Enzymatic Hydrolysis of Pretreated Newspaper Having High Lignin Content for Bioethanol Production

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Recovered papers are suitable biomass sources for conversion into sugars that can be used in bioethanol production. However, paper materials with a high lignin content have been found to be recalcitrant to enzymatic hydrolysis. To address this issue, several biomass pretreatment methods were employed to evaluate their efficiency on the conversion of newspaper with high lignin content to sugar. Autohydrolysis, a hot water treatment, was identified to adversely affect sugar conversion, presumably as a result of pore collapse under high-temperature pretreatment. Flexo ink, used in newspaper printing, had no effect on the enzymatic hydrolysis, with or without autohydrolysis. The ink was still detachable after autohydrolysis, as measured by hyperwashing. Compared to untreated newspaper, separate treatments of either mechanical refining or a non-ionic surfactant (sorbitan polyoxyethylene monooleate) improved the sugar conversion by 10% at enzyme dosages of 2 and 8 FPU/g substrate. The combination of both refining and surfactant resulted in the highest sugar conversions, *i.e.*, 46.3%, 56.7%, and 64.1% at 2, 4, and 8 FPU/g enzyme dosages, respectively. Oxidative pretreatment (oxygen, 100 °C) marginally increased the sugar conversion, whereas alkaline and green liquor ($NaCO_3$ and Na_2S) pretreatments (at 160 °C) had either no effect or decreased the sugar conversion. Based on the results of the pretreatments, higher pretreatment temperatures of newsprint negatively impacted subsequent enzyme hydrolysis.

Keywords: Newspaper; Autohydrolysis; Pretreatment; Enzymatic hydrolysis; Refining; Surfactant; Ink; Accessible pore volume

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INTRODUCTION

Among the many kinds of feedstock for bioethanol production, lignocellulosic biomass presents advantages because of its low cost, widespread availability, environmental friendliness, and sustainability (Solomon *et al*. 2007; Kumar *et al*. 2008). Waste papers, among other biomass materials, present a unique opportunity for the production of bioethanol. Shortening of paper fibers during the recycling process decreases the paper quality; thus, waste paper is usually converted into lower grades of paper. It has been reported that the maximum ratio of paper-to-paper recycling is 65% (Ikeda *et al*. 2006). This means that an unavoidable portion of cellulose fines are disposed, collected, dried and burnt to produce energy, or applied to fields as an additive to soil (Duff and Murray 1996). This portion contains an underutilized carbohydrate fraction that can be enzymatically converted into fermentable sugar for ethanol production. In this case, waste papers, compared to other lignocellulosic biomasses, may provide both environmental and financial benefits (Chen *et al*. 2012, 2014).

Lignocellulosic biomass generally needs to be pretreated to soften the biomass, open the cellular structure, and make the cellulose fraction more accessible to enzyme digestion. Presently, pretreatment processes primarily use chemicals for the catalysis process (Hamelinck *et al*. 2005). Pretreatments such as dilute acid, alkaline, ammonia fiber explosion (AFEX), steam explosion, organic solvent, carbon dioxide explosion, and others have been applied to prepare cellulosic biomass for enzymatic hydrolysis (Kaar *et al*. 1998, 2000; Zheng *et al*. 1998; Kurakake *et al*. 2001; Alizadeh *et al*. 2005; Pan *et al*. 2006). In addition, mechanical pretreatments have been found to be effective and complementary to chemical pretreatments (Chen *et al*. 2012; Jones *et al*. 2013).

Newspaper, an example of a waste paper material, is rich in cellulose (40 to 55%) and hemicellulose (25 to 40%), but has a relatively high lignin content (18 to 30%), compared to other lignocellulosic waste materials (Sun and Cheng 2002). Extensive research in the past two decades has been performed towards developing an enzymatic hydrolysis process for the conversion of newspaper into ethanol (Saxena *et al.* 1992; Duff *et al*. 1995; Wu and Ju 1998; Kim and Moon 2003; Kim and Chun 2004a; Kim *et al.* 2007; Lee *et al*. 2010a; Xin *et al*. 2010). Non-ionic surfactants such as sorbitan polyoxyethylene monooleate (Tween® 80) and sodium dodecyl sulfate (SDS) have been reported to enhance the enzymatic hydrolysis of newspaper (Kim *et al*. 2007; Xin *et al*. 2010). Sorbitan polyoxyethylene monooleate showed approximately 7% higher digestibility than sorbitan polyoxyethylene monolaurate (Tween® 20). In another study (Lee *et al*. 2010a), organosolv pretreatment at a high boiling point and the solvent ethylene glycol was studied on newspaper bioconversion. A glucan conversion of 94% was achieved at the optimum conditions (2% sulfuric acid at 150 °C for 15 min) identified in that study. Ammonia fiber explosion (AFEX) and ammonia treatment were found to contribute a marginal improvement to the enzymatic hydrolysis of newspaper (Wang *et al.* 2012). Ammonia-H_2O_2 was reported to be very efficient on newspaper pretreatment, thereby greatly increasing the susceptibility to enzymatic digestion (Kim and Moon 2003). In addition, oxidative lime, using $Ca(OH)_2$ and O_2, was found to be efficient (Wang *et al*. 2012); the sugar yield improvement was 33% to 78% at a 7.5 FPU/g cellulase (Celluclast® 1.5L) enzyme dose. Ultrasound-assisted alkaline pretreatment with optimized conditions was reported to achieve 80% delignification (Subhedar and Gogate 2014).

As mentioned above, chemical-based pretreatments, with or without high temperature, have been utilized prior to enzymatic hydrolysis of newspaper, and high enzyme dosages (typically greater than 10 FPU per oven dry g of substrate) have been utilized to achieve ideal sugar yields. It is estimated from conference presentations and discussions with enzyme producers that enzyme dosages in the range of 4 to 5 FPU/g can be economically attractive (*i.e.*, enzyme costs contribute to around $0.50 per gallon of ethanol) (Chen *et al*. 2014). Most research has used enzymes at non-practical dosages for actual implementation. Thus, our studies focused on more economically viable dosages.

Because newspaper has already been chemically and/or physically pretreated during the paper-making process, it does not require as extensive pretreatment as wooden or herbaceous biomasses (Kim and Moon 2003; Xin *et al*. 2010). Accordingly, a relatively mild pretreatment such as autohydrolysis followed by enzymatic hydrolysis for newspaper, deserves attention. Autohydrolysis provides a chemical-free, corrosion-limited, minimal waste, environmentally friendly, and low-cost pretreatment technology for fermentable sugar production from lignocellulosic materials (Garrote *et al*. 1999; Lee *et al*. 2009). The primary sugar component in lignocellulose, depolymerized and solubilized in autohydrolysis, is xylan (Dekker and Wallis 1983; Saska and Ozer 1995). The sugars are produced from the catalysis of hydronium ions from water ionization and from *in situ*-generated acids (such as acetic, uronic, and phenolic acids) that cleave the heterocyclic ether bonds of the xylan backbone, depolymerizing it into xylooligomers and xylose (Garrote *et al*. 1999).

