxic, mutagenic, persistent, and bioaccumulating and are therefore very harmful to biological systems.

Due to concerns about the short- and long-term environmental effects of chlorinated organic compounds, regulatory agencies in many countries have imposed limits on their discharge. In order to prevent production of organochlorine compounds, the most commonly used enzymes in bleaching are hydrolytic enzymes such as xylanases. Prebleaching of kraft pulps using xylanase has provided many advantages to pulp mills such as improving environmental performance, reducing bleaching cost, increasing productivity, and enhancing pulp properties. This technology has been well-received worldwide (Nguyen *et al.* 2008).

The use of enzymes in pulp bleaching is known as biobleaching. The use of xylanase for biobleaching yields pulps with high brightness and saves bleaching chemicals (Bajpai and Bajpai 1999). Xylanase is also widely used in the bleaching of non-woody pulps (Bajpai and Bajpai 1996; Chauhan *et al.* 2006; Roncero *et al.* 2003b; Shirkolaee *et al.* 2008). The use of *Bacillus coagulans* xylanase on three non-woody pulps (wheat straw, rice straw, and jute) was explored using a TCF bleaching sequence (Chauhan *et al.* 2006). A maximum brightness gain of 5.1 points was achieved in rice straw pulp at an initial pH value of 8.5. In the case of wheat straw pulp, maximum brightness gains of 4.4 points has been obtained only after bleaching stage with the enzyme-treated pulp at a pH of 8.5. Similarly, in the cases of jute straw pulps, maximum brightness gains of 4.0 points were obtained at pH values of 7.0. Biobleaching (*i.e.*, enzymatic pre-treatment/prebleaching of pulp with xylanases such as endo-1,4-β-xylanase, EC 3.2.1.8.) before chemical bleaching is an alternative and cost-effective way to reduce the consumption of bleaching chemicals (especially chlorine) in pulp mills. It further works to minimize the formation of toxic chlorinated organic substances in bleach plant effluent (Shatalov and Pereira 2008; Suurnakki *et al.* 1997).

The application of xylanase is often referred to as “prebleaching” or “bleach boosting” because it enhances the effects of bleaching chemicals by breaking the xylan network, which helps in removing the trapped lignin from the pulp rather than removing lignin directly or attacking lignin-based chromophores (Bajpai and Bajpai 1997). Roncero *et al.* (2003c) also reported effects of enzyme pretreatment before the ozone bleaching. They worked with two different pulps, eucalyptus and straw pulp and treated both pulp with enzyme treatment (X stage) before oxygen delignification (O) followed by ozone (Z stage) bleaching and compared individually with reference pulp which was not treated with enzyme before O stage and followed by Z stage. They observed that there was no significant effect of enzyme pretreatment on the elimination of lignin during the Z stage with straw pulp, while with eucalyptus O pulp and XO pulp, decrease in lignin was parallel, but the difference in the kappa number was maintained at 1.5 in XO pulp and 2.0 in O pulp. On the basis of these results they concluded that the X treatment eliminates a certain amount of lignin that cannot be eliminated in the Z stage.

The aim of enzymatic bleaching depends on mill conditions and may be related to environmental demands, reduction of chemical costs, and improvement and maintenance of product quality. The concept of using xylanase enzymes to increase the efficiency of bleaching pulp was introduced in 1986 (Viikari *et al.* 1986) and commercialized in 1991.

Bleaching with xylanase requires strict control of parameters including pH, temperature, and retention time (Farrell *et al.* 1996; Suurnakki *et al.* 1997). The performance of enzymes depends heavily on the types of the raw material, the pulping process, brown stock washing, and the bleaching sequence. The xylan content in the pulp depends on the performance of the digester. In conventional kraft pulping, the xylan content depends on the effective alkalinity. Bleaching using xylanase, as compared to conventional bleaching, increases bleaching efficiency and decreases the need for bleaching chemicals.

There are, however, some disadvantages of using xylanase in the bleaching process. It has been observed in mills that it is responsible for accelerated corrosion of equipment and increased time for maintenance of the brown stock (Tolan *et al.* 1996**)**, a very common problem with enzymatic bleaching. In mills, sulfuric acid is used in huge amounts to lower the pulp's pH for more effective application of enzymes. This results in corrosion of flexible steel facilities. Thus, the effects of xylanase prebleaching are limited due to its indirect operation and problems related to strength properties. It was observed in a study by Bajpai *et al.* (2006) that enzymatic bleaching results in reduced tear strength of the final paper.

The production and use of paper has a number of adverse effects on the environment by generating pollutants. In 2011, Keefe and Teschke reported that wood derivatives dissolved in the pulping liquors, including oligosaccharides, simple sugars, low molecular weight lignin derivatives, acetic acid, and solubilized cellulose fibres, are the main contributors to both biological oxygen demand (BOD) and chemical oxygen demand (COD). Compounds that are toxic to aquatic organisms include chlorinated organic (AOX) generated from bleaching, especially kraft pulp. Contaminated wastewater from pulp and paper mills may cause of death for aquatic organisms, allow bioaccumulation of toxic compounds in fish, and impair the taste of downstream drinking water. Sulphur compounds generated in paper industries are the main cause of mucous membrane irritation and headache in human being. Particulate matters are the main cause of respiratory problems in children. Those paper mills, which are using chemical methods for producing pulp particularly in kraft pulping, generate more pollutants in air.

MECHANISM OF XYLANASE ACTION IN PULP AND PAPER

Xylanase enzymes remove xylan by breaking the link between cellulose and lignin. During subsequent stages of the bleaching process, lignin is readily eliminated by the breakage of its links with cellulose (Woolridge, 2014; Roncero *et al.* 2005; Torres *et al.* 2000; Pham *et al.* 1995; Turner *et al.* 1992). It is known that kraft pulping causes precipitation of short xylan chains onto the surface of fibers. These xylans re-deposit on the fiber and act as a physical barrier against penetration by bleaching agents (Kantelinen *et al.* 1993). Re-deposited xylan becomes an obstacle for bleaching chemicals and results in increased consumption of chlorine dioxide (ClO_2) in the first bleaching stage. Xylan physically entraps the lignin, influencing fiber swelling (Shobhit *et al.* 2005).

Xylanases catalyze the hydrolysis of xylans and therefore can hydrolyze precipitated xylans. This results in a reduction of the xylan concentration on the secondary wall of the fiber surface during enzymatic treatment with xylanase, particularly in hardwood pulps. Reducing the xylan concentration increases the permeability of fiber surfaces, improving bleachability (Paice *et al.* 1992; Bajpai *et al.* 1994; Viikari *et al.* 1996; Gliese *et al.* 1998; Shah *et al*. 2000; Torres *et al.* 2000; Roncero *et al.* 2000a). Another mechanism of the action of xylanases involves hemicellulases. According to this mechanism, hemicellulases cleave hemicellulose bonds near their points of attachment to lignin. It is possible that this improves the extraction of trapped lignin from the pulp. Xylanases are thought to promote efficient pulp bleaching *via* the hydrolysis of the re-precipitated xylan on the fiber.

Henriksson and Teeri (2009) suggested a possible mechanism for xylanase action in pulp and paper, wherein:

- Lignin that is covalently bound to xylan (LCC) or lignin entrapped physically by xylan can be extracted from the fiber after xylanase treatment;
- Xylanase treatment partly removes the xylan layer that is re-precipitated onto the fiber surface and opens more spaces for bleaching chemicals to enter the fiber (Sharma *et al.* 2007); and
- Xylanase treatment removes the region with high hexenuronic acid content and thereby reduces the consumption of bleaching chemicals.

According to Roncero *et al.* (2003a), hexenuronic acids can cause significant reversion of brightness in TCF bleached pulp, a problem which can be combatted by the application of xylanases (Cadena *et al.* 2010). In addition, it has been observed that the kappa number, which reflects the lignin content in pulp, also decreases with xylanase treatment as xylanases are responsible for better penetration of bleaching chemicals after removing hexenuronic acid form the pulp.

However, certain characteristic features are desired in xylanases to facilitate their use in the pulping and bleaching processes. These include:

- Minimum cellulolytic activity to avoid hydrolysis of cellulose fibers (Archana and Satyanarayana 2003);
- Low molecular mass to facilitate their diffusion into the pulp fibers; and
- High yield of enzymes through cost-effective processes (Niehaus *et al.* 1999).

