Differences in bleaching responses from fungal- versus bacterial-derived enzymes

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ABSTRACT: Several mills in North America have been successful in using xylanase enzymes expressed from *Trichoderma reesei* (a fungus) as part of their bleaching sequence for many years. These mills process hardwood and softwood species, with and without oxygen delignification. These mills also use three-, four-, and five-stage bleaching sequences. North American mills tend to report increased pulp brightness ceilings and decreased bleaching costs as benefits associated with the application of enzymes in the bleaching process. Laboratory testing suggests that eucalyptus pulp is highly susceptible to fungal- and bacterial-derived enzyme bleaching and should result in significant cost savings in South American mills. At least four different mills in South America have attempted to perform enzyme bleaching trials using bacterial-derived enzymes. Each of these mill trials resulted in significantly increased operating costs and/or unsustainable operating conditions. More recently, one of these South American mills performed a short trial using a commercially available, fungal-derived enzyme. This trial was technically successful. This report attempts to determine why the South American mill experiences with bacterial-derived enzymes have been poor, while North American mills and the one South American mill trial have had good results with fungal-derived enzymes. Operating conditions and trial goals for the North and South American mills also were examined.

Application: By better understanding the differences between bacterial- and fungal-produced enzymes, mills should be able to choose commercial applications that minimize negative performance aspects associated with enzyme bleaching applications.

Enzymatic bleaching has been in commercial operation in North America for many years. Typically, these enzymes have been obtained from fungal sources such as *Trichoderma reesei*. The use of enzymes in kraft pulp bleaching has been well documented to either reduce the total amount of oxidant required to obtain a given pulp brightness [1-5] or to increase the final pulp brightness for a given oxidant charge [6-8]. Early enzyme biobleaching applications used blends of natural enzymes that attacked a broad spectrum of materials in the pulp. Most enzyme-assisted bleaching uses xylanases that hydrolyze xylan. Xylanases have been isolated from a number of microorganisms, including eukaryotes (e.g., the fungi *Trichoderma* spp., *Aspergillus* spp.) [9,10] and prokaryotes of both filamentous (i.e., *Actinomycetes*) [11-14] and non-filamentous varieties [15]. The use of xylanases from fungi in pulp bleaching can be problematic because some species produce cellulases along with the desired hemicellulases, and even small amounts of cellulase can negatively affect important pulp properties, including yield, viscosity, and strength properties. Other sources of xylanases have been obtained from *Bacillus circulans*, *Escherichia coli*, and other bacterial sources.

Early enzyme applications used naturally occurring blends of enzymes as produced by the organism being used to secrete them. When used in pulp bleaching, these blends substantially and negatively affected pulp yield and strength. Frequently, these naturally occurring enzyme blends contained high levels of cellulase enzymes. Recognizing the potential commercial applications, enzyme producers have conducted wide-spread screening of known hemicellulase-producing microorganisms and successfully isolated species that generate essentially cellulase-free crude enzyme preparations. Alternately, enzyme producers have developed purification methods to produce cellulase-free commercial products. The use of these formulations has led to enhanced pulp properties, such as increased viscosity and improved beatability [16]. Even with the improvements in enzyme preparation and purification for commercial applications, published literature and some laboratory studies suggest that the use of enzymes will reduce pulp yield [2,3]. Furthermore, commercial applications of xylanases can still have trace amounts of cellulases, which can reduce yield.

The exact mechanism used in improving bleaching results is not well understood. What is understood is that removal of xylan and xylan complexes from fiber either reduces the kappa number of the treated pulp [17] or renders the fiber more susceptible to lignin removal in subsequent bleaching stages [6]. In general, xylanase enzymes do only very specific things; they partially remove xylan from the fiber and reduce the degree of polymerization of the residual xylan [18]. During the production of kraft pulp, residual lignin and lignin