Although autohydrolysis of various lignocellulosic biomasses for ethanol production has been extensively studied (Dekker and Wallis 1983; Grethlein and Converse 1991; Garrote *et al*. 1999; Vázquez *et al.* 2005; Öhgren *et al*. 2007), very limited research has been conducted on the autohydrolysis of newspaper for bioethanol production. In addition, some studies have reported that ink had no effect on enzymatic hydrolysis (Spano *et al*. 1976; Rivers and Emert 1988; Duff *et al.* 1995). On the other hand, the negative influence of ink on enzyme digestibility has been emphasized for newspaper fibers in some studies (Kim *et al.* 2006; Kuhad *et al.* 2010). Therefore, another objective of this study was to examine the effect of flexo ink, one of the most commonly used newspaper inks, on the enzymatic hydrolysis of newspaper. In this study, the effects of autohydrolysis, oxygen, alkaline, and green liquor (GL) pretreatments on newspaper were investigated. Surfactant addition and mechanical refining were also evaluated pertaining to their effects on saccharification. The objectives of this study were to identify the limitations and inhibitions involved in the bioconversion of newspaper into fermentable sugar and to explore treatments to overcome such recalcitrance.

EXPERIMENTAL

Materials

Newspaper (issued in January, 2011) and the same unprinted roll-ends were obtained from The Raleigh News & Observer (Raleigh, NC, USA). For the samples subjected to hyperwashing and enzymatic hydrolysis, newspaper with a moisture content of 7% was soaked and repulped using a TAPPI disintegrator (Testing Machines Inc., Amityville, NY) for 15,000 revolutions (5 min). The pulp used for enyzymatic hydrolysis was then centrifuged, fluffed, and stored in a cold room in sealed plastic bags for at least 24 h prior to moisture content determination. Newspaper samples subjected to autohydrolysis were shredded into 0.5-cm × 5-cm strips and stored in air-tight plastic bags prior to pretreatment.

Deinking

Disintegrated newspaper (either before or after autohydrolysis) was subjected to a hyperwashing step to analyze the amount of non-detached or redeposited ink. Hyperwashing was conducted in a washing trough with a 200-mesh (74-µm) filter screen using abundant tap water for 10 min. Effective residual ink concentration (ERIC) was measured using a Technidyne Color Touch 2 ISO Model (Technidyne Corporation, New Albany, IN) according to the TAPPI standard method T567 pm-97 (1997). This technique measures reflectance at a 950-nm infrared wavelength from a paper sample over a black backing (Jordan *et al.* 1994). The ink detachment parameter (Ink_D) was calculated to determine the percentage of eliminated ink during the hyperwashing step according to the ERIC values of original newsprint handsheets and of pretreated newsprint handsheets.

The ink detachment parameter (InkD) is given as follows (Pèlach *et al*. 2003):

$$Ink_D = \frac{ERIC_O - ERIC_P}{ERIC_O} \times 100\% \tag{1}$$

The detachable ink removal determines the percent of the defined detachable ink that was removed,

$$\text{Detachable ink removal (\%)} = \frac{ERIC_O - ERIC_P}{ERIC_O - ERIC_U} \times 100\% \tag{2}$$

where $ERIC_O$ is the effective residual ink concentration (ERIC) of the original newsprint handsheets (ppm), $ERIC_P$ is the ERIC of pretreated newsprint handsheets (ppm), and $ERIC_U$ is the ERIC of unprinted newsprint handsheets (ppm). Note that $ERIC_O$ consists of the freshly applied ink to the newsprint, as well as any ink coming with the recycled fibers in the newsprint. Note that $ERIC_U$ is the ink that exists in the newspaper from residual ink that was contained in recycled fiber; this ink was assumed to be non-detachable because it was not removed in a prior deinking operation.

Pretreatments

Four pretreatment methods were evaluated in this study: autohydrolysis, oxygen, alkaline, and green liquor (GL). All of the pretreatments were carried out in a 1.5-L stainless steel rotating bomb digester (Thermcraft, Winston-Salem, NC, USA).

Autohydrolysis was carried out with 100 oven dry (OD in g) newspaper samples per bomb digester and loaded with six volumes (by weight) of deionized water at 160 °C for two retention times (30 min and 60 min). Another set of autohydrolysis experiments was conducted at 180 °C for 60 min. For some conditions, 3% or 5% (v/w) acetic acid, based on the oven-dry newsprint, was added to supplement autohydrolysis.

Oxygen pretreatment was conducted using the sodium hydroxide concentrations of 3% and 4% of the OD weight of the newspaper. The oxygen pressure was set to 100 psig (6.9 MPa) at 100 °C for 1 h, and the newspaper consistency was 10%.

Sodium hydroxide was used in the alkaline pretreatment condition at 4% and 6% of the OD weight of the newspaper. The pretreatment conditions were 160 °C for 1 h with 10% consistency based on OD weight of the newspaper.

The GL solution was prepared by mixing sodium carbonate with sodium sulfide to obtain a sulfidity ($Na_2S/(NaOH + Na_2S)$ all as Na_2O) of 25%, which is commonly used in most kraft mills in the United States. The total titratable alkali (TTA) ($NaOH + Na_2S + Na_2CO_3$ all as Na_2O) charge on the OD weight was 4%. The GL pretreatment conditions were set to 160 °C for 1 h with 10% consistency based on the OD weight of the newspaper.

After pretreatment, bomb digesters were cooled in a water bath. Pretreated samples were filtered through cheese cloth, and the first filtrates were collected for further determination of pH and sugar content. After the first filtration, samples were washed thoroughly and collected for future experiments, including handsheet making, post-pretreatment (*i.e.*, hyperwashing) and enzymatic hydrolysis. For chemical composition analysis, the first filtrates were subjected to a post-acid hydrolysis by adding 72% (w/w) H_2SO_4 to a H_2SO_4 concentration of 4% (w/w). The samples were then incubated in an autoclave for 1 h at 121 °C. The acid-hydrolyzed samples were neutralized with calcium carbonate prior to the sugar analysis step.

Refining

The newspapers were subjected to 2,500 to 10,000 revolutions of refining in a PFI mill (Hamjern Maskin A/S, Hamar, Norway) using 30 g of the OD pulp at 10% consistency, following TAPPI procedure T 248 cm-85 (1993). The pulps were then filtered through cheese cloth, fluffed, evenly divided, sealed in plastic bags, and stored in a cold room until further testing.

Enzymatic Hydrolysis

Enzymatic hydrolysis was carried out with a consistency of 5% (w/v) at 50 °C for 48 h and 180 rpm in an environmental incubator shaker (New Brunswick Scientific, Edison, NJ). A concentration of 50 mM of acetate buffer (pH 4.8) with 0.3% (w/v) sodium azide was used. The dry matter content of the treated solid samples and raw materials were determined using an infrared moisture analyzer (Mettler LJ16, Greifensee, Switzerland). Cellulase from *Trichoderma reesei* (NS50013), β-glucosidase from *Aspergillus niger* (NS50010), and hemicellulase (NS50014) preparations were supplied by Novozymes A/S (Bagsværd, Denmark). The ratio of cellulase to glucosidase to hemicellulase was 1 to 0.3 to 0.3. The activity of the cellulase (NS50013) was determined to be 80.5 filter paper units (FPU)/mL, according to methods described by Ghose (1987). Cellulase enzyme loadings of 2, 4, 8, and 20 FPU/g per substrate were evaluated. In some cases, the enzymatic hydrolysis was supplemented with a non-ionic surfactant, sorbitan polyoxyethylene monooleate (Tween® 80), at a concentration of 0.4% (v/v), to evaluate its effect on the enzymatic hydrolysis of newspaper.

