

PRETREATED CENTRAL APPALACHIAN HARDWOOD RESIDUES AND THEIR POTENTIAL FOR BIOENERGY PRODUCTION

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(Received February 2012)

Abstract. Hardwood residues of yellow-poplar (*Liriodendron tulipifera*) and northern red oak (*Quercus rubra*) were pretreated with two alkaline solutions: 1) ammonium hydroxide and sodium hydroxide (ASO) and 2) hydrogen peroxide/ammonium hydroxide and sodium hydroxide (PASO) mixtures. After pretreatment, particulates of yellow-poplar, northern red oak, and pulp were enzymatically hydrolyzed with cellulases (Accellerase 1000 [Genencor International, Rochester, NY]) for comparisons of glucose production. ASO pretreatment increased the lignin solubility of yellow-poplar and northern red oak by an average of 8.6%. PASO pretreatment performed better than ASO pretreatment with respect to lignin decrease in the pretreated wood. Sugar yield after enzymatic hydrolysis ranged from 70-81 mg/mL for PASO-pretreated yellow-poplar and northern red oak residues, respectively, and 50-62 mg/mL for ASO-pretreated yellow-poplar and northern red oak.

Keywords: Biomass, alkali, pretreatment, hydrolysis, lignocellulose, biofuel.

INTRODUCTION

The price of transportation fuel has steadily increased since 1979 with more than 250% of that increase caused by increased demand, supply shortage, and political instability in some major oil-producing nations of the world. Statistics show that the price of fuel may continue to increase in the US for the foreseeable future (EIA 2011).

Currently, a significant proportion (45%) of electricity and transportation fuels in the US is generated from nonrenewable sources through direct combustion of coal and imported crude oil (USDOE 2010). The US, with a population

of more than 300 million people, consumed 20 million barrels of oil per day, an amount just a little less than one-quarter of the world's daily demand (Clean Fuels Development Coalition [CFDC] 2007). Cellulosic ethanol could be a promising alternative fuel option because it is renewable, ecologically friendly, and possesses the potential to meet one-third of the current US transportation fuel needs (Perlack et al 2005).

Logging and mill residue statistics from the US Forest Service, timber product output (TPO 2008), showed that existing wood residues are capable of supporting bioenergy plants in the Central Appalachian region (West Virginia and surrounding states). West Virginia produces an estimated 4.5 Tg of wood fiber in the form of industry and forest residue comprised of 35.4 Tg of chips, saw particulates, and bark and 1.1 Tg

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of logging residues per year (Wang et al 2006). These available biomass resources can assist in fulfilling the long-term energy independence goal of the US, which is to replace 30% of the current transportation fuel demand (about 227.1 billion liters per year) with forest and agricultural biomass resources of 1.2 dry Tg (CFDC 2007).

Cellulases produced from a host of microorganisms can be used to hydrolyze wood into fermentable sugars provided the limiting factors (recalcitrants) such as lignin, extraneous substances, and anatomical constraints are removed from wood through an appropriate pretreatment method. Pretreatment alters the structure of cellulose, decreases cellulose crystallinity, increases surface area, and solubilizes hemicelluloses and lignin (Mosier et al 2005). Overcoming recalcitrance of lignocellulosic for the conversion of wood carbohydrates into fermentable sugars for production of liquid fuels is one of the principal techno-economic problems for bioenergy production (Demain et al 2005). Pretreatment of woody biomass includes biological, physical, and chemical methods (eg dilute acid, steam explosion, organosolv, ammonia, and alkali pretreatment methods) (Mosier et al 2005). To date, all these pretreatment methods have limitations for large-scale production of biofuels under the current economic situation.

Alkaline solutions have been reported to cause cellulosic fiber swelling and deacetylation and increased water/chemical impregnation and digestibility of wood (Constanza and Constanza 2004). Ammonia pretreatment such as ammonium fiber explosion (AFEX) is effective in cellulose mesmerization, lignin reduction, and micro and macro accessibility of cellulases in grasses (Mosier et al 2005). Hydrogen peroxide is also reported to promote hemicelluloses and lignin removal during the pulping process of softwoods (Tymouri et al 2005; Wyman et al 2005; Chundawat et al 2007).

