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Pretreatments and energy potentials of Appalachian hardwood residues for biofuel production

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**PRETREATMENTS AND ENERGY POTENTIALS OF APPALACHIAN HARDWOOD
RESIDUES FOR BIOFUEL PRODUCTION**

by

Adebola Bamikole Adebayo

**Dissertation Submitted to the
Davis College of Agriculture, Natural Resources, and Design
at West Virginia University
in partial fulfillment of the requirements
for the degree of**

**Doctor of Philosophy
in
Forest Resources Science**

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Division of Forestry and Natural Resources

**Morgantown, West Virginia
2010**

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hydrolysis**

Abstract

PRETREATMENTS AND ENERGY POTENTIALS OF APPALACHIAN HARDWOOD RESIDUES FOR BIOFUEL PRODUCTION

by Adebayo Bamikole Adebola

Continuous increase in crude oil price and environmental issues related to toxic emissions from the use of petroleum products has necessitated the need to find an alternative renewable source of energy in woody biomass. Accordingly, properties, chemical pretreatments, and enzymatic hydrolysis of logging residues to fermentable sugars were evaluated in this study. Logging residue specimens of yellow-poplar (*Liriodendron tulipifera*) and red oak (*Quercus rubra*) were collected from fifteen previously harvested sites across West Virginia and then analyzed for their physical, chemical, and thermal properties. Results indicated that logging residues of yellow-poplar and red oak were dried in nature to a moisture content that ranged from 7.4% to 39%. Chemical analysis showed higher extractives and lignin contents for decayed wood samples collected in 2005 harvested sites. Heating value showed no significant difference between sapwood and heartwood residues of undecayed and decayed logging residues.

The effects of low alkali mixtures and hydrogen peroxide solutions at 80°C over variable cook time were evaluated on sound and decayed wood residues of yellow-poplar and red oak using two chemical treatments: (1) ammonium hydroxide and sodium hydroxide (ASO) and (2) hydrogen peroxide/ ammonium hydroxide and sodium hydroxide (PASO) mixture. Effects of ultrasonication, microwave and hydrogen peroxide prior to enzymatic hydrolysis was also evaluated on yellow poplar residue. Results indicated wood hydrolysis rate increases with an increase in sodium hydroxide concentration and cook time for ASO and PASO chemical

treatments on all wood residues. All observed alkali treatments showed little or no effect on the decomposition of lignin content of wood residues. Measured sugar content after alkali treatments and enzymatic hydrolysis ranged from 50 to 81 mg/ml from ASO/PASO treated yellow poplar and red oak residues. Enzymatic hydrolysis of ASO and PASO treated residues hydrolyzed approximately 61% of the original wood content, while over 80% of hardwood pulp fibers and paper wastes were digested to sugar.

Ultrasonication reduced wood particles into nano scale sizes. During the chemical pretreatment wood, constituents were progressively hydrolyzed (ranged from 20-100%) with increases in temperature within 10 minutes of cook time in the microwave into high molecular weight sugars. Hydrolysis of wood particles in the pretreatment chemical was directly proportional to temperature and time, regardless of particle size. Sugar production via enzymatic hydrolysis from pretreated yellow-poplar residue gave comparable glucose yield range of 80 to 110 mg/ml when compared to lignin free cellulose.

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CHAPTER 1: INTRODUCTION

Energy is the basic driving force that makes survival, growth, and movement of all life forms possible; it is required by plants in the form of solar energy to transform carbon dioxide and water into sugar-the basic building block of plant cell walls. Energy is also required by members of the animal kingdom to grow, reproduce, and perform work. It is also crucial in our modern society for heat and electricity generation, manufacturing, transportation, employment, as well as the overall economic growth and development of industrialized nations.

Currently, a significant proportion (57%) of electricity (DOE 2010) and transportation fuels are generated in the United States from non renewable sources through direct combustion of coals and imported crude oil. In 2006, the U.S. with a population of 300 million people, consumed 20 million barrels of oil per day, an amount just a little less than one quarter of the worlds' daily demand (Ethanol Fact Book 2007). The price of transportation fuel has been on the increase since 1979 with over 250% change due to increased demand, supply shortage, and political instability in the oil producing nations of the world. Statistics show that the price of fuels may continue to increase for the foreseeable future in the United States as a result of overdependence on dwindling, nonrenewable fuel resources (USDOE 2005).

One promising solution to the above challenges is the use of waste bio-renewable resources such as woody biomass to produce energy. Cellulosic biomass sources are a good alternative fuel option because they are abundant and renewable, ecologically friendly, and possess the potential to displace one-third of the current U.S. transportation fuel demand (Perlack et al. 2005). Wood and wood fiber products accounts for 60% of the total municipal solid wastes generated in the United States (USEPA 1997). In addition, logging and mill residues represent a significantly under utilized source of biomass feedstock. For example, West Virginia produces

about 2.4 million dry tons of wood residues per year, in the form of residue by-products which includes 39,000 tons of chips, sawdust, bark, and 1.2 million tons of logging residues per year (Wang et al. 2006). To date, most of the abundant biomass resources is under utilized for bioenergy due to lack of economically proven conversion technology. One of the biggest challenges to the bio-conversion of wood into fuel is the recalcitrant nature of wood lignin. The difficulty with lignocellulosic hydrolysis is due to the complex association between cellulose and hemicelluloses, with a surrounding covalently bonded lignin matrix.

The conversion of lignocellulosics has been studied for over 120 years (Sherrard and Kressman, 1945). Several biomass conversion methods like dilute or concentrated acid-catalyzed hydrolysis (Lynd et al. 1991), organosol alcohol pulping (Pazner and Cheng 1982), complete liquefaction of wood cell wall components (cellulose, hemicellulose, and lignin) under supercritical conditions (350⁰C/43 MPa). (Minami and Saka 2002), enzymatic hydrolysis of pretreated wood into sugar, wood gasification and pyrolysis had been previously reported. However, these research milestones have not been developed to commercial success, due low sugar yield, emphasis on agricultural crops, and waste disposal problems resulting from environmentally unsafe chemicals. Accordingly, this study was designed to address the following objectives; 1) Evaluate the effects of environmental exposure on the physical, chemical, and heat related properties of logging residues of the two most abundant Appalachian hardwood species, and their potential for bioenergy production, 2) Application of green chemicals and environmentally safe pretreatment processes such as ultrasonic sound and microwave to reduce wood particle size and enhance chemical penetration prior to enzymatic hydrolysis and ethanol production, 3) Evaluate the effects of low concentration alkali and hydrogen peroxide solutions at 80°C over variable cook time on yellow-poplar (*Liriodendron*

tulipifera) and red oak (*Quercus rubra*) hardwood residues and determination of the combined effects of chemical pretreatments on cellulase enzyme digestibility of wood residues for economically high yield sugar production.

References

Ethanol fact Book .2007. A compilation of information about ethanol .

<http://www.ethanolcrossamerica.net/pdfs/2007EthanolFactBook.pdf>. Last accessed in June 20. 2009.

DOE (Department of Energy) 2010. Electric Power.

<http://www.energy.gov/energysources/electricpower.htm>. Last accessed in Jan. 18, 2010.

Lynd, L.R., J.H. Cushman, R.J. Nichols, and C.E. Wyman. 1991. Fuel ethanol from cellulosic biomass. *Science* 251:318-323.

Paszner L and Chang PC.1981. Organosolv delignification and saccharification process for lignocellulosic plant materials. CA patent 1.100.266.

Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ and Erbach DC. 2005. Biomass as feedstock for bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. A Joint Study Sponsored by U.S. Department of Energy and U.S. Department of Agriculture. 2005; 78pp.

USEPA, Characterization of Municipal Solid Waste in the United States. 1997. Update. United States Environmental Protection Agency, Washington, D.C.

USDA. Highlights of 2000 West Virginia Forest Inventory. Northeastern FIA.

<http://www.fs.fed.us/ne/fia/state/wv/wvhlite00.PDF>. 2000. Last accessed in October 24, 2006.

USDOE (U.S. Department of Energy). 2005. Monthly energy review, April. Energy Information Administration, U.S. Dept. of Energy, Washington DC.

Sherrard, E.C., Kressman, FW. 1945. Review of processes in the United States prior to World War II. *Ind. Eng Chem.* 37,5-8.

Wang J, Grushecky S, and McNeel J., 2006. Biomass Resources, Uses, and Opportunities in West Virginia. Division of Forestry and Natural Resources, West Virginia University Morgantown . 103 pp.

CHAPTER 2: ASSESSMENT OF APPALACHIAN HARDWOOD RESIDUE PROPERTIES AND POTENTIALS FOR BIOENERGY UTILIZATION ¹

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Abstract

Logging residue specimens of yellow-poplar and red oak were collected from fifteen previously harvested sites across West Virginia. Sites were selected with respect to the varying soil compositions based on the USDA soil survey, as well as to the average annual precipitation regions. Wood specimens were then analyzed for their physical, chemical, and thermal properties. Results indicated that logging residues of yellow-poplar and red oak were dried in nature to a moisture content that ranged from 7.4% to 39%. Yellow-poplar sapwood specific gravity reduced by 15% and 26.5% on average after two years and three years of ground contact respectively. Red oak sapwood specific gravity was lower than its heartwood by 26.6%, 25.3%, and 8.2% for 2005, 2006, and 2007 harvest years respectively. Chemical analysis showed higher extractives and lignin contents for decayed wood samples collected in 2005 harvested sites. Heating value showed no significant difference between sapwood and heartwood residues of undecayed and decayed. Our analysis on the potential sugars available showed that about 85-90% of 1.02 billion kg of sugars derived from wood residues in West Virginia can be fermented to ethanol or butanol.

2.1 Introduction

The current price of crude oil is over 250 percent greater than ten years ago. Statistics show that the price of fuels and petrochemical products may continue to increase indefinitely in the United States as a result of overdependence on dwindling nonrenewable petroleum resources. Overdependence on petroleum products has consistently been on the increase (USDOE 2005). For example, net petroleum product imports increased 16.8 % in 2005, crude oil and natural gas imports increased by 5.3 % and 3.8 % respectively.

One promising solution to the above challenges is the use of waste bio-renewable resources such as woody biomass to produce energy. There exist abundant wood residues in the United States for conversion into fuels (Perlack et al. 2005). For example, West Virginia produces about 2.41 billion kg of wood residues annually, that comprised of 1.34 billion kg of red oak residues, 1.3 kg/m² of yellow-poplar, and 0.3 kg/m² of maple species with (Grushecky et al. 2006, Wang et al. 2006).

There is an increasing demand for more a sustainable utilization of biomass residue through direct combustion, gasification, and fermentation technologies to complement the use of nonrenewable coal resources in the Appalachian region. This is of a particular interest because wood is renewable and environmentally friendly compared to coal.

One problem with utilizing logging wastes is decay. Previous studies indicate that weathering and decay resulting from extended exposure in the environment affect the physical, chemical, and anatomical properties of wood (Anderson et al. 1990, Wilhelmsson 2006). However, the magnitude of the effects of environmental exposure of Appalachian hardwoods is poorly understood with no concerted documentation for logging residues. This study addresses

the need for better understanding of the effects of environmental exposure on the physical, chemical, and heat related properties of logging residues of the two most abundant Appalachian hardwood species, and their potential for bioenergy production.

2.2 Materials and methods

During the summer of 2007, log residues were collected based on a 5 x 3 x 2 x 2 factorial experiment in a completely randomized design with ten replicates. In this design, the four main factors considered are five forest district locations, year of harvest (2005, 2006, and 2007), species [red oak (*Querus rubra*) and yellow-poplar (*Liriodendron tulipifera*), and location of the specimen within the stemwood (sapwood or heartwood). Logging residues were collected from previously harvested sites within each of the five forest district locations in the state. Throughout the entire state, collection areas were selected from the major soil types based on the USDA soil survey, and average annual precipitation. Within each distinct location, three harvested sites were randomly selected from a list of all harvests in 2005, 2006, and 2007 using the West Virginia Division of Forestry logging notification forms. (WVDOF 2005). At each collection area, three discs (102 mm thick) of large end diameter range of 203-253 mm were collected respectively for red oak and yellow-poplar species. This diameter range was based on a survey conducted in 2002, where average diameter inside bark of red oak and yellow-poplar logging residues were about 203 to 253 mm respectively (Grushecky et al. 2006).

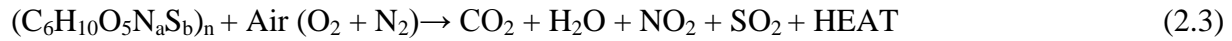
Methods

After the residues were collected from the designated sites, each specimen was processed into block sizes of 10 mm x 10 mm x 40 mm for moisture content and specific gravity determination using the gravimetric method.

The amounts of lignin, extractives, and holocellulose in the wood were determined in accordance with ASTM designation: D 1110-84; 1106-96 (ASTM 2001). Proximate analysis, i.e. content of volatile matter, carbon, and ash in wood, was determined according to ASTM designation: D3 172 (ASTM 2006) and D 1102-84 (ASTM 2001). All pulverized samples used for heat value determination were oven dried to zero percent moisture content. One gram of each sample was placed inside an oxygen bomb calorimeter (Parr 6300) for heat determination. After running each sample through the bomb calorimeter, corrections were made based on the Parr bomb calorimeter gross and net heat of combustion equations (2.1,2.2) (ASTM D-240, Parr 2007) and combustion equation(2.3) for hydrogen, nitric, sulfuric acids, ignition wire and tread for the final computation of the gross and net heating value of each wood specimen (ASTM E 711-87, 2006). At the completion of all physical, chemical, proximate, and thermal tests on the logging residues, analysis of variance (ANOVA) with Duncan multiple comparison test was performed using SPSS statistical software.

$$H_c = (WT - e_1 - e_2 - e_3)/kg \quad (2.1)$$

$$H_n = 1.8H_c - 92.7H \quad (2.2)$$



Where:

- H_c = Gross heat combustion (KJ/kg)
- T = Observed Temperature rise ($^{\circ}C$)
- W = Energy equivalent of calorimeter used (KJ/kg)
- e_1 = Heat produced by burning nitrogen in air (J)
- e_2 = Heat produced by formation of sulfuric acid in bomb (J)
- e_3 = Heat produced by heating wire and tread (J)
- m = mass of sample (Kg).
- H_n = Net heating value (KJ/kg).
- H = Hydrogen (%).

2.3 Results and discussion

2.3.1 Physical properties

The measured physical properties (specific gravity and moisture content) of yellow-poplar and red oak logging residues from the 2005-2007 harvests are given in Tables 2.1 and 2.2. Moisture content in the logging residue samples was significantly different ($P < 0.0001$) among district locations, harvest year, between species, heartwood, and sapwood. Moisture content differences were relatively low among year of harvest and species with minimal variation between heartwood and sapwood within each district. 2007/2006 residues had significantly higher moisture contents than 2005 residues ($P < 0.0001$); moisture content of heartwood was significantly higher than sapwood ($P < 0.0001$). Generally, our results indicate that logging residues of yellow-poplar and red oak have been air dried in the environment to a moisture content range of 7.4 -39% due to cyclical exposure to humidity and temperature over an extended period of time. The only exception to the low moisture trend observed above was in red oak wood residue with 72% sapwood moisture content. This high moisture content was a result

of increased precipitation prior to the wood residue collection. Although all the logging residues across the state were naturally dried to about the same moisture content, the hygroscopic nature of wood drives it to absorb moisture until it reaches equilibrium with the prevailing environmental conditions. The moisture range of 7.4-39% observed in this study is lower than the green wood moisture content range of 80 % to 100%, and is advantageous with respect to transportation cost, processing, drying, and wood combustion.

A significant ($P < 0.0001$) decreasing trend in sapwood specific gravity was demonstrated with increased exposure on site (Figure 2.1) as previously reported for various hardwood species (Smith et al. 2007). Differences between sapwood and heartwood specific gravity of yellow-poplar and red oak residues (Tables 2.1 and 2.2) were significant ($P < 0.0001$). There were approximately three percent differences between heartwood and sapwood specific gravities of yellow-poplar wood in the 2007 harvest year, while 2006 and 2005 harvest samples showed significant ($P < 0.0001$) reductions in sapwood specific gravity values when compared to heartwood. Yellow-poplar sapwood specific gravity was reduced by an average of 15% after two years (2006) and 26.5% after three years (2005) of ground contact when compared with its heartwood samples. Similarly, the specific gravities for samples of red oak sapwood were lower than their heartwood by 26.6%, 25.3%, and 8.2% for 2005, 2006 and 2007 harvest years, respectively. The reason for the higher reduction of specific gravity in 2006-2005 samples could be attributed to greater sapwood decay from extended exposure to favorable environmental condition for growth and development of bio-deteriorating agents and weathering (Highley 1995, FPL 1999).