Specific Surface Area (SSA) and Total Pore Volume by N_2-BET

Specific surface areas (SSA) of freeze-dried newspaper fibers were measured according to a nitrogen adsorption, Brunauer–Emmett–Teller (BET) method (Gemini VII 2390, Micrometrics, Atlanta, Georgia, USA). Adsorption was performed with an evacuation rate of 300 mmHg/min for 1 min, equilibration time of 5 s, and relative pressures of 0.05, 0.11, 0.18, 0.24, and 0.30. The total pore volume at the single point p/p0 = 0.5 on the adsorption curve was used.

Sugar Analysis

Compositional analysis of all the treated materials was conducted using the NREL Standard Procedure (Sluiter *et al*. 2004). Sugar concentration from enzymatic hydrolysate and filtrate from autohydrolysis were determined using a high-performance liquid chromatography (HPLC) system (Agilent 1200, Agilent, Santa Clara, CA) equipped with a refractive index detector on a Pb-loaded cation exchange column of Shodex Sugar SP0810 (8x300 mm, Showa Denko, Tokyo, Japan). All HPLC samples were filtered through 0.45-µm HV filters (Millipore, Bedford, MA), and a volume of 20 µL was injected. The mobile phase for the column contained Milli-Q water moving at a flow rate of 0.5 mL/min. The system was equipped with a de-ashing refill cartridge (Bio-Rad 125-0118, Bio-Rad, Hercules, CA). Sugar conversion and sugar recovery were calculated as follows:

$$\text{Sugar conversion} = \frac{\text{Sugar released (g)} \times 0.9}{\text{Carbohydrate content in the treated material (g)}} \times 100\% \tag{3}$$

$$\text{Sugar recovery} = \frac{\text{Sugar released (g)} \times 0.9}{\text{Carbohydrate content in the untreated material (g)}} \times 100\% \tag{4}$$

The factor 0.9 is the conversion factor from mono sugars to oligomers. Newspaper yield from autohydrolysis was calculated based on the starting material (OD weight). Separate sugar reductions were calculated based on the sugar analysis of the prehydrolysate liquor from autohydrolysis, relative to the sugar in the untreated newspaper samples.

Surface Lignin Content by XPS

The surface lignin content of the newspaper was measured using X-ray photoelectron spectroscopy (XPS). The newspaper samples (untreated and pretreated) were made into handsheets using the TAPPI T205 method (2002) without pressing. Square samples of approximately 1.1 cm × 1.1 cm were cut from sheets and extracted using acetone. The extraction process lasted for 8 h using a 250-mL Soxhlet apparatus with a condensate drip rate of approximately 2 drops per s, with the purpose of removing extractable impurities from the newspaper sheets. The newspapers were then subjected to surface lignin measurements by XPS with Mg-Kα excitation (1254 eV) and a PHOIBIS 150 hemispherical analyzer (SPECS, Berlin, Germany) at 10^{-10} mbar pressure. Energy calibration was established by referencing to adventitious carbon (C1s line at 285.0 eV binding energy). Surface carbon and oxygen content were evaluated to calculate the surface lignin content based on the method proposed by Gray *et al.* (2010). A bleached softwood sample with no lignin content was prepared using the same procedures as referenced in the calculation.

Accessible Pore Volume by DSC

The accessible pore volume, interpreted by the accumulation of freezing bound water (FBW), was measured by differential scanning calorimetry (DSC) Q100 (TA Instruments, New Castle, DE), and analyzed based on the work of Park *et al*. (2006). Freezing point depression is reflective of the phenomenon that water in small pores freezes at lower temperatures than does bulk water. The depressed freezing temperature has a reciprocal relationship with the pore diameter. Therefore, the accessible pore volume can be determined by calculating the accumulation of freezing bound water (FBW) in different pore diameters, based on the Gibbs-Thomson equation. In this research, the pulps with approximately 35% consistency were sealed in a hermetic aluminum pan and frozen at -80 °C for 10 min. After this, the temperature was elevated to -40 °C to determine the melting point of the wet fibers. This was tested using several heating increments from -40, -30, -20, -15, -10, -6, -4, -2, -1.5, -1.1, -0.8, -0.5, -0.2 to -0.1 °C with a rate of 1 °C/min; the samples were maintained isothermally at each target temperature until the heat flow returned to the baseline (this required trials for pulps treated differently). The freezing bound water in pore sizes of 1.3 to 396 nm were calculated based on the integration of the heat-adsorbing segments described above.

RESULTS AND DISCUSSION

Composition of Raw Newspaper

The newspaper tested in this study consisted of 75.5% carbohydrates (58.3% glucan, 8.31% xylan, 0.59% galactan, 8.89% arabinan and mannan), 0.66% acid-soluble lignin, 16.0% klason lignin, 2.70% ash, and 0.57% extractives. The composition of the newspaper in this study had cellulose, hemicelluloses, and lignin contents within the range of those from other studies, as shown in Table 1. Also included in Table 1 are average biomass compositions for hardwood and softwood.

Table 1. Average Biomass Compositions of Untreated Newspaper *versus* Typical Lignocelluloses from Wood

Biomass	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Newspaper	58.0	17.7	16.7
Newspaper[a]	60.3	16.4	12.4
Newspaper[b]	53.3	26.3	11.2
Newspaper[c]	40-55	25-40	18-30
Softwood[c]	45-50	25-35	25-35
Hardwood[c]	40-55	24-40	18-25

Source: [a] (Lee *et al*. 2010a), [b] (Xin *et al*. 2010), [c] (Sun and Cheng 2002)

Effect of Ink and Surfactant on Enzymatic Hydrolysis

Various pretreatments were conducted to compare the sugar conversion efficiency during the enzymatic hydrolysis process with that of original printed newspaper. As shown in Fig. 1, original newspaper and hyperwashed newspaper achieved similar sugar conversion levels at all enzyme dosages. The ERIC values (Table 2) of these two substrates showed that hyperwashing removed 53.6% of the total ink from the original newspaper, corresponding to 81.2% of the detachable ink, as the paper contained non-detachable inks from recycled fibers, contributing to 228.3 ppm ERIC. The slight amount of residual ink in unprinted newspaper may appear because newspaper is made partially or entirely from recycled paper, which contains non-detachable ink. Negative enzyme inhibition of detachable ink was not observed upon sugar conversion based on the time-dependent enzymatic hydrolysis (4 FPU/g enzyme dose) data generated over the 108 h period (Fig. 2). This was in agreement with the untreated and hyperwashed newspapers, which exhibited similar enzyme hydrolysis results, as shown in Fig. 1. This suggests that the quantities of ink present neither covers the substrate to the extent that it will interfere with enzyme access to the substrate nor does it appreciably bind to the enzyme.