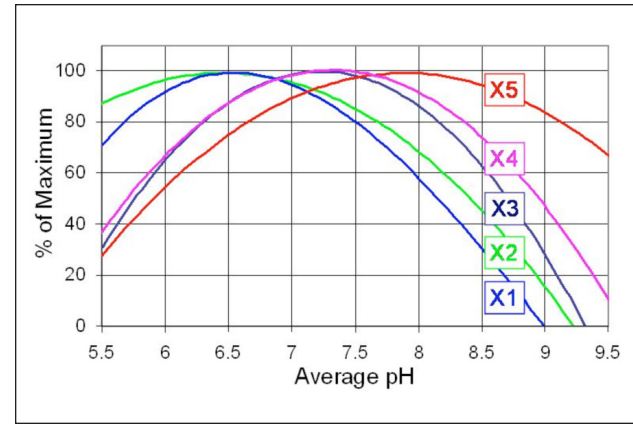
derivatives are either covalently attached to carbohydrate moieties in the pulp [19] or entrapped in the pulp matrix by reprecipitated xylan located on the fiber surface [20]. Hexenuronic acid groups also might be bonded to xylan chains and contribute to measured kappa number (false lignin) [21].

Multistage, oxidative chemical-based bleaching processes are used to extract the residual lignin and hexenuronic acid groups and brighten the resulting bleached pulp. The application of endoxylanase before oxidative chemical treatment can facilitate subsequent removal of residual lignin, thus reducing the demand for chlorine dioxide in the bleaching sequence [22]. The exact mechanism for improved lignin removal is not known. The breakdown of xylan within the pulp matrix might open the fiber structure sufficiently to allow enhanced access of bleaching chemicals and easier removal of larger residual lignin fragments [23]. Alternately, hemicellulase activity might remove lignin carbohydrate complexes (LCCs) from the pulp fiber [17,19]. Finally, xylanase activity might simply remove reprecipitated xylan from the kraft pulp surface and release entrapped lignin [20]. Xylans containing hexenuronic acids also might be removed, thus lowering the measured kappa number, which results in lowered chlorine dioxide consumption during bleaching.

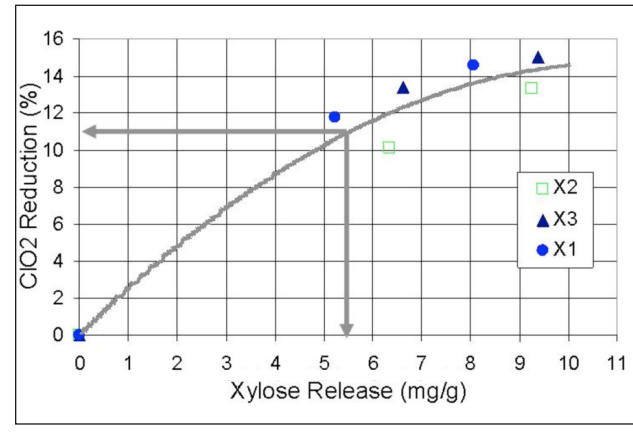
A challenge to understanding of enzyme mechanisms is that not all xylanase enzymes exhibit the same type or level of bleaching effect [17]. Some xylanases have been reported to significantly reduce the lignin content of the treated fiber [16]. Other xylanases have little or no effect on kappa number, but still result in significant oxidant reduction to obtain a target brightness [5]. Of the xylanase enzymes currently in use, the fungal enzymes tend to enhance oxidative chemical effectiveness, while the bacterial enzymes tend to produce a significant kappa reduction [21]. The fungal enzymes are thought to not exhibit the same level of tenacity toward carbohydrate binding domains; hence, they are less aggressive than bacterial based enzymes [24]. In fact, only two family 11 enzymes have been identified to have substrate binding domains. These are xylanases produced from the filamentous bacterium *Streptomyces lividans* and *Thermomonospora fusca* [25]. The *T. fusca* bacterial enzyme binds to both cellulose and insoluble xylan, but the enzyme has activity against only xylan [25]. Thus, fungal-based enzymes tend to produce a lesser kappa drop and a lesser degree of hemicellulose removal for a given charge than corresponding bacterial-based enzymes.