Decay as evaluated by environmental exposure time, visual observation of significant color changes and weight loss on wood from inside bark to the pith ranged from 36-100% for the

2005 harvest and 35-76% for the 2006 harvest for yellow-poplar residues. Lower decay ranging from 10-25% was observed for red oak residues (Tables 2.1 and 2.2). Based on the observed decay, heartwood samples of yellow-poplar and red oak were found to be more resistant to decay than their sapwood counterparts. The reason for this difference in decay patterns between these two hardwood species could be due to the presence of chemical substances in red oak residues that is toxic to bio-deteriorating organisms. It has been previously reported that the heartwood of some tree species such as oak, Douglas-fir, and certain pines were found to be more resistant to decay due to their contents of phenol, terpenes, and alkaloids (Scheffer and Cowling 1966). These structural and nutritional differences combine to form a mechanism of natural resistance to microbial deterioration (Highley 1995, FPL 1999). Wood also decays faster in the humid and warm environment of the Appalachian hardwood forests due to the mechanical breakdown by insects and borers, and biochemical breakdown by white and brown-rot fungi (Scheffer and Cowling 1966).

2.3.2 Chemical properties

Measured chemical properties (extractives, lignin, and holocellulose) contents of yellow-poplar and red oak residues (Tables 2.3 and 2.4) generally agreed with that obtained in a previous study (White 1987). Differences in extractive contents were highly significant ($P < 0.0001$) between heartwood and sapwood across year of harvest, district location, and wood species. The extractives of 2005 residues were about 20% more than the extractives of undecayed wood residues in the 2006 and 2007 harvest years. Figure 2.2 depicts one exceptionally high extractive content (17%) for 2005 yellow-poplar residues. Lower

holocellulose content, at the expense of proportionately higher extractives and lignin contents, was observed in the decayed sapwood samples of yellow-poplar and red oak residues than in fresh residues from the 2007 harvest. The lower holocellulose content observed on decayed wood (Figure 2.3) was a result of microbial mediated biochemical changes as previously reported (Rowell 1984, Pandey and Nagveni 2007). During fungi growth on wood, cellulose and hemicellulose are the desired carbon sources for the energy required for growth and development of the fungi. Holocellulose is biochemically broken down into six-carbon sugars for enzymatic hydrolysis, leading to reduced holocellulose content in decayed wood residues. This also explains the higher sapwood extractive contents of decayed yellow-poplar and red oak wood samples as remnants of decomposed holocellulose and weathered lignin content leached out together with the remaining extractives during wood solubility tests.

The observed holocellulose content (ranging from 60-73%) is a representative of the potential amount of sugars (five and six carbon sugars) which can be converted via a host of cost effective enzymatic and fermentation processes into ethanol, butanol, and other liquid fuels. In addition, extracted lignin can also be efficiently utilized as an emulsifier in animal feed, raw material in the production of vanillin, in pharmaceuticals, as a fragrance in perfumes products, and a binder in ceramic and wood composite industries (Northey 1992).

Assuming 60% of all the annual dry wood residues of 2.41 billion kg in West Virginia can be economically accessed, this residue would potentially support about five (5) ethanol plants or other biofuel plants based on a feasibility study by the Quincy Library Group (QLG 1997), for plant size processing 725,600 kg (800 dry tons) per day. On the basis of our chemical analysis, about 1.02 billion kg of sugars, 0.36 billion kg of lignin, and 0.06 billion kg of

extractive content could be generated from 60% of the total annually generated logging waste of 2.41 billion kg dry wood residue in West Virginia.

Based on a biomass to ethanol feasibility study in California, approximately 85-90% of 1.02 billion kg of six carbon sugars (glucose) derived from wood can be fermented to ethanol at the bench scale using *Saccharomyces cerevisiae* (Kadam et al. 2000). The results obtained on specific gravity (SG), effect of environmental exposure (E) or decay factor, and exposure length (T) was used to develop a predictive equation (2.4) for the future analysis of biomass to ethanol production in West Virginia.

$$SG = 0.72 - 0.20E + 0.002T \quad (r^2 = 0.48, RMSE = 0.071, P = 0.0001) \quad (2.4)$$

Where:

E = 0.75, 0.50, 0.25, 0.0 for 3, 6, 9, and over 9 years decay factor

T = 0, 24, 36... for year 1, 2, and 3

A ten year simulation using equation (2.4) to predict yellow-poplar wood loss due to effect of environmental exposure, indicated an increase in biomass accumulation at a decreasing rate leading to over 50% reduction in total logging residue when compared to fresh residues (i.e. assuming no wood decay) in West Virginia (Figure 2.4). Although a significant amount of logging residue would be lost to decay, enough biomass still exists (about 1.5 billion kg/year) for sugar production to support potential ethanol plants. However, there would be a need for two other industries to consume wastes (about 83% loss of the original solid wood residues before

final conversion to ethanol) generated from extractives, lignin, and solid substrates from fermentation.

2.3.3 Proximate analysis and higher heating value

Volatile matter, fixed carbon, and ash content of decayed and undecayed wood residue samples were less variable among year of harvest and between heartwood and sapwood of yellow-poplar and red oak (Tables 2.3 and 2.4). Volatile matter averaged around 80%, fixed carbon around 20%, and ash content at about 1% (Tables 2.3 and 2.4). These were in agreement with values reported previously (Sjostrum 1981, Demirbas 1997). The only exception to this general trend was in 2007 red oak heartwood residue with a fixed carbon content of 27.5%. Although red oak had more wood elements per unit volume than yellow-poplar, and more material in undecayed wood than decayed (Tables 2.1 and 2.2), there were no observable significant differences found in the volatile contents due to the particulate nature of the samples used for the proximate analysis. These woods yielded about the same proximate content, but in reality, a less dense wood like yellow-poplar or decayed yellow-poplar wood would contain more wood materials in proportion to its specific gravity to provide an equivalent proximate content of undecayed wood. For example, decayed yellow-poplar wood of specific gravity 0.23 is 50% less dense than an undecayed wood sample. Equal volumes of the decayed versus undecayed wood when processed into dust will result in the decayed sample having 50% less wood compared to the undecayed yellow-poplar sample. Due to this constraint, a carbonization experiment at temperature range of 150 to 750 °C was carried out using uniform size blocks versus wood dust for undecayed (2007 harvest) and decayed (2005 harvest) yellow-poplar residue. At

temperature range of 300-600°C result showed significant differences ($P < 0.0001$) between decayed and undecayed wood blocks and wood dust residues with each sample corresponding inversely to specific gravity of wood; i.e. less dense decayed yellow-poplar residue had the highest volatile content followed by undecayed yellow-poplar and red oak residues (Figure 2.6).

The mean and standard deviation of the gross higher heating values of yellow-poplar and red oak were determined using a Parr oxygen bomb calorimeter (Tables 2.3 and 2.4), which were generally consistent with other reported values (Ince 1979, White 1987). The heating value showed no significant differences ($P > 0.09$) between sapwood and heartwood residues of 2007 harvest site, between decayed (2005 and 2006) and undecayed (2007) logging residues. Higher heating values of yellow-poplar and red oak sapwood residues of 2005 harvest were 3.2% and 3.5% higher than their 2007 sapwood residues respectively. A t-test comparison between yellow-poplar and red oak residues failed to show any significant differences ($P = 0.056$), while Duncan multiple comparison test indicated that the 2005 residues were significantly different from 2006/2007 residues in heating value. It is evident from this study that the heating value of red oak wood was not significantly higher (about 1%) than yellow-poplar residues, and that duration of residue on site within four years of harvest had a significant effect on the higher heating value of red oak and yellow-poplar residues (Figure 2.5).

Observed higher heating values of decayed residues of red oak and yellow-poplar were contrary to the expected result. It is expected that the reduction in holocellulose content through the various activities of bio-deteriorating agents would indirectly lower the heating value of decayed wood residues. But a more critical look at the chemistry of wood decay process also reveals that the reduction in holocellulose content resulted in a proportionate increase in high calorific lignin content plus accumulated layers of dead bio-deteriorating organisms. The cell

walls of wood decay fungi are composed of 80-90% high calorific polysaccharides, with proteins, lipids, and polyphosphates (Madigan et al. 2003). Lignin, with a heating value of 9.8-10.8 kJ (10,000-11,000 Btu/lb), was reported to have a strong correlation to higher heating value of wood, whereas cellulose and hemicelluloses has a heating value of only 7.9 kJ (8,000 Btu/lb) (Baker 1983, White 1987). These additions to the lignin content could have resulted in the slight increases in the higher heating values of decayed wood residues versus undecayed.

2.4 Conclusions

Our results indicated that logging residues of yellow-poplar and red oak was dried on site to a moisture range of 7.4% to 39% due to the long cyclic exposure to humidity and temperature after one to three years of harvest. A decreasing trend in sapwood specific gravity with duration on site due to activities of bio-deteriorating organisms was observed. Yellow-poplar sapwood specific gravity was reduced by 15% and 26.5% on average after two years (2006) and three years (2005) of ground contact, respectively. Similarly, red oak specific gravity for sapwood was lower than its heartwood by 26.6%, 25.3%, and 8.2% for 2005, 2006, and 2007 harvest years respectively.

Chemical properties of yellow-poplar and red oak wood residues were less variable between heartwood and sapwood and across year of harvest, except in samples which experienced increased fungal decay due to length of exposure. Higher extractive and lignin content at the expense of lower holocellulose content were observed in decayed (2005 harvest) sapwood residues of yellow-poplar and red oak woods.

The range of values obtained for volatile and fixed carbon and ash content were in agreement with previous findings (Sjostrum 1981, Demirbas 1997). Observed volatile content

was about 80%, 20% for carbon, and about 1% for ash content in both species, regardless of their decay condition. The heating values showed no significant differences between sapwood and heartwood of decayed and undecayed residues. The heating value of red oak wood is not significantly higher (about 1%) than yellow-poplar residues, and that the duration of residue on site within four years of harvest significantly affects the higher heating values of red oak and yellow-poplar residues.

On the basis of our chemical analysis, about 1.02 billion kg of sugars, 0.36 billion kg of lignin, and 0.06 billion kg of extractive content could be generated from 60% of the total annually generated logging waste of 2.41 billion kg dry wood residue in West Virginia. A ten years simulation to predict yellow-poplar wood loss due to effect of environmental exposure, and residue potential indicated an increase in biomass accumulation at a decreasing rate leading to over 50% reduction in total logging residue when compared to fresh residues. Assuming 60% of all residues can be economically transported; accumulated biomass has potential to support five ethanol plants. Residue loss because of decay points to a need for on-site storage facility or prompt removal of logging residues from the forest site after two years. There is also need for complementary industries to consume wastes (about 83% loss of the original solid wood residues) generated from extractives, lignin and solid fermentation substrates.

The residues of the two hardwood species used in this study can be used as feedstock or serve as substitute species for the production of fermentable sugars, lignin, and synthetic gases (a raw material for Fisher Tropsch Process) for the production biofuels, bio-chemical products, and electricity generation through direct combustion of wood owing to their comparable physical and heat related properties. This close property similarities between red oak and yellow-poplar is a

positive indication for effective utilization of logging waste resources in the Appalachian region for bioenergy.

References

- Anderson EL, Pawlak Z, Owen NL, and Feist WC. 1990. Infrared study of wood weathering part I: softwoods. *Applied Spectroscopy* 45(4):641-647.
- ASTM (American Society for Testing and Materials). 2006. D3: 172, E 711-87. Annual Book of ASTM Standards, West Conshohochen , PA. 4.10 (4), 1429 pp.
- _____. 2001. D: 1110-84; 1106-96;1102-84. Annual Book of ASTM Standards, West Conshohochen , PA. 4.10 (4): 708 pp.
- Baker AJ .1983. Wood fuel properties and fuel products from woods. Fuel management and utilization seminar,. Michigan State Univ. East Lansing, MI. 14-25.
- Demirbas A .1997. Calculation of higher heating values of biomass fuels. *Fuel* 76(5):431-434.
- Filley TR, Cody G, Goodell B, Jellison J, Noser C, Ostrofsky A .2002. Lignin demethylation and polysaccharide decomposition in spruce sapwood degraded by brown-rot fungi. *Org. Geochem.* 33:111–124.
- FPL (Forest Prod. Lab) .1999. Wood handbook—Wood as an engineering material. Gen. Tech. Rep. FPL–GTR–113. Madison, WI : U.S. Department of Agriculture, Forest Service, Forest Products Laboratory. 463 pp.
- Grushecky ST, McGill DW, and Anderson RB (2006) Inventory of wood residues in southern West Virginia. *Northern Journal of Applied Forestry* 23(1): 47-52.
- Highley T L .1995. Comparative durability of untreated wood in use above ground. *International Biodeterioration and Biodegradation.* Elsevier Science limited. 409-419.
- Ince PJ .1979. How to estimate recoverable heat energy in wood or bark fuels. USDA Forest Serv. Gen. Tech. Rep. FPL 29. Forest Prod. Lab. Madison, WI. 5 pp.

- Kadam KL, Robert J, Andy A, Quang AN, Mark AY, Francis MF .2000. Softwood forest thinnings as a biomass source for ethanol production: a feasibility study for California. *Biotechnol. Prog.* 2000, 16, 947-957.
- Madigan MT, Martinko JM, and Parker J .2003. *Brock Biology of Microorganisms*. Tenth Edition, Pearson Education, Inc. Upper Saddle River, NJ. 1019 pp.
- Northey RA .1992. *Low-Cost Uses of Lignin, Emerging Technology if Materials and Chemicals from Biomass*, ACS Symposium Series 476, Washington, D.C.
- Parr 2007. 6300 Calorimeter Operating instruction Manual. 11pp.
- Pandey KK and Nagveni HC .2007. Rapid characterization of brown and white rot degraded chir pine and rubberwood by FTIR spectroscopy. *Holz Roh Werkst.* 65: 477-481.
- Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ, and Erbach DC .2005. *Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply*. April 2005. A Joint Study Sponsored by U.S. Department of Energy and U.S. Department of Agriculture. 78 pp.
- QLG .1997. California Energy Commission, California Institute of Food and Agriculture, Plumas Corporation, TSS Consultants, and National Renewable Energy Laboratory. *Northern California Ethanol Manufacturing Feasibility Study*, Quincy Library Group: Quincy CA.
- Rowell RM .1984. *The chemistry of solid wood*. *Advances in Chemistry Series No. 207*. American Chemical Society Washington DC. 540 pp.
- Scheffer C and Cowling EB .1966. Natural resistance of wood to microbial deterioration. *A Rev. Phytopathol.* 4:147-170.

- Sjöström E .1981. Wood chemistry: fundamentals and applications. New York:
Academic Press. 300 pp.
- Smith KT, Shortle WC, Jellison J, Connolly J, and Schilling J .2007. Concentrations of
Ca and Mg in early stages of sapwood decay in red spruce, eastern hemlock, red maple,
and paper birch. *Can. J. For. Res.* 37 (5):957-965.
- USDOE (U.S. Department of Energy) (2005) Monthly energy review, April. Energy
Information Administration, U.S. Dept. of Energy, Washington DC.
- Wang J, Grushecky S, and McNeel J .2006. Biomass Resources, Uses, and
Opportunities in West Virginia. Division of Forestry and Natural Resources, West
Virginia University Morgantown . 103 pp.
- White RH .1987. Effect of lignin content and extractives on the higher heating value of
wood. *Wood and Fiber science* 19(4):446-452.
- Wilhelmsson L .2006. Two models for predicting the number of annual rings
in cross-sections of tree stems. *Scandinavian Journal of Forest Research* 21(7):37-47.
- WVDOF .2005. West Virginia silvicultural best management practices for controlling
soil erosion and sedimentation from logging operations. WVDOF-TR-05-3.
West Virginia Division of Forestry, Charleston, West Virginia. 29 pp.
- Zerbe JI .2006. Thermal energy, electricity, and transportation fuels from wood. *For.
Prod. J.* 56(1): 1-14.

Table 2.1 Specific gravity (SG) and percentage moisture content (MC) of Yellow-poplar by district and year of harvest

Year of Harvest	Forest District				
	1	3	4	5	5
2005					
Dia. (mm)	203.0	216.0	229.0	178.0	191.0
% Decay	50	36	53	100	53
Sap SG	0.28 (0.05)	0.31 (0.13)	0.43 (0.05)	0.34 (0.05)	0.38 (0.05)
Sap MC	10.76 (0.73)	16.6 (1.33)	11.60 (1.31)	39.33 (8.79)	7.45 (1.10)
Heart SG	0.48 (0.07)	0.56 (0.05)	0.45 (0.06)	0.43 (0.08)	0.48 (0.06)
Heart MC	10.57 (0.34)	24.48 (1.85)	12.56 (0.86)	33.47 (6.8)	8.0 (0.81)
2006					
Dia. (mm)	229.0	203.0	241.0	178.0	178.0
% Sap/Decay	40	35	42	76	46
Sap SG	0.42 (0.038)	0.45 (0.09)	0.49 (0.04)	0.42 (0.05)	0.56 (0.03)
Sap MC	18.80 (1.20)	21.23 (6.87)	9.25 (1.63)	10.85 (1.38)	8.40 (0.43)
Heart SG	0.49 (0.03)	0.56 (0.05)	0.47 (0.10)	0.45 (0.04)	0.67 (0.06)
Heart MC	25.09 (3.78)	25.77 (1.93)	10.84 (0.71)	11.97 (1.33)	8.40 (0.67)
2007					
Dia. (mm)	229.0	229.0	254.0	203.0	254.0
% Sap	55	35	50	50	40
Sap SG	0.49 (0.05)	0.44 (0.03)	0.46 (0.20)	0.44 (0.02)	0.49 (0.04)
Sap MC	17.09 (2.5)	25.05 (1.22)	15.78 (7.37)	7.41 (0.42)	7.75 (0.30)
Heart S.G	0.48 (0.03)	0.49 (0.07)	0.51 (0.13)	0.41 (0.02)	0.43 (0.06)
Heart MC	16.49 (2.5)	26.17 (7.69)	14.37 (5.53)	7.18 (2.00)	7.35 (0.86)

() standard deviation.