The effect of surfactants, especially sorbitan polyoxyethylene monooleate, on newspaper saccharification has been studied by several researchers. A sugar conversion improvement of 10% to 35% was reported based on enzyme type and dosage, as well as sorbitan polyoxyethylene monooleate concentration (Castanon and Wilke 1981; Wu and Ju 1998; Xin *et al*. 2010). Improvements in sugar conversion using sorbitan polyoxyethylene monooleate (0.4%, v/v) were observed herein (Fig.1). In hyperwashed newspaper, during enzymatic hydrolysis, sorbitan polyoxyethylene monooleate improved sugar conversion by 7.3%, 8.0%, and 6% (all by subtraction) at enzyme dosages of 2, 4, and 8 FPU/g, respectively. This is presumably due to the ability of the surfactant to reduce adsorption on lignin (Eriksson et al. 2002).

In addition, the enzyme digestibility of the newspaper was increased by lab-scale mechanical refining, as shown in Fig. 1. Refining shortened and fibrillated the fibers, creating a higher accessible surface area for enzyme digestion (Hubbe *et al*. 2007). Refined newspapers achieved the same sugar conversion compared to the newspapers containing sorbitan polyoxyethylene monooleate. Compared with the original newspapers at various enzyme doses, improvement in sugar conversion was about 10% with refining and/or surfactant usage at all dosages of enzyme studied.

This improvement in sugar conversion with the addition of surfactant agrees with the results of Xin *et al*. (2010). When surfactant was applied, non-productive binding of cellulase to lignocellulose was reduced by blocking of the hydrophobic sites on the fiber's surface (Berlin *et al*. 2006). Also, surfactant was reported to stabilize the enzyme and positively affect enzyme-substrate interactions to improve digestibility (Kim *et al*. 2006). The combination of both refining and surfactant resulted in the highest sugar conversions, *i.e.*, 46.3%, 56.7%, and 64.1% at enzyme dosages of 2, 4, and 8 FPU/g, respectively.

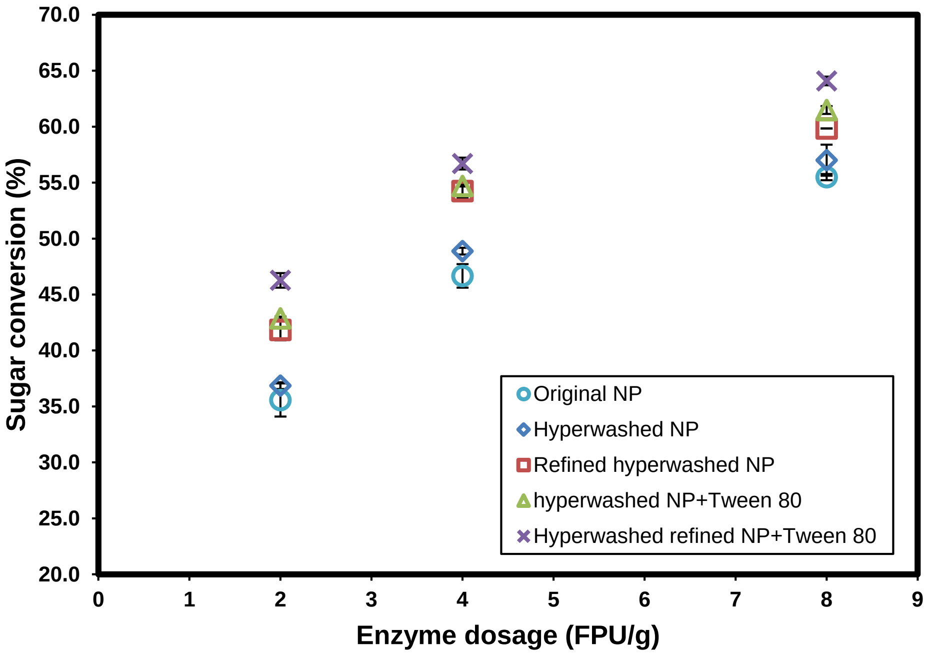

Fig. 1. Sugar conversion of newspaper with various pretreatments at enzyme dosages of 2, 4, and 8 FPU/g for 48 h. Each data point is the average of two replicates; error bars depict ± 1 standard error of the mean.

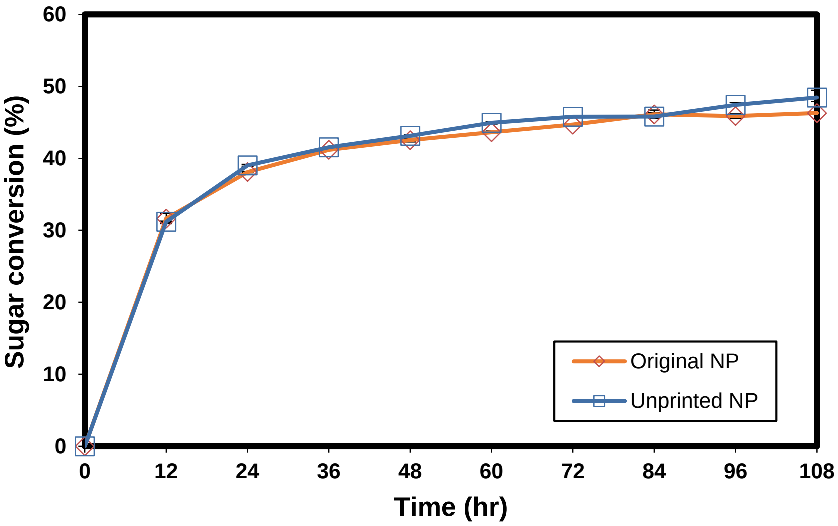

Fig. 2. Sugar conversion of the original/printed newspaper and unprinted newspaper at 4 FPU/g cellulase. Each data point is the average of two replicates; error bars depict ± 1 standard error of the mean.

ERIC values of unprinted/printed newspaper and newspaper with the hyperwashing step before or after autohydrolysis were measured, as shown in Table 2. Hyperwashing before autohydrolysis removed 53.6% of the total ink or 81.2% of the detachable ink, assuming the ink in the unprinted newspaper was non-detachable. Ink was not removed during the autohydrolysis pretreatment, which was a high-temperature process. Handsheets were made from fiber with modest washing and filtration after autohydrolysis, and the ERIC values remained the same compared to that of original newspaper. Therefore, the flexo ink was not solubilized or removed during high-temperature pretreatment. It has been reported that ink becomes stickier during high-temperature treatment and clings to fibers, making it difficult to remove (Kim and Chun 2004b). However, hyperwashing after autohydrolysis removed a similar amount of ink to hyperwashing alone, which indicated that the flexo ink, after high-temperature treatment, was still detachable.