BLEACHING IN NORTH AMERICA WITH FUNGAL XYLANASE

Currently, at least 12 North American mills are using fungal-derived enzymes as part of their bleaching sequence. These mills encompass most aspects of traditional pulp bleaching. Hardwood and softwood kraft pulping, with and without oxygen delignification, and three-, four-, or five-stage bleach plants have found economic and operational advantages through enzyme application [10,17,22,26]. To be considered

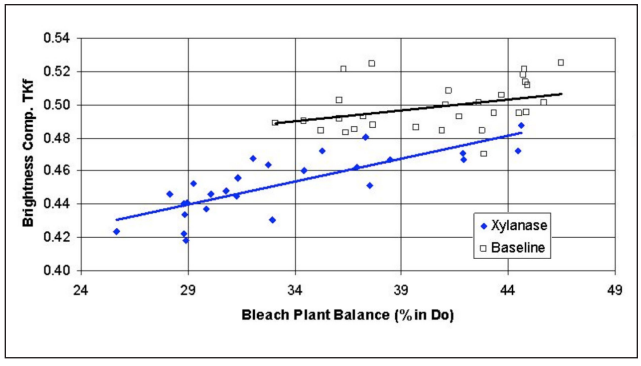


1. Effect of pH on the optimal performance of various xylanases enzymes [26].

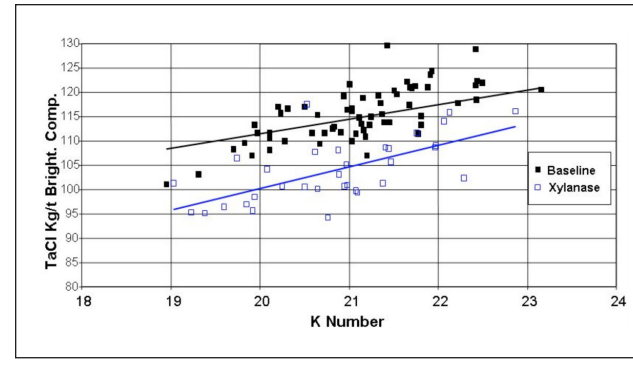


2. Chlorine dioxide reduction obtained as a function of the amount of xylose released in the enzyme stage for three different xylanase enzymes. Typically, for a hardwood pulp, a minimum of 5.5 mg/g of xylose release is required to obtain an economically viable enzyme application [23,26].

a viable candidate for enzyme application, a mill must overcome several hurdles. In general, a mill will have to have an enzyme application point with good mixing and at least 15 min retention time afterwards. The pH during retention preferably should be in the 6.0 to 8.0 range. It is important to match the optimal pH with the type of enzyme being used. **Figure 1** shows laboratory results reporting enzyme performance as a function of the system pH for several different xylanase enzymes [26]. Depending on the type of enzyme, application temperature, and retention time, pH conditions as wide as 5.5 to 9.0 may be acceptable [23]. Generally, the application temperature should be in the range of 40°C to 80°C. Finally, the pulp needs to be susceptible to enzymes. Pulp susceptibility is determined by measuring the xylose release in laboratory treated pulp samples. Successful xylanase applications typically require a minimum xylose release of 5.5 mg of xylose per gram of fiber treated for hardwood pulps and 3.5 mg/g for softwood fiber [23,26]. **Figure 2** shows the percentage chlorine dioxide reduction obtained as a function of xylose release from the fiber for three different xylanase en-



3. Effect of bleach plant balance on total sequence kappa factor required to bleach to a constant target brightness with and without enzymes. North American hardwood mill with four-stage bleaching.



4. Total active chlorine (TaCl) required to bleach to a target pulp brightness with and without enzymes. North American softwood mill with a five-stage bleach plant.

zymes. Laboratory bleaching was performed to a target brightness to determine the amount of chlorine dioxide reduction obtained. When all of these conditions are met, a mill may perform enzyme bleaching trials.