Table 2.2 Specific gravity (SG) and percentage moisture content (MC) of red oak by district and year of harvest.

Year of Harvest	Forest District				
	1	3	4	5	5
2005					
Dia. (mm)	229.0	229.0	254.0	229.0	191.0
% Decay	32	17	10	44	53
Sap SG	0.49 (0.09)	0.43 (0.06)	0.48 (0.08)	0.55 (0.07)	0.39 (0.05)
Sap MC	11.66 (4.86)	10.16 (0.82)	12.44 (1.30)	72.11 (11.0)	7.45 (1.10)
Heart SG	0.66 (0.04)	0.72 (0.14)	0.73 (0.10)	0.64 (0.05)	0.48 (0.06)
Heart MC	20.78 (3.14)	13.18 (0.97)	18.44 (1.94)	28.28 (3.08)	8.06 (0.85)
2006					
Dia. (mm)	229.0	229.0	254.0	203.0	178.0
% Sap/Decay	10	17	15	25	23
Sap SG	0.54 (0.03)	0.47 (0.09)	0.58 (0.07)	0.52 (0.08)	0.57 (0.03)
Sap MC	11.75 (0.66)	9.60 (0.51)	14.00 (1.67)	15.39 (3.03)	8.40 (0.43)
Heart SG	0.62 (0.03)	0.72 (0.04)	0.74 (0.08)	0.90 (0.05)	0.67 (0.06)
Heart MC	16.85 (1.16)	9.40 (0.92)	15.84 (5.03)	38.08 (5.59)	8.40 (0.67)
2007					
Dia. (mm)	241.0	241.0	254	229.0	254.0
% Sap	10	17	10	9	20
Sap SG	0.61 (0.10)	0.70 (0.14)	0.60 (0.05)	0.52 (0.03)	0.49 (0.05)
Sap MC	18.84 (2.98)	12.54 (0.64)	14.06 (1.41)	7.76 (0.52)	7.76 (0.31)
Heart S.G	0.70 (0.07)	0.74 (0.05)	0.62 (0.01)	0.56 (0.04)	0.43 (0.06)
Heart MC	27.57 (2.22)	13.97 (0.66)	17.36 (1.02)	8.46 (0.94)	7.52 (0.07)

() standard deviation.

Table 2.3 Chemical and heat related properties of yellow-poplar by year of harvest.

Properties	Year of Harvest		
	2005	2006	2007
% Sap-decay range	53-100	40-70	40-50
Sapwood Chemical (%)			
Extractives	11.00 (3.26)	8.29 (1.62)	8.00 (1.14)
Holocellulose	67.94 (7.31)	73.08 (4.93)	72.67 (2.3)
Lignin	32.06 (7.31)	25.25 (3.70)	27.33 (2.3)
Proximate analysis (%)			
Volatile	80.90 (2.71)	80.77 (1.03)	82.5 (3.77)
Fixed carbon	19.1 (2.71)	19.23 (1.03)	17.5 (3.77)
Ash	0.62 (0.2)	0.62 (0.24)	0.64 (0.26)
Heat value (KJ/kg)			
Net	7.99 (0.54)	7.71 (0.26)	7.74 (0.40)
Gross	8.54 (0.54)	8.26 (0.26)	8.27 (0.40)
Heartwood Chemical (%)			
Extractives	8.30 (1.46)	8.30 (1.16)	9.28 (2.4)
Holocellulose	71.18 (6.41)	72.1 (3.34)	71.71 (6.4)
Lignin	27.65 (5.95)	27.92 (3.34)	28.29 (6.4)
Proximate analysis (%)			
Volatile	80.27 (1.70)	81.23 (1.27)	80.82 (1.07)
Fixed carbon	19.73 (1.70)	18.77 (1.27)	19.18 (1.07)
Ash	0.67 (0.34)	0.59 (0.32)	0.62 (0.24)
Heat value (KJ/kg)			
Net	7.85 (0.27)	7.65 (0.21)	7.72 (0.15)
Gross	8.39 (0.27)	8.19 (0.21)	8.27 (0.15)

() standard deviation.

Table 2.4 Chemical and heat related properties of red oak wood by year of harvest.

Properties	Year of Harvest		
	2005	2006	2007
% Sap-decay range	10-53	10-46	10-40
Sapwood Chemical (%)			
Extractives	11.3 (3.1)	11.5 (3.1)	8.5 (1.7)
Holocellulose	58.7 (6.8)	60.7 (3.3)	70.4 (4.2)
Lignin	41.4 (6.8)	39.3 (3.3)	29.6 (4.2)
Proximate analysis (%)			
Volatile	79.2 (7.8)	79.6 (1.2)	79.5 (1.0)
Fixed carbon	21.3 (7.9)	20.7 (1.1)	20.7 (1.1)
Ash	0.58 (0.28)	0.84 (0.16)	0.34 (0.07)
Heat value (KJ/kg)			
Net	8.10 (0.39)	7.82 (0.20)	7.79 (0.16)
Gross	8.65 (0.39)	8.37 (0.20)	8.34 (0.16)
Heartwood Chemical (%)			
Extractives	12.4 (4.0)	11.6 (1.4)	9.9 (1.2)
Holocellulose	67.4 (8.5)	60.1 (4.9)	68.2 (5.9)
Lignin	32.6 (8.5)	39.9 (4.9)	31.8 (5.9)
Proximate analysis (%)			
Volatile	81.5 (7.2)	78.5 (1.1)	79.0 (1.0)
Fixed carbon	19.1 (7.4)	21.8 (1.2)	27.5 (1.0)
Ash	0.58 (0.28)	0.66 (0.82)	0.23 (0.09)
Heat value (KJ/kg)			
Net	7.88 (0.25)	7.61 (0.25)	7.81 (0.22)
Gross	8.42 (0.25)	8.15 (0.25)	8.35 (0.22)

() standard deviation.

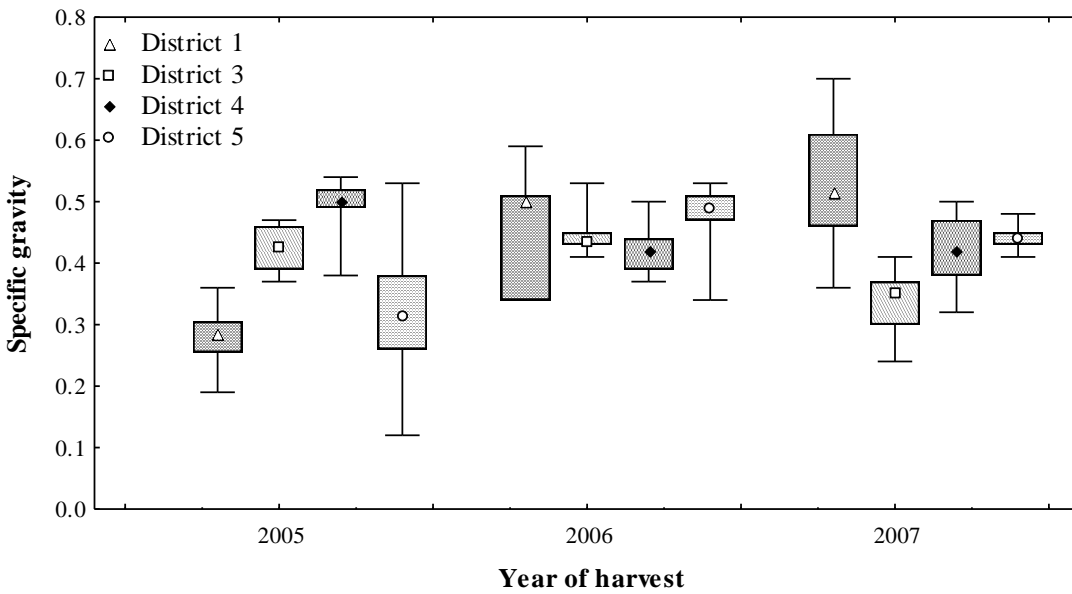


Figure 2.1 Yellow-poplar (sapwood) specific gravity variability among districts and year of harvest.

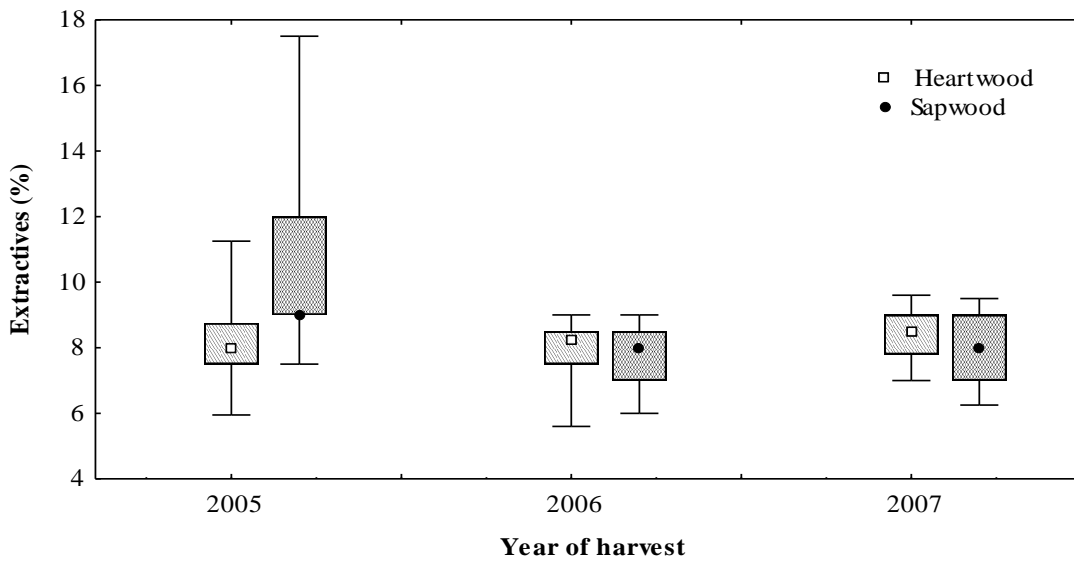


Figure 2.2 Variability of yellow-poplar extractive content from five districts by year of harvest and location in sapwood (sap), heartwood (heart) of tree bole.

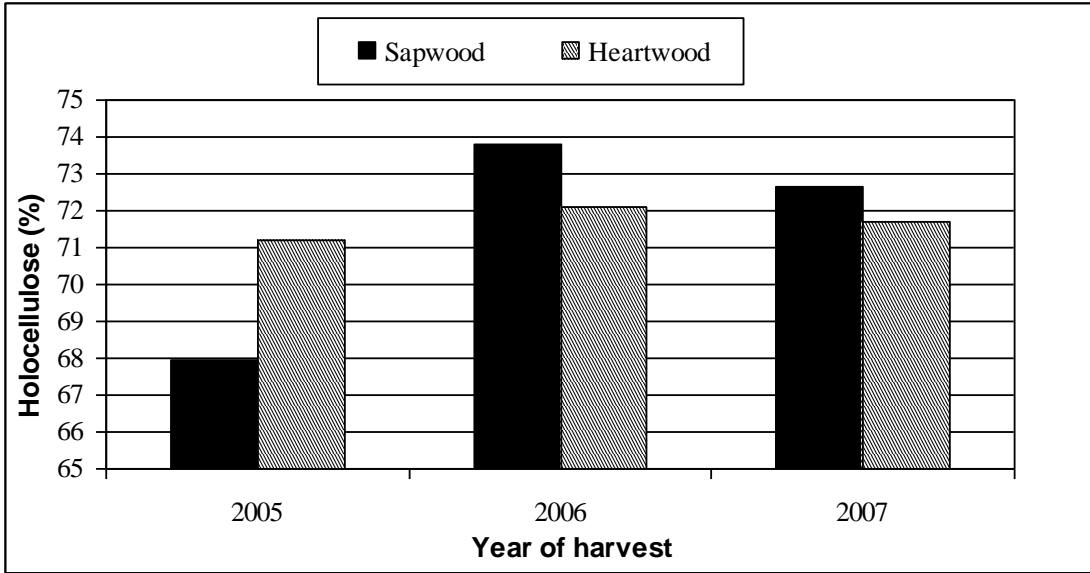


Figure 2.3 Variability of yellow-poplar holocellulose content, across the districts by year of harvest and location in wood (sapwood vs. heartwood).

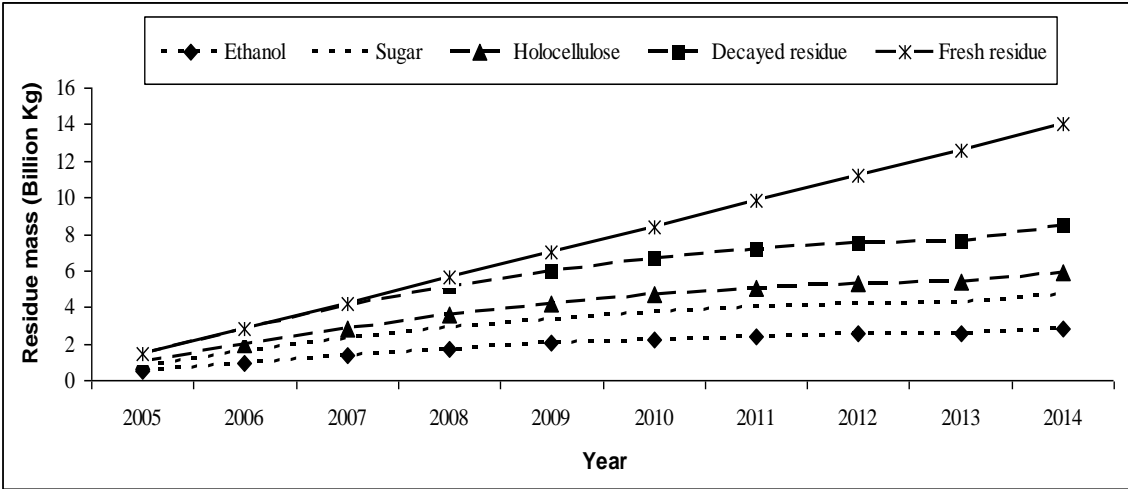


Figure 2.4 Simulation of 10 years for potential residue accumulation and conversion to ethanol.

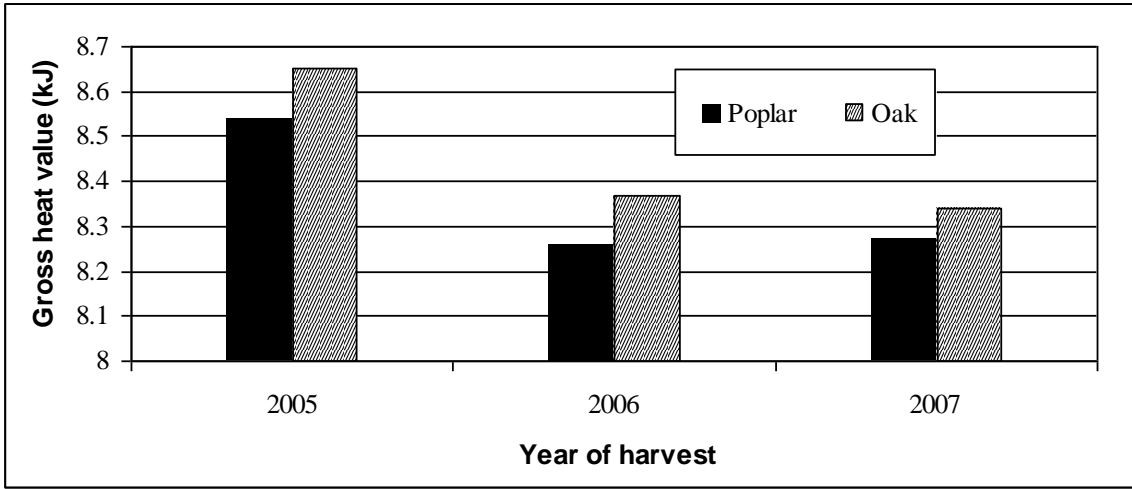


Figure 2.5 Gross higher heating value of yellow-poplar and red oak sapwood residues by year of harvest.