Table 2. ERIC Values of Newspaper with Various Treatments

Samples	ERIC (ppm)	Ink detachment parameter (Ink_D) (%)	Detachable ink removal (%)
Unprinted NP	228.3	/	/
Original NP	672.2	/	/
Hyperwashed NP	311.8	53.6	81.2
160 °C 30 min	662.7	1.4	2.1
160 °C 60 min	670.1	0.3	0.5
160 °C 30 min+hyperwashing	332.5	50.5	76.5
160 °C 60 min+hyperwashing	363.5	45.9	69.5

Autohydrolysis

Autohydrolysis pretreatment is a hydrothermal process that treats lignocellulosic materials under a high-pressure steam at a temperature range of 130 to 260 °C for several seconds to hours (Lee *et al*. 2010b). Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short retention time or lower temperature and longer retention time. It has been reported that lower temperature and longer retention time treatment is more favorable for improving subsequent enzymatic hydrolysis (Sun and

Cheng 2002). Autohydrolysis depolymerizes the major part of the hemicellulose from the solid material. During this pretreatment process, extractives from the solid phase, mostly ash and proteins, are solubilized in solution. In addition, partial dissolution of lignin takes place, as well as the generation of by-products, including furfural and 5-hydroxy-2-methylfurfural (HMF) (Taherzadeh and Karimi 2007; Lee *et al.* 2009; Pu *et al*. 2011).

In general, the pretreatment yields (dry matter basis) decreased with time, temperature, and acetic acid addition, as shown in Table 3. The sugar recovery of original, hyperwashed, and unprinted newspaper showed that longer retention time solubilized more oligo-sugars, and most sugars generated in the filtrates existed as oligomers. At 160 and 180 °C, the main oligo-sugar in the filtrates was hemicellulose, with only trace amounts of glucose detected. The addition of 3% or 5% acetic acid accelerated the solubility of the sugars, which may be due to the heterocyclic ether bonds of the xylan backbone, which were cleaved to give compounds of a lower polymerization degree *via* the catalysis of acetic acid (from cleavage of the acetyl groups of the hemicellulose or from supplementation) (Vázquez *et al*. 2005).

Table 3. Reaction Time, Yield, pH, Sugar (g), and (%) Recovered in Filtrate during Autohydrolysis

Conditions	Time (min)	Yield (%)	pH of filtrate	Sugar in filtrate (g)[a]			Sugar recovered in filtrate (%)[e]
				G[b]	H[c]	T[d]	
Original NP	30	94.1	4.76	0.05	0.41	0.46	0.61
				0.25	2.47	2.72	3.59
	60	93.6	4.50	0.61	0.38	0.99	1.31
				0.92	1.98	2.9	3.83
Hyperwashed NP	30	93.1	4.16	0.06	0.45	0.51	0.68
				0.26	2.28	2.54	3.36
	60	91.4	3.76	0.09	0.56	0.65	0.86
				0.33	2.75	3.08	4.07
Unprinted NP	30	95.0	5.34	0.05	0.29	0.34	0.45
				0.28	1.69	1.97	2.60
	60	95.3	5.28	0.06	0.34	0.40	0.53
				0.30	1.93	2.23	2.95
Unprinted NP+3% AA[f]	60	88.2	3.47	0.20	0.82	1.01	1.34
				0.67	3.61	4.28	5.65
Unprinted NP+5% AA	60	87.2	3.26	0.28	0.99	1.26	1.67
				0.82	4.38	5.20	6.86
Unprinted NP 180 °C	60	87.8	4.12	0.02	0.26	0.28	0.37
				0.32	1.81	2.13	2.81

Note: All the other samples were treated at 160 °C except the "Unprinted NP 180 °C".

[a] Sugar (g) in autohydrolysis filtrate is based on 100 g raw newsprint and sugar recovery is based on total sugar in the raw material. Sugar data before and after 4% H2SO4 hydrolysis is listed for each condition.

[b] G: glucan.

[c] H: xylan and other oligo-sugars.

[d] T: total oligo-sugars dissolved in filtrate.

[e] Average % sugar recovered in filtrate based on carbohydrate content in starting materials.

[f] AA: acetic acid.

The compositions of original newspaper and autohydrolyzed newspaper are listed in Table 4. In general, autohydrolysis had very little impact on the composition of the fibers. Few variations were observed on total carbohydrate and lignin content. The main sugar loss was observed in xylan. Hyperwashing removed about half of the ash content.

In this study, the sugar conversion of the newspapers after autohydrolysis decreased compared to the control groups, which were newspapers without pretreatment (Fig. 3). The sugar recovery also decreased because the solubilization of carbohydrates in autohydrolysis was considered to be a loss of potential sugar. Even with the addition of acetic acid, enzyme digestibility was unchanged, although more hemicellulose was recovered in the filtrate. The sugar conversion of newspaper with the autohydrolysis pretreatment plus hyperwashing was lower than that of the original newspaper, indicating that a decrease in sugar conversion occurred after autohydrolysis; this was not attributed to the effects of high temperature on the change in composition or distribution of ink in the fibers. In addition, autohydrolysis at a higher temperature (180 °C) had a greater negative effect on enzymatic hydrolysis compared to autohydrolysis at 160 °C. When considering that autohydrolysis had very little effect on composition, the decreases in enzymatic hydrolysis are likely to originate from structural changes in the fibers themselves, such as the redistribution of lignin components as observed in this laboratory for autohydrolysis of coastal Bermuda grass (Lee *et. al.* 2010b).

Table 4. Compositions of Fibers from Autohydrolyzed Newspaper

Substrate	Total Carb (%)	Glu (%)	Xyl (%)	Gal (%)	Ara+ Man (%)	Ash (%)	Acid Soluble Lignin (%)	Klason lignin (%)	Extrac -tives (%)	Mass Balanc e (%)
Original NP	75.7	58.3	8.3	0.6	8.9	4.71	0.7	16.0	0.57	98.0
Original NP 160 °C 30min	73.7	58.7	4.4	0.52	10.1	4.64	0.54	17.0	N/D	95.9
Original NP 160 °C 60min	74.4	59.3	4.2	0.50	10.4	4.35	0.54	16.9	N/D	96.3
Hyperwashed NP 160 °C 30min	79.4	62.3	5.2	0.77	11.2	2.42	0.50	15.7	N/D	98.0
Hyperwashed NP 160 °C 60min	77.2	61.8	4.8	0.72	9.9	2.30	0.50	15.8	N/D	95.8
Unprinted NP 160 °C 30 min	76.7	59.9	5.2	0.98	10.7	4.43	0.52	16.6	N/D	98.2
Unprinted NP 160 °C 60 min	76.3	59.4	5.0	1.17	10.7	4.31	0.48	16.4	N/D	97.5
Unprinted NP 3% AA 160 °C 60min	77.1	61.8	4.4	0.90	10.1	3.33	0.45	16.8	N/D	97.7
Unprinted NP 5% AA 160 °C 60min	77.4	62.2	4.4	1.12	9.7	2.53	0.44	17.2	N/D	97.6
Unprinted NP 180 °C 60 min	81.2	64.6	2.7	1.4	10.5	4.01	0.48	17.1	N/D	102.7

Higher enzyme dosages were attempted, and the results confirmed that autohydrolysis had a negative influence on enzymatic hydrolysis, as shown in Table 5. Even at the 20 FPU/g enzyme dose, sugar conversion decreased from 64% to 59%, and the conversion of all types of sugars decreased.