Figure 3 shows the effect of reducing the percentage of applied oxidant in the first stage of the bleaching sequence on the total kappa factor (TKf) required to obtain a constant final pulp brightness. As the percentage of applied chlorine dioxide in the first stage is reduced, the total chlorine dioxide savings resulting from enzyme application increases. Typically, full brightness results can be obtained in the D_1 stage even with the substantially reduced front-end chemical charge. Well-performing mills have reduced their total chlorine dioxide requirement by as much as 10%–24% through the proper application of xylanase enzymes. **Figure 4** shows data from a North American softwood mill operating with and without enzymes. Note the significant reduction in total active chlorine when enzymes are used in the constant final brightness bleaching sequence. Note also that no significant change in kappa number occurred between the baseline and after enzyme treatment. No change in effluent biological oxygen demand (BOD) has been reported from the application of enzymes [27] in the North American bleach plants.

BLEACHING WITH A BACTERIAL XYLANASE IN SOUTH AMERICA

Unlike North American mills, mills in South America are not currently using enzyme bleaching in their commercial bleach plants. At least five mill enzyme trials (four eucalyptus and one radiata pine) have been performed. All of these trials have been definitive failures. Four of these mill trials (three eucalypt and one radiata pine) were conducted on fiber lines containing oxygen bleaching and having a final brownstock press in the final washing position post-enzyme treatment. The mill configurations are fairly closed, with enzyme stage filtrate being returned through the brownstock washing system and ultimately being recycled through the oxygen bleaching system. In each case, initial results suggested that enzyme application was performing as expected. A brightness increase in the D_1 stage was noted. Then, after a short period of operation, the performance of the oxygen delignification system started to deteriorate because of a significant and rapid increase in the total organic carbon (TOC) and chemical oxygen demand (COD) returning from the enzyme treatment stage. As the TOC and COD increased and the oxygen stage performance deteriorated, additional oxidant (chlorine dioxide) was required in the first stage of the bleaching sequence. The resulting increase in first-stage oxidant more than offset the potential savings of oxidant resulting from the enzyme treatment.

The fifth mill (eucalypt) trial was performed at a mill with a relatively open effluent configuration. The enzyme treatment was performed in a high density chest feeding the first bleaching stage, with no washing between the enzyme treatment and the first chlorine dioxide stage. The effluent from the first stage of bleaching was sewered to the waste treatment plant. Upon enzyme application, signs of a successful application were seen at the start of the process. A brightness increase in the D1 stage was noted. A significant reduction in chlorine dioxide was realized in the D1 stage, while maintaining the target brightness. Within 24 h of operation, the trial had to be canceled because of excessive BOD and COD entering the waste treatment plant from the bleach plant. Shortly after the elimination of enzymes from the bleaching sequence, the BOD and COD levels going to waste treatment returned to normal. The higher TOC and COD in the bleach plant effluent might have been caused by solublized xylans resulting from the enzyme treatment.

BLEACHING WITH FUNGAL XYLANSE IN SOUTH AMERICA

After the failed bacterial-based xylanase trial at the radiata pine mill, a second trial was performed using a commercially available *Trichoderma sp.* derived enzyme typically used in North America. Again, at the start of the trial, a significant brightness increase was noted in the D_1 stage and about 12% of the total applied chlorine dioxide was able to be eliminated from the bleaching sequence. A very slight increase in TOC was noted in the effluent used in the brownstock washing and

no negative effect was noted in the oxygen delignification stage performance. This trial was scheduled to be very short, and although technically successful, has not been repeated.

None of the trials (failed or technically successful) were performed over a long enough period to fully optimize and categorize the full effect of chemical savings associated with enzyme application. In general, the one successful trial obtained a significant reduction in total applied oxidant and all of the trials experienced an initial significant increase in D_1 stage brightness.