XYLANASE WITH A LACCASE MEDIATOR SYSTEM

There has been a wealth of research regarding the use of xylanase with a laccase mediator system. It has been reported that xylanase modifies surfaces of the pulp fiber and results in increased penetration by bleaching agents (Roncero *et al.* 2000b; 2005; Salles *et al.* 2005). An enzyme pretreatment stage (X) with milder application conditions for laccase mediator systems (L) was introduced. At high levels of the variables in this system (the XLE sequence, where E is an alkaline extraction stage) the kappa number dropped by 55%, 11% more than with the LE sequence, and an ISO brightness gain of 6% was found in all XLE-treated pulp samples compared to the corresponding LE sequence pulps (Valls *et al.* 2010a).

Another study was done on oxygen-delignified eucalyptus kraft pulp under optimal conditions for a laccase mediator treatment. Experiments were run with and without a xylanase pretreatment using a statistical plan for the dose of laccase, the dose of mediator (1-hydroxybenzotriazole, HBT), and the reaction time. Kappa number and brightness results showed that some lignin in the pulp remained inaccessible and the bleaching system started to remove or alter other chromophoric compounds present in the pulp. The optimum points for the LE and XLE sequences were achieved at the lowest HBT dose, highest laccase dose, and at a reaction time of 3.4 or 4.6 h. The xylanase pretreatment increases enzyme access to cellulose fibers, thereby boosting the effect of the laccase mediator system in reducing the residual lignin content and releasing more hexenuronic acids (Valls and Roncero 2009). Further, it has been reported that increasing HBT dose affects the kappa number and brightness. It was also seen that low HBT doses provide the better quality pulp. Another advantage of using a lower dose of HBT is that it reduces laccase inactivation and effluent toxicity (Valls *et al.* 2010a).

COMMERCIALLY AVAILABLE XYLANASES

Enzymes are produced by natural sources (typically fungal or bacterial strains), which are available on the market for the production of bleached pulp with higher brightness and lower kappa number. These enzymes are also applicable for reducing brightness reversion and improving physical strength. Mainly, bacterial and fungal strains are used to produce xylanase for pulp and paper industries. In 2002, Subramaniyan and Prema reported that bacterial xylanase have more advantages over fungal xylanase due to their alkaline-thermostable xylanase producing trait. In general, the optimum pH and temperature of bacterial xylanases are slightly higher than the optimal pH and temperature of fungal xylanases, which is a suitable characteristic in most industrial applications, especially in the pulp and paper industries (Ratanachomsri *et al.* 2006; Khasin *et al.* 1993). *Bacillus* strains are attractive producers of high levels of extracellular cellulase free xylanases stable at both high temperature and alkaline pH (Nagar *et al.* 2013). The usual drawbacks of fungal xylanases are that their optimal activity occurs in a pH range that is too low and too narrow for direct treatment of brownstock pulp. The ideal xylanase should maintain all or most of its activity through as broad pH range as possible. Ideally, the optimal pH range should allow brownstock to be treated with no acidification. Bacterial xylanases fulfill the criteria of having lower residual cellulase activity (which would reduce the fibre strength and pulp yield) in comparison of fungal xylanases (Ledoux *et al.* 1993).

Valls *et al.* (2010b) reported that the enzymatic stage removed 14% of Hex-A as a result of xylanase hydrolyzing xylans on fiber surfaces. The effects of commercially available xylanases (Pulpzyme HC, Irgazyme-10 and 40S, and Bleachzyme-B and F, *etc.*) were also examined by Bajpai and Bajpai in 1996 with respect to subsequent bleaching and the improvement of pulp quality. They observed that the enzyme treatments led to a decrease in extraction stage kappa number by 0.4 to 1.2 units relative to untreated pulp. The brightness gain in final pulp under the same total bleaching chemical charge was 0.8 to 1.5 units with Bleachzyme-B, Cartazyme HS-10, and Irgazyme-40S. The maximum brightening effect was noted in the cases of Bleachzyme F, Cartazyme HS-10, and Irgazyme-40S. In this study, about 20% reduction in chlorine was observed on the basis of the kappa number at the extraction stage with both enzymes.

In 2001, Bajpai and Bajpai tested six different enzymes under a wide range of pH values, incubation temperatures, incubation times, and enzyme doses at 5 to 10% stock concentration. After enzyme treatment the pulp was bleached using a CEHED (where C-chlorine, E-extraction, H-hypochlorite, and D-chlorine dioxide) bleaching sequence.

Observations indicated that the levels of pentosans were high in the unbleached pulp after mild hydrolysis, resulting in lesser energy consumption and a higher pulp yield. The treatment also resulted in reduced pollutant release into the prehydrolyzate liquor, higher pulp brightness, and reduced consumption of bleaching chemicals.

APPLICATIONS OF XYLANASE IN PULP AND PAPER

Effect of Xylanase Treatment on Carbohydrate Composition

Hydrolysis of xylan occurs in the presence of the xylanases used in enzymatic treatment (Roncero *et al.* 2005). Hydrolysis was found to be more dramatically affected when the enzymatic treatment was done prior to oxygen delignification (ODL). Enzymatic treatment by itself yielded a 13.4% reduction in xylan content and a 15.5% reduction when used in conjunction with oxygen delignification. The authors also studied the influence of xylanase on the carbohydrate composition. Results obtained from XRD indicated that the ratio of crystalline and amorphous regions was affected in both cases by enzymatic treatment and oxygen delignification. The degree of crystallinity was increased.

Limited hydrolysis of the xylan network is often sufficient to facilitate the subsequent chemical removal of lignin without sacrificing yield. The viscosity of the pulp is also improved as a result of xylanase treatment. However, the viscosity of the pulp is adversely affected when cellulase activity is present as it increases the degradation of cellulose (Jeffries 1992). Therefore, cellulase activity by the enzyme preparation is undesirable in enzymatic bleaching.

Shatalov and Pereira (2008) studied two commercial enzymes (Ecopulp and Pulpzyme) using *Eucalyptus globulus* pulp bleached with an XQPPP bleaching sequence (where X-xylanase treatment, Q-pulp chelating, and P-hydrogen peroxide). The main polysaccharide constituent was xylose and the change in its content generally reflected the bleaching behavior of the heteroxylan. Xylanase pre-treatment with Ecopulp was more effective in removing xylose during the three stages of peroxide bleaching after enzymatic treatment, dissolving about 9% of xylan while pulpzyme dissolved only 5.8%. Both xylanase preparations caused additional xylan removal, in comparison to the reference pulp during each subsequent peroxide bleaching stage. They also noted lignin removal by 65.4% and 63.7% with enzyme treated with Ecopulp and Pulpzyme, respectively, and 58.0% and 57.9% was noted for and corresponding control pulps, within the specified range of peroxide charge of 3-9%. Peroxide also affects the viscosity of the bleached pulp. In the sequence XQPPP, viscosity drop was observed due to enhanced degradation of lignin associated carbohydrates and cellulose under deep delignification of enzyme treated pulps.

A study by Shatalov and Pereira (2009) on the removal of lignin compounds in *E. globulus* pulp with three-stage peroxide bleaching after xylanase pretreatment with Ecopulp and Pulpzyme was completed. Lignin content was determined as a Klason and acid soluble lignin according to T 222 om-88 and UM 250 TAPPI standards. Results are shown in Table 1.

Table 1. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on the Reduction of Lignin in *E. globulus* Kraft Pulp*

Bleaching stage		Lignin (%, Oven dry pulp)	
		Ecopulp	Pulpzyme
X	Control	3.33	3.06
	Enzyme	2.98	2.85
XQP	Control	1.95	1.87
	Enzyme	1.57	1.60
XQPP	Control	1.63	1.61
	Enzyme	1.24	1.30
XQPPP	Control	1.40	1.29
	Enzyme	1.01	1.03
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira (2009)			

The reduction of lignin content after xylanase pretreatment of the pulp also depends on the types of raw material used, as shown in Table 2.