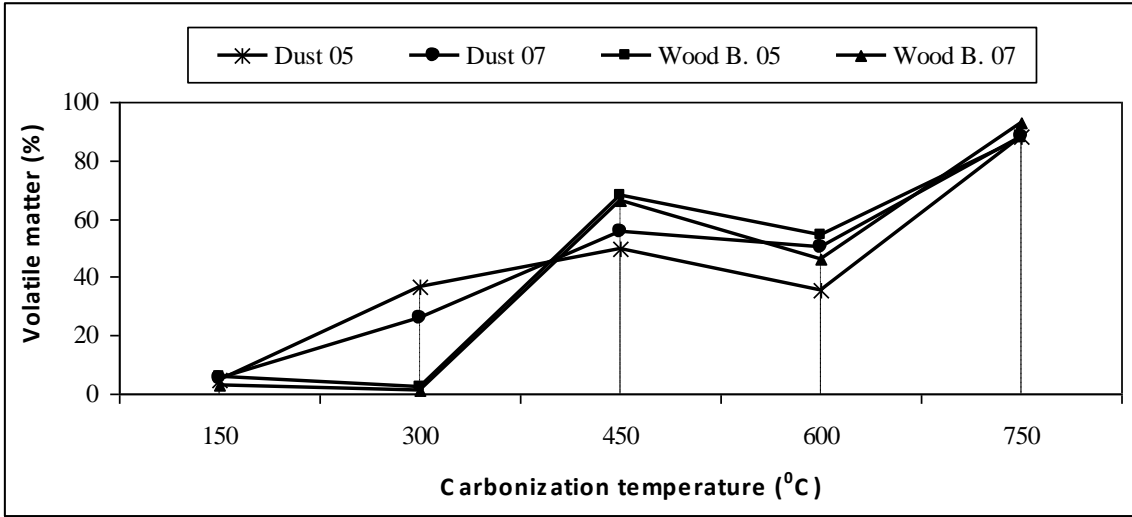


Figure 2.6 Carbonization of solid wood blocks (Wood B.) versus wood dust (Dust) of 2005 and 2007 harvested yellow-poplar wood.

**CHAPTER 3: ALKALI PRETREATED CENTRAL APPALACHIAN HARDWOOD
RESIDUES FOR SUGAR PRODUCTION**

Abstract

The effects of low alkali mixtures and hydrogen peroxide solutions at 80°C over variable cook time were evaluated on sound and decayed wood residues of yellow-poplar and red oak using two chemical treatments: (1) ammonium hydroxide and sodium hydroxide (ASO) and (2) hydrogen peroxide/ ammonium hydroxide and sodium hydroxide (PASO) mixture. ASO and PASO treated residues, hardwood pulp, and papers were enzymatically hydrolysed for comparisons in terms of sugar production. Results showed wood hydrolysis rate increased with increase in sodium hydroxide concentration and cook time for ASO and PASO chemical treatments on all wood residues. All observed alkali treatments showed little or no effect on the decomposition of lignin content of wood residues. Measured sugar content after alkali treatments and enzymatic hydrolysis ranged from 50 to 81 mg/ml from ASO/PASO treated yellow poplar and red oak residues. Enzymatic hydrolysis of ASO and PASO treated residues hydrolyzed approximately 61% of the original wood content, while over 80% of hardwood pulp fibers and paper wastes were digested to sugar.

3.1 Introduction

Overdependence on petroleum products has consistently been on the increase over the past twenty years. According to the U.S. Energy Information Administration statistics, net petroleum product imports increase was 16.8% in January 2005, crude oil net imports increase was 5.3% (USDOE 2005). In 2006, the U.S. with a population of 300 million people consumed 20 million barrels of oil per day, an amount just a little less than one quarter of the worlds' daily demand (Ethanol fact book .2007). Cellulosic ethanol could be a promising alternative fuel option because of the relative abundance of biomass resources (Perlack et al. 2005), and the potential to displace one-third of the current U.S. transportation fuel demand (Perlack et al. 2005). Wood and wood fiber products accounts for 60% of the total municipal solid wastes generated in the United States (USEPA 1997). In addition, logging and mill residues represent a significantly under-utilized source of biomass feedstock for a variety of value-added biochemical products and energy. For example, West Virginia produces an estimated 4.5 billion kg of wood fiber, or 2.6^{13} KJs per year, in the form of industry and forest residue comprising of 35.4 million kg of chips, sawdust, bark, and 1.1 billion kg of logging residues per year (Wang et al. 2006). To date, the abundant wood residues in West Virginia is yet to be fully utilized for bioenergy.

One of the biggest challenges to the enzymatic conversion of wood to sugars is due to the presence of recalcitrant wood lignin. The difficulty with lignocellulosic hydrolysis is due to the complex association between cellulose and hemicelluloses, with a surrounding covalently bonded lignin matrix. Cellulase enzymes produced from a host of microorganisms can be used to hydrolyze wood into fermentable sugars provided the appropriate pretreatment is used. Pretreatment alters the structure of cellulose, reduce cellulose crystallinity, increases surface area, solubilizes hemicelluloses and lignin (Mosier et al. 2005, Demain et al. 2005). The use of

cellulase enzymes for cellulose breakdown to glucose offers an economical high sugar yield (Lynd 1996, Wyman 1999). However, the reactivity of cellulase is slow, thus requiring an appropriate pretreatment methodology and high enzyme loadings to attain high sugar yields (Wooley et al. 1999). Ammonia has great ability to remove lignin with low hemicellulose solubility, while hydrogen peroxide promotes effective hemicellulose and lignin removal (Dale et al. 1985, Chundawat et al. 2007). Presently, the use of ammonia and hydrogen peroxide as an alternative wood pretreatment method is yet to be fully utilized in the conversion of wood to sugar due to limited studies on sound and decayed hardwood residues. Accordingly, this study was designed to evaluate the effects of low concentration alkali and hydrogen peroxide solutions at 80°C over variable cook time on yellow-poplar and red oak hardwood residues. In addition, the combined effects of the chemical treatments on cellulase enzyme digestibility of wood residues were also examined for economically high yield sugar production.

3.2 Materials and methods

3.2.1 Experimental design and pretreatments

This study was set up as a three factor (chemical, cook time, and species of wood residue) factorial experiment with five replicates in completely randomized design. The two chemical mixtures used were (1) a mixture of 29% ammonium hydroxide and sodium hydroxide (ASO) at various concentrations (0.1, 0.2, and 0.3g per 25ml of ammonium hydroxide solution) and (2) a mixture of equal volumes of 29% ammonium hydroxide and 3% hydrogen peroxide at varying concentrations of sodium hydroxide (0.1, 0.2, 0.3g per 25ml of ammonium hydroxide solution) (PASO). Cook temperature of 80⁰C, time duration of 3, 6, 12 hours, and two predominant wood residues (yellow-poplar (*Liriodendron tulipifera*) and red oak (*Querus rubra*)) in the central Appalachian hardwood region were used during the experiments in addition to the chemicals. Logging residues of red oak and yellow-poplar were collected in West Virginia from harvested forest sites of years 2005 and 2008. Mild cook temperature of 80⁰C was chosen in this experiment so as to prevent degradation of sugars during hydrolysis. Sound wood residues collected from the 2008 forest site and decayed residues from the 2005 forest site were pulverized into small sizes to pass through mesh #60 (i.e. 90% or more of wood materials of 250 mm particle size passed through mesh #60). After pulverization, a portion of the residue was used to determine the amounts of lignin, extractives, and holocellulose in the wood in accordance with ASTM designation: D 1110-84; 1106-96 (ASTM 2001). For each chemical mixture, one gram of oven dried wood particles was mixed with the appropriate alkali treatments and cooked for 3, 6, and 12 hours.

At the end of the cook time, solid wood residues were separated from the mixture and oven dried for 24 hours to determine the proportion of un-dissolved wood and residual lignin

content. The liquid extracts resulting from the chemical reaction were analyzed using high performance liquid chromatography (HPLC) to determine the types and proportion of dissolved five-six carbon sugars present. The acid insoluble lignin content of the solids was determined using the National Renewable Energy Laboratory (NREL) protocol (Templeton and Ehrman 1995)

3.2.2 Enzymatic hydrolysis and sugar analysis

AccelleraceTM 1000 product of Genencor International, Inc. Rochester NY, was used in this experiment to further evaluate the effects of ASO/PASO treatment for sugar production. AccelleraceTM 1000 is a commercial biomass enzyme produced from genetically modified *Trichoderma reesei* (Genencor 2008). Enzymatic hydrolysis was done with 0.15g wood of 6 hour treated ASO and PASO yellow-poplar and red oak residues, pulp fiber, and waste paper samples per 0.24 ml of Accellerace enzyme inside an incubator at 50°C for 72 hours in accordance with the NREL protocol (Selig et al. 2008) The waste paper used was a bleached kraft pulp commonly used in printing. This waste paper is assumed to contain less than 1% lignin content in accordance with the previous report that bleached kraft wood pulp has low lignin content of 0.1% (Saariaho et al. 2003).

Extracts after chemical treatment and enzyme hydrolysis were filtered through a 0.4- μm sieve and diluted in water for sugar analysis. The parameters for HPLC system used were as follows: (1) detector: refractive index; (2) column: sugar pack I; (3) injection volume: 10 μL ; (4) number of injections per vial: 3; (5) mobile phase: HPLC grade water; (6) flow rate: 0.2 mL/minute; (7) column temperature: 60°C; (8) detector temperature: 38°C; and (9) run time: 20

minutes. At the completion of the experiment, analysis of variance (ANOVA) and least squares difference (LSD) procedures were run in SPSS 12.0 statistical package to ascertain significant differences among experimental treatments.

3.3 Results and discussion

Measured wood chemical properties (extractives, lignin, and holocellulose) of yellow-poplar and red oak residues were presented in Table 3.1. These results generally agreed with that obtained in a previous study (Sjostrom 1994). There were differences between wood species, sound and decayed wood residues. Lignin content of decayed residues was about 20% more than the sound wood residues. Lower holocellulose content, at the expense of proportionately higher extractives and lignin contents, was observed in the decayed sapwood samples of yellow-poplar and red oak residues rather than in sound residues. The lower holocellulose content observed on decayed wood was a result of changes in climatic conditions and microbial mediated biochemical changes (Pandey and Nagveni 2007, Adebola et al. 2009).

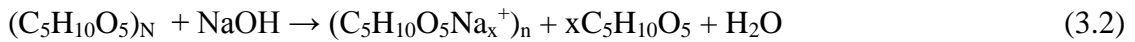
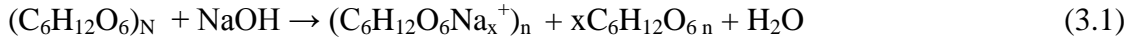
Results on the alkali treatment of wood residues (Table 3.2) indicated that PASO depolymerize wood more than ASO treatment. Hydrolysis of wood was also observed with increases in sodium hydroxide concentration over cook time (Figures 3.1 and 3.2). Of all treatment combinations, the highest hydrolysis rate observed (40.7 % for red oak residue) was at 0.3g sodium hydroxide concentration and for 6 hours of cook time (Figure 3.2). Percentage of hydrolyzed red oak wood was higher than yellow-poplar for both ASO and PASO chemical treatments. The observed differences in hydrolysis between these hardwood residue species could be a result of the existing structural and chemical variability (Table 3.1). Structurally, red

oak is a ring porous wood, that is it contains many large vessels in the early wood, and smaller vessels in the latewood, while yellow-poplar is diffuse-porous (i.e. many small vessels across growth increment) (Bruce Hoardley 1990). Accordingly, it is probable that the large pores in red oak facilitated the efficient penetration of the chemical treatments thus leading to increased hydrolysis when compared to the limitations imposed by the small pores of yellow-poplar wood.

Decayed wood of yellow-poplar and red oak failed to show higher wood hydrolysis than sound woods despite the presence of weakened phenolic bonds in the decayed wood lignin. Dissolved yellow-poplar wood after ASO treatment ranged from 20 to 33%, while the ASO treatment showed a range of 21 to 34% for dissolved sound and decayed red oak wood respectively. The dissolved component of wood was holocellulose. This assertion was based on the post chemical analysis of wood extracts and wood residues after alkali treatment, which reveals glucose sugar (Figure 3.3) and lignin content ranging from 29 to 47% for yellow-poplar and 30 to 35% for red oak (Tables 3.1 and 3.3). In the above reaction, sodium hydroxide in conjunction with the alkalis depolymerized these hardwood residues into liquid substances in agreement with a previous report that sodium hydroxide can change the structure of cellulose into less crystalline and short length fibers by mercerization process (Johnson 1979). Sodium hydroxide dissolves cellulose and hemicelluloses substituting hydroxyl ions in cellulose with sodium ions thus weakening the bonds.

The reaction of sodium hydroxide with cellulose and hemicelluloses is outlined in equations (3.1 and 3.2). The analysis of ASO and PASO liquid hydrolysis extracts after heat treatment revealed predominantly the presence unknown chemicals and minute content of glucose sugar (Figure 3.3). The measured sugar content was about the same among the various wood residues used for the experiment. Accordingly, 2.1 to 2.7 mg/ml and 2.3 to 2.7 mg/ml of

dissolved glucose sugar was observed for PASO treated yellow poplar and red oak residues respectively, while 1.5 to 2.7 mg/ml and 2.4 to 2.7 mg/ml was observed for ASO treated yellow poplar and red oak residues respectively. The low sugar concentration observed in this report could be due to degradation actions of hydrogen peroxide solution on wood sugars as previously reported that hydrogen peroxide can increase depolymerization of holoceluloses but with small recoverable sugars in liquid (Kim et al. 2000).



3.3.1 Effects of hydrogen peroxide

The presence of hydrogen peroxide in the alkali mixture of sodium hydroxide and ammonium hydroxide (i.e. PASO treatment) increased the effectiveness of the alkali treatment in the depolymerization of wood residues with a range of 21 to 36% for yellow-poplar wood and 25 to 41% for red oak wood residues (Table 3.2). Hydrogen peroxide, a strong oxidizing agent, is the prime factor responsible for the increased depolymerization of wood holocellulose in this reaction as previously reported (Kim et al. 2000). During the reaction, some of hydrogen peroxide could have been consumed, dissolved in water, and some converted to hydroxyl radicals (equations 3.3, 3.4, 3.5) via an ionic pathway (Nascimento et al. 1995). These free hydroxyl radicals are the agents of the delignification action of hydrogen peroxide (Nascimento 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, ketones, and carbonyl groups (Mussatto et al. 2008). The formation of hydroperoxide (HC (O) OOH) was reported to be responsible for breaking the aromatic chains in the complex lignin structures through an internal rearrangement mechanism (Nascimento et al. 1995).

3.3.2 Effect of ammonium hydroxide

Ammonium hydroxide solution was less effective for wood hydrolysis when compared to mixed alkali treatments of ASO and PASO. However, a twelve-hour cook time treatment of wood residues with only ammonium hydroxide solution also resulted in depolymerization of wood residue. Dissolved wood after the 12 hour treatment ranged from 18 to 24% and 21 to 33% for yellow-poplar and red oak, respectively (Table 3.2). Consequently, the lignin content of wood residues (Table 3.3) after ammonium hydroxide treatment remained relatively the same when compared with lignin content of sound wood (Table 3.1). It is anticipated that ammonium hydroxide will react with phenolic molecules, incorporate nitrogen into wood lignin, thus resulting in lignin oxidation; that is oxygen consumption and carbon dioxide elimination from

wood leading to degradation of aromatic rings (Capanema 2006). The two-sided action of ammonia that is the dual removal of wood hemicellulose and lignin has been reported that an increase in ammonia concentration from 0.5 to 20% resulted in increased hemicellulose recovery from 19% to 30% and an increase in lignin removal from 34 to 66% (Kim et al. 2000). Conversely, the observed experiment outcome and assumptions did not completely agree with some previous findings that ammonium hydroxide or ammonia water is effective in delignification and preservation of cellulose with no significant loss of holocellulose (Dale et al. 1985, Chundawat et al. 2007) The possible explanation for the observed delignification could be as a result of temperature, concentration of ammonia, and inhibitory properties of wood chemicals such as terpenes, wax and extractives in the hardwood species used in this experiment. This could also be as a result of surface modification of lignin by hydrogen peroxide resulting in the formation of ketone (CHO) and carboxylic acid (CHOOH) (Lu et al. 2001).

3.3.3 Enzymatic hydrolysis

The pattern of enzymatic hydrolysis of ASO and PASO treated wood, untreated wood, hardwood pulp fiber, and waste paper after 72 hours duration was summarized in figure 3.4. The untreated wood was less hydrolyzed when compared to treated hardwood residues of yellow-poplar and red oak, pulp and waste paper. The observed enzymatic hydrolysis in this study was in accordance with the enzyme manufacturers speculation that accellerace enzyme applies synergetic activities of its coenzymes (exoglucanase, endoglucanase, hemicellulase, and beta-glucosidase) to convert complex lignocellulosic biomass into fermentable sugars (Figure 3.3b) through the breaking down of $\beta(1\rightarrow4)$ -glycosidic bonds of cellulose (Genencor 2008).

Digestion of cellulose was significantly low ($p=0.0001$) in untreated wood (10-12%) when compared to ASO and PASO treated wood. A relatively higher digestion of over 60% was also observed for ASO and PASO treated hardwood residues, while 90% and 80% conversion was observed for hardwood pulp fiber and waste paper, respectively.