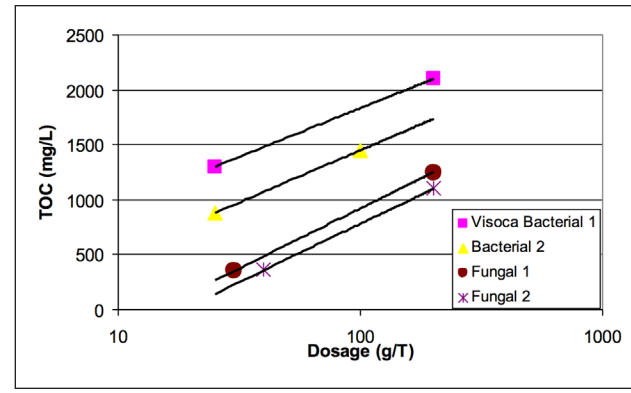
DISCUSSION

Initially, the difference in bleach plant behavior between the North American and South American mills was puzzling because enzyme applications have been used successfully in fairly closed oxygen delignification-containing fiber lines of similar configurations within North America and Europe. Enzymes also have been used successfully in North America without affecting the BOD entering the waste treatment plant [27]. Careful comparison of these successful North American applications with the South American applications revealed one major difference: the type of xylanase enzyme used in the respective applications differed. In the North American and European applications, a highly purified fungal-based xylanase was used in the enzyme stage application. In each of the failed South American mill trials, a bacterial xylanase was used.

Another difference for the eucalyptus mills is that, according to laboratory testing with fungal-based enzymes, eucalyptus fiber tends to be much more reactive towards xylanase treatment than are corresponding mixed southern U.S. hardwoods. A fungal enzyme application of only 60 mL/ton of eucalyptus fiber was required to obtain a 12% chlorine dioxide reduction, while a dosage of 150 mL/ton of mixed southern U.S. hardwood fiber of the same enzyme was required to obtain the same percentage chlorine dioxide reduction.

Xylan structures are complex, long-chain, heterogeneous branched polymers found within the cell wall. The acetylated xylan of hardwoods and the arabinoxylan of softwoods are the two major forms of xylan in wood [28]. These two types of xylan have single 4-O-methyl-α-D-glucuronic acid substituents, occurring on approximately 10% and 18% of their xylosyl residues, respectively [24]. The acidic unit is attached to C-2 of the xylosyl residues, whereas O-acetyl and o-L-arabinofuranosyl substituents tend to occur on C-3; none of the xylosyl residues have more than one branch. The acetyl substituents occur on approximately 70% of the xylosyl residues in hardwood xylans, whereas the arabinosyl substituents occur on approximately 12% of the xylosyl residues in softwood xylans. Hardwood and softwood xylans have a reducing end group consisting of a rhamnosyl, a galacturonosyl, and a xylosyl residue [29,30]. Some xylans have branches containing various combinations of arabinosyl, galactosyl, glucuronosyl, and xylosyl residues [31-34].

Xylan appears to be a major link between lignin and other

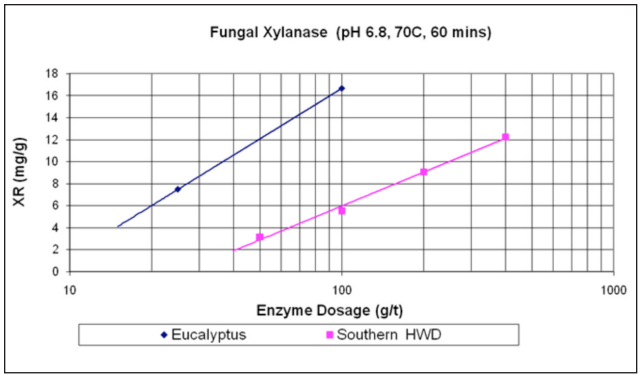


5. Total organic carbon (TOC) released at various dosages of both bacterial and fungal xylanase treatments. One set of data was obtained from work by Borges [46].

carbohydrates in secondary woody cell walls. It is a major carbohydrate component in many isolated phenoliccarbohydrate complexes [35-37], likely covalently linked to phenolic residues via its arabinosyl [38,39] and glucuronosyl [35,39] residues. The high phenolic content in some of these complexes [35,36,40] suggests that the carbohydrates are linked to lignin fragments. However, the phenolic substituents also might cross-link xylan molecules and xylan to other polysaccharides [35]. Linkages between xylan and pectic substances also have been suggested [40,41], as have xylan-glucan-protein complexes [40].