Table 2. Effect of Xylanase Pretreatment on Kappa No. Reduction in Bagasse, Rice Straw, and Wheat Straw*

Sample		Enzyme dose (U/g Pulp)	Decrease in Kappa No.
Bagasse	Xylanase (*T. lanuginosus*)	1	0.4
		5	0.7
		10	0.8
	Xylanase HS	1	1.1
		5	1.3
		10	1.9
Rice straw	Xylanase (*T. lanuginosus*)	1	0.3
		5	0.6
		10	0.6
	Xylanase HS	1	0.8
		5	0.8
		10	0.9
Wheat straw	Xylanase (*T. lanuginosus*)	1	0.2
		5	0.6
		10	0.7
	Xylanase HS	1	0.5
		5	0.9
		10	1.7
* Xylanase pretreatment was carried out at 60 °C for 3 h in sodium citrate buffer (pH 6.5)			
* Xylanase HS pretreatment was carried out at 60 °C for 3 h in citrate/phosphate buffer (pH 5.5)			
* Based on data from Shirkolaee *et al.* (2008).			

Effect of Xylanase Treatment on Fiber Morphology

Roncero *et al.* (2000a) concluded that xylanase treatment of kraft pulps is responsible for opening pores in the cell walls of fibers. Some morphological changes such as cracks, flakes, holes, filaments, and peeling are caused by enzyme treatment. These cracks and holes allow for the diffusion of larger lignin macromolecules, as reported by some authors (Paice *et al.* 1995; Wang *et al.* 1997). According to Roncero *et* *al.* (2005) xylanase treatment improves the accessibility of bleaching chemicals to the pulps by increasing diffusion to outward movement of degraded lignin fragments. This results in the removal of less-degraded lignin fragments from the cell wall, yielding a reduction in kappa number and enhanced brightness. The viscosity of pulp also increases in xylanase-treated pulps as compared to untreated pulps.

With attention to the effects of enzymatic treatment, eucalyptus pulp has been studied to determine effects of the treatment on fiber morphology after TCF and ECF bleaching sequences. Xylanase changes the surface of the fiber as observed in an analysis done by scanning electron microscopy (SEM). Treated fibers have rough surfaces with splits (*i.e.*, are more open), which in turn increases contact between the bleaching agent and the substrate (Roncero *et al.* 1999; 2000a; Viikari *et al.* 1986).

In another study, it was reported that the effects of enzymatic treatment on fiber surfaces were more evident in the earlier bleaching stages. Unbleached eucalyptus kraft pulps were treated with xylanase (Roncero *et al.* 2000a). There was a remarkable flaking found in the fibers of enzyme treated pulps. Many flakes and filaments of material detached from their surface. In contrast, smoother fiber surfaces were seen in untreated pulps. The group also studied untreated (O-Pulp) and xylanase-treated (XO-Pulp) pulps after the ODL stage. Fibers with very smooth surfaces were observed in untreated (O-Pulp) pulp. In contrast, fibers with a remarkable peeling effect were observed in treated pulp (XO-Pulp). The XO-Pulp appeared to continue undergoing a peeling process in which xylans were removed as flakes. These flakes of material removed from the surface caused the surface modification.

Effect of Xylanase on Hexeneuronic Acid Content

Hexenuronic acid (Hex-A) is widely distributed among natural polysaccharides such as heparin, chondroitin, and lepidimoide (Adorjan *et al.* 2006). During the alkaline pulping process, about 75 to 90% of 4-O-methyl-glucuronic acid side groups (MeGlcA) linked to heteroxylan are lost and the residual MeGlcA are almost completely (83 to 88%) converted to unsaturated hexenuronic acid (Hex-A or 4-deoxy-l-threo-hex-4-enopyranosyluronic acid) *via* the intermediate product, 4-O-methyliduronic acid (Shatalov and Pereira 2008; Chauhan *et al.* 2006; Beg *et al.* 2001; Bim and Franco 2000; Senior *et al.* 1999; Farrell *et al.* 1996).

The alkali charge and the H-factor are the main variables that influence the formation of hexenuronic acid during kraft pulping. Considering *Eucalyptus globulus* wood as a raw material for kraft pulp, the alkali charge is the main factor that contributes to the formation and degradation of hexenuronic acid during pulping. During the cooking process, hexenuronic acids form covalent bonds with lignin (Vuorinen *et al.* 1999). Hex-A is formed in kraft cooking from the methyl glucoronic acid side group found in xylans. It plays an important role in bleaching because of its undesired neutralization of electrophilic bleaching chemicals such as chlorine dioxide, ozone, and peracids, increasing the consumption of these chemicals. Bajpai *et al.* (2005) reported that the Hex-A percentage in particular raw material depends on the growing region and its species. As in hardwood unbleached pulp, it is found in the range of 7.1 to 30.5 mmol/kg, *Casuarina* pulp having the highest and Subabul pulp has the lowest Hex-A content. In bamboos, it is found in the range of 3.1 to 6.6 mmol/kg; Assam bamboo having the highest and Maharashtra bamboo having the lowest Hex-A content.

Hexenuronic acid protects xylan against terminal depolymerization reactions, thus preserving the yield of the pulping process. However, in extreme temperature and alkali dosage conditions, these composites, as well as other polysaccharides, suffer alkaline hydrolysis and are degraded. Hexenuronic acid also suffers hydrolysis under acidic conditions as it is vulnerable to the attack of electrophilic oxidants (Marechal *et al.* 1993). Since the discovery of hexenuronic acid structures in kraft pulps (Vuorinen *et al.* 1999), several strategies have been proposed for removing the composites from the pulp during the bleaching phase, which are based on an acid hydrolysis stage conducted at a temperature of about 80 to 100 °C and a pH of about 3.0 (Almeida 2004).

Shatalov and Pereira (2009) used chemical pulps to determine that the impact of hexenuronic acids on xylanase aided biobleaching. They found that xylanase assisted in direct pulp brightening. This was presumed to be due to Hex-A removal with solubilized xylooligosaccharide fractions. A strong positive correlation was established between the xylanase bleach boosting effect and the bleaching profile of Hex-A. The effects of alkali and oxygen extractions of kraft pulp on xylanase aided bleaching were studied as well. The group noted an improvement in final brightness of up to 1.4 to 2.1 units with reductions in Hex-A and the kappa number of xylanase-pretreated pulps compared to the corresponding control pulps (Wong *et al.* 2001).

According to Shatalov and Pereira (2009), the carbohydrate derived chromophores have a pronounced effect on brightness development of chemical pulps during xylanase aided bio-bleaching. The xylanase assisted direct pulp brightening was caused by HexA removal with solubilized xylooligosaccharide fractions. Strong positive correlation was established between xylanase bleach boosting effect and bleaching profile of HexA. The results are summarized in Table 3.

Table 3. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on Hexenuronic Acid Content in *E. globulus* Kraft Pulp*

Bleaching stage		Hexenuronic acid content (µmol/g dry pulp)	
		Ecopulp	Pulpzyme
X	Control	50.45	49.16
	Enzyme	43.13	43.69
XQP	Control	44.13	44.04
	Enzyme	34.69	35.92
XQPP	Control	39.81	40.19
	Enzyme	32.19	33.77
XQPPP	Control	36.60	37.14
	Enzyme	30.63	31.88
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira 2009.			

Effect of Xylanases on Bleaching Chemical Consumption

A number of reports on the reduction of bleaching chemical usage with xylanase enzymes (alone or in combination of other enzymes) in the enzymatic bleaching of pulp are available. According to Chakar *et al.* (2000), hexenuronic acids contributed 33 to 67% of the kappa number of hardwood kraft pulps, whereas for softwood kraft pulps, hexenuronic acids contributed only 5 to 12% of the pulp’s kappa number. Xylan contains hexenuronic acid, which consumes bleaching chemicals, resulting in more consumption of chemicals during bleaching. Xylanase treatment removes regions with high contents of hexenuronic acid, thereby decreasing the consumption of bleaching chemicals.

Results obtained from laboratory studies and mill trials indicate savings in total active chlorine of about 20 to 25% (Bim and Franco 2000; Senior and Hamilton 1992; 1992a,b; 1993; Senior *et al.* 1999; Shobhit *et al.* 2005; Tolan and Canovas 1992). Xylanase enzymes hydrolyze the xylan re-precipitated onto the fiber surface and therefore improve fiber permeability to bleaching reagents (Kantelinen *et al.* 1993). Compared to chemical bleaching, enzymatic bleaching is beneficial in that it reduces chlorine consumption by 10% when bleaching wheat straw pulp to the same brightness and kappa number (Lin *et al.* 2013).