Sugar analysis after enzymatic hydrolysis showed that 72 to 81 mg/ml and 70 to 78 mg/ml of dissolved glucose sugar was observed for PASO treated yellow poplar and red oak residues respectively, while 52 to 62 mg/ml and 50 to 62 mg/ml was observed for ASO treated yellow poplar and red oak residues respectively. The highest glucose concentration of 112 to 115 mg/ml was observed from hardwood pulp (Figure 3.4).

The reason for the lower conversion of ASO and PASO treated wood residues can be attributed to their high lignin content (Table 3.5) as previously reported that 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 depolymerize wood during the pretreatment phase and simultaneously weaken the lignin bonds in wood to a level sufficient enough for the cellulase enzyme to penetrate and digest cellulose molecules in the wood. The high digestibility of hardwood pulp fiber and waste paper is an indication that lignin, hemicelluloses, and extractive contents are the main inhibitors to the efficient digestion of wood using accellerace enzyme.

Wood residue conversion to fermentable sugars is presented in figure 3.6. By combining ASO/PASO treatment with enzymatic hydrolysis, about 30% of equivalent of initial mass of pretreated wood was obtained for sugar from yellow-poplar and red oak wood residues, leaving

about 21% of unknown chemicals, 22% lignin, and 27% undissolved holocellulose content. An advantage of ammonium solution is that residual ammonia is also useful for the production of less inhibitory compounds and nutrient for yeast growth during fermentation (Tymouri et al. 2005).

3.3.4 Potential sugar and ethanol production from wood residues

Logging and mill residues statistics from U.S. Forest Service, timber product output (TPO 2008) showed that existing abundant wood residues are capable of supporting bio-energy plants in the central Appalachian hardwood region (West Virginia and its surrounding states) (Table 3.5). These available biomass resources will assist in fulfilling the long term energy independence goal of the U.S. which is to displace 30% of the current transportation fuel demand (about 60 billion gallons per year) with forest and agricultural biomass resources of 1.2 trillion dry kilograms (Ethanol fact book 2007).

In Table 3.5; potential pulp, sugar, ethanol yields, and wastes were calculated based on the industrial pulp yield (50%), sugar yield from ASO/PASO via enzymes (30%), and fermentation productivities. Assuming that pulp and paper mills are to be used to produce kraft pulp directly for sugar production, through enzymatic pathways or through ASO/PASO/enzymatic approach, about one third of the total available wood residues can be converted to sugars equivalent to 0.12-1.8 billion dry kilograms of wood residues. Based on the conversion rate of 907 kg (one dry ton) wood biomass to 67 gallons of ethanol (Aden et al. 2002), the above estimated biomass would yield a total sum of 245 to 406 million gallons of ethanol across the Central Appalachian region. This ethanol production potential from cellulosic

biomass would boost the local economy, generate new household income, improve tax revenue, and create new jobs by a factor of 20 in accordance to the report that a 40 million gallons ethanol production per year would increase community's economy by \$110 million, create household income of \$19.6 million, tax revenue, and about 700 jobs (Ethanol fact book 2007).

On a one-to-one scale, 245- 406 million gallons of ethanol will replace an equal amount of imported crude oil into the U.S and indirectly reduce reliance on imported petroleum, offset part of the U.S. trade deficit (since crude oil accounts for about 31% of U.S. deficit), and reduce wealth transfer to foreign countries (GAO 1991, U.S. Census Bureau 2006).

Today in the U.S., 41% of greenhouse emission is as a result of gasoline and diesel fuel consumption (Jim 2007). According to the American Lung Association, "transportation sources such as burning gasoline and diesel are responsible for 55.8% of outdoor air pollution. "Gasoline emission includes 77.3% carbon monoxide, 44.5% nitrogen oxides, 3.3% sulfur oxides, 35.6% of volatile organic compounds (VOCs), 26.3% of particulate matter, and 26.6% of lead" (American Lung Association 2009). Ethanol production in the Appalachian region can help reduce environmental pollution, prevent micro-climatic changes, and health hazards associated with gasoline greenhouse gas emissions.

3.4 Conclusions

Measured chemical properties of wood residues generally showed differences among species, sound, and decayed wood residues of yellow-poplar and red oak. Percentage lignin of decayed residues was higher than in sound undecayed wood residues. Results on thermochemical

conversion of wood residues during ASO/PASO pretreatment indicated that PASO treatment degrade wood residue more than ASO treatment.

Hydrolysis of wood during ASO/PASO pretreatment increased with increases in sodium hydroxide concentration over cook time. Of all treatment combinations, the highest hydrolysis rate observed was with PASO treatment at 0.3g sodium hydroxide concentration and 6 hours cook time. Dissolved cellulose and hemicelluloses obtained from ASO ranged from 17.7 to 33% for yellow-poplar, 20 to 33% for decayed yellow-poplar/red oak, and 24 to 33.7% for sound red oak. In PASO treatment, dissolved cellulose and hemicelluloses ranged from 21 to 36% for yellow-poplar, 23.7 to 38% for decayed yellow-poplar, 22.7 to 39.3% for decayed red oak, and 25.3 to 40.67% for sound red oak. All observed alkali treatments showed little or no effect on decomposition of wood lignin with low sugar yield.

Sugar yield resulting from enzymatic hydrolysis of ASO, PASO treatments failed to show any significant difference between species and the condition of wood residue (sound versus decayed wood). 61% of the original ASO/PASO treated wood residue was converted to fermentable sugar, while over 80% of hardwood pulp fibers and paper wastes were hydrolyzed. Biomass production in West Virginia and environs can produce enough ethanol to positively affect the environment, benefit the economies of these states, and generate tax revenue, and jobs.

References

- USDOE (U.S. Department of Energy), 2005. Monthly energy review, April. Energy Information Administration, U.S. Dept. of Energy, Washington DC.
- Ethanol fact book, 2007. A compilation about ethanol fuel.
- <http://www.ethanolcrossamerica.net/pdfs/2007EthanolFactBook.pdf>. Last accessed June 20, 2009
- Perlack RD, Wright LL, Turhollow AF, Graham RL, Stokes BJ and Erbach DC, 2005. Biomass as feedstock for bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. April 2005. A Joint Study Sponsored by U.S. Department of Energy and U.S. Department of Agriculture. 78pp.
- USEPA, Characterization of Municipal Solid Waste in the United States. Update. United States Environmental Protection Agency, Washington, D.C.1997
- USDA. Highlights of 2000 West Virginia Forest Inventory. Northeastern FIA, 2006.
- <http://www.fs.fed.us/ne/fia/state/wv/wvhlite00.PDF>. 2000. Last accessed, October 24, 2006.
- Wang J, Grushecky S, and McNeel J, 2006. Biomass Resources, Uses, and Opportunities in West Virginia. Division of Forestry and Natural Resources, West Virginia University Morgantown . 103 pp.
- Mosier N, Wyman C, Dale B., 2005. Bioresource. Technol. 96: 673-686.
- Demain AL, Michael N, and David J H, Wu, 2005. Cellulase, clostridia and ethanol. Microbiology and Molecular biology Reviews, Mar. 69:124–154.
- Lynd LR. Overview and evaluation of fuel ethanol from cellulosic biomass: Technology, economics, the environment, and policy. Ann Rev Energy Environ 1996; 21:403–465.

- Wyman CE.,1999. Biomass ethanol: Technical progress, opportunities, and commercial challenges. *Ann Rev Energy Environ.* 24:189–226.
- Wooley R, Ruth M, Glassner D, Sheehan J, 1999. Process design and costing of bioethanol technology: A tool for determining the status and direction of research and development. *Biotechnol Prog* 15(5):794–803.
- Dale B. Henk L. and Shiang, M.Dev, 1985. *Ind. Microbiol.* 26, 223-233.
- Chundawat SPS, Venkatesh B. and Dale BE, 2007. *Biotechnol. Bioeng.* 96 219-231.
- ASTM. (American Society for Testing and Materials), 2001. D: 1110-84; 1106-96;1102-84. *Annual Book of ASTM Standards, West Conshohochen , PA. Section 4, volume 4.10, 1429pp.*
- Templeton D, Ehrman T, 1995. Determination of acid-Insoluble lignin in biomass. *Laboratory Analytical Procedure No.003, National Renewable Energy Laboratory. Golden, CO.*
- Genencor, 2008. Accelerace™ 1000, Cellulase enzyme complex for lignocellulosic biomass hydrolysis. *Technical Bulletin #1 Saccharification. www.genencor.com. May 18, 2008.*
- Selig M, Weiss N, and Ji Y, 2008. Enzymatic saccharification of lignocellulosic biomass. *Laboratory analytical procedure (LAP). NREL/TP-510-42629.*
- Saariaho A, Skelainen A, MARI M, Nuopponen, and Vuorinen T., 2003.”Ultra violet resonance Raman spectroscopy in lignin analysis: Determination of characteristic vibrations of p-Hydroxyphenyl, guaiacyl, and syringyl lignin structures,” *Appl. Spectrosc.*, 57:58–66.
- Sjostrom E. *Wood Chemistry Fundamentals and Applications.* 1994;212 pp.
- Pandey KK and Nagveni HC., 2007. Rapid characterization of brown and white rot degraded chir pine and rubberwood by FTIR spectroscopy. *Holz Roh Werkst.* 65: 477-481.

- Adebola AB, Wang J, Benjamin D., Joseph MF, and James PA, 2009. Assessment of Appalachian hardwood residue properties and potentials for bioenergy utilization. *Wood and Fiber Science*. 41(1): 1-10.
- Bruce Hordley, 1990. *Identifying Wood: Accurate Results with Simple Tools*. Taunton Press, Box 5506, Newtown, CT 06470-5506, 240 pp.
- Johnson DJ., 2000. "High-temperature stable and high-performance fibres", in: *Applied Fiber Science*, Vol. 3, F. Happey (Ed.), Academic Press, London, New York, 1979;Chapter 3, 116 pp.
- Kim SB, Yum DM and Park SC. *Biores. Technol*, 2000. 72: 289-294.
- Nascimento EA, Antonia EH and Morais SJ., 1995. Photochemical hydrogen peroxide bleaching of eucalyptus organosolv pulp. *Chem. Soc.* 6:365-371.
- Rahmawati N, Ohashi Y, Honda Y, Kuwahara M, Fackler K, Messner K, Watanabe T., 2005. Pulp bleaching by hydrogen peroxide activated with copper 2,2'-dipyridylamine and 4-aminopyridine complexes. *Chem. Eng J.* 112:167-171.
- Mussatto SI, Dragone G, Roberto IC, 2008. Hydrogen peroxide bleaching of cellulose pulps obtained from brewer's spent grain. *Cellulose* 15:641-649.
- Capanema, Ewellyn, A, 2006. Oxidative Ammonolysis of Technical Lignins. Part 4. Effects of the Ammonium Hydroxide Concentration and pH. *Journal of Wood Chemistry and Technology*. 26: 95-109.
- Lu L, Zhou Xian-Tao, Zhao De-Sheng, Qiu Yu-Gui, 2001. Structural changes of lignin caused by oxidative ammonolysis. *Huaxue xuebao*. 60:176-179.
- Mansfield SD, Mooney C., and Saddler JN.,1999. *Biotechnol Prog.* 15, 804-816

Tymouri F, Laureano-Perez L, Alizadeh H. Dale BE., 2005. Optimization of the ammonia fiber explosion (AFEX) treatment parameters for enzymatic hydrolysis of corn stover.

Bioreour Technol 96(18): 2014-2018.

TPO (Timber Product Output).2008. <http://srsfia.fia.srs.fs.fed.us/php/tpo2/tpo.php>

Aden A, Ruth M, Ibsen K, Jechura J, Neeves K, Sheehan J, and Wallace B, 2002.

Lignocellulosic biomass to ethanol design and economic utilizing co-current dilute acid pretreatment and enzymatic hydrolysis for corn stover. National Renewable Energy Laboratory (NREL). Technical report NREL/TP-510-32438

GAO (Government Accountability Office), Southwest Asian, 1991. Cost of Protecting U.S. Interests, GAO/NSIAD-91-250.

U.S. Census Bureau, Foreign Trade Division, 2006.

Jim Woolsey, 2007. Testimony before the Senate Finance Committee.

American Lung Association (www.lungusa.org). 2009. Last accessed in march 17 2009.

Table 3.1 Chemical components (%) of yellow-poplar and red oak residues by duration of exposure.

Properties	Duration of environmental exposure (years)		
	3-4 ^a	2 ^b	1 ^b
Yellow-poplar			
Extractives	9.90 (3.26)	7.78 (1.62)	7.41 (1.14)
Holocellulose	61.21 (7.31)	68.54 (4.93)	70.37 (2.3)
Lignin	28.88 (7.31)	23.68 (3.70)	22.22 (2.3)
Red oak			
Extractives	10.18 (3.1)	10.31 (3.1)	7.80 (1.7)
Holocellulose	52.88 (6.8)	54.44(3.3)	65.25 (4.2)
Lignin	37.30(6.8)	35.25 (3.3)	26.88 (4.2)

() standard deviation.

^aDecayed wood residue.

^bSound wood residue.

Table 3.2 Dissolved yellow poplar and red oak holocellulose contents (%) after ASO and PASO pretreatments.

Cook Time (hours)	NaOH (g)	ASO (%)				PASO (%)			
		Yellow-poplar	Yellow-poplar*	Red oak	Red oak*	Yellow-poplar	Yellow-poplar*	Red oak	Red oak*
3	0.1	19.67	24.33	28.33	24.11	21.33	23.67	25.33	26.33
	0.2	24.67	29.33	30.67	28.22	23.67	28.67	29.33	30.00
	0.3	23.67	31.67	30.67	28.67	25.67	29.67	33.67	33.33
6	0.1	26.33	27.33	27.67	27.11	27.67	27.67	27.00	28.33
	0.2	28.67	28.67	28.33	28.56	33.00	35.33	34.00	35.67
	0.3	32.67	32.67	33.67	33.00	36.33	38.33	40.67	39.33
12	0.0	17.67	20.33	24.33	20.78	21.00	25.33	25.33	32.67
	0.1	19.00	24.00	26.33	25.67	32.00	38.00	37.00	31.67
	0.2	24.33	28.33	29.00	31.67	33.00	32.33	29.33	22.67
	0.3	24.67	25.00	27.33	31.33	27.33	26.33	26.33	20.67

*Decayed residue.

Table 3.3 Yellow-poplar and red oak wood residues content (%) after ASO pretreatment.

Time (hours)	NaOH (g)	Yellow-poplar (%)			Red oak (%)		
		Lignin	Holocellulose*	Holocellulose**	Lignin	Holocellulose*	Holocellulose**
3.00	0.1	29.00	19.67	43.33	32.00	28.33	31.67
	0.2	37.11	23.67	39.33	33.33	30.67	28.00
6.00	0.1	31.33	26.33	34.34	35.56	27.67	28.77
	0.2	35.33	32.67	24.00	32.22	33.67	26.11
12.00	0.0	29.67	17.60	44.73	32.00	24.33	35.67
	0.1	39.00	19.00	34.00	27.78	26.30	37.92
	0.3	47.00	24.67	20.33	30.00	27.33	34.67

*dissolved holocellulose content.

**undissolved holocellulose content.

Table 3.4 Yellow-poplar and red oak wood residues content balance after PASO pretreatment.

Time (hours)	NaOH (g)	Yellow-poplar (%)			Red oak (%)		
		Lignin	Holocellulose*	Holocellulose**	Lignin	Holocellulose*	Holocellulose**
3.00	0.1	23.33	21.30	46.87	25.33	21.00	45.17
	0.2	23.33	25.67	42.50	33.67	25.67	32.16
6.00	0.1	21.11	27.67	42.72	27.00	27.67	36.83
	0.2	24.17	36.30	31.03	40.67	36.33	14.50
12.00	0.0	23.00	21.00	47.50	25.33	21.00	45.17
	0.1	24.44	32.00	35.06	37.00	33.00	21.50
	0.3	25.56	27.33	38.61	26.33	27.00	38.17

*dissolved holocellulose content.

**undissolved holocellulose content.

Table 3.5 Potential sugar production from total available (TA) residues in West Virginia and neighboring states.

State	Wood residues (billion dry kg)			TA	Kraft Pulp			ASO/PASO	
	Logging	Mill	Total		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

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

TA-Total available wood residues.