Although ammonia pretreatment (eg AFEX), an acclaimed promising pretreatment method, works well for grasses, it is less effective for

woody biomass (Mosier et al 2005). However, combining ammonia with hydrogen peroxide could be more effective for hardwood pretreatment prior to enzymatic hydrolysis than the individual chemicals. Accordingly, this study was designed to 1) investigate effects of alkali mixtures and hydrogen peroxide solutions at 80°C with variable cook times on hydrolysis of yellow-poplar and northern red oak hardwood residues; 2) compare sugar yields of the two hardwood residues to that of pulp; and 3) evaluate the sugar production potential of available biomass resources in West Virginia and neighboring states.

MATERIALS AND METHODS

Yellow-poplar and northern red oak hardwood logs were collected from the West Virginia University Experimental Forest. Microcrystalline cellulose (pulp) was purchased from FMC BioPolymer Company (Philadelphia, PA). Other chemicals were 29% (wt/v) ammonia (Sigma-Aldrich, St. Louis, MO), 3% wt/v hydrogen peroxide (Sigma-Aldrich), 98% anhydrous sodium hydroxide (Sigma-Aldrich), and Accellerase 1000 cellulase (Genencor International, Rochester, NY).

Figure 1 summarizes the pretreatment process and chemical analysis. Yellow-poplar and northern red oak hardwood logs were converted to flakes, dried at $103 \pm 2^\circ\text{C}$ for 48 h, and milled into wood particulates passing through #60 mesh (ie more than 90% wood materials of 250- μm particle size passed through mesh #60). A milled portion of the wood particulates was used to determine the amount of holocellulose in the wood in accordance with ASTM (2001). Insoluble lignin content of the wood particulates was determined using the National Renewable Energy Laboratory (NREL) protocol (Templeton and Ehrman 1995).

Two sets of chemical pretreatments were used: 1) ammonium hydroxide + sodium hydroxide (ASO), which is comprised of 0.1, 0.2, and 0.3 g of sodium hydroxide solubilized into 25 mL ammonium hydroxide; and 2) hydrogen peroxide + ammonium hydroxide + sodium hydroxide (PASO), which contains 0.1, 0.2,

