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Use of xylanase and arabinofuranosidase for arabinose removal from unbleached kraft pulp

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Abstract

Preparations of arabinofuranosidase and xylanase, respectively from *Aureobasidium pullulans* and *Trichoderma longibrachiatum*, were used to remove selectively xylose and arabinose from kraft pulp. The equilibrium moisture content of pulps treated with both enzymes, at varying relative humidities, revealed a consistently lower percent moisture content at all humidity set points. Shorter fiber lengths indicated some deterioration when pulp was exposed to high concentrations of both enzymes.

Introduction

The use and study of hemicellulases, particularly xylanase, in biobleaching has increased significantly over the last two decades (Royer & Nakas 1989, 1990, 1991, Senior *et al.* 1991, Royer *et al.* 1992, Beg *et al.* 2001). Less well examined have been the L-arabinofuranosidases which hydrolyze the linkage between arabinose substituents and xylan. Arabinofuranosidases occur intracellularly or extracellularly in a number of higher fungi (Saha *et al.*1998, Nogawa *et al.* 1999, Koseki *et al.* 2003), or as membrane-bound and/or extracellular enzymes from bacteria (Debeche *et al.* 2000, Degrasi *et al.* 2003). These enzymes are produced by growth on complex oligoxylan-containing substrates such as beet pulp or oat spelt xylan, and can occur either as monomeric or multimeric proteins with molecular masses varying from 38 to 495 kDa (Saha 2000). Research on arabinofuranosidases have only recently become the focus of lignocellulose-based research groups (Saha 2000) and are still to be commercially applied in the pulp and paper industry.

The residual moisture present in paper affects important properties such as tensile strength and decay resistance. Previous research has shown that the removal of xylan from fibers reduced moisture adsorption (Salmén 1998). Additionally, arabinose residues have specifically been shown to be responsible for sorption of moisture between 20 and 50% relative humidity (Cöpür 2002). The objective of this research was to determine if the enzymatic removal of arabinose and arabinose–xylose linked oligomers from the hemicellulose complex of kraft pulp resulted in diminished hygroscopicity.

Materials and methods

Organisms and enzyme production

Aureobasidium pullulans NRRL Y-12974 was obtained from the ARS culture collection

(National Center for Agriculture Utilization Research, Peoria, Il.). The α-L-arabinofuranosidase activity assay has been previously described (Saha *et al*. 1998). The extracellular proteins were harvested from the culture supernatant by centrifugation at 16,000 × *g* for 30 min after the liquid culture had been incubated on a rotary shaker (125 rpm) at 28 °C for 5 days. The supernatant was concentrated using a 100 kDa YM regenerated cellulose membrane (Fisher, Pittsburgh, Pa) fitted in an Amicon ultrafiltration cell.

Trichoderma longibrachiatum was grown on solid medium consisting of 3.9% (w/v) potato/dextrose/agar and 2% (w/v) oat spelt xylan. Following incubation on a rotary shaker at ambient temperature for 10–14 days, the culture was harvested from the production medium (Royer & Nakas 1989) by centrifugation at 6000 × *g* for 30 min. Production medium consisted of (g l^{-1}) NH_4SO_4, 0.5; KH_2PO_4, 1; KCl, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.1; yeast extract, 0.5 and 2 ml trace elements. Trace minerals added were (mg l^{-1}) $FeSO_4 \cdot 7H_2O$, 5.0; $MnSO_4 \cdot H_2O$, 1.4; $ZnCl_2$, 1.7, and $CoCl_2$, 2. Vitamins added were (mg l^{-1}) biotin, 0.005; inositol, 2; calcium pantothenate, 0.2; pyridoxine hydrochloride, 0.2 and thiamine, 0.2. Carbon sources were added at 1% (w/v). The supernatant was filtered through a 0.45 μm membrane and concentrated using an Amicon ultrafiltration cell fitted with a 10 kDa PM regenerated cellulose membrane. The latter was used as the xylanase preparation and assayed for xylananse and cellulase activity as described by Royer & Nakas (1989).

A unit of enzyme activity is defined as one μmol product (*p*-nitrophenol from *p*-nitrophenyl α-L-arabinofuranoside for arabinofuranosidase, and xylose, measured as release of reducing sugar from oat spelt xylan for xylanase) per min per ml of enzyme solution (Royer & Nakas 1989, Saha *et al*. 1998). Both enzyme substrates were obtained from Sigma-Aldrich.