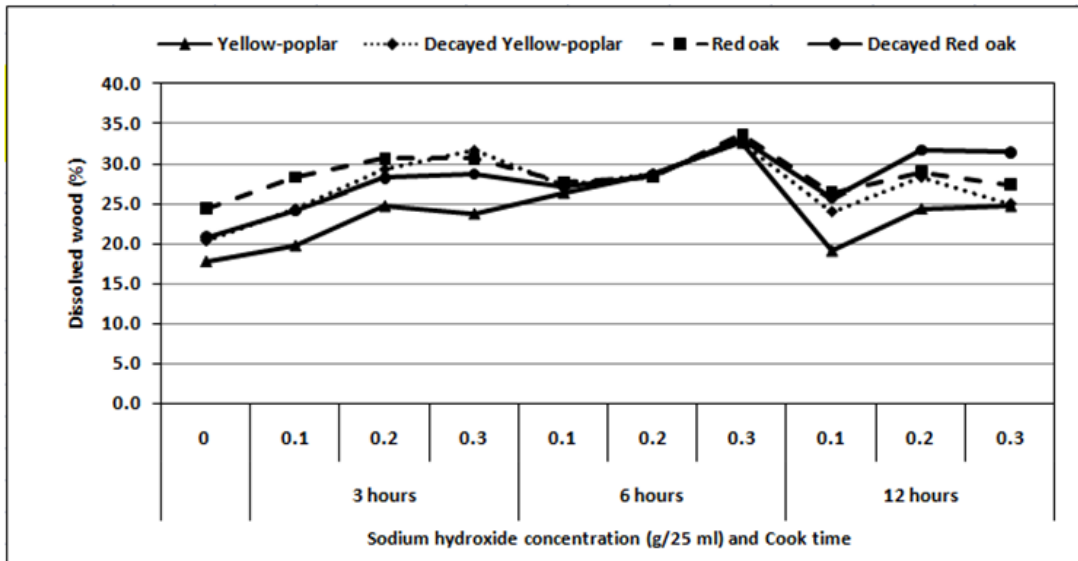


Figure 3.1 Dissolved wood holocellulose content after ASO pretreatment.

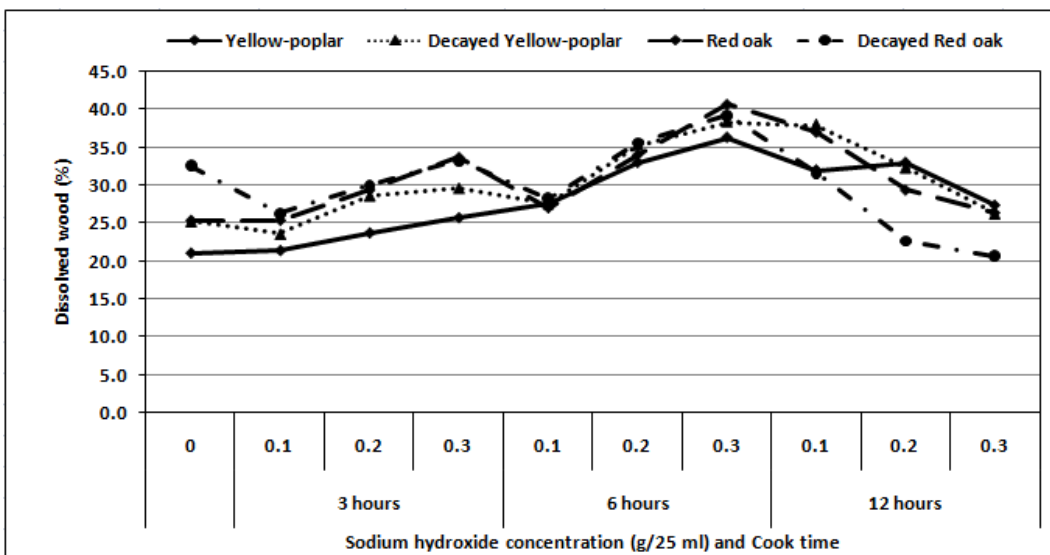


Figure 3.2 Dissolved wood holocellulose content after PASO pretreatment.

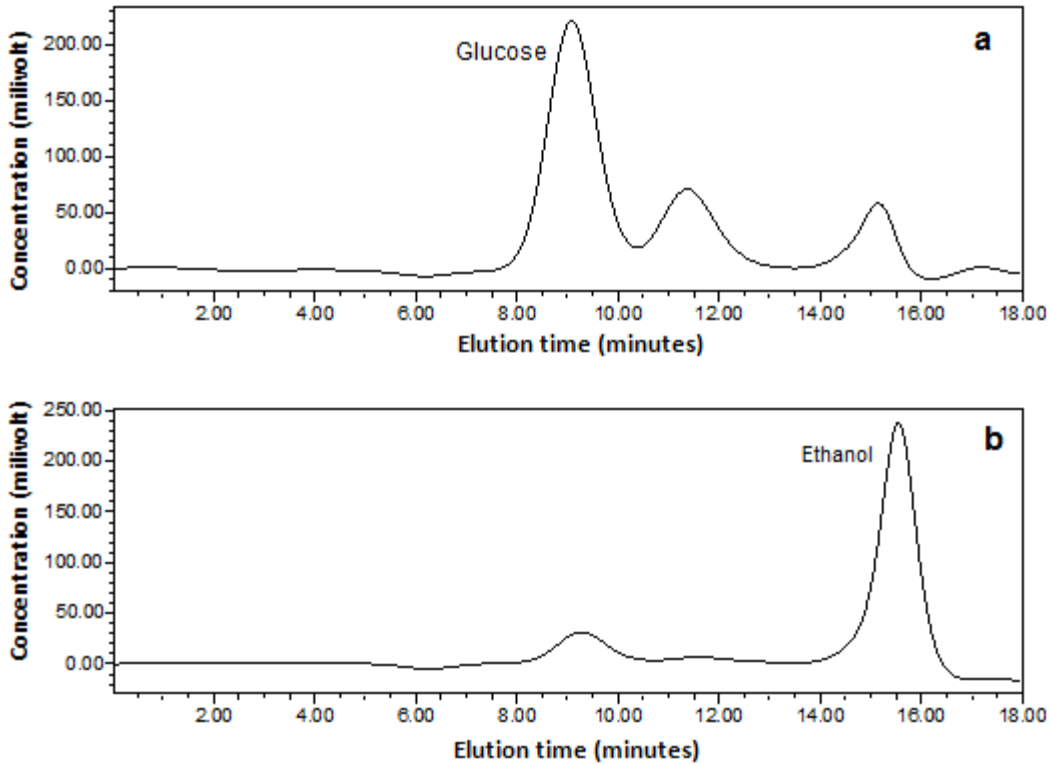


Figure 3.3 HPLC chromatogram of glucose sugar from alkali treated yellow poplar residue (a), and fermented sugar extract after enzyme hydrolysis (b).

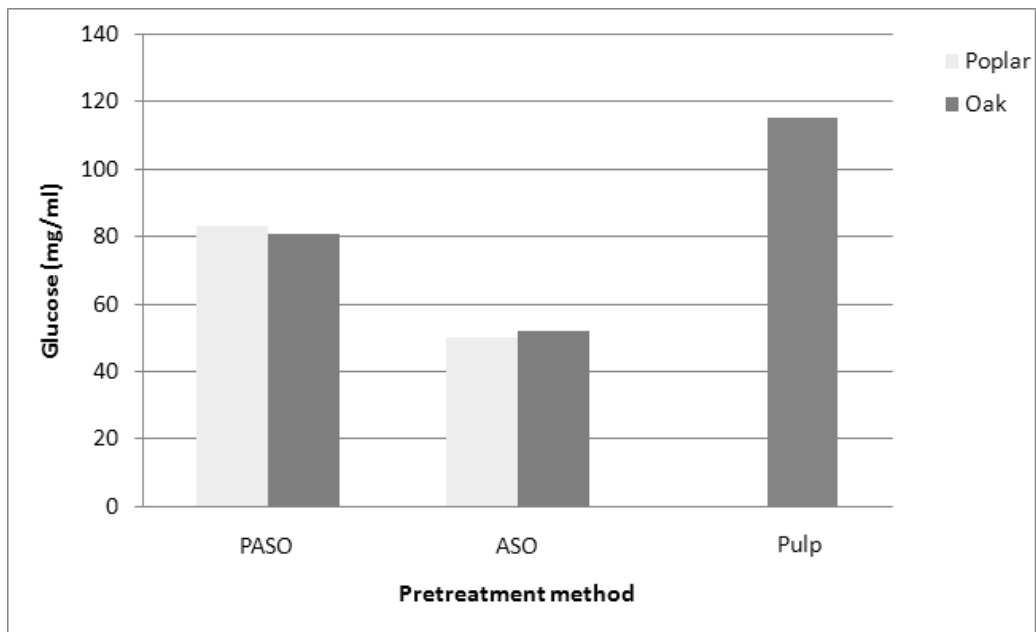


Figure 3.4 Glucose sugar yield after ASO and PASO pretreatment on yellow poplar residue and red Oak.

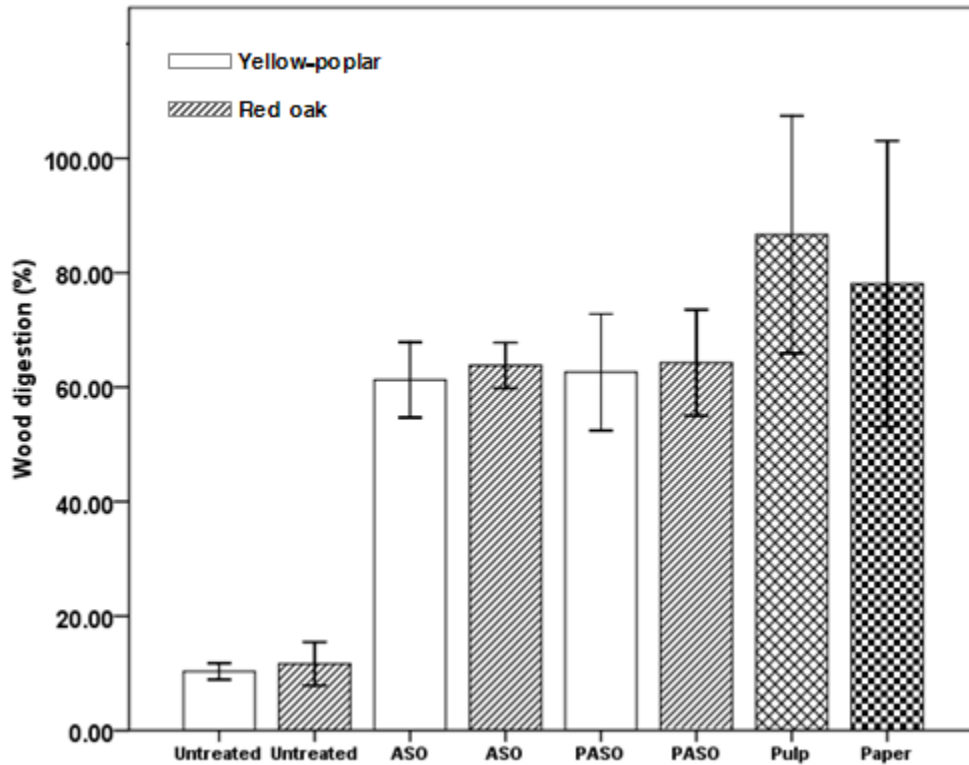


Figure 3.5 Enzymatic hydrolysis of ASO and PASO treated red oak and yellow-poplar residue, hardwood pulp and paper after 72 hours.

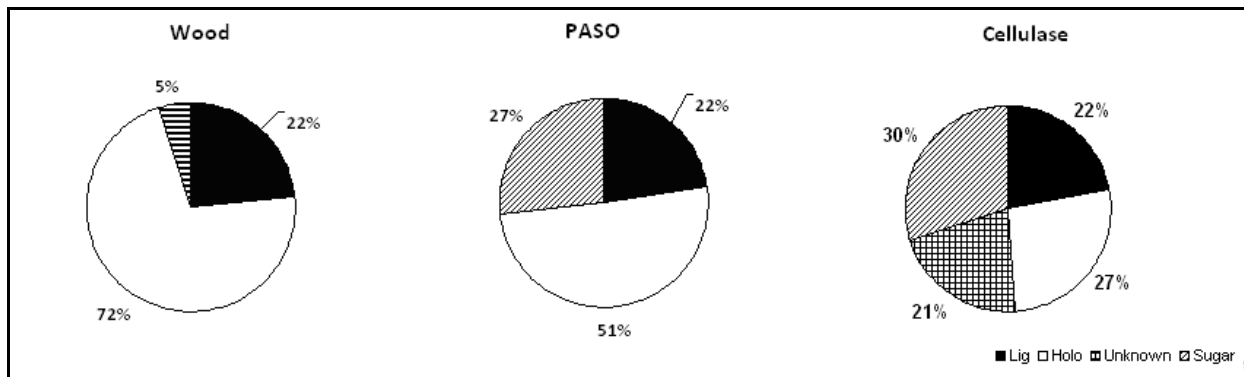


Figure 3.6 Changes in yellow-poplar holocellulose, extractives, and lignin content after PASO treatment to enzymatic (cellulase) hydrolysis.

**CHAPTER 4: ENZYMATIC HYDROLYSIS OF ULTRASONICATED AND
HYDROGEN PEROXIDE PRETREATED YELLOW POPLAR RESIDUE FOR SUGAR
PRODUCTION**

Abstract

Effects of wood pretreatment with ultrasonic/microwave and hydrogen peroxide chemical at various heating conditions prior to enzymatic hydrolysis for sugar production was evaluated in this study. It was generally observed that during the chemical pretreatment that wood constituents were progressively hydrolyzed with increase in temperature. The observed hydrolysis rate ranged from 20-30% at low depolymerization phase, 45-60% at mid phase, and 60-100% at the hyper phase for all category of pretreated wood residue within 0-10 minutes of cook time in the microwave into high molecular weight sugars. Hydrolysis of wood particles in the pretreatment chemical was directly proportional to temperature and time, regardless of particle size. Sugar production via enzymatic hydrolysis from pretreated yellow-poplar residue gave comparable glucose yield range of 80 to 110 mg/ml when compared to lignin free cellulose. The enzymatic hydrolysis was significantly affected by chemical pretreatment, pH, and time.

Key words: Enzyme; Glucose sugar; Hydrogen peroxide; Hydrolysis; Fermentation, Pretreatment; Ultrasonication; Wood residue

4.1 Introduction

The United State of America with a population of 260 million people consumes more than 140 billion gal/year of gasoline (Don et al., 2007), an amount which is about three times more than that consumed in China-the most populated nation of the world and about 67 times more than that consumed in Nigeria (Africa's number one producer of oil). The increase in oil consumption over the years in the world will continue to push up the price of gasoline leading to more economic instability. It therefore behooves us to find a renewable substitute that can supplement the current nonrenewable fuels sources. Biomass comprised of forest and agricultural resources is a good alternative for bio-fuel production fuels (Perlack et al., 2005). Lignocellulosic ethanol has the potential to displace more than 30% of gasoline consumption, reduce dependency on imported fuels, stabilize income, and reduce carbon emission (Office of Technology Assessment, 1993; Don, 2007). One significant advantage of cellulosic ethanol is that it reduces greenhouse gases emission by 85% (Wang, 2005).

The conversion of lignocellulosic biomass has been studied for over 120 years (Sherrard and Kressman, 1945); dilute or concentrated acid-catalyzed hydrolysis was performed in the 90s (Lynd et al., 1991), later enzymatic hydrolysis methods of wood conversion was developed. Other method includes wood liquefaction with ethanol under supercritical conditions (Minami and Saka 2002). However, these research milestones have not been developed to commercial success, due in part to the emphasis on agricultural crops, low sugar yield, and waste disposal problem resulting from unfriendly environmental chemicals.

It has been indicated in some previous study that cellulose fibers can be reduced to micro and nano scales particles sizes and that the degree of reduction is a function of time (Cheng and

Wang, 2007; Cheng et al., 2009; Wang and Cheng, 2009). High-intensity ultrasonication can produce very strong mechanical oscillating power due to cavitation. Cavitation is capable of disaggregating wood microstructures by weakening liquids intermolecular forces to form free radical for wood depolymerization (Kim and Lee, 2002). Previously, sonication equipment had been used exclusively on processed wood components like cellulose fibers, but yet to be applied as a pretreatment method for material processing for biofuel production.

This study was aimed at application of green chemical and environmentally friendly pretreatment process of ultrasonic sound and microwave to reduce wood particles size and enhance chemical penetration prior to enzymatic hydrolysis and fermentation. Specifically, the objectives of this study were to (1) evaluate the combined pretreatment impact of ultrasonic, microwave, and green chemical on wood including the effect of ultrasonication on lignin removal; (2) examine the cook time and pH on enzymatic hydrolysis; and (3) assess the fermentability of sugars produced.