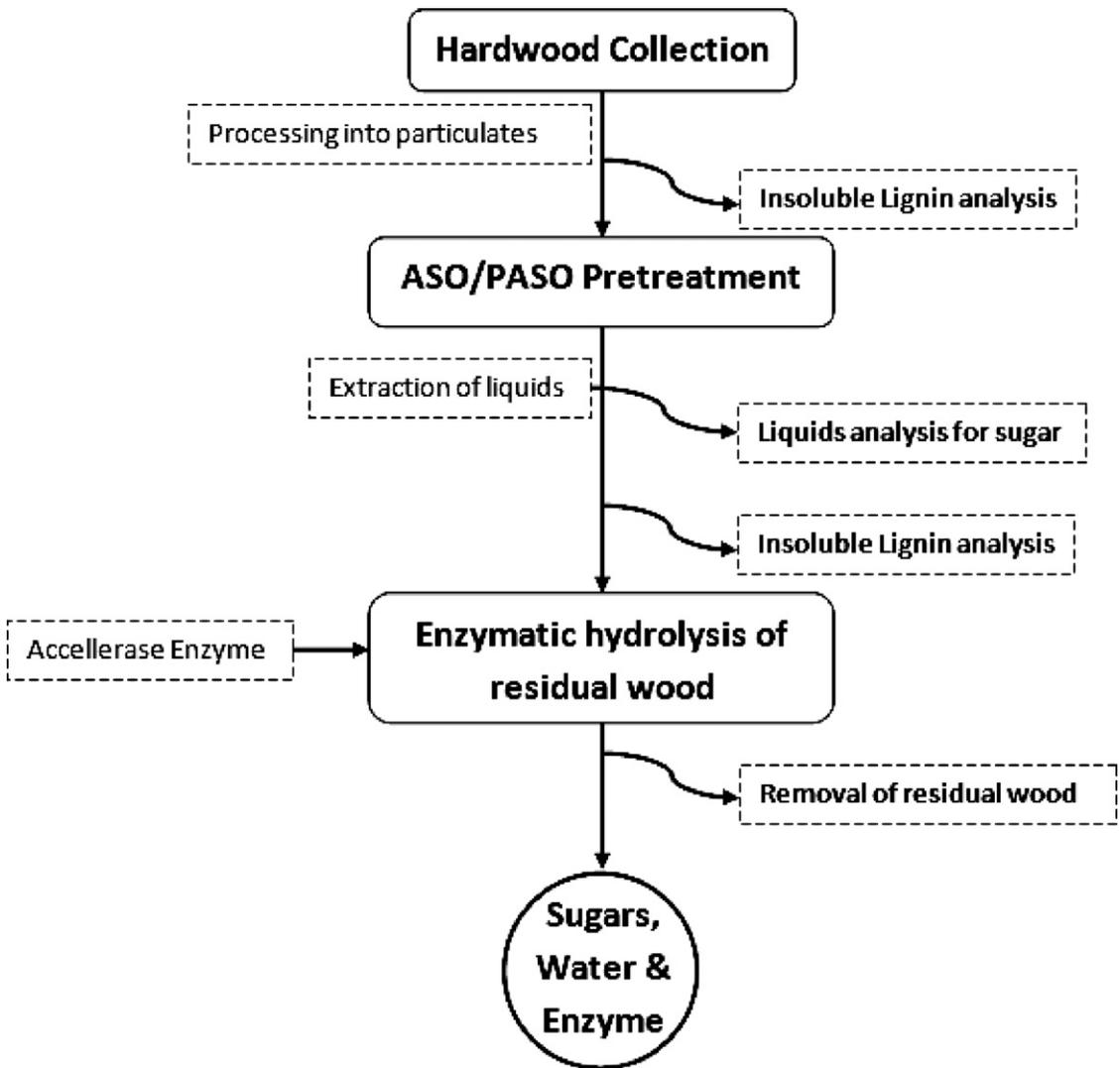


Figure 1. Flow diagram of ammonium hydroxide and sodium hydroxide (ASO) and hydrogen peroxide/ammonium hydroxide and sodium hydroxide (PASO) pretreatment process and chemical analysis.

and 0.3 g of sodium hydroxide solubilized into 25 mL ammonium and sodium hydroxide (3% wt/v of diluted hydrogen peroxide) mixture at 1:1 volume ratio.

These chemical pretreatments were applied on 1-g wood particulates of yellow-poplar and northern red oak at 80°C in a water bath for 3, 6, and 12 h. The mild cook temperature of 80°C was chosen in this study to prevent degrada-

tion of sugars during the pretreatment process (Wyman et al 2005). At the end of the pretreatment process, the insoluble fraction from yellow-poplar and northern red oak wood was filtered off and oven-dried at $103 \pm 3^\circ\text{C}$ for 24 h to determine insoluble wood fraction. Each filtrate was analyzed for its monomeric sugar content using high-performance liquid chromatography (HPLC) (Waters 2695 Alliance) equipped with a 2414 refractive index detector and a sugar pak

column. HPLC parameters for sugar analysis were as follows: 1) injection volume: 10 μ L; 2) number of injections per vial: 3; 3) mobile phase: HPLC-grade water; 4) flow rate: 0.2 mL/min; 5) column temperature: 60°C; 6) detector temperature: 38°C; and (7) run time: 20 min.

Accellerase enzyme was used in this study to convert wood cellulose into fermentable sugars through the breaking down of glycosidic bonds of cellulose. Accellerase enzyme produced from genetically modified *Trichoderma reesei* is brown in color with a pH of 4.8-5.2. It contains exoglucanase, endoglucanase, hemicellulase, and β -glucosidase. Endoglucanase activity of 25,000 carboxymethylcellulose (CMC) and beta-glucosidase activity of 400 paranitrophenyl-B-D-glucopyranoside (pNPG) was standardized on CMC and pNPG, respectively (Genencor 2008). Enzymatic hydrolysis was done with 0.15-g wood particulates of 6-h-pretreated ASO and PASO yellow-poplar, northern red oak residues, and pulp using 0.24 mL of accellerase enzyme at 50°C for 72 h as per NREL (Selig

et al 2008). The pulp was assumed to contain less than 1% lignin in accordance with the previous report that bleached Kraft wood pulp has a low lignin content of 0.1% (Saariaho et al 2003).

RESULTS AND DISCUSSION

Table 1 showed differences between yellow-poplar and northern red oak wood constituents (lignin and holocellulose) in accordance with previous findings (Sjostrom 1993; Adebola et al 2009). Analysis of variance on pretreatment of the hardwood residues indicated that PASO depolymerized more wood holocellulose than ASO pretreatment ($p = 0.0214$). Of all pretreatment combinations, the highest wood depolymerization rate (40.7% for northern red oak particulates) occurred at 0.3-g sodium hydroxide concentration and 6 h of pretreatment.