In a different study, Bajpai and Bajpai (2001) examined six commercial enzymes (Pulpzyme HB, Bleachzyme F, Irgazyme 40 S, VAI Xylanase, and Cartazyme HS-10) to determine their effects on bleaching chemical consumption. They found that xylanase pretreatment of pulp is responsible for reducing chemical consumption *via* removal of hexenuronic acid from the pulp, which results in removing of trapped lignin in the pulp.

A mill-scale study on xylanase prebleaching of hardwood pulp conducted by Thakur *et al.* (2012) concluded that it reduced bleaching chemical requirements by 15%. In their work, enzymatic treatment was carried out at a pH of 9 to 10 and a temperature of 50 to 60 °C. Reductions in kappa number from 23.0-25.0 to 21.0-22.0 were observed. After the chlorine (C) stage, a kappa number drop from 7.0-8.0 to 6.0-7.0 was achieved. After the first extraction (E_P) stage, a drop from 5.0-5.9 to 4.0-5.0 was noted, and in the second extraction stage, a drop from 3.6-3.8 to 3.0-3.5 was observed, with a brightness gain of 2.0 to 3.0 units in each stage. The average chlorine charge in the mill was 5 kg/t pulp before treatment. It was reduced to 4 kg/t pulp and the hypochlorite flow rate was reduced from 75% to 60-65% (15 m^3/h to 13 m^3/h). Therefore, hypochlorite consumption was reduced from 45 kg/t pulp to 38-40 kg/t pulp, a hypochlorite savings of 10 to 12% while maintaining the target ISO brightness of 82 to 83%. In that way it was achieved to reduce 15% of chlorine in the C and H stages on the plant scale using hardwood pulp.

Shirkolaee *et al.* (2008) reported that xylanase pretreatment reduces the consumption of chlorine dioxide as shown in Table 4.

Table 4. Effect of Xylanase Pretreatment on Chlorine Dioxide Consumption in a DED Bleaching Sequence*

Brightness (%)						Reduction of chlorine dioxide (kg/t)
Bagasse pulp		Rice straw pulp		Wheat straw pulp		
Xylanase (*T. lanuginosus*)	Xylanase HS	Xylanase (*T. lanuginosus*)	Xylanase HS	Xylanase (*T. lanuginosus*)	Xylanase HS	
79.5	79.7	79.1	79.9	79.2	79.6	Control
80.6	81.7	81.1	82.0	80.1	80.8	0.0
79.9	81.1	80.8	81.7	80.0	80.4	0.5
78.9	80.5	80.6	81.5	79.7	80.2	1.0
79.7	80.2	80.6	81.6	79.8	79.9	1.5
79.5	80.0	79.8	81.0	79.6	79.7	2.0
79.1	79.8	79.3	80.5	79.2	79.6	2.5
78.7	79.8	79.1	80.1	79.2	79.1	3.0
78.0	79.7	N.D*	79.9	79.1	N.D*	3.5
N.D*	79.1	N.D*	79.9	79.0	N.D*	4.0
* D_1 (Chlorine dioxide) conditions: 10% consistency, 67 °C, 113 min						
* E (alkali extraction) conditions: 10% consistency, 67 °C, 67 min						
* D_2 (Chlorine dioxide) conditions: 10% consistency, 67 °C, 180 min						
* Based on data from Shirkolaee *et al.* (2008).						

Effect of Xylanase on Pulp Brightness

To assess the effect of xylanases on the peroxide bleachability of *Eucalyptus globulus* kraft pulp, unbleached industrial eucalyptus kraft pulp was treated with two commercial xylanase preparations: Ecopulp® TX-200A and Pulpzyme® HC (endo-1, 4-β-xylanase activity; EC 3.2.1.8). The pulp was bleached using a totally chlorine-free (TCF), three-stage hydrogen peroxide bleaching sequence (QPPP, where Q is a pulp chelating stage and P is a hydrogen peroxide bleaching stage) without oxygen pre-delignification. The change in pulp properties after each bleaching stage was examined and compared with those of control samples treated identically except without the addition of enzyme. Hydrogen peroxide bleaching was used, though it is generally used as a separate bleaching stage incorporated into multistage, industrial bleaching sequences (Shatalov and Pereira 2008).

Jimenez *et al.* (1996) reported that biobleaching of wheat straw pulp yielded a brightness gain of 2.4 points for enzyme peroxide bleaching and 3.0 points in the case of enzyme peroxide active chlorine bleaching. Xylanase post-treatment of bleached hardwood kraft pulp resulted in significantly reduced yellowing. In spite of the reduction of yellowing, yield could suffer significantly from enzymatic treatment (Simeonova *et al.* 2007). The effects of Pulpzyme HC, a commercial enzyme, were also studied as potential post-treatment enzymes for use after bleaching processes. They yielded a 1.5% brightness improvement, reductions in PC number of up to 15%, and a 10 to 15% decrease in Hex-A. These effects were possibly due to hydrolysis of xylan located on the fiber surface but were probably due to the extraction of stabilized quinine chromophoric structures otherwise retained by the pulp.

Bajpai and Bajpai (1996) reported the effects of various cellulase-free commercial xylanases on the brightness of pulps. Their results are shown in Table 5.

Table 5. Increase in the Brightness of Pulps using Various Cellulase-free Commercial Xylanases in a C_DEHD Bleaching Sequence*

Sample Tested	Reaction pH	Reaction temp. (°C)	Reaction time (h)	Enzyme dose (IU/g)	Brightness (%ISO)	Increase in brightness (%ISO)
Control	---	---	3.0	---	87.1	---
Bleachzyme B	7 to 7.5	40 to 50		10.0	87.9	0.8
Bleachzyme F	6 to 6.5	45 to 50		6.0	88.6	1.5
VAI xylanase	5 to 7	50 to 60		3.5	88.4	1.3
Ecopulp-X200	5 to 6	50 to 55		10.0	88.5	1.4
Cartazyme HS-10	4 to 5	40 to 55		13.0	88.6	1.5
Pulpzyme HC	8 to 9	60 to 70		14.0	88.0	0.9
Pulpzyme HB	7 to 8	45 to 55		14.0	88.2	1.1
Irgazyme-10	5.5 to 6	40 to 50		12.0	88.5	1.4
Irgazyme 40S	7 to 8	50 to 60		7.5	88.6	1.5
* C_D-Chlorination; E-Extraction; H-Hypochlorite; D-chlorine dioxide						
* Based on data from Bajpai and Bajpai (1996).						

Xylanase-aided three-stage hydrogen peroxide bleaching also affected the brightness of *E. globulus* pulp, as shown in Table 6.

Table 6. Effect of Xylanase-aided Three-stage Hydrogen Peroxide Bleaching on the Brightness of *E. globulus* Kraft Pulp*

Bleaching stage		Brightness (%ISO)	
		Ecopulp	Pulpzyme
X	Control	42.4	42.0
	Enzyme	43.9	43.2
XQP	Control	75.8	74.8
	Enzyme	77.9	76.9
XQPP	Control	81.2	80.1
	Enzyme	82.9	81.5
XQPPP	Control	85.0	84.5
	Enzyme	86.4	85.7
* X-Enzymatic pretreatment; Q-Chelating; P-Peroxide bleaching			
* Based on data from Shatalov and Pereira (2009).			

Effect of Xylanases on Pulp Yield

Cellulase-free xylanases are more favorable for enzymatic bleaching, as hydrolysis of cellulose components results in reducing yield and viscosity of pulps. Cheng *et al.* 2013 worked on isolation of cellulase-free crude xylanase (*S. griseorubens* LH-3) and used it in enzymatic bleaching. They observed that xylanase treatments of eucalyptus kraft pulps did not cause significant reduction in pulp yield due to non-degradation of cellulose, as there was no cellulase activity present in their isolated xylanase enzyme. According to Gubitz *et al.* 1997, 16% loss of yield was observed due to the presence of cellulase in the fungal xylanase extract, whereas Manimaran *et al.* 2009 reported that treatment of bagasse pulp with cellulase-free xylanase extract yielded a loss of only 2.5%.