Commercially available bacterial-based xylanse treatments tend to be more aggressive towards sugar removal than fungal-based enzyme treatments [24,25,42]. By cleaving xylan-based molecules at different locations along the xylan backbone, the bacterial-based xylanases tend to more aggressively reduce the kappa number of the treated pulp, whereas the commercial fungal-based xylanases tend to maintain or only slightly reduce the kappa number of the treated pulp [21]. Significant levels of kappa reduction suggest that the bacterial xylanases cleave lignin and xylan structures from the fiber in the form of lignin-carbohydrate complexes (LCCs), while the fungal xylanase enzymes more selectively cleave xylose oligomers from the fiber surface [43]. Other possible mechanisms in the kappa number reduction might include the removal of xylan that is associated with lignin via a hydrogen-bonding complex or through lignin leaching, which might result in a small kappa number drop. About one third to one half of the total kappa number drop reported in some studies [44] might result from the removal of about 5-10 mmol/kg pulp of hexenuronic acid (roughly corresponding to 0.4 to 0.9 kappa number unit reduction) via enzyme treatment.

Bacterial-based enzymes tend to exhibit extremely powerful carbohydrate binding domains, which result in the aggressive removal of xylose oligomers and LCCs [24]. Fungal-based enzymes do not have the same tenacity of carbohydrate binding domains, so they tend to be less aggressive toward carbohydrate removal than bacterial-based enzymes. **Figure 5** shows a laboratory investigation comparing TOC release as a



6. Susceptibility of eucalyptus and mixed southern hardwoods towards xylanase enzyme application as determined by sugar release as a function of enzyme dosage under identical reaction conditions.

function of enzyme dosage. Figure 5 shows that bacterial enzyme application does indeed release significantly higher levels of TOC as compared with fungal xylanases. Additionally, recent work by Collodette et al. [44] has shown that bacterial xylanase treatment increases the TOC and COD in simulated open effluent systems.

Based on the kappa number reduction occurring in the bacterial xylanase treatment, a significant amount of LCC removal likely is a major cause of increased effluent COD and TOC in open systems and decreased oxygen delignification efficiency in the closed systems used in the South American mills. The lignin released via the bacterial-based enzyme treatment might not contain sufficient amounts of phenolic lignin to react with oxygen, and so is nonreactive in the oxygen delignification stage [45,46]. Another potential mechanism is that the more aggressive fungal-based xyloses free more xylan material, which then encases the fiber and lignin in the oxygen stage, shielding it from reacting via favorable interactions between carbohydrate hydroxyl groups with the π-bonds in the aromatic ring. This mechanism would be similar to the reaction of cyclodextrans used with dyes to stabilize them against chemical attack [47].

Laboratory investigations treating eucalyptus pulps with fungal-based xylanases have suggested that eucalyptus fiber is highly susceptible to enzyme treatment. **Figure 6** shows the amount of xylose released for a eucalyptus pulp and a mixed southern hardwood pulp under identical laboratory treatment conditions. The data in Figure 6 show that at the same level of enzyme application, significantly more sugar is released from the eucalyptus fiber than from the mixed southern hardwood fiber. Figure 6 suggests that relatively low dosage treatments with extremely xylan-selective enzymes could result in the enzyme-related bleaching cost reductions realized in North America, without negatively affecting the oxygen bleaching stages of the South American fiber lines. The one successful fungal xylanase trial on radiata pine also suggests that this technique may be successful.

By using extremely low doses of enzymes and obtaining similar levels of sugar release typically encountered in North American applications, it might be possible to control the total amount of TOC and BOD released and sent back to the oxygen bleaching stage, while successfully realizing enzyme related benefits in the bleach plant. Further laboratory investigations should be performed to select the proper fungal xylanase.