Table 5. Sugar Conversion of Unprinted Newspaper at the 20 FPU/g Enzyme Dose With and Without Autohydrolysis

Pretreatment Conditions	Carbohydrate conversion (%)	Glucan conversion (%)	Xylan conversion (%)	Galactan conversion (%)	Ara+Man conversion (%)
Unprinted NP	64.5	64.1	98.1	75.1	47.5
Unprinted NP 160 °C 30 min	59.2	57.7	83.9	38.6	47.3
Unprinted NP 160 °C 60 min	59.1	57.5	79.7	49.1	46.5

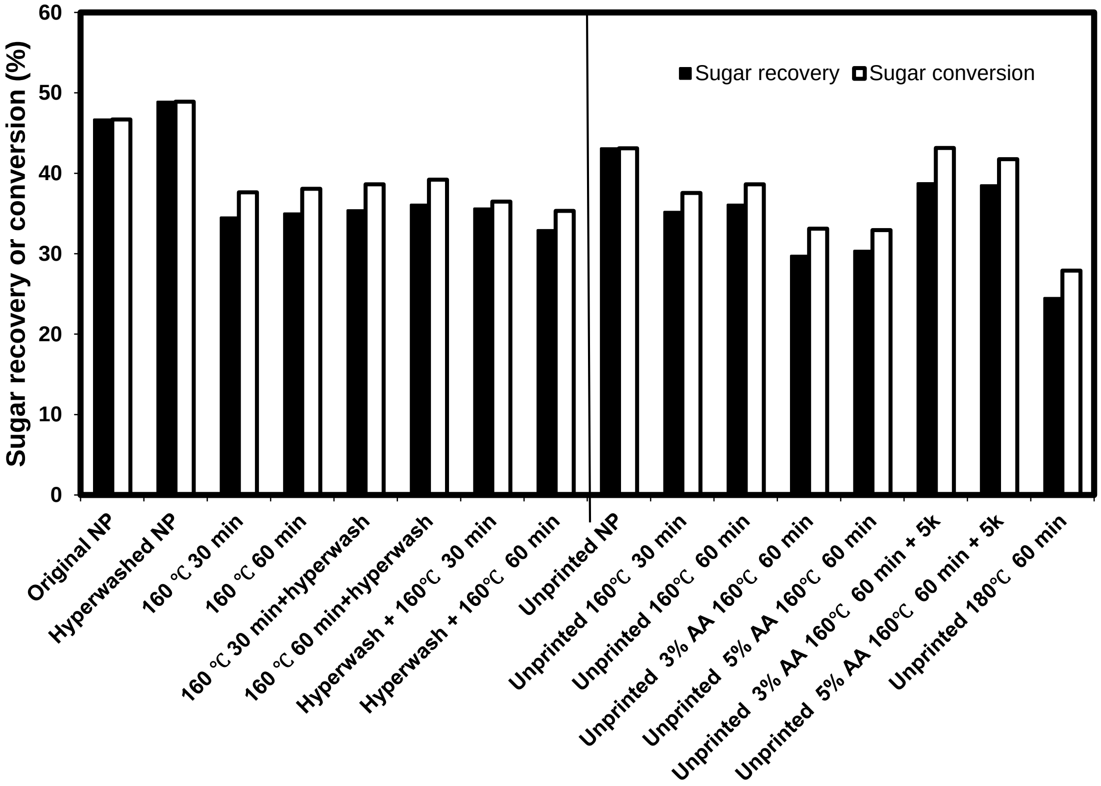

Fig. 3. Sugar conversion and sugar recovery of the newspaper at the 4 FPU/g enzyme dose under various treatments. Note that the vertical line separates printed newspaper and unprinted newspaper under various treatments. 5k: 5000 revolutions of PFI refining. Each data point is the average of two replicates; the average of the standard error of the mean for these results is 0.5 %.

Autohydrolysis of hardwoods and non-wood biomass has been shown to increase the enzymatic conversion of the residual solids (Lee *et al*. 2009; Romaní *et al*. 2011; Ertas *et al*. 2014). Newspaper is mostly produced from softwood; this was confirmed by the average fiber length and bordered pits of the fibers (Usta 2005) (Fig. 4). Hardwood is generally 20 to 40 μm in width, while softwood is 30 to 50 μm in width (Smook 1982). The average width of the newspaper fibers in this study was measured to be 44 μm by sampling 40 fibers under the microscope. The newspaper fiber from mechanical pulping has a very different morphology than the closely compacted fibers hydraulically connected by pits in wood. The mechanical pulping of wood generates liberated fibers with most of the lignin remaining after pulping and existing on the surface of the individual fibers as parts of the middle lamella. This difference in morphology between mechanical pulp and the original wood may also play a role in the different responses to autohydrolysis.

Autohydrolysis is a pretreatment method that predominantly affects hemicelluloses (Heitz *et al*. 1991). It is also reported that lignin transformation and relocation to the surface is observed in wheat straw (Kristensen *et al.* 2008) and coastal Bermuda grass (Lee *et al.* 2010b) during autohydrolysis. To examine the possibility of lignin relocation in the pretreated newspaper, three separate samples, unprinted NP, unprinted NP with autohydrolysis at 160 °C for 60 min, and unprinted NP with autohydrolysis augmented with 5% acetic acid at 160 °C for 60 min, were selected for surface lignin measurement by XPS, as shown in Table 6.

Fig. 4. Microscopic image of a representative newspaper softwood fiber (scale = 100 µm)

The corrected molecular ratio of oxygen to carbon (N_0/N_C) can be estimated from the measured value using the following equation (Gray *et al*. 2010):

$$\frac{1}{(N_0/N_C)_{\text{pulp,corrected}}} = \left[\frac{2.88 * (N_0/N_C)_{\text{cellulose,measured}} - 0.833}{1.88 * (N_0/N_C)_{\text{cellulose,measured}}}\right] * \left[\frac{1}{(N_0/N_C)_{\text{pulp,measured}}} + 1\right] - 1 \tag{5}$$

Using this equation and the O:C ratio for cellulose (0.833) and lignin (0.335), the surface lignin content can be estimated (Gray *et al.* 2010). Compared to the control group, unprinted newspaper with 0.202 segment mole fraction and 22% weight fraction of surface lignin content, autohydrolysis of the newspaper with or without acetic acid did not significantly change the estimated surface lignin content (Table 6). Hence, lignin relocation was not evident with these measurements and therefore cannot be identified as the sole cause for the negative impact of autohydrolysis on the sugar conversion.

Table 6. Surface Lignin Content of Newspaper Samples

Samples	$(N_o/N_c)_m$	$(N_o/N_c)_c$	Segment mole fraction of lignin (S_L)	Weight fraction of lignin (W_L)
Unprinted NP	0.758	0.686	0.202	22%
Unprinted NP 160 ℃ 60 min	0.766	0.693	0.192	21%
Unprinted NP 5% AA 160 ℃ 60 min	0.744	0.674	0.222	24%

Note: $(No/Nc)_m$: measured molecule ratio of oxygen and carbon; $(No/Nc)_c$: corrected molecule ratio of oxygen and carbon.