4.2 Material and methods

4.2.1 Pretreatments

The pretreatment experiment was implemented based on 3 x 5 x 3 factorial, completely randomized design. Three factors were wood sample of different mesh sizes (60, 100, and ultrasonicated sample), temperature (120, 140, 160, 170, and 180°C), and cook time (0, 10, 30 minutes). On each combined treatment, 0.5 grams of yellow-poplar residue were mixed with 15 ml of 3% hydrogen peroxide solution and heated in a Microwave Accelerated System (MARS), CEM corporation, North Carolina, USA) to the specified heating conditions and time. A portion of the wood (those retained on 60 mesh size) was ultrasonicated with an ultrasonic processor

(VCX 750, Sonics & Materials, Inc., Newtown, CT) for 10, 20, 40, 60, 80, 100 minutes to determine the degree of microfibrillation. After sonication, degree of micro-fibrillation was evaluated using an indirect method called water retention value (WRV) in accordance with the procedure of a previous article (Cheng and Wang 2007). WRV, a percent ratio of the water in wood after centrifuged (W) to the dry weight of the sample (W^*) (equation 4.1), has been previously reported to be a measure of degree of homogenization, microfibril surface and volumetric phenomena (Herrick et al., 1983). Particle size distributions were analyzed using optical and scanning electron microscope (SEM: HITACHI S-4000).

$$\text{WRV} = ((W - W^*) / W^*) \times 100 \quad (4.1)$$

The ultrasonic treatment was combined with hydrogen peroxide in a microwave heating system to significantly remove recalcitrant lignin molecules from wood prior to enzymatic hydrolysis. One significant advantages of microwave when compared with conventional heating method is that it heats materials from the inside thus conserving energy and time. It also improves enzymatic hydrolysis by accelerating chemical reactions process (Zhu et al., 2005). After heat and chemical treatment, liquid extracts from the mixture were analyzed with alliance high performance liquid chromatography (HPLC) system and thermo-Finnigan LTQ FT mass spectrometer (Perkin Elmer Clarus 500 GC/MS) for chemical constituents. The solid residue collected were oven dried and used for enzymatic hydrolysis. The amounts of lignin and holocellulose in the wood were determined in accordance to National Renewable Energy Laboratory (NREL) (Templeton and Ehrman 1995).

4.2.2 Enzymatic hydrolysis

ACCELLERASE™ 1000” enzyme (Genencor International, Inc., Rochester, NY) was used in this experiment to further evaluate the effects of the pretreatment on wood for sugar production. ACCELLERASE™ 1000 is a commercial biomass enzyme produced from genetically modified *Trichoderma reesei*. This enzyme contains exoglucanase, endoglucanase, hemi-cellulase and beta-glucosidase. The endoglucanase activity of 25,000 CMU and beta-glucosidase activity of 400 pNPG was standardized on carboxymethylcellulose (CMC) and para-nitrophenyl-B-D-glucopyranoside (pNPG) respectively. ACCELLERASE™ 1000” enzyme color is brown with pH of 4.8 - 5.2. (Genencor, 2008).

The enzymatic hydrolysis experiment was also set up as a 5x3x3 factorial experiment in randomized complete block design (five substrates, three enzyme loading, and three cook time) in order to evaluate the effects of wood pretreatment, enzyme loading, pH, and cook time. The treatment combinations were divided into two blocks by pH levels (4.0, 4.5). The five substrates comprised of two pretreated (ultrasonicated/ hydrogen peroxide) yellow-poplar residues (samples treated at 120-140°C), two laboratory prepared yellow-poplar pulp (Pulp-1, Pulp-2), and one commercial hardwood pulp (Pulp-3). The reason why only low heat treated samples of 120-140°C was used was because at temperatures above this level, significant proportions of the wood residues were already hydrolyzed.

Prior to enzymatic hydrolysis, a hot water treatment at 150°C temperature was applied on all treatments for one hour duration to remove extractives, starches, and residual chemical after pretreatment. Pulp-1 was prepared in the laboratory with sodium chlorite in accordance to Pulp and Paper Association procedures (Wise et al., 1946). While Pulp-2 was prepared by removing extractives and hemicellulose content with dilute acid hydrolysis method, following with

significantly more sodium chlorite chemical. The second experimental factor comprised of three enzyme loadings (0.1, 0.2, 0.3 ml product/g glucose). Samples of 0.2 g from each of the treatment combinations were enzymatically hydrolyzed in an incubator at 50°C for 72 hours according to the NREL protocol (Selig et al., 2008). This protocol was modified using a different buffer (potassium biphthalate buffer with a pH of 4.1). Samples were taken from each treatment combination at 24, 48, and 72 hours of duration for sugar analysis.

4.2.3 Sugar and fermentation analysis

Sugar analysis was performed at each stage of experiment (that is after hydrogen peroxide pretreatment and enzymatic hydrolysis stages) with HPLC. Samples for sugar analysis were replicated three times in an auto sampler vial for analysis. The parameters for HPLC system used are as follows: detector -- refractive index, column-- sugar pack I, injection volume-- 10 µL, number of injections per vial-- 4, mobile phase-- HPLC grade water, flow rate-- 0.2 ml/minute, column temperature-- 60°C, detector temperature-- 38°C, run time-- 15minutes. HPLC parameters used for ethanol were similar to that for sugar except for different flow rate (0.4 ml/minutes) and temperature (42°C). At the end of the experiment, sugars produced from each treatment were fermented for 48 hours using Saccharomyces cerevisiae to ascertain the fermentability of the sugars produced into ethanol in an incubator and a 2.0 liters BioFlo 110 modular bench-top reactor (New Brunswick Scientific Instruments).

4.3 Results and discussion

4.3.1 Characterizations of wood particles after pretreatments

Particle size distributions of ultrasonicated yellow-poplar residues were similar to those untreated with sound wave (Figure 4.1). The particles were predominantly of 12.0 microns in size with a mean of 37.0 ± 56 microns, and a range of 9.0- 400 microns, respectively (Figure 4.2). The water retention value (WRV) increased with increasing of ultrasonication time (Figure 4.3). The minimum WRV value observed was 90% for unsonicated wood, while the WRV for sonicated samples ranged from 100-160%. Although the values observed are over 100% lower than that previously reported for bleached pulp, however, the result still indicated an increasing trend with ultrasonication time in conformity with the previous study on lyocell fibers (Cheng et al. 2007). The increasing WRV trend observed here is an indication that proportions of the wood particles were reduced in size (Cheng et al., 2007).

4.3.2 Hydrolysis of pretreated wood particles

It was generally observed that during the chemical pretreatment that wood constituents were progressively hydrolyzed with increases in temperature (Figure. 4.4). It specifically indicates three major phases in wood depolymerization; low depolymerization phase (0-140°C), mid depolymerization phase (>140-160°C) and high-depolymerization phase (>160°C). At the high stage, wood depolymerization occurred at a much faster rate and correspondingly with the rate of temperature changes when compared with the first two phases. The observed hydrolysis ranged from 20-30% at low depolymerization phase, 45-60% at mid phase, and 60-90% for the high-phase for all category of wood within 0-10 minutes cook time. Decomposition of wood particles in the pretreatment chemical was more of a direct function of changes in cook temperature, time, and chemical, regardless of particle size or ultrasonication time.

Ultrasonication of wood did not significantly influence hydrolysis yield when compared to untreated wood (Figure 4.5). The reason why the particle size and ultrasonication did not significantly affect the degree of hydrolysis could be because hydrogen peroxide aided by temperature was able to penetrate and breakup the 1, 4- β -glycosidic and phenol-phenol bonds in cellulose and lignin polymers. Hydrogen peroxide, as a strong oxidizing agent, is the prime factor responsible for the increased depolymerization of wood holocellulose in this reaction as previously reported (Kim et al., 2000). During the reaction, some of hydrogen peroxide was converted to hydroxyl radical (equations 4.3-4.5) via an ionic pathway (Nascimento et al., 1995). These free hydroxyl radicals are the agents of the wood decomposition action of hydrogen peroxide (Rahmawati, 2005).



Anion (HOO^-) produced by hydrogen peroxide is a strong nucleophile and a major active agent in peroxide reactivity. The formation of hydroperoxide ($\text{HC}(\text{O})\text{OOH}$) was reported to be responsible for the breakdown of the complex aromatic lignin chains through internal rearrangement of lignin structures (Nascimento et al. 1995). The combined effect of heat and hydrogen peroxide on lignin removal as evaluated after the treatments is presented in figure 4.6. The result indicated low reduction in lignin content with increasing cook temperature when compared with the original lignin content and those previously reported (Adebola et al. 2009).

Holocellulose content of wood was the most significantly reduced wood content when compared to the rate of lignin removal. This result shows that hydrogen peroxide treatment in combination with temperature have stronger affinity for carbonyl groups removal in wood than phenolic groups in wood lignin.

The analysis of the liquid hydrolytes after the hydrolysis failed to show the presence glucose. A further analysis of the liquid extract with mass spectrometer indicated predominantly the presence of high molecular weight sugars with molecular mass to charge (m/z) ratio range from 214 to 795.5 m/z (figure 4.6). The most abundant of these sugars is $C_{21}H_{43}O_4$ with a molecular weight of 359.3 m/z, followed by an unknown compounds with a molecular weight 739.6 m/z, $C_{30}H_{59}O_3N_{10}$, $C_{19}H_{37}O_2N_6$.

4.3.3 Enzyme hydrolysis and fermentation

The ACCELLERASE™ 1000 enzyme complex converted pretreated yellow-poplar residues and hardwood pulp into glucose. The endoglucanases (endo -1, 4- β -glucanase) contained in the cellulase enzyme assist in breaking down crystalline regions of cellulose fiber, exoglucanase (exo-1,4- β -glucanase) removes cellobiose from the cellulose polymer, while beta-glucosidases convert cellobiose into glucose (Genencor, 2008). The pattern of substrate conversion was illustrated after 72-hour cook time duration (figure 4.7). Digestion of cellulose was low in treated and untreated wood (15-42%) when compared to bleached hardwood pulp (80%). The reason for lower conversion of pretreated and untreated wood can be attributed to the higher lignin content in wood (figure 4.8) as previously reported that wood hydrolysis increases with high delignification (Kim et al., 2000). This conclusion was further strengthened by the

observation of higher digestion of a low-lignin content (about 1%) hardwood pulp fiber, which indicated that lignin is a major inhibitor to the enzymatic digestion of wood with ACCELLERACE™ 1000 enzyme.

Glucose production varied among five substrates and across three levels of ACCELLERACE™ 1000 enzyme (Tables 4.1, 4.2, and figure 4.9). Glucose yields achieved from pretreated yellow-poplar residue (substrates Y120 and Y140) at 0.1 ml enzyme loading, pH 4.5 for 24 hours duration were 83.81 mg/ml and 41.90 mg/ml respectively, while those from pulp ranged from 18.86-30.38 mg/ml. The highest glucose yield was obtained from substrate “Pulp-1” at 111.54 mg/ml at 0.3 ml enzyme loading and 72 hours cook time. A comparable glucose yield was also obtained from pretreated yellow-poplar residues (Y120, Y140) with pulp at 0.2 enzyme loading for 72 hours of cook time. Sugar production increased significantly with increase in enzyme loading during the 24 hours cook time for most of the substrates used, except for substrate (Y120). The variability existed in the glucose yield can be ascribed to the specific nature in which enzyme functions. Enzymes could be very specific during wood hydrolysis reactions. Their activities could be easily impaired by substrate morphology (i.e. surface area, differences in size distribution) (figure 4.1). Chemical bonds resulting from previous treatments, phenol-phenol bonds in lignin, concentration, low accessibility of enzyme to substrate active sites, and reaction conditions-pH and temperature (David and Fornasier, 1986) were also observed in this study. Specifically, the glucose yield from Pulp-2 was lower than that from other substrates because Pulp-2 contained significantly more residual sodium chlorite during the delignification process.

The effect of pH reduction from pH 5.4 to 4.0 was as evidenced by significant decrease in sugar production in all the substrates. The average differences by comparing “pH to pH”,

“substrate to substrate”, and “enzyme level to enzyme level” for 24, 48, and 72 hours of cook time duration ranged from 4-83% of decreases in glucose production (Table 4.2). The decreases of glucose yields could be due to the deactivation of ACCELLERACE™ 1000 enzyme by formation of inhibitors (David and Fornasier, 1986).

It has been observed that glucose production increases at an increasing rate for a short time to reach a maximum during enzymatic hydrolysis but approaches zero for about 24 hours (David and Fornasier, 1986). Similarly in this study, the effect of cook time across the various treatment combinations indicated in general a decreasing trend of sugar production after 24 hours. The only exception to this general trend was in substrates Y120 and Pulp-1. Glucose production rate after 24 hours (i.e. 48-72 hours) at 0.1 ml enzyme loading ranged from low to zero for most substrates (Table 4.2). This is an indication of low reactivity of ACCELLERACE™ 1000 enzyme due to depletion of the initial dose applied on the substrates. Another possible reason for the low glucose yield could be due to the cellobiose formation during enzymatic hydrolysis (Wu and Lee, 1997) and difference in cellulose concentration to sugar production as previously reported that lower cellulose loading results in higher sugar yield (Elwyn, 1955). All substrates with corresponding enzyme loading, pH, and time indicated that the glucose produced can be fermented to ethanol with *Saccharomyces cerevisiae* as indicated in figure 4.10.

To improve the conversion efficiency of the pretreated yellow poplar from this study, we propose to use a new model entitled “**H**emicelluloses hydrolysis, **c**yclic hydrogen peroxide pretreatment (or any other viable pretreatment), and **e**nzyme replacement (**HeCyPE**).” This model involves a prior extraction of hemicelluloses which constitutes about 25-50% of wood (Sun and Cheng, 2002) using an effective hemicelluloses hydrolysis method like dilute acid

hydrolysis, followed by repeated hydrogen peroxide pretreatment/ enzymatic hydrolysis (figure 4.11). The replacement of enzyme at predetermined period (24-hour) needs to be conducted to recover glucose sugar from wood cellulose that constitutes about 40-50% of wood (Sjostrom, 1993; Sun and Cheng, 2002; Adebola et al., 2009). Dilute acid hydrolysis has been proven to be very effective in hemicellulose sugars recovery up to 85% of xylose (Maloney et al., 1984). By combining these two approaches, pentose and hexose sugar recovery could be maximized from yellow-poplar residues.

4. 4 Conversion efficiency and future of bio-energy conversion process

Assuming 1000 Ib of biomass waste produced through photosynthesis, by considering the three main conversion pathways (Direct combustion of biomass to generate electricity, Pyrolysis of biomass to bio-oil, charcoal, and gases, and Pretreatment/ enzymatic hydrolysis of wood/fermentation to ethanol) (Figure 4.12), the most efficient approach would be pyrolysis and direct combustion process with about 100% conversion efficiency of initial biomass. Fast pyrolysis of biomass was found to produce 60-75 %, 15-25 %, and 10-20 % of bio-oil, charcoal, and noncondensable gases respectively without waste (Dinesh et. al. 2006). Pyrolysis and direct combustion of wood is about 70% more efficient than the ethanol conversion rout where about 25% of lignin and 35% of yeast waste (after fermentation) is generated.

Although the higher heating value of conventional fuel (ethanol, petroleum etc) is about 40% higher than pyrolysis oil (10,000 - 13,000 Btu/lb (about 26 MJ/kg)) compared to 18,000-19,000 Btu/lb (42-44 MJ/kg), but by adding the heat values of the other pyrolysis product; charcoal (11,000-13,500 BTU/lb) and gases (200-500 BTU/scf) (Knight et al. 1976), a

comparable or higher energy would be obtained from pyrolysis process. Another advantage of wood pyrolysis process is its simplicity and the potential to produce a spectrum of products that can be utilized for transportation, cosmetics, pharmaceuticals and other chemical industries. Conversion of wood through direct combustion is the simplest conversion method but heat generated per unit mass (7,000-8,000 BTU/lb)(Adebola et al. 2009) is significantly lower than energy generated from pyrolysis products and ethanol. Its end product is also limited to heat and electricity generation thus making this approach a less suitable biomass utilization process to meet diverse energy uses.

The likely question we would face in the nearest future on the appropriate biomass conversion process would be largely driven by one question; which is how can we efficiently utilize available biomass to meet our diversified energy and chemical needs? This question is an indirect comparison between single product (combustion-electricity, fermentation-ethanol) versus multiple products (pyrolysis) to meet diversified energy needs.

4.5 Conclusions

The evaluation of the effects of ultrasonication, wood particle size, and green chemical at various temperatures and times indicated that increase in WRV with time increased microfibrillation of yellow-poplar residues. However, this outcome failed to show any significant effect on wood pretreatment and subsequent hydrolysis at higher temperatures.

It was generally observed that during the chemical pretreatment wood constituents were progressively hydrolyzed with increase in temperature. The observed hydrolysis rate ranged from 20-90% for all categories of wood samples within 10 minutes of cook time. Hydrolysis of wood

particles in the pretreatment chemical was directly proportional to temperature and time, regardless of particle sizes. HPLC analysis of the extracts indicated no glucose molecules, while mass spectrometric analysis showed a spectrum of high molecular weight sugars.

Pretreatment of yellow-poplar residues had significant impact on glucose sugar release during enzymatic hydrolysis because glucose produced from chemically treated residues at 120°C in MARS showed comparable yield with a lignin free commercial pulp. Production of glucose was predominantly influenced by the nature of substrate, substrate morphology, pH, cook time and enzyme loading.

The limitation of the methodology used in this study is that, at increased pretreatment temperature, wood was increasingly hydrolyzed into a host range of sugars that are not fermentable. However, the potentials of this method is that the chemical used in the pretreatment is green with minimal environmental impacts. It does not require the use of toxic, non-environmental friendly inorganic chemicals for lignin treatment prior to enzymatic hydrolysis. Application of microwave technology is faster than most conventional heating sources and is capable of ensuring deeper penetration of pretreatment chemicals at wood sorption sites. The new conversion model (HeCyPE) is proposed with the potential to increase wood sugar recovery to the maximum theoretical yield.