Thakur *et al.* 2012 studied the effect of enzymatic pre-treatment on hardwood and nonwood kraft pulps of eucalyptus and bagasse. They charged 500g/t dose of Pulpzyme HC (from Novozymes) in X stage. Pulp after enzymatic treatment was carried out for ECF bleaching. They observed pulp yield loss of 0.5% in eucalyptus while 0.6% in bagasse pulp in comparison to control. Pulp kappa number was reduced by 4.2% and 14.0% in eucalyptus and Bagasse pulp respectively as compare to control. Brightness gain was observed of 1.20 and 2.17 units in eucalyptus and bagasse pulp respectively.

Lian *et al.* 2011 reported the combined effect of a xylanase-laccase system on the same dosage levels on pulp yield loss with or without refining of the pulp. A significant reduction in pulp yield, about 1.8%, from 96.6% to 94.8% was obtained with the Laccase/Xylanase System (LXS) without refining. Correspondingly, pulp yield loss, about 2.5% from 95.9% to 93.4% was observed in addition of refining. This is probably due to the fact that fines are lost which generated during refining.

Blomstedt *et al.* (2010) studied the selective hydrolysis of xylan using xylanase, Ecopulp TX 200 A from the AB Enzyme, Finland. A dose of 200 nkat/g xylanase in X stage resulted in pulp yield loss of 0.58% of pulp dry weight. They concluded that the filtrate from the xylanase treatment mainly contained sugars originating from xylan, indicating that the used commercial xylanase was applicable for the selective hydrolysis of xylan.

Cheng *et al.* (2013) found the influence of enzymatic treatment on peroxide bleaching in respect of yield and viscosity drop in pulp. In set 1, they treated the pulp with xylanase (X stage) at 20 IU/g of dry pulp followed by bleaching with hydrogen peroxide (P stage) at 3.0%, while in set 2, they bleached the pulp with hydrogen peroxide only at 3.6%. They concluded that yield and viscosity of eucalyptus pulp were higher in set 1 by 2.13% and 1.8%, respectively as compared to set 2. Chemical reduction of 17% was also observed with the use of xylanase enzyme in biobleaching of eucalyptus pulp.

Effect of Xylanases on Paper Properties

The modification of the fiber structure of bleached hardwood pulp is a very attractive means for improvement of paper properties. Enzymatic treatment with xylanases modifies the structure and characteristics of fibers, resulting in improved hydration, internal fibrillation, and delamination.

The enzyme-treated pulp showed unchanged or improved strength properties (Kim and Paik 2000; Tolan and Guenette 1997; Tolan *et al.* 1996; Viikari *et al.* 1991; 1993) and was easier to refine than the untreated reference pulps (Wong *et al.* 1999). During the papermaking process, hemicelluloses strengthen inter-fibril bonding and have a favorable effect on the physical properties of fibers themselves. The removal of xylan during an enzymatic bleaching stage interferes with the strength of treated pulps. The loss in burst and tensile strength (32 to 40% and 11 to 25%, respectively) was more notable than that of tear strength (8.2 to 10.3%). The tear resistance measures the work required to tear the paper. The length of fibers and the linkages between them are factors that may affect the tear resistance (Batalha *et al.* 2011). The difference in strength properties, particularly tear strength, between enzyme-treated and untreated pulps is normally minimized by pulp refining (Roncero *et al.* 2005; Wong *et al.* 1996). This may be due to superior external fibrillation of treated pulps after enzymatic elimination of re-deposited xylan from the surface of the fibers (Roncero *et al.* 2005; Shatalov and Pereira 2008). Similar results were also found in a study by Roncero *et al.* (2003b) wherein enzymatically treated hardwood pulps obtained a higher tear resistance value and kept the same tensile index compared to the untreated reference pulp. Znidarsic *et al.* (2009) worked on hardwood pulp and reported that better external fibrillation is observed in the case of the enzyme-treated pulp. Such increased fibrillation favors increased tear resistance. With refining, these fibrils were then probably more or less removed from the fiber surface, causing a weaker tear resistance.

A study was done on a commercial enzyme (Bleachzyme F) by Bajpai and Bajpai (1996) to determine its effects on the physical strength properties of bamboo pulp. Their results are shown in Table 7.

It was also reported (Shatalov and Pereira 2008) that limited degradation of carbohydrates during the bleaching process could be the cause of similar changes in the physical properties of xylanase-treated, peroxide-bleached pulps compared to untreated, unbleached eucalyptus pulps.

A notable work regarding both enzymatic treatments and ultrasonic processes was carried out to determine their effects on the tensile strength of paper (Batalha *et al.* 2011). Tensile strength is related to the durability and utility of the paper. For example, packaging papers are subject to direct tension forces.

Table 7. Effects of Bleachzyme F on the Physical Strength Properties of Bamboo Pulp*

Parameter	Bleachzyme F							
	A*				B*			
	C_DEHD		XC_DEHD		C_DEHD		XC_DEHD	
°SR	30	40	30	40	30	40	30	40
PFI revolution (No.)	1800	2800	1895	2900	1800	2800	1895	2900
Bulk (cm^3/g)	1.65	1.45	1.60	1.40	1.61	1.40	1.58	1.40
Tensile index (N.m/g)	56.72	60.60	57.40	61.54	55.45	59.60	57.78	61.99
Burst index (kN/g)	3.706	3.795	3.667	4.070	3.65	3.82	3.75	3.92
Tear index ($mN.m^2$/g)	7.74	7.61	7.74	7.04	7.60	7.50	7.80	7.11
Double fold (No.)	53	110	78	115	50	105	80	120
A*-Same chemical dose in XC_DEHD as in control								
B*-20% less chlorine dose in XC_DEHD compared to the control								
* Based on data from Bajpai and Bajpai (1996).								

In this work it was also observed that combined enzymatic and ultrasonic treatments resulted in 48.0 and 12.1% increases in MOE and TEA compared to the initial pulp, respectively. It was also shown that the ultrasonic treatment improved opacity when the ultrasound was applied before xylanase treatment.

Effect of Xylanases on the Effluent Characteristics

The bleaching of kraft pulp is responsible for the generation of a large effluent volume in paper mills. Organochlorine compounds, contaminants generated during chlorine-based bleaching, are major components within this effluent (Vidal *et al.* 1997). Presently, the paper industry is looking for new bleaching processes in order to minimize the impact of these effluents on the environment. In the 1990s, the use of enzymes in pre-bleaching stages was intended to improve effluent quality, particularly by reducing the amount of organochlorine compounds (AOX) in the effluent (Faleiros 2008).

Xylanase is very efficient in reducing the consumption of bleaching chemicals (Call and Mucke 1997) such as chlorine or chlorine dioxide. It can lower the AOX in the filtrates by as much as 25% while increasing the brightness of the pulp (Atik *et al.* 2006; Hart and Harry 2005; Manji 2006; Saleem and Akhtar 2002).

In 1991, it was determined that after xylanase pre-bleaching of softwood, the biochemical oxygen demand (BOD) of the filtrate increased by almost two times as compared to non-treated pulp. Similarly, the chemical oxygen demand (COD) and total organic carbon (TOC) were increased, and the ratio of BOD to COD was significantly higher for the xylanase pre-bleaching filtrates, indicating that the effluents were more biodegradable (Senior and Hamilton 1991). In spite of the increase in the COD of the enzymatic prebleaching stage filtrates, treatment of the generated effluents was efficient in aerobic bioreactors. The COD removed was found to be above 85%, similar to the reference. Increases in the organic matter contents of the filtrates led to higher aeration and energy demands in the treatment plant. Moreover, the final COD of the treated effluents from enzymatic pre-bleaching stages was higher than in those generated in conventional bleaching sequences (Borges *et al.* 2010).

Xylanase-treated pulp has significantly lower levels of AOX in its effluents as compared to the effluents of conventionally bleached control pulps (Viikari *et al.* 1986).

In a study by Shobhit *et al.* (2005), it was observed that enzymatic pretreatment of pulp reduced AOX levels by 20 to 30%. During the enzyme treatment period, the amount of AOX being discharged into the receiving waters decreased from 2.4 kg/air-dry metric ton to 2.2 kg/air-dry metric ton.

Effect of Mill Operations on Xylanase Performance

Mill operations also affect the performance of the xylanase enzyme. The performance of xylanase depends on the types of raw materials, the pulping process, and the bleaching sequence (Tolan and Guenette 1997). Among raw materials, the important distinction is between hardwoods and softwoods. The percentage of bleaching chemicals saved by xylanase treatment is greater for hardwoods than for softwoods as the xylan content is greater in hardwood. Under favorable treatment conditions, the decrease in chlorine chemicals is about 20% for hardwoods and 15% for softwoods.