Results on the alkali pretreatment of wood particulates (Table 2) indicated that PASO depolymerized more wood than ASO pretreatment. Hydrolysis of wood was also observed to increase

Table 1. The components of yellow-poplar and northern red oak wood residues (%) after ASO pretreatment.^a

Time (h)	Sodium hydroxide (g)	Yellow-poplar (%)		Northern red oak (%)	
		Lignin	Holocellulose	Lignin	Holocellulose
3.00	0.0	24 \pm 0.02	64 \pm 0.00	34 \pm 0.03	60 \pm 0.02
	0.1	29 \pm 0.04	63 \pm 0.04	32 \pm 0.05	60 \pm 0.05
	0.3	37 \pm 0.08	60 \pm 0.10	33 \pm 0.00	65 \pm 0.01
6.00	0.1	31 \pm 0.08	60 \pm 0.10	35 \pm 0.02	56 \pm 0.02
	0.3	35 \pm 0.04	56 \pm 0.04	32 \pm 0.02	60 \pm 0.02
12.00	0.1	39 \pm 0.02	53 \pm 0.02	28 \pm 0.04	62 \pm 0.04
	0.3	47 \pm 0.06	45 \pm 0.06	30 \pm 0.10	61 \pm 0.01

^a Six replicates.

ASO, ammonium hydroxide and sodium.

Table 2. Yellow-poplar and northern red oak wood residues content balance after PASO pretreatment.^a

Time (h)	Sodium hydroxide (g)	Yellow-poplar (%)		Northern red oak (%)	
		Lignin	Holocellulose	Lignin	Holocellulose
3.00	0.0	25 \pm 0.02	64 \pm 0.02	34 \pm 0.03	60 \pm 0.02
	0.1	23 \pm 0.03	65 \pm 0.02	33 \pm 0.04	61 \pm 0.04
	0.3	23 \pm 0.00	65 \pm 0.01	33 \pm 0.05	58 \pm 0.05
6.00	0.1	21 \pm 0.02	66 \pm 0.02	27 \pm 0.02	63 \pm 0.02
	0.3	24 \pm 0.01	66 \pm 0.02	27 \pm 0.06	41 \pm 0.06
12.00	0.1	24 \pm 0.01	66 \pm 0.02	27 \pm 0.02	51 \pm 0.02
	0.3	26 \pm 0.05	64 \pm 0.04	26 \pm 0.15	64 \pm 0.10

^a Six replicates.

PASO, hydrogen peroxide/ammonium hydroxide and sodium hydroxide.

with sodium hydroxide concentration during pretreatment time ($p = 0.0017$) (Figs 2 and 3). The only exception to the observed general trend of increased wood depolymerization during pretreatment time was the 12-h pretreatment time, which showed a decrease. Explanation for this behavior is currently unknown. Of all treatment combinations, the highest depolymerization rate observed (40.7% for red oak residue) was at 0.3-g sodium hydroxide concentration and 6 h of cook time (Fig 3).

The percentage of hydrolyzed northern red oak wood was higher than that of yellow-poplar for both ASO and PASO pretreatments. The observed differences in hydrolysis between these hardwood species residues could be a result of existing structural and chemical variability (Tables 1 and 2). Structurally, northern red oak wood is ring-porous and contains many large vessels in the early wood and smaller vessels in the late-wood, whereas yellow-poplar wood is diffuse-porous (ie many small vessels across growth