References

- Adebola, B.A., Wang, J., Dawson-Andoh B. E., McNeel, J. Armstrong, J.P, 2009. Assessment of Appalachian hardwood residue properties and potentials for bioenergy utilization. *Wood and Fiber Science*. 41(1), 74-83.
- Anton Paar Instruments.2009. www.anton-paar.com. Last accessed on May 14, 2009.
- Cheng, Q., Wang, S., Han, Q., 2009. A novel method to isolate fibrils from cellulose fibers by high intensity ultrasonication. Part II: Fibril characterization. *Journal of Applied Polymer Science*. 115, 2756–2762.
- Cheng, Q., Wang, S., Rial, T.G., Lee, S.H., 2007. Physical and mechanical properties of polyvinyl alcohol and polypropylene composite materials reinforced with fibril aggregates isolated from regenerated cellulose fibers. *Cellulose*. 14,593–602.
- David, C. and R. Fornasier, 1986. Utilization of Waste Cellulose. Kinetic Study of the Enzymatic Hydrolysis of Spruce Wood Pretreated by Sodium Hypochlorite. *Macromolecules*. 19, 552-557.
- Dinesh Mohan, Charles U. Pittman, and Philip H. Steele. 2006. Pyrolysis of Wood/Biomass for Bio-oil: A Critical Review. *Energy & Fuels* 2006, 20, 848-889.
- Don, S. Reed, J., Dewitt, P., 2007. Investors Focus on Opportunities In Cellulosic Ethanol Production. *Pulp and Paper*.
http://findarticles.com/p/articles/mi_qa3636/is_200705/ai_n19431588,
Last accessed on Jan. 10, 2009.

- Genencor, 2008. Accellerace™ 1000, Cellulase enzyme complex for lignocellulosic biomass hydrolysis. Technical Bulletin #1 Saccharification. www.genencor.com. Last accessed May 18, 2008.
- Kim, S.B, Yum, D.M. and Park, S.C., 2000. Step-change variation of acid concentration in a percolation reactor for hydrolysis of hardwood hemicelluloses. *Biores. Technol.* 72, 289-294.
- Kim H. and Lee J.W., 2002. Effect of ultrasonic wave on the degradation of polypropylene melt and morphology of its blend with polystyrene. *Polymer.* 43,2585-2589.
- Kitchaiya, P., Intanaku, P. and Krairiksh, M., 2003. Enhancement of Enzymatic Hydrolysis of Lignocellulosic Wastes by Microwave Pretreatment Under Atmospheric Pressure. *Journal of wood chemistry and technology.* 23, 217–225.
- Knight, J. A.; Bowen, M. D.; Purdy, K. R. Pyrolysis. 1976. A method for conversion of forestry wastes to useful fuels. Presented at the Conference on Energy and Wood Products Industry, Forest Products Research Society, Atlanta, GA, 1976.
- Lynd, L.R., Cushman, J.H., Nichols, R.J., and Wyman, C.E., 1991. Fuel ethanol from cellulosic biomass. *Science* 251, 318-323.
- Mark, T. Maloney, Thomas, W. Chapman, Andrew J.B., 1984. Dilute acid hydrolysis of paper birch: Kinetics studies of xylan and acetyl-group hydrolysis. *Biotechnology and Bioengineering.* Vol. 27, Issue 3, 355-361.
- Mussatto, S.I., Dragone, G, Roberto, I.C., 2008. Hydrogen peroxide bleaching of cellulose pulps obtained from brewer's spent grain. *Cellulose.* 15,641–649.

- Minami, E. and Saka, S., 2002. A comparative study of decomposition of behaviors between hardwood and softwood in supercritical methanol. *J. Wood Sci.* 49,73-78.
- Nascimento, E.A., Antonia, E.H., and Morais, S.J., 1995. Photochemical hydrogen peroxide bleaching of eucalyptus organosolv pulp. *Chem. Soc.*6, 365-371.
- Office of Technology Assessment, 1993. Potential environmental impacts of bioenergy crop production. Background Paper TOA-BP-E-118. U.S. Congress, OTA, Washington, D.C. 71 pp.
- Perlack, R.D., L. L. Wright, A.F. Turhollow, R.L. Graham, B.J. Stokes, and D.C. Erbach, 2005. Biomass as feedstock for bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. A Joint Study Sponsored by U.S. Department of Energy and U.S. Department of Agriculture. 78pp.
- Rahmawati, N, Ohashi, Y., Honda, Y., Kuwahara, M., Fackler, K., Messner, K, Watanabe T., 2005. Pulp bleaching by hydrogen peroxide activated with copper 2,2'-dipyridylamine and 4-aminopyridine complexes. *Chem. Eng J.*112,167–171.
- Saariaho A, Skelainen A, MARI M, Nuopponen, and Vuorinen T., 2003. "Ultra violet resonance Raman spectroscopy in lignin analysis: Determination of characteristic vibrations of p-Hydroxyphenyl, guaiacyl, and syringyl lignin structures," *Appl. Spectrosc.*57,58–66.
- Selig M, Weiss, N., and Ji, Y., 2008. Enzymatic saccharification of lignocellulosic biomass, Technical report NREL/TP-510-42629. 7p.
- Sherrard, E.C., Kressman, F.W., 1945. Review of processes in the United States prior to World War II. *Ind. Eng Chem.* 37,5–8.
- Sjostrom, E., 1981. *Wood chemistry: Fundamentals and applications.* Academic Press,

- New York, NY. 300 pp.
- Templeton, D, Ehrman, T., 1995. Determination of acid-Insoluble lignin in biomass. Laboratory Analytical Procedure No.003, National Renewable Energy Laboratory. Golden, CO.1995.
- Wang, J, Grushecky S, McNeel J., 2006. Biomass resources, uses, and opportunities in West Virginia. Division of Forestry and Natural Resources, West Virginia University, Morgantown, WV. 103 pp.
- Wang, M., 2005. "Updated Energy and Greenhouse Gas Emissions Results of Fuel Ethanol." Argonne National Laboratory. September 26-28.
- Wang, S., Cheng, Q., 2009. A novel method to isolate fibrils from cellulose fibers by high intensity ultrasonication. Part I: Process optimization. *Journal of Applied Polymer Science*. 113, 1270–1275.
- Wu, Z. and Y.Y. Lee, 1997. Inhibition of the enzymatic hydrolysis of cellulose by ethanol *Biotechnology Letters*, 19, 977–979.
- Yoshikuni, T., Tanaka, N., Seung-Hwan, L., Takashi, E., 2008. Pretreatment of Eucalyptus Wood Chips for Enzymatic Saccharification Using Combined Sulfuric Acid-Free Ethanol Cooking and Ball Milling. *Biotechnology and Bioengineering*, 99, 75-85.
- Yuhuan, L., Roger R., Xiangyang, L., Fei, Y., Paul, C., Shaobo, D., Yuhong, L., Vance, M., Tom, Y., 2005. Preparation ion of biopolymers from liquefied corn stover. *Transactions of the CSA E*. 21, 116-120.
- Zhu, S. Wu, Y., Yu, Z., Zhang, X., Li, H. Gao, M., 2005. Enhancing enzymatic hydrolysis

of rice straw by microwave pretreatment. *Chemical Engineering Communications*, 192, 1559–1566.

Table 4.1 Sugar production and fermentability potentials of ACCELLERACETM 1000 enzyme treated yellow poplar residue at 0.1-0.3 ml product /glucose, 4.5 pH level, 50⁰C for 72 hours.

Time	Enzyme(ml)	Sugar production(mg/ml)				
		Y120	Y140	Pulp-1	Pulp-2	Pulp-3
Lignin (%)	0.0	18	18	3	3	1
24 hrs	0.1	83.81	41.90	18.86	30.38	78.97
	0.2	17.70	54.58	53.43	15.77	84.49
	0.3	72.81	63.75	23.62	35.10	90.72
48 hrs	0.1	83.81	14.07	23.36	41.90	93.27
	0.2	109.16	12.92	25.53	45.15	99.52
	0.3	96.97	14.21	23.01	42.60	99.17
72 hrs	0.1	70.19	14.21	23.01	42.60	103.99
	0.2	101.20	101.55	101.34	14.98	106.58
	0.3	48.92	23.99	111.54	15.78	109.20
Digestion (%)	0.0	26.83	26.33	59.83	58.70	84.67
Fermentability	0.0	+	+	+	+	+

+ indicates that ethanol was produced from sugars resulting from enzymatic hydrolysis

Table 4.2 Sugar production and fermentability potentials of ACCELLERACETM 1000 enzyme treated yellow poplar residue at 0.1-0.3 ml product /glucose, 4.0 pH level, 50⁰C for 72 hours.

Time	Enzyme(ml)	Sugar production(mg/ml)				
		Y120	Y140	Pulp-1	Pulp-2	Pulp-3
Lignin (%)	0.0	18	18	1	1	1
24 hrs	0.1	14.25	13.62	4.30	7.33	59.89
	0.2	16.40	18.44	27.81	29.60	81.50
	0.3	72.81	63.62	23.60	35.10	85.75
48 hrs	0.1	18.93	22.59	42.78	32.27	87.41
	0.2	95.02	19.28	32.13	32.30	92.23
	0.3	92.75	15.96	35.13	36.56	93.90
72 hrs	0.1	39.61	22.94	15.78	35.17	99.84
	0.2	108.60	10.00	41.45	38.00	104.03
	0.3	102.28	18.37	40.37	42.30	90.27
Digestion (%)	0.0	40.67	48.67	58.50	71.33	82.00
Fermentability	0.0	+	+	+	+	+

+ indicates that ethanol was produced from sugars resulting from enzymatic hydrolysis

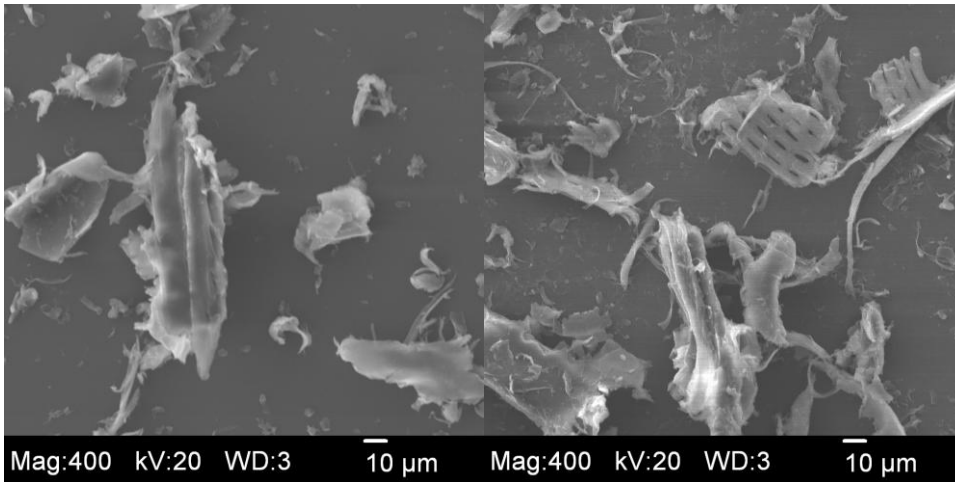


Figure 4.1 SEM (Hitachi S-4000) image of particle size distribution of untreated (left) and ultrasonicated (right) yellow-poplar residue before chemical treatment in microwave accelerated system (MARS).

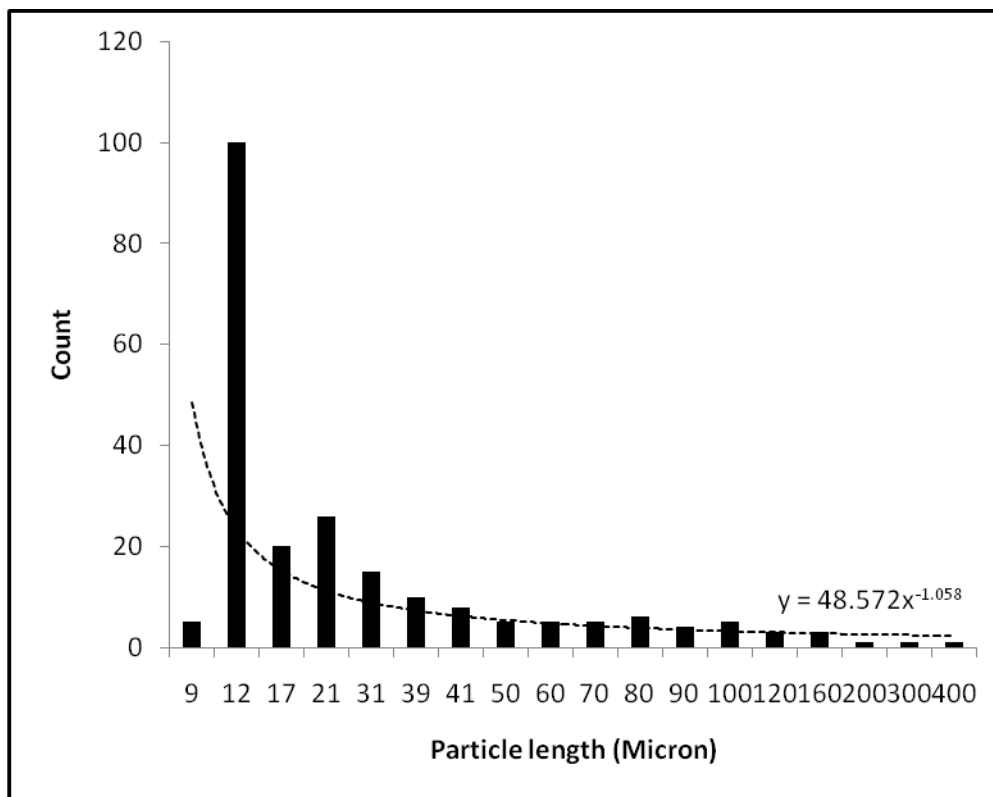


Figure 4.2 Particle size (length) distribution of 80 minutes ultrasonicated yellow-poplar residue before chemical treatment in microwave accelerated system (MARS).

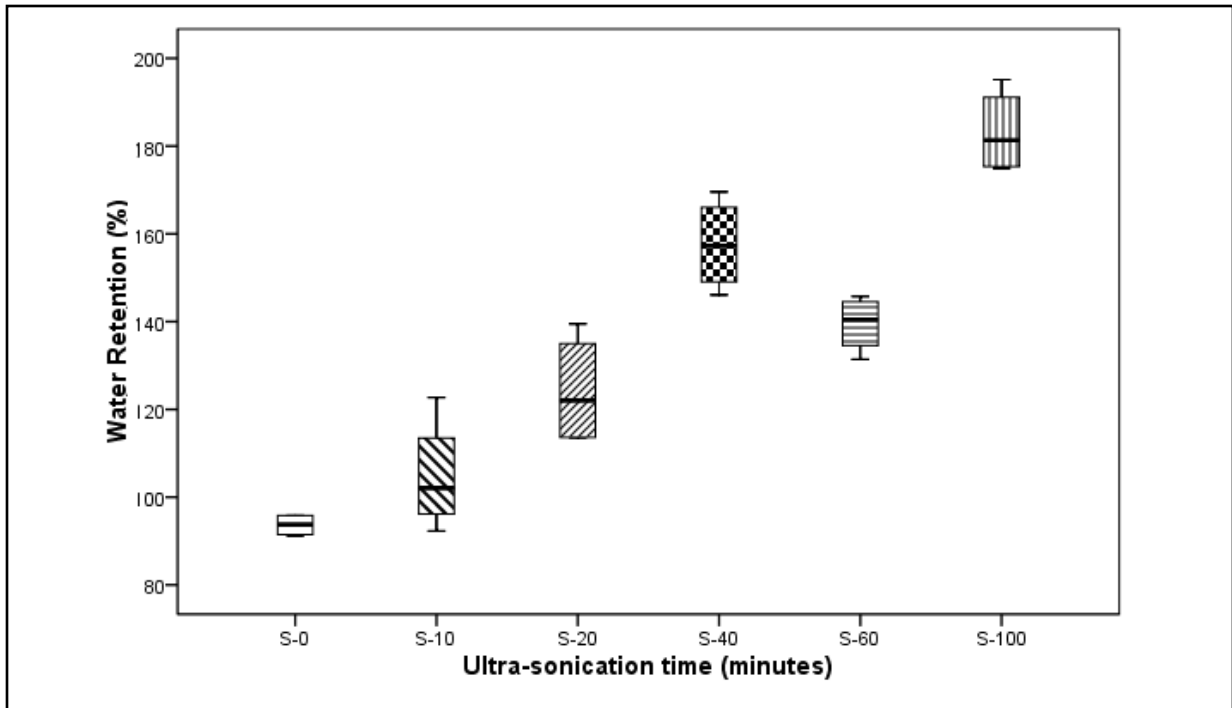


Figure 4.3 Increased micro-fibrillation of yellow poplar residue as measured with water retention value over ultrasonication time.