The pore volume accessible to enzymes has also been used to explain differences in the enzyme digestibility of biomass (Yu *et al*. 2011). To investigate the effect of accessible pore volume on newspaper saccharification, freezing bound water measurements on the control and autohydrolysis-treated newspaper samples were performed, as shown in Fig. 5. After treating newspaper at 160 °C for 60 min with only water, total pore volume was dramatically reduced. This could be mainly attributed to the collapse of pores with diameters larger than 30 nm. The same reduction in pore volume was determined for autohydrolysis conditions at higher temperatures or with acetic acid addition, as shown in Fig. 5. The pore collapse observed was consistent with the lower sugar conversion level exhibited post-autohydrolysis.

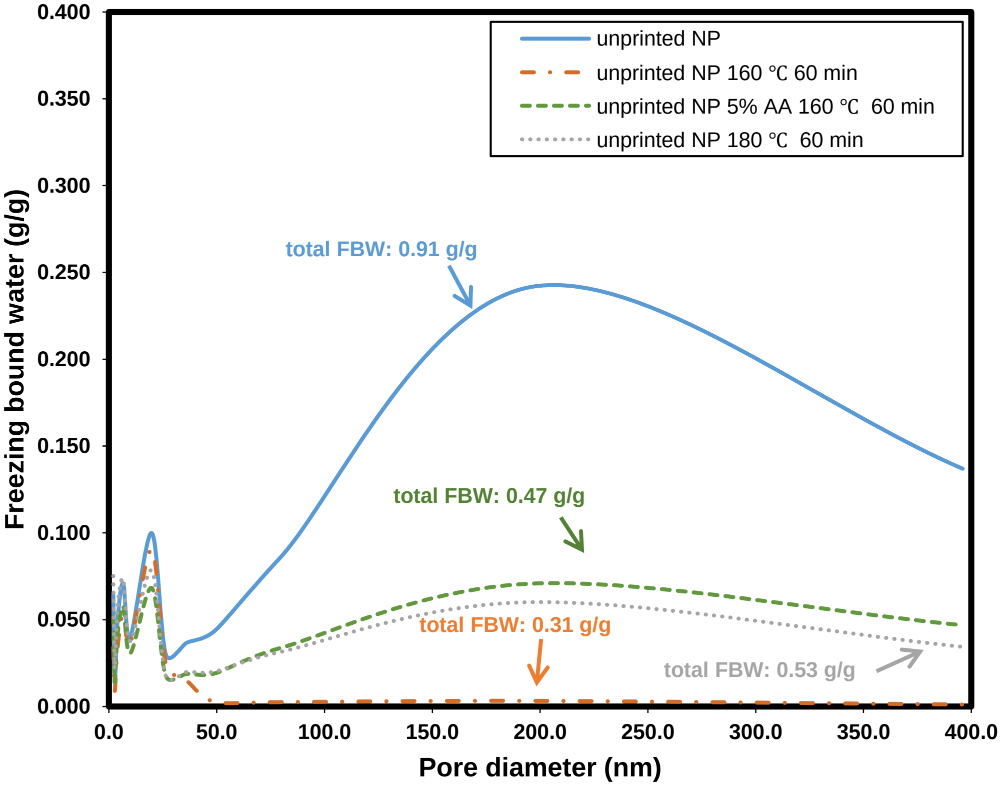

Fig. 5. Pore diameter distribution and total accessible pore volume expressed by freezing bound water (FBW) of unprinted and autohydrolyzed newspaper

Effect of Mechanical Refining on Enzymatic Hydrolysis

It was expected that mechanical refining would result in an increase in internal delamination and external fibrillation, which would in turn increase the accessible fiber area to enzyme, thus enhancing the enzymatic hydrolysis efficiency. This result has been shown previously for wood pulps such as chemically pulped and bleached recycled copy paper fibers (Chen *et al.* 2012). The unprinted newspaper was refined using a laboratory PFI mill with 0, 2,500, 5,000, 7,500, and 10,000 revolutions. Escalation of refining intensity resulted in an increase in specific surface area (SSA) and total pore volume, as shown in Fig. 6. Refining of unprinted newspaper fibers resulted in considerably higher surface areas, which have been reported to determine the rate and degree of hydrolysis of the substrate (Nazhad *et al*. 1995). The sugar conversion at 4 FPU/g was improved by refining to 43% and 56% using 10,000 revolutions of PFI.

BET nitrogen adsorption was used to measure the SSA of material in the dry state. Although SSA from the BET method does not represent the actual SSA of newspaper fiber in the wet state, it should be representative of the wet state. It is known that mechanical fiber does not shrink to a large degree upon drying, relative to chemically pulped fiber. This is due to the lignin content of the mechanical fiber. Also, the newsprint in this study had already been refined and dried prior to re-wetting during the autohydrolysis/enzyme hydrolysis and subsequent drying processes.

It is known that the shrinkage of fiber occurs to the greatest extent on the first drying cycle and less on subsequent cycles. For these reasons, the newspaper studied using the BET method was assumed to have minimal shrinkage prior to the BET analysis. The increases in SSA and total pore volume were consistent with increasing sugar conversion, as was expected, because refining increases the accessible area of the substrate for enzyme hydrolysis.

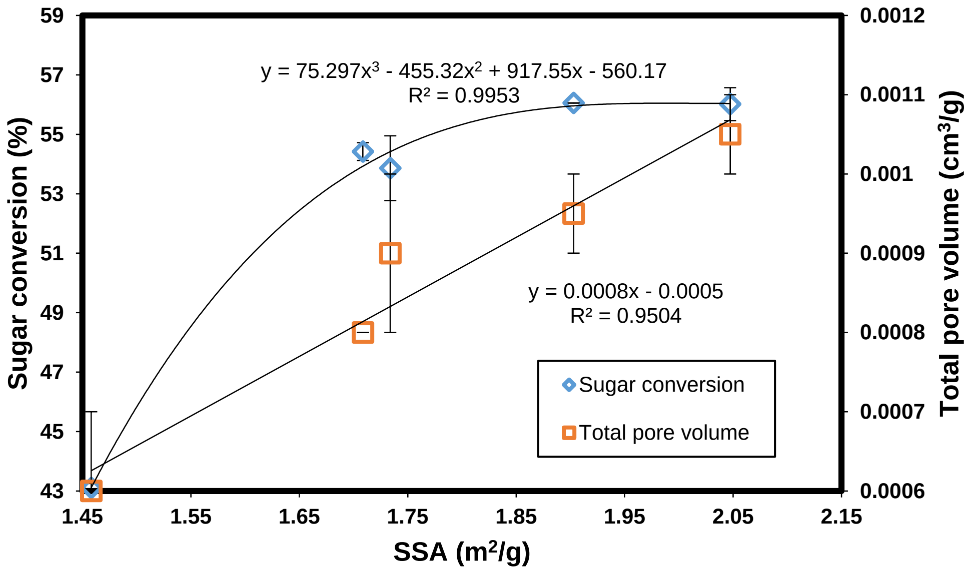

Fig. 6. Sugar conversion of unprinted newspaper at 4 FPU/g with various refinements *versus* the corresponding specific surface area (SSA) and total pore volumes. Each data point is the average of two replicates; error bars depict ± 1 standard error of the mean.