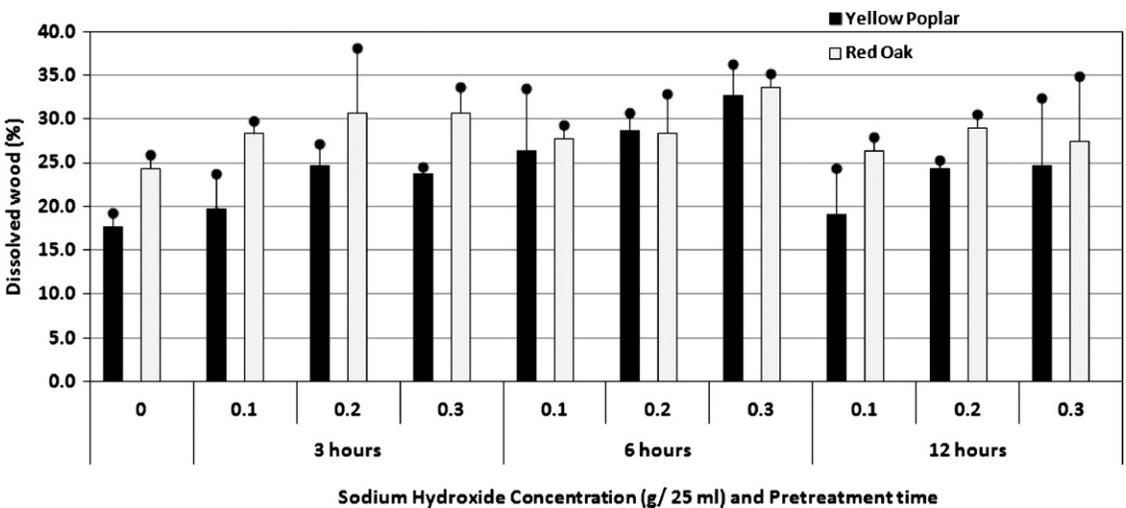


Figure 2. Dissolved wood content after ammonium hydroxide and sodium hydroxide (ASO) pretreatment.

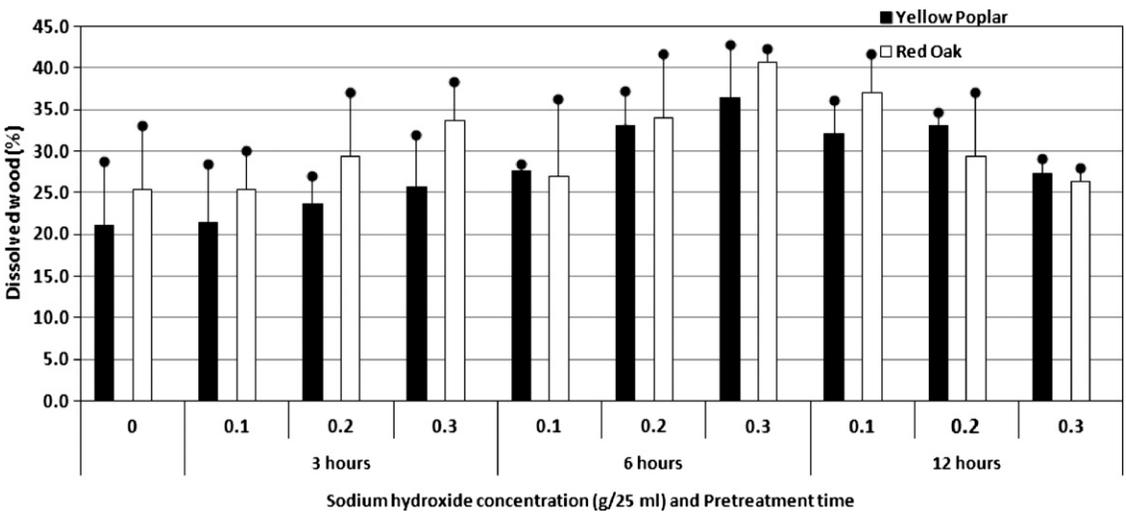


Figure 3. Dissolved wood content after hydrogen peroxide/ammonium hydroxide and sodium hydroxide (PASO) pretreatment.

increment) (Bruce 1990). Accordingly, the large pores in northern red oak probably facilitated efficient penetration of the chemicals, leading to increased depolymerization.

Dissolved wood after ASO treatment ranged from 20-33% and from 21-34% for yellow- poplar and northern red oak, respectively. The dissolved component of wood was holocellulose. This assertion was based on the postchemical analysis of the liquid portion after the alkali pretreatment, which revealed glucose sugar (Fig 4). Other sugars (five-carbon) in wood hemicelluloses may have been degraded or changed to other unidentifiable compounds in the sugar pak column used for HPLC sugar analysis. In this reaction, sodium hydroxide depolymerized these hardwood residues in agreement with a previous report that sodium hydroxide can change the structure of cellulose into less crystalline and short-length fibers by a mercerization process and breaking the hydrogen bonds in hemicellulose and cellulose (Johnson 2000; Zheng et al 2009).

Analysis of liquid hydrolysis extracts after the pretreatment revealed predominant presence of wood sugars (Fig 4). The measured sugar con-

tent was about the same among the various wood residues used for the study. Accordingly, 2.1-2.7 and 2.3-2.7 mg/mL of glucose sugar occurred for PASO-pretreated yellow-poplar and northern red oak residues, respectively, whereas 1.5-2.7 and 2.4-2.7 mg/mL occurred for ASO-treated yellow-poplar and northern red oak residues, respectively. The low sugar concentration observed in this study could have been caused by degradation actions of hydrogen peroxide solution on wood sugars as previously reported that hydrogen peroxide can increase depolymerization of holocellulose but with small recoverable sugars in liquid (Kim et al 2000).