The xylan content of the pulp significantly depends on digester performance. For example, sulfite pulping destroys most of the xylan, so sulfite pulp is not suitable for enhanced bleaching with enzymes. In conventional kraft pulping, the xylan content depends strongly on the effective alkalinity. The bleaching sequence that is being used by the mill is also equally important to the performance of the xylanase in enzymatic bleaching.

CONCLUSIONS AND FUTURE PROSPECTS

Chlorine and alkaline extraction stages have historically been used as the main stages for the bleaching of kraft pulp in the paper industry. These stages generate effluent with high levels of corrosive chloride that cannot be recycled back into the chemical recovery furnace. Generated effluent from these stages contains large amounts of hazardous chemicals in the form of chlorinated organic compounds, which are known to have mutagenic and carcinogenic effects. Presently, regulatory agencies are very concerned with protecting the environment from these pollutants (Vidal *et al.* 1997). The environmental effects of these chlorinated organic compounds have driven pulp mills to seek out new bleaching technologies that reduce or eliminate the consumption of these hazardous chemicals during the bleaching process.

Enzymes are eco-friendly in nature and could be used as a substitute for these hazardous chemicals in the pulp bleaching process. Xylanases, which are capable of reducing the consumption of hazardous chemicals, could prove cost-effective. Byproducts generated from biochemical reactions of microorganisms are generally non-hazardous in nature. Therefore, enzymes produced *via* microbial sources have become alternatives to polluting chemical technologies. However, implementation of these enzymes on the industrial level is still a challenge for the pulp and paper industry.

Bleaching process parameters such as temperature and pH act as limiting factors preventing the best possible use of bleaching chemicals. High pH and temperature are favorable for bleaching processes. Many xylanase-producing commercial strains which are highly active and stable at high pH and temperatures are available. Still, an innovative approach should be explored for the screening of such novel xylanolytic microbial strains. Such an approach would need to be able to work at high pH and temperature within cost-effective processes on an industrial scale. Microbial and recombinant DNA methods for obtaining xylanase enzymes with new properties must be explored so that enzymes can be commercialized easily. When enzymes become cost-effective to produce and use, the paper industry will enjoy benefits like the prevention of environmental degradation and reduction of health hazards.

In order to bring about such a revolution in paper production and industrial applications, microbiologists, biotechnologists, and biochemists should work cooperatively with the paper industry towards a pollutant-free future.

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REFERENCES CITED

Adorjan, I., Jaaskelainen, A. S., and Vuorinen, T. (2006). "Synthesis and characterization of the hexenuronic acid model methyl 4-deoxy-beta-L-threo-hex-4-enopyranosiduronic acid," *Carbohydrate Research* 341(14), 2439-2443.

Allison, R. W., and Clark, T. A. (1994). "Effect of enzyme pre-treatment on ozone bleaching," *TAPPI Journal* 77(7), 127-134.

Almeida, F. S. D. (2004). "Influence of alkali charge on hexenuronic acid formation and pulping efficiency for lo-solids cooking of eucalyptus," *Engineering, Pulping and Process Control Division, TAPPI Technical Conference*, Chicago, pp. 1-13.

Archana, A., and Satyanarayana, T. (2003). "Purification and characterization of a cellulose free xylanase of a moderate thermophile *Bacillus licheniformis* A99," *World Journal of Microbiology and Biotechnology* 19(1), 53-57.

Atik, C., Imamoglub, S., and Bermekc, H. (2006). "Impact of xylanase pre-treatment on peroxide bleaching stage of biokraft pulp," *International Biodeterioration & Biodegradation* 58(1), 22-26.

Bajpai, P., Bhardwaj, N. K., Bajpai, P. K., and Jauhari, M. B. (1994). "The impact of xylanases on bleaching of eucalyptus kraft pulp," *Journal of Biotechnology* 38(1), 1-6.

Bajpai, P., and Bajpai, P. K. (1996). "Application of xylanases in prebleaching of bamboo kraft pulp," *TAPPI Journal* 79(4), 225-230.

Bajpai, P., and Bajpai, P. K. (1997). "Microbial xylanolytic enzyme system: Properties and Applications," *In:* Neildleman, S., Laskin, A., (Eds.), *Advances in Applied Microbiology*, Vol. 43, Academic Press, New York, NY, pp. 141-194.

Bajpai, P., and Bajpai, P. K. (1999). "Time for enzymes in pulp bleaching," *In paper International* 3(4), 17-19.

Bajpai, P., and Bajpai, P. K. (2001). "Bleaching of dissolving kraft pulp with xylanase enzyme," *8th International Conference on Biotechnology in the Pulp and Paper Industry*, Finland, pp. 310.

Bajpai, P., and Bajpai, P. K. (2001). "Development of a process for the production of dissolving kraft pulp using xylanase enzyme," *APPITA Journal* 54(4), 381-384.

Bajpai, P. K., Bajpai, P., Anand, A., Sharma, N., Mishra, O. P., and Vardhan, R. (2005). "Hexenuronic acids in different pulps and its removal effects on bleaching and pulp properties," *7th International Conference on Pulp, Paper and Conversion Industry*, India, pp. 393-405.

Bajpai, P., Anand, A., and Bajpai, P. K. (2006). "Bleaching with lignin-oxidizing enzymes," *In: Biotechnology Annual Review,* Vol. 12, *Elsevier B.V.*, Amsterdam, Chapter 10: pp. 349-378.

Batalha, L. R., Silva, J., Jardim, C., Oliveira, R., and Colodette, J. (2011). "Effect of ultrasound and xylanase treatment on the physical-mechanical properties of bleached eucalyptus kraft pulp," *Natural Resources* 2(2), 125-129.

Beg, Q. K., Kapoor, M., Mahajan, L., and Hoondal, G. S. (2001). "Microbial xylanases and their industrial applications: A review," *Applied Microbiology and Biotechnology* 56(3-4), 326-338.

Bim, M. A., and Franco, T. T. (2000). "Extraction in aqueous two phase systems of alkaline xylanase produced by *Bacillus pumilus* and its application in kraft pulp bleaching," *Journal of Chromatography B: Biomedical Sciences and Applications* 743(1-2), 349-356.

Blomstedt, M., Asikainen, J., Lahdeniemi, A., Ylonen, T., Paltakari, J., and Hakala, T. K. (2010). "Effect of xylanase treatment on dewatering properties of birch kraft pulp," *Bioresources* 5(2), 1164-1177.

Borges, M. T., Silva, C. M., Colodette, J. L., Alves, L. B., Rodrigues, G. R., Lana, L. C., and Tesser, F. (2010). "Effect of eucalyptus kraft pulp enzyme bleaching on effluent quality and bio-treatability," *Pulp & Paper Canada* 111(4), 23-26.

Cadena, E. M., Vidal, T., and Torres, A. L. (2010). "Influence of the hexenuronic acid content on refining and ageing in eucalyptus TCF pulp," *Bioresource Technology* 101(10), 3554-3560.

Call, H. P., and Mucke, I. (1997). "History, overview and applications of mediated lignolytic systems, especially laccase-mediator-systems (Lignozym®-process)," *Journal of Biotechnology* 53(2-3), 163-202.

Chakar, F. S., Allison, L., Donough, T. J., and Ragauskas, A. J. (2000). "Evaluation of hexenurunic acids in U.S. kraft pulps," *6th European Workshop on Lignocellulosics and Pulp*, Bordeaux, France, pp. 1-6.

Chauhan, S., Choudhury, B., Singh, S. N., and Ghosh, P. (2006). "Application of xylanase enzyme of *Bacillus coagulans* as a prebleaching agent on non-woody pulps," *Process Biochemistry* 41(1), 226-231.

Cheng, X., Chen, G., Huang, S., and Liang, Z. (2013). "Biobleaching effect of crude xylanase from *Streptomyces griseorubens* LH-3 on eucalyptus kraft pulp," *BioResources* 8(4), 6424-6433.

Faleiros, M. (2008). "Chemicals come to an alliance with the sector eco-efficiency," *O PAPEL Journal*, pp. 36-38.

Farrell, R. L., Viikari, L., and Senior, D. (1996). "Enzyme treatment of pulp" in *Pulp Bleaching, Principles and Practice*," C. W. Dence and D. W. Reeve, (eds.), *TAPPI Press*, Atlanta, G.A., Chapter 7, pp. 365-377.