Removal of hemicellulose from pulp

Softwood pulp samples were obtained from the UPM-Kymmene/Miramichi Mill, New Brunswick, Canada. Eight samples of pulp, equivalent to 2 g dry wt, were each suspended in 200 ml 50 mM sodium acetate buffer, pH 5.0. Each suspension was mixed and incubated in a water bath at 50 °C for 2 h. The reaction was terminated by filtration through a Buchner funnel followed by an additional 2 l deionized water to ensure that released carbohydrates and enzymes were removed from the fibers. A 100 mg sub-sample of each pulp treatment was air-dried and subjected to complete hydrolysis with sulfuric acid and prepared for NMR analysis (Kiemle *et al*. 2004). The carbohydrates present in pulp samples were identified and quantified by proton NMR analysis using a Bruker AVANCE™ 600 MHz NMR system (Bruker Biospin Corp., Billerica, Ma).

Pulp responses to humidity variations and fiber analysis

Enzyme treated pulp samples (1 g oven-dry wt) were incubated in a controlled humidity chamber at 23 °C for desorption and adsorption through one full cycle of a sequential decrease in relative humidity from 80%, to 50%, to 20%, and a stepwise increase returning to 80% as previously described (Çöpür 2002). Portions of pulp samples were suspended (0.002% w/v) in distilled water and fiber dimensions determined using a Kajaani FS-100 fiber analyzer (Kajaani Electronics Ltd., Finland).

Results

Treatment of pulp samples with 1000 units of α-L-arabinofuranosidase released 15 mg reducing sugar/g pulp while treatment with 1000 units xylanase released 106 mg reducing sugar/g pulp in 2 h. Treatment of pulp with 1000 units of both enzymes released carbohydrate at a linear rate giving in excess of 225 mg reducing sugar/g pulp (Figure 1).

The weight % carbohydrates remaining in pulp after enzyme treatments, based on compositional characterization with proton NMR, are shown in Table 1. Pulp samples treated with 1000 U of α-L-arabinofuranosidase exhibited 0.4% (w/w) arabinose compared to 0.5% (w/w) for the control pulp samples. Treatment of pulp samples with xylanase (1000 U) and xylanase in conjunction with α-L-arabinofuranosidase (1000 U of each) exhibited only 0.3% (w/w) arabinose.

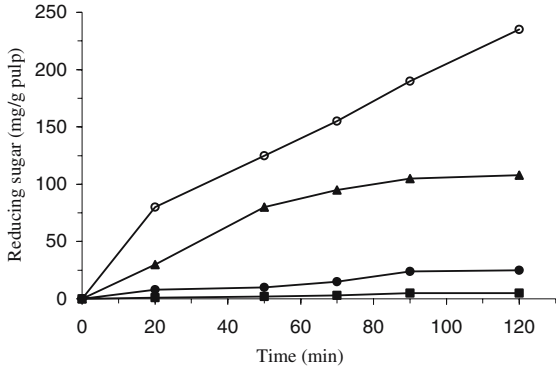

Fig. 1. Release of reducing sugars from a 5% (w/v) pulp suspension in 50 mM sodium acetate buffer pH 5.0 treated with enzymes. The untreated pulp is represented by ■, ● represents pulp treated with α- L-arabinofuranosidase (1000 units), ▲ represents pulp treated with 1000 units of xylanase, and ○ represents pulp treated with both α-L-arabinofuranosidase (1000 units) and xylanase (1000 units).

Xylanase used in conjunction with α-L-arabinofuranosidase consistently lowered the equilibrium moisture content at all humidity set points (Table 2). Kajaani fiber analysis showed that the % fines (particles less than 0.2 mm in length) for the α-L-arabinofuranosidase-treated pulp was similar to the control pulp samples, but the sample treated with both enzymes increased the % fines three-fold (40% compared to 13% for the control, data not shown). Plotting the fiber length distribution showed that the original pulp, the control, and the α-L-arabinofuranosidase-treated pulp samples exhibited a comparable distribution pattern. The fiber length distribution of the xylanase-treated pulps showed some minor variability while the distribution curve for samples treated with both enzymes shifted significantly toward shorter length fibers (Figure 2).

Table 1. Weight % residual carbohydrate in enzyme-treated pulp.

Carbohydrates	Control	A[a]	X[b]	A + X[c]
Glucose	85.4	84.1	86.9	85.6
Mannose	7.3	7.5	6.3	7.4
Xylose	6.6	7.7	6.2	6.4
Galactose	0.3	0.2	0.3	0.3
Arabinose	0.5	0.4	0.3	0.3

Pulp treated with: [a]1000 units of α-L-arabinofuranosidase, [b]1000 units of xylanase, and [c]1000 units of each enzyme at 50 °C for 2 h.