Effects of Hydrogen Peroxide

The presence of hydrogen peroxide in the alkali mixture of sodium hydroxide and ammonium hydroxide increased the effectiveness of PASO pretreatment in the depolymerization of wood residues with a range of 21-36% for yellow-poplar and 25-41% for northern red oak residues (Fig 3). Hydrogen peroxide, a strong oxidizing agent, is the prime factor responsible for the increased depolymerization of wood holocellulose

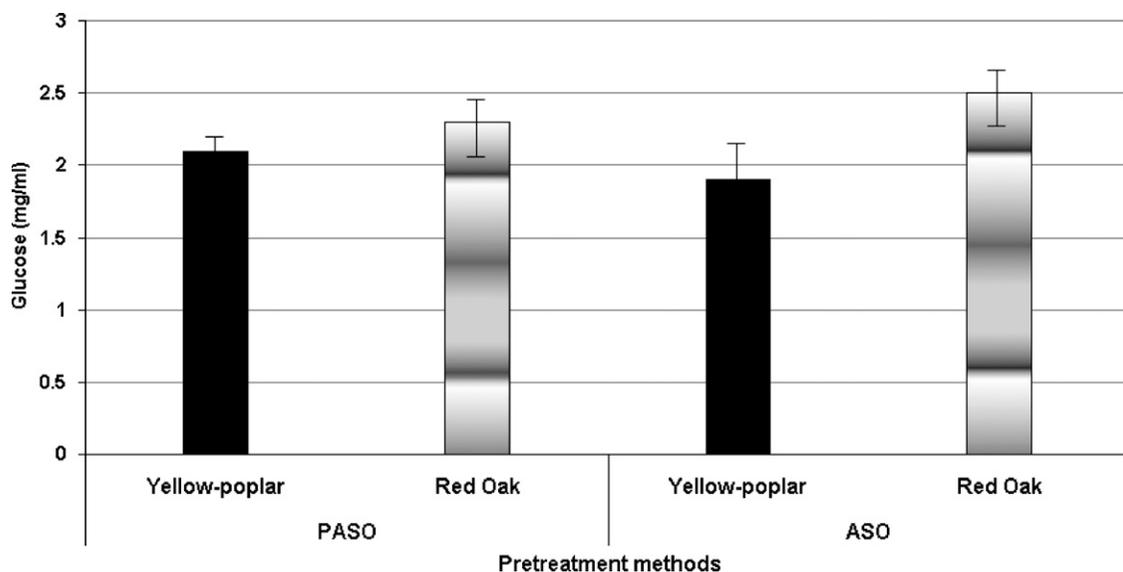


Figure 4. Glucose yield from liquid extract after ammonium hydroxide and sodium hydroxide (ASO) and hydrogen peroxide/ammonium hydroxide and sodium hydroxide (PASO) pretreatment of yellow-poplar and northern red oak.

in this reaction as previously reported (Kim et al 2000). During the reaction, some hydrogen peroxide could have been consumed, dissolved in water, or converted to hydroxyl radicals (Eqs 1, 2, and 3) via an ionic pathway (Nacimento et al 1995). These free hydroxyl radicals are the agents of the delignification action of hydrogen peroxide (Nacimento et al 1995; Rahmawati et al 2005).



In alkaline media such as the type used in this study, hydrogen peroxide dissociates into hydrogen peroxide anion (HOO^-). This anion is a strong nucleophile, a major active agent in peroxide reactivity, which converts unsaturated wood aldehydes and ketones to hydroperoxide and carboxylic acids (Mussatto et al 2008). The formation of hydroperoxide ($\text{HC}[\text{O}]\text{OOH}$) was reported to be responsible for breaking the aromatic chains in the complex lignin structures through an internal rearrangement mechanism (Nacimento et al 1995).

Effect of Sodium Hydroxide

ASO pretreatment showed little or no direct impact on solubility of yellow-poplar lignin during the pretreatment process; on average, yellow-poplar lignin increased by 43% across the various pretreatment conditions. This was caused by the selective depolymerization action of ammonium hydroxide (in ASO pretreatment) as the easily dissolvable components such as holocellulose lose proportions of their initial composition to lignin, therefore resulting in increased lignin composition (Table 1). In other words, increased lignin composition implied minute or nondelignification of yellow-poplar wood. Conversely, ASO pretreatment decreased northern red oak lignin composition by an average of 8.6% across the various pretreatment conditions (Table 1). PASO pretreatment performed better than ASO pretreatment with respect to

lignin solubility as average percentage decrease of lignin composition was 5.7 and 8.6% for yellow-poplar and northern red oak wood, respectively (Table 2).