Gliese, T., Kleemann, S., and Fischer, K. (1998). "Investigations on mechanism and kinetics of xylanase on prebleaching," *Pulp and Paper Canada* 12(99), 171-174.

Gubitz, G., Haltrich, D., Latal, B., and Steiner, W. (1997). "Mode of depolymerisation of hemicellulose by various mannanases and xylanases in relation to their ability to bleach softwood pulp," *Applied Microbiology and Biotechnology* 47(6), 658-662.

Hart, P. W., and Harry, S. F. (2005). “Statistical determination of the effects of enzymes on bleached pulp yield,” *TAPPI Journal* 4(8), 3-6.

Henriksson, G., and Teeri, T. (2009). “Biotechnology in the forest industry,” In: *Pulp and Paper Chemistry and Technology Volume 1. Wood Chemistry and Wood Biotechnology*, Ek, M., Gellerstedt, G., and Henriksson, G., (eds.), *Walter de Gruyter*, pp. 273-300.

Jeffries, T. W. (1992). “Enzymatic Treatments of Pulps,” In: Rowell, R. M., Schultz, T. P., and Narayan, R., (eds.), *Emerging Technologies for Materials and Chemicals from Biomass*, ACS Symposium Series 476, American Chemical Society, Washington, D.C., pp. 313-329.

Jimenez, L., Martinez, C., Maestre, F., and Lopez, F. (1996). “Biobleaching of pulp from agricultural residues with enzymes,” *Bioprocess Engineering* 14(5), 261-262.

Kantelinen, A., Hortling, B., Sundquist, J., Linko, M., and Viikari, L. (1993). “Proposed mechanism of the enzymatic bleaching of kraft pulp with xylanases,” *Holzforschung* 47(4), 318-324.

Keefe, A., and Teschke, K. (2011). “Environmental and public health issues,” *Encyclopedia of Occupational Health and Safety*, International Labor Organization, Geneva.

Khasin, A., Alchanati, I., Shoham, Y. (1993). “Purification and characterization of a thermostable xylanase from *Bacillus stearothermophilus* T-6,” *Applied and Environmental Microbiology* 59(6), 1725–1730.

Kim, D. H., and Paik, K. H. (2000). “Effect of xylanase pre and post treatment on oxygen bleaching of oak kraft pulp,” *Journal of Industrial and Engineering Chemistry* 6(3), 194-200.

Ledoux, P., Detroz, R., DeBuyl, E., Throughton, N., Shetty, J., and Presley, J. R. (1993). “Use of bacterial xylanase in chlorine free bleaching sequences,” Pulping conference, TAPPI Proceedings 1057-1065.

Lian, H. L., You, J. X., and Lian, Z. N. (2011). “Effect of machanochemistry on biobleaching of wheat straw pulp with laccase/xylanase treatment,” International Conference on Agricultural and Natural Resources Engineering, Advances in Biomedical Engineering 3-5, 44-51.

Lin, X. Q., Han, S. Y., Zhang, N., Hu, H., Zheng, S. P., Ye, Y. R., and Lin, Y. (2013). “Bleach boosting effect of xylanase A from *Bacillus halodurans* C-125 in ECF bleaching of wheat straw pulp,” *Enzyme and Microbial Technology* 52(2), 91-98.

Manimaran, A., Kumar, K. S., Permaul, K., and Singh, S. (2009). “Hyper production of cellulase-free xylanase by *Thermomyces lanuginosus* SSBP on bagasse pulp and its application in biobleaching,” *Applied Microbiology and Biotechnology* 81(5), 887-893.

Manji, A. H. (2006). “Extended usage of xylanase enzyme to enhance the bleaching of softwood kraft pulp,” *TAPPI Journal* 5(1), 23-26.

Marechal, A. (1993). “Acid extraction of the alkaline wood pulps (kraft or soda/AQ) before or during bleaching, reason and opportunity,” *Journal of Wood Chemistry and Technology* 13(2), 261-281.

Nagar, S., Jain, R. K., Thakur, V. V., and Gupta, V. K. (2013). “Biobleaching application of cellulase poor and alkali stable xylanase from *Bacillus pumilus* SV-85S,” *3 Biotechnology* 3(4), 277-285.

Nguyen, D., Zhang, X., Jiang, Z. H., Audet, A., Paice, M. G., Renaud, S., and Tsang, A. (2008). "Bleaching of kraft pulp by a commercial lipase: Accessory enzymes degrade hexenuronic acids," *Enzyme and Microbial Technology* 43(2), 130-136.

Niehaus, F., Bertoldo, C., Kahler, M., and Antranikian, G. (1999). "Extremophiles as a source of novel enzymes for industrial applications," *Applied Microbiology and Biotechnology* 51(6), 711-729.

Paice, M. G., Bourbonnais, R., Reid, I. D., Archibald, F. S., and Jurasek, L. (1995). "Oxidative bleaching enzymes: A review," *Journal of Pulp and Paper Science* 21(8), 280-284.

Paice, M. G., Gurnagul, N., Page, D. H., and Jurasek, L. (1992). "Mechanism of hemicellulose directed prebleaching of kraft pulp," *Enzyme and Microbiological Technology* 14(4), 272-276.

Pham, P. L., Alric, I., and Delmas, M. (1995). "Incorporation of xylanase in total chlorine free bleach sequences using ozone and hydrogen peroxide," *APPITA Journal* 48(3), 213-217.

Ratanachomsri, U., Sriprang, R., Sornlek, W., Buaban, B., Champreda, V., Tanapongpipat S., and Eurwilaichitr, L. (2006). "Thermostable Xylanase from Marasmius sp.: Purification and Characterization," *Journal of Biochemistry and Molecular Biology* 39 (1), 105-110.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (1999). "Study the influence of xylanase on the fibre surfaces by SEM," *In: Proceedings of Microscopy as a Tool in Pulp and Paper Research and Development*, Stockholm, Sweden, pp. 27-30.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2000a). "Effects of xylanase treatment on fibre morphology in totally chlorine free bleaching (TCF) of eucalyptus pulp," *Process Biochemistry* 36(1), 45-50.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2000b). "Using xylanase before oxygen delignification on TCF bleaching. Influence on fibre surfaces by SEM," *Process Biochemistry* 36(1-2), 45-50.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2003a). "Effect of xylanase on ozone bleaching kinetics and properties of eucalyptus kraft pulp," *Journal of Chemical Technology and Biotechnology* 78(10), 1023-1031.

Roncero, M. B., Torres, A. L., Colom, J. F, and Vidal, T. (2003b). "TCF bleaching of wheat straw pulp using ozone and xylanase, Part A: Paper quality assessment," *Bioresource Technology* 87(3), 305-314.

Roncero, M. B., Torres, A. L., Colom, J. F, and Vidal, T. (2003c). "TCF bleaching of wheat straw pulp using ozone and xylanase, Part B: Kinetic studies," *Bioresource Technology* 87(3), 315-323.

Roncero, M. B., Torres, A. L., Colom, J. F., and Vidal, T. (2005). "The effect of xylanase on lignocellulosic components during the bleaching of wood pulps," *Bioresource Technology* 96(1), 21-30.

Saleem, M., and Akhtar, M. S. (2002). "Biobleaching of kraft pulp by xylanase produced by *Bacillus subtilis*," *International Journal of Agriculture and Biology* 4(2), 242-244.

Salles, B. C., Medeiros, R. G., Bao, S. N., Silva, F. G., and Filho, E. X. F. (2005). "Effect of cellulase free xylanases from *Acrophialophora nainiana* and *Humicola grisea* var. thermoidea on eucalyptus kraft pulp," *Process Biochemistry* 40(1), 343-349.

Senior, D. J., and Hamilton, J. (1991). "Use of xylanases for the reduction of AOX in kraft pulp bleaching," *CPPA Environmental Conference*, Quebec, Canada, pp. 310-314.