Discussion

The enzyme concentrations were chosen based on preliminary data that showed increased release of reducing sugar when the enzyme concentration was increased from 500 to 1000 units of enzyme per reaction but no further rate increase at enzyme concentrations greater than 1000 units per reaction (Francis 2002). The simultaneous use of α-L-arabinofuranosidase and xylanase resulted in a greater release of reducing sugar from pulp as compared to the additive effects of using α-L-arabinofuranosidase and xylanase independently (Figure 1), suggesting a synergistic enzyme interaction. Examples of such synergism in the degradation of arabinoxylan have been observed with *Aspergillus awamori* (Koseki *et al.* 2003), and *Aspergillus oryzae* (Hashimoto *et al.* 2003) preparations. Previous investigations into the action of hemicellulases on pulp fibers have shown that limited hydrolysis of carbohydrates is achieved (Suurnakki *et al.* 1995). The non-selective adsorption of enzymes onto pulp fibers and the possible inhibitory effect of materials in the pulp, are also factors to be considered. Xylanases have been found to adsorb non-selectively to the cellulose component in pulp and significant loss in enzyme activity has also been observed when the enzyme was incubated with unbleached pulp fibers (Senior *et al.* 1989).

The consistently lower moisture content observed in pulp treated with both arabinofuranosidase and xylanase was expected as the removal of xylan from fibers reduces the degree of moisture adsorption (Salmén 1998). This supports the hypothesis that arabinose and xylose residues are responsible for sorption in the 20–

Table 2. Equilibrium moisture content (percent) of pulp samples at varying relative humidities (RH)[a].

RH	80%	50%	20%	50%	80%
C	15.95	9.25	5.29	8.47	13.35
A	15.29	9.11	5.35	8.15	12.96
X	16.06	9.09	5.56	8.47	13.33
A+X	15.00	8.27	4.56	7.52	12.85

[a]All values are the average of duplicate experiments for control pulp (C), arabinofuranosidase-treated pulp (A), xylanase-treated pulp (X), and arabinofuranosidase + xylanase-treated pulp (A + X). One thousand units of each enzyme were used for each treatment and in combination treatments.

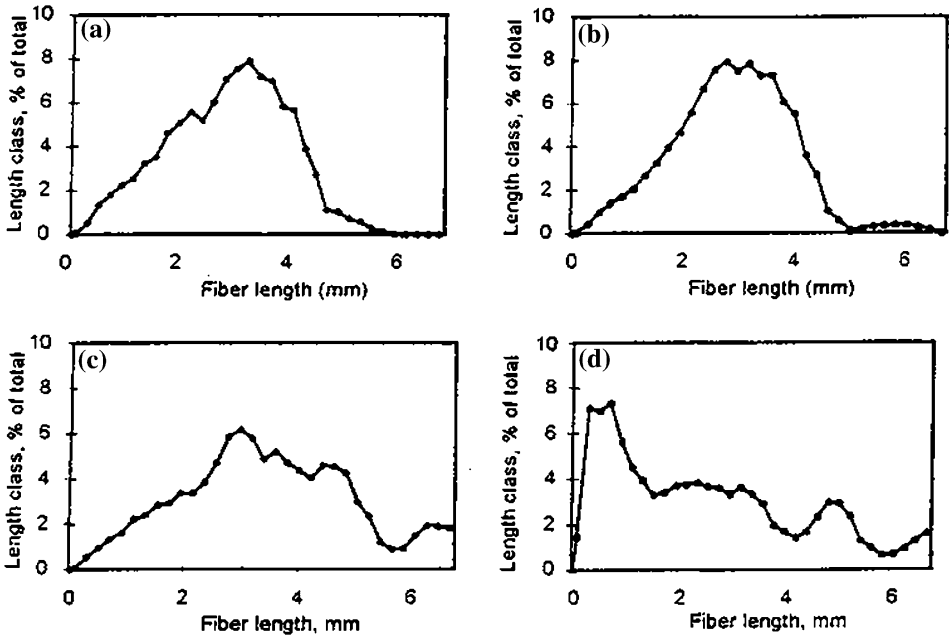

Fig. 2. Fiber length distribution of pulp samples after enzyme treatment (1000 U of each enzyme): control (a), α-L-arabinofuranosidase-treated (b), xylanase-treated (c), and pulp treated with both enzymes (d).

80% humidity range and specific removal of arabinose should result in lower moisture gains. Exposure to 1000 units of each enzyme simultaneously also resulted in a higher percentage of fines and shorter fiber lengths, indicating some deterioration in the structural integrity of the pulp. This emphasizes the necessity for careful optimization to achieve selective carbohydrate removal while preserving fiber strength and integrity.

Arabinose represents the lowest individual carbohydrate concentration in pulp. Its removal would therefore represent very low losses in overall yield yet resulting in a substantial decrease in fiber hygroscopicity. The hygroscopic nature of pulp is of considerable practical importance as it may negatively impact important properties such as dimensional stability and strength, machinability, adhesiveness, and decay resistance. This study shows that moisture adsorption in pulp was reduced by the selective enzymatic removal of xylose and arabinose and also illustrates that careful adjustment of enzyme dosage to match pulp type will be required to optimize selective carbohydrate removal and moisture properties while preserving yield and structural integrity.

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