Fiber coarseness, an important parameter in papermaking, is defined as the mass of the fiber per unit length. Fibers with high coarseness have thick cell walls, which would be expected to have lower enzyme hydrolysis rates than fibers with very thin cell walls (and higher SSA). It has been proposed that refining will reduce coarseness by breaking off parts of the fiber wall, leaving lower mass in the fiber wall (Table 7). Also, fine material should contribute to lower coarseness of the pulp overall. It was determined that decreases in coarseness correlated with increased sugar conversion. However, this was just a correlation; decreased fiber length and increased fines also correlated with an increase in sugar conversion upon refining.

Table 7. Refined Fiber Properties by Different Refining Intensities and Corresponding Sugar Conversion at the 4 FPU/g Enzyme Dose

Refining revolutions	Fines, %	Mean length, mm	Coarseness, mg/m	Sugar conversion, %
0	8.33±0.27	1.32±0.03	0.145±0.001	43.1±0.2
2500	9.21±0.08	1.09±0.007	0.135±0.001	54.4±0.3
5000	9.16±0.13	1.09±0.002	0.131±0.002	56.1±0.1
7500	9.01±0.26	1.17±0.002	0.133±0.005	53.9±1.1
10000	9.32±0.09	1.08±0.006	0.110±0.002	56.0±0.6

Impact of Oxygen, Alkaline, and GL Pretreatments on Enzymatic Hydrolysis

As discussed above, autohydrolysis at 160 °C for 30 and 60 min did not improve the enzymatic hydrolysis of newspaper. Even with the addition of acetic acid, this treatment was not effective on newspaper. Thus, other pretreatments were attempted, such as oxygen, alkaline, and GL, which were expected to remove lignin and thus improve enzyme accessibility and efficiency.

The oxygen pretreatment (100 °C) achieved a 97.4% total weight yield with 3% NaOH and 93.3% yield with 4% NaOH when compared to the starting material using unprinted newspaper. The oxygen pretreatments did not appreciably change the chemical composition of the newspaper, as shown in Table 8. Green liquor (160 °C) and alkaline pretreatments (160 °C), with 4% or 6% NaOH, partially removed lignin from the starting newspaper, and pretreatment yields of 86%, 82%, and 80% were obtained, respectively.

Compared to the sugar conversion of unprinted newspaper, only the oxygen pretreatment marginally improved enzymatic digestibility, from 43% to 46% and from 55% to 60% at enzyme dosages of 4 and 8 FPU/g, respectively (Fig. 7). This was notable because the oxygen pretreatment did not remove as much lignin as the GL and alkaline pretreatments, and thus might not be expected to achieve a higher digestibility rate. Further, the oxygen pretreatment was the only one occurring at a relatively low temperature (100 °C). This suggests that the elevated pretreatment temperatures in autohydrolysis, GL, and alkaline pretreatments may be the cause for the negative effect. The NaOH dose of 3% or 4% in the oxygen pretreatment was insignificant in terms of sugar conversion.

Table 8. Pretreatment Compositions and Yields (Based on Treated Newspaper) using Oxygen, Alkaline, and GL Pretreatments

Substrate	Temper-ature (ºC)	Yield (%)	Total Carb (%)	Glu (%)	Xyl (%)	Gal (%)	Ara+ Man (%)	Ash (%)	Acid Soluble Lignin (%)	Klason lignin (%)
Unprinted NP	/	/	75.7	58.3	8.3	0.6	8.89	2.70	0.66	16.0
Oxygen (3% NaOH)	100 °C	97.4	77.9	57.4	7.8	1.3	11.4	3.69	0.66	17.2
Oxygen (4% NaOH)	100 °C	93.3	81.8	59.7	8.5	2.0	11.6	3.48	0.66	16.2
Green liquor	160 °C	86.3	85.1	64.3	6.9	2.0	11.9	3.92	0.78	13.0
Alkaline (4% NaOH)	160 °C	81.7	86.1	65.9	7.3	1.8	11.1	4.15	0.75	13.1
Alkaline (6% NaOH)	160 °C	80.3	88.3	68.9	7.7	1.4	10.3	3.89	0.76	11.7

Green liquor is an effective pretreatment for hardwood in terms of enzymatic hydrolysis (Jin *et al*. 2010). However, GL alone is not efficient for the improvement of sugar conversion of softwoods (Wu *et al.* 2010). Although newspaper has already been through the pulping and papermaking process, which presumably makes fiber more amenable for enzyme digestion, GL was not able to improve sugar conversion because the newspaper used in this case was primarily made of softwood. Lastly, alkaline pretreatments at 4% and 6% NaOH showed lower sugar conversion than the untreated NP, where these two conditions presented similar performance in enzymatic hydrolysis.

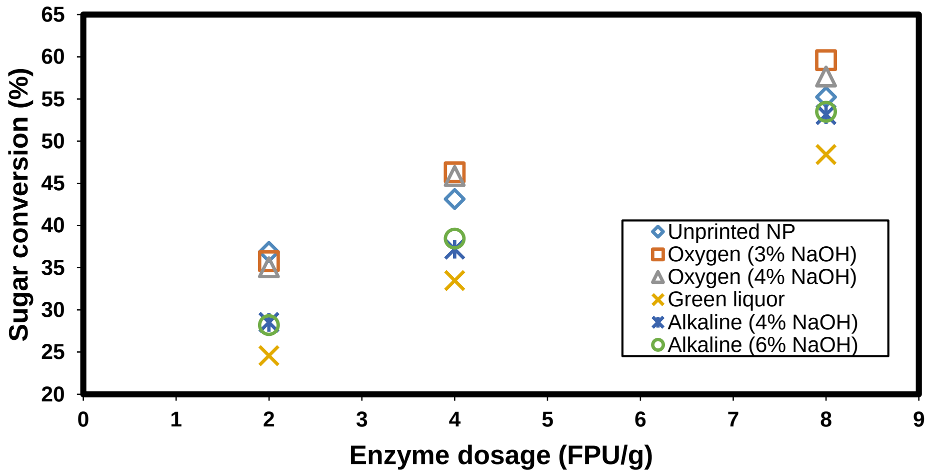

Fig. 7. Sugar conversion in unprinted newspaper, oxygen, green liquor (GL), and alkaline pretreated newspaper. Each data point is the average of two replicates; the average of the standard error of the mean for these results is 0.4 %.

CONCLUSIONS

1. Mechanical refining and a non-ionic surfactant, sorbitan polyoxyethylene monooleate, positively influenced the enzymatic hydrolysis of newspaper. Increasing the refining intensity resulted in the increase of specific surface area (SSA) and total pore volume, which positively correlated with sugar conversion. However, refining also caused increases in fines and decreases in coarseness that correlated with sugar conversion.
2. Autohydrolysis at 160 °C and 180 °C had a negative effect on enzyme hydrolysis. The same trend was found for autohydrolysis with the addition of acid. Of the oxygen, alkaline, and green liquor (GL) pretreatments, only the oxygen pretreatment marginally increased the sugar conversion rate; the alkaline and GL pretreatments had the opposite effect. Interestingly, the oxygen pretreatment was the only pretreatment performed at a lower temperature (100 °C). There appeared to be an inverse relationship between temperature of pretreatment and subsequent sugar conversion, as the lowest sugar conversion was achieved with the highest autohydrolysis temperature.

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