The two-sided action of ammonia (the dual removal of wood hemicellulose and lignin) has been reported to increase ammonia concentration from 0.5-20%, resulting in increased hemicellulose recovery from 19-30% and an increase in lignin removal from 34-66% (Kim et al 2000). The observed experimental outcome and assumptions did not agree with previous findings that ammonium hydroxide or ammonia water was effective in delignification and preservation of cellulose with no significant loss of holocellulose (Dale et al 1985; Chundawat et al 2007). Specifically, lignin degradation was achieved through reactivity of ammonium hydroxide with phenolic molecules of lignin and incorporation of nitrogen into wood lignin (ie lignin oxidation or oxygen consumption and carbon dioxide elimination from wood), leading to degradation of lignin aromatic rings (Capanema 2006).

A possible explanation for the observed delignification could be the low pretreatment temperature, ammonia concentration, inhibitory actions of minuscule wood chemicals (eg wax, oil, and extractives), and surface modification of lignin by hydrogen peroxide to form additional inhibitory chemicals such as ketone (CHO) and carboxylic acid (CHOOH) (Lu et al 2001).

Enzymatic Hydrolysis

The pattern of enzymatic hydrolysis of ASO- and PASO-pretreated wood, untreated wood, and pulp after 72 h is summarized in Figs 4 and 5. The untreated wood was less hydrolyzed compared with pretreated hardwood residues of yellow-poplar and northern red oak and untreated pulp. The observed enzymatic hydrolysis was in accordance with the enzyme manufacturers' speculation that accellerase enzyme applies synergetic activities of its coenzymes (exoglucanase, endoglucanase, hemicellulase, and beta-glucosidase) to convert complex lignocellulosic biomass into fermentable sugars through

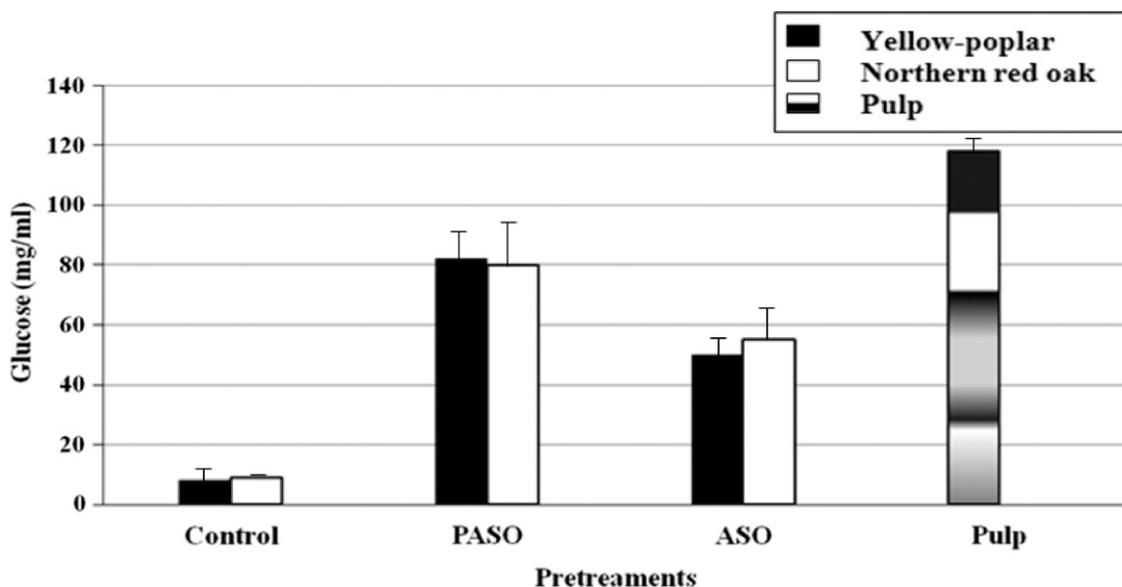


Figure 5. Glucose yield of pretreated yellow-poplar and northern red oak after enzymatic hydrolysis (ammonium hydroxide and sodium hydroxide [ASO]; hydrogen peroxide/ammonium hydroxide and sodium hydroxide [PASO]).

the breaking down of $\beta(1\rightarrow4)$ -glycosidic bonds of cellulose and hemicelluloses (Genencor 2008).