SUMMARY

To date, millions of tons of commercial enzyme treated pulp have been produced successfully in North American mills. Enzyme application has resulted in cost savings in three- and four-stage bleach plants and in fiber lines with and without oxygen delignification. At least six mill-scale trials have been conducted with enzymes in South American fiber lines containing oxygen delignification. Four of these trials have been conducted on eucalyptus fiber lines and two were conducted on a radiata pine fiber line with oxygen delignification. All four of these South American trials have been unsuccessful. Shortly after the beginning of each of these trials, the TOC and COD entering the oxygen delignification stage of the process rapidly escalated and decreased the efficiency of the stage. The resulting higher kappa number pulp leaving the oxygen bleaching stage and entering the bleach plant produced a substantial increase in the chlorine dioxide demand in the D_0 stage, offsetting any potential savings associated with the use of enzymes. In one set of trials conducted in a more open mill system, the application of enzymes substantially increased the BOD and COD entering the waste treatment plant, thereby causing the trial to be terminated prematurely.

Laboratory work performed with fungal-based enzymes has shown that eucalyptus fiber is extremely susceptible to xylanase application and produces significant levels of chlorine dioxide reduction with small dosages of xylanase, resulting in significant economic savings for the eucalyptus mills. The deterioration of the oxygen delignification stage performance resulting from increased COD entering the stage eliminates this savings.

Until recently, the mill-scale trials conducted in South America have been performed with bacterial-based xylanase enzymes instead of fungal-based enzymes. Laboratory work has shown that these bacterial-based enzymes are more aggressive in sugar removal than corresponding fungal-based enzymes. Significant levels of kappa number reduction have been reported when fiber is treated with bacterial-based enzymes versus no or minimal reported kappa number reduction with fungal-based enzymes. By cleaving the xylan molecules at a location that liberates substantial LCC complexes, the bacterial-based enzymes may substantially increase the TOC in the resulting enzyme stage effluent. It would appear that these LCCs are highly resistance to oxygen attack under typical oxygen delignification operating conditions. As noted, other mechanisms may be attributed to this outcome. The liberated lignin likely does not contain sufficient amounts of phenolic lignin to be reactive to oxygen delignification.

More recently, a short, three-week mill trial was conducted in which a fungal-based enzyme was applied in a radiata pine mill. A trial using a commercial bacterial-based enzyme in a mill with a very similar bleach plant configuration and a similar pulp supply had been unsuccessful. The fungal-based enzyme did produce about a 12% reduction in total chlorine dioxide demand without negatively affecting the operation of the oxygen delignification stage. Plans to continue this trial were stopped because of water restrictions within the mill and a desire to perform various pulping trials with other wood species. At this time, it is believed that further fungal-based enzymes trials will occur. **TJ**

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ABOUT THE AUTHORS

I have been involved with enzyme bleaching for several years, starting with one of the first full-time commercial installations in the United States. Over the years, it has become apparent that different commercial enzymes behave differently in the bleach plant. The current work attempts to determine some of the underlying causes of these differences in performance. It attempts to reconcile differences in bleaching enzyme (xylanase) performance and in bleach plants in North and South America. Comparing and contrasting observed bleach plant performance with various laboratory testing and known differences in different sources of xylanases has allowed several performance discrepancies to be explained.

The current work compares different commercially available xylanases under both industrial and laboratory conditions. Industrial conditions were obtained from several different mills associated with multiple companies. Careful consideration of mill confidentiality along with obtaining specific operating conditions was a difficult but essential part of this research effort.

Several substantial differences appear to exist in the bleaching performance of fungal- versus bacterial-produced commercially available xylanase enzymes. These performance metrics include effects on kappa number reduction, pulp yield, and organic loading to the waste treatment plant, among others. Despite these differences, both sources of xylanase enzymes can substantially improve bleach plant performance with respect to the amount of oxidant required to obtain a target brightness.

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Hopefully, the current work will be used as a starting point for enzyme suppliers to better understand sources of potential negative performance effects of their products on bleach plant operations. Improved understanding of performance metrics could lead to improved engineered enzyme applications in the future.

Ideally, the next step would be for specific enzyme suppliers to improve their understanding of different sources of enzymes on bleaching performance and tailor their product offerings towards beneficial performance.

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