Senior, D. J., and Hamilton, J. (1992a). "Bleaching with xylanases brings biotechnology to reality," *Pulp and Paper* 66(9), 111-114.
Senior, D. J., and Hamilton, J. (1992b). "Reduction in chlorine use during bleaching of kraft pulp following xylanase treatment," *TAPPI Journal* 75(11), 125-130.
Senior, D. J., and Hamilton, J. (1992c). "Use of xylanases to decrease the formation of AOX in kraft pulp bleaching," *Journal of Pulp and Paper Science* 18(15), 165-168.
Senior, D. J., and Hamilton, J. (1993). "Xylanase treatment for the bleaching of softwood kraft pulps: The effect of chlorine dioxide substitution," *TAPPI Journal* 76(8), 200-206.
Senior, D. J., Hamilton, J., Taiplus, P., and Torvinen, J. (1999). "Enzyme use can lower bleaching costs, aid ECF conversions," *Pulp and Paper* 73(7), 59-62.
Shah, A. K., Cooper, D., Adolphson, R., and Eriksson, K. E. L. (2000). "Xylanase treatment of oxygen bleached hardwood kraft pulp at high temperature and alkaline pH levels gives substantial savings in bleaching chemicals," *Journal of Pulp and Paper Science* 26(1), 8-11.
Sharma, A., Adhikari, S., and Satyanarayana, T. (2007). "Alkali thermostable and cellulase free xylanase production by an extreme thermophile *Geobacillus thermoleovorans,"* *World Journal of Microbiology and Biotechnology* 23(4), 483-490.
Shatalov, A. A., and Pereira, H. (2008). "Effect of xylanases on peroxide bleachability of eucalypt (*E. globulus*) kraft pulp," *Biochemical Engineering Journal* 40(1), 19-26.
Shatalov, A. A., and Pereira, H. (2009). "Impact of hexenuronic acids on xylanase-aided bio-bleaching of chemical pulps," *Bioresource Technology* 100(12), 3069-3075.
Shirkolaee, Y. Z., Talebizadeh, A., and Soltanali, S. (2008). "Comparative study on application of *T. lanuginosus* SSBP xylanase and commercial xylanase on biobleaching of non wood pulps," *Bioresource Technology* 99(16), 7433-7437.
Shobhit, M., Satish, K., and Rao, N. J. (2005). "Action of xylanase prebleaching on wheat straw and oxygen delignified wheat straw soda pulps - Probable mechanisms," *59th Appita Annual Conference and Exhibition: Incorporating the 13th ISWFPC,* Auckland, New Zealand, pp. 631-638.
Simeonova, G., Sjodahl, R., Ragnar, M., Lindstrom, M. E., and Henriksson, G. (2007). "On the effect of a xylanase post treatment as a means of reducing the yellowing of bleached hardwood kraft pulp," *Nordic Pulp and Paper Research Journal* 22(2), 172-176.
Spence, K., Tucker, J., and Hart, P. W. (2009). "Comparison of various hardwood kraft pulp pre-bleaching techniques," *TAPPI Journal* 8(4), 10-14.
Subramaniyan, S., Prema, P, (2002). "Biotechnology of microbial xylanases: enzymology, molecular biology, and application," *Critical Reviews in Biotechnology* 22(1), 33-64.
Suurnakki, A., Tenkanen, M., Buchert, J., and Viikari, L. (1997). "Hemicellulases in the bleaching of chemical pulps," *In:* Scheper, T., (Ed.), *Advances in Biochemical Engineering / Biotechnology*, Vol. 57, *Springer Verlag*, Berlin, Germany, pp. 261-287.
Thakur, V. V., Jain, R. K., and Mathur, R. M. (2012). "Studies on xylanase and laccase enzymatic prebleaching to reduce chlorine based-chemicals during CEH and ECF bleaching," *BioResources* 7(2), 2220-2235.
Tolan, J. S., and Canovas, R. V. (1992). "The use of enzymes to decrease the chlorine requirements in pulp bleaching," *Pulp and Paper Canada* 93(5), 39-42.

Tolan, J. S., Olson, D., Dines, R. E. (1996). "Survey of mill usage of xylanase," *In:* Jeffries, T. W., Viikari, L. (eds.), *Enzymes for Pulp and Paper Processing*, ACS Symposium Series 655, American Chemical Society, Washington, D.C., pp. 25-35.

Tolan, J. S., and Guenette, M. (1997). "Using enzymes in pulp bleaching: Mill applications," in: Scheper, T., (Ed.), *Advances in Biochemical Engineering / Biotechnology*, Vol. 57, Springer Verlag, Berlin, Germany, pp. 289-310.

Torres, A. L., Roncero, M. B., Colom, J. F., Pastor, F. I. J., Blanco, A., and Vidal, T. (2000). "Effect of a novel enzyme on fibre morphology during ECF bleaching of oxygen delignified *Eucalyptus* kraft pulps," *Bioresource Technology* 74(2), 135-140.

Turner, J. C., Skerker, P. S., Burns, B. J., Howard, J. C., Alonso, M. A., and Andres, J. L. (1992). "Bleaching with enzymes instead of chlorine: Mill trials," *TAPPI Journal* 75(12), 83-89.

Valls, C., and Roncero, M. B. (2009). "Using both xylanase and laccase enzymes for pulp bleaching," *Bioresource Technology* 100(6), 2032-2039.

Valls, C., Vidal, T., and Roncero, M. B. (2010a). "Boosting the effect of a laccase-mediator system by using a xylanase stage in pulp bleaching," *Journal of Hazardous Materials* 177(1-3), 586-592.

Valls, C., Vidal, T., and Roncero, M. B. (2010b). "The role of xylanases and laccases on hexenuronic acid and lignin removal," *Process Biochemistry* 45(3), 425-430.

Vidal, G., Soto, M., Field, J., Mendez, P. R., and Lema, J. M. (1997). "Anaerobic biodegradability and toxicity of wastewaters from chlorine and total chlorine-free bleaching of eucalyptus kraft pulps," *Water Research* 31(10), 2487-2494.

Viikari, L., Ranua, M., Kantelinen, A., Sundquist, J., and Linko, M. (1986). "Bleaching with enzymes," *Proceedings of the 3rd International Conference on Biotechnology in the Pulp and Paper Industry*, Stockholm, Sweden, pp. 67-69.

Viikari, L., Kantelinen, A., Ratto, M., and Sundquist, J. (1991). "Enzymes in pulp and paper processing," *Enzymes in Biomass Conversion* Chapter 2: Vol. 460, pp. 12-21.

Viikari, L., Tenkanen, M., Buchert, J., Ratto, M., Bailey, M., Siikaaho, M., and Linko, M. (1993). "Hemicellulases for industrial applications," in: *Bioconversion of Forest and Agricultural Wastes*, Saddler J. (ed.), CAB International, Wallingford, pp. 131-182.

Viikari, L., Suurnakki, A., and Buchert, J. (1996). "Enzyme-aided bleaching of kraft pulps: Fundamental mechanisms and practical applications," *Enzymes for Pulp and Paper Processing* 655, 15-24.

Vuorinen, T., Fagerstrom, P., Buchert, J., Tenkanen, M., and Teleman, A. (1999). "Selective hydrolysis of hexenuronic acid groups and its application in ECF and TCF bleaching of kraft pulps," *Journal of Pulp and Paper Science* 25(5), 155-162.

Wang, L., Jiang, L. K., and Argyropoulos, D. S. (1997). "Isolation and characterization of lignin extracted from softwood kraft pulp after xylanase treatment," *Journal of Pulp and Paper Science* 23(2), 47-51.

Wong, K. K. Y., Nelson, S. L., and Saddler, J. N. (1996). "Xylanase treatment for the peroxide bleaching of oxygen delignified kraft pulps derived from three softwood species," *Journal of Biotechnology* 48(1-2), 137-145.

Wong, K. K. Y., Kibblewhite, R. P., and Signal, F. A. (1999). "Effect of xylanase and dosage on the refining properties of unbleached softwood kraft pulp," *Journal of Wood Chemistry and Technology* 19(3), 203-212.

Wong, K. K. Y., Allison, R. W., and Spehr, S. (2001). "Effect of alkali and oxygen extractions of kraft pulps on xylanase-aided bleaching," *Journal of Pulp and Paper Science* 27(7), 229-234.

Woolridge, E. M. (2014). "Mixed Enzyme Systems for Delignification of Lignocellulosic Biomass," *Catalysts* 4(1), 1-35.

Znidarsic, P. P., Rutar, V., and Ravnjak, D. (2009). "The effect of enzymatic treatments of pulps on fiber and paper properties," *Chemical and Biochemical Engineering* 23(4), 497-506.

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