Digestion of cellulose was significantly low ($p = 0.0001$) in untreated wood (10-12%) compared with ASO- and PASO-pretreated wood. A relatively higher digestion of more than 60% was also observed for ASO- and PASO-treated hardwood residues, whereas 90% conversion was observed for pulp (Fig 5).

Sugar analysis after enzymatic hydrolysis showed 72-81 and 70-78 mg/mL dissolved glucose sugar for PASO-treated yellow-poplar and northern red oak residues, respectively, whereas 52-62 and 50-62 mg/mL was observed for ASO-treated yellow-poplar and northern red oak residues, respectively. The highest glucose concentration of 112-115 mg/mL was observed from hardwood pulp (Fig 5).

The reason for the lower conversion of ASO- and PASO-pretreated wood residues is their high lignin content (Tables 1 and 2). Wood digestion with enzyme increases with hemicellulose and lignin removal (Mansfield et al 1999). In this study, ASO and PASO alkali treatments served a dual purpose of wood depolymerization and

as well a wood pretreatment before enzymatic digestion. It is also interesting to know the unique mechanism through which these alkali treatments operate. It is probable that these treatments selectively depolymerized hemicellulose during the pretreatment phase and simultaneously weakened the lignin bonds in wood to a level sufficient enough for the cellulase enzyme to penetrate and digest cellulose molecules in the wood.

Bioenergy Production Potential

Table 3 shows total available residues in West Virginia and neighboring states (TPO 2008). The last five columns of this table contain calculated Kraft pulp (based on industrial pulp yield of 50%) ethanol yields and sugar yield from ASO/PASO. The ASO and PASO enzymatic approach would potentially convert about one-third of the TA wood residues in West Virginia and neighboring states to sugars (Table 3). Using the 50% industrial ethanol conversion rate, estimated biomass would potentially yield a total sum of 927-1537 million liters of ethanol across West Virginia and neighboring states. This ethanol production potential from

Table 3. Potential sugar production from total available (TA) residues in West Virginia and neighboring states.^a

State	Wood residues (dry Tg) Kraft pulp ASO/PASO								
	Logging	Mill	Total	TA	Pulp	Sugar	Waste	Sugar	Waste
Kentucky	2.10	1.55	3.65	2.37	1.07	1.18	1.20	0.71	1.66
Maryland	0.41	0.22	0.63	0.41	0.18	0.20	0.21	0.12	0.29
Ohio	0.86	0.35	1.21	0.79	0.36	0.39	0.40	0.24	0.55
Pennsylvania	2.60	1.63	4.23	2.75	1.24	1.36	1.39	0.82	1.92
Virginia	3.03	2.90	5.93	3.86	1.74	1.91	1.95	1.16	2.70
West Virginia	1.34	0.94	2.28	0.91	0.41	0.45	0.46	0.27	0.64

^a Source: US Forest Service Timber Output Map Maker (TPO 2008).

ASO, ammonium hydroxide and sodium hydroxide; PASO, hydrogen peroxide/ammonium hydroxide and sodium hydroxide.

cellulosic biomass (151.42 million liters ethanol production per year) would increase the local economy by \$110 million, create household income of \$19.6 million, increase tax revenue, and provide about 700 jobs (CFDC 2007).

On a one-to-one scale, 927-1537 million liters of ethanol would replace an equal amount of imported crude oil into the US and indirectly decrease reliance on imported petroleum, offset part of the US trade deficit (because crude oil accounts for about 31% of US deficit), and decrease wealth transfer to foreign countries (GAO 1991).

CONCLUSIONS

The effects of two alkali pretreatments (ASO and PASO) on energy production potential of yellow-poplar and northern red oak residues were evaluated at 80°C with variable time and alkaline hydrolysis conditions. Results indicated that both pretreatments weakened lignin bonding sites in yellow-poplar and red oak hardwood particulates.

Glucose produced during enzymatic hydrolysis of hardwood residue was low in untreated wood compared with ASO- and PASO-pretreated wood. Sugar yield after enzymatic hydrolysis ranged from 70-81 mg/mL for PASO-pretreated yellow-poplar and red oak residues, whereas a range of 50-62 mg/mL was observed for ASO-pretreated yellow-poplar and red oak residues. Available biomass in West Virginia and neighboring states could potentially be used to produce lignocellulosic ethanol or other biofuels, which would

benefit the economy, tax revenue, and jobs creation in the central Appalachian region.

ACKNOWLEDGMENTS

This manuscript is published with the approval of the Director of West Virginia Agricultural and Forestry Experimental Station as Scientific Article No. 3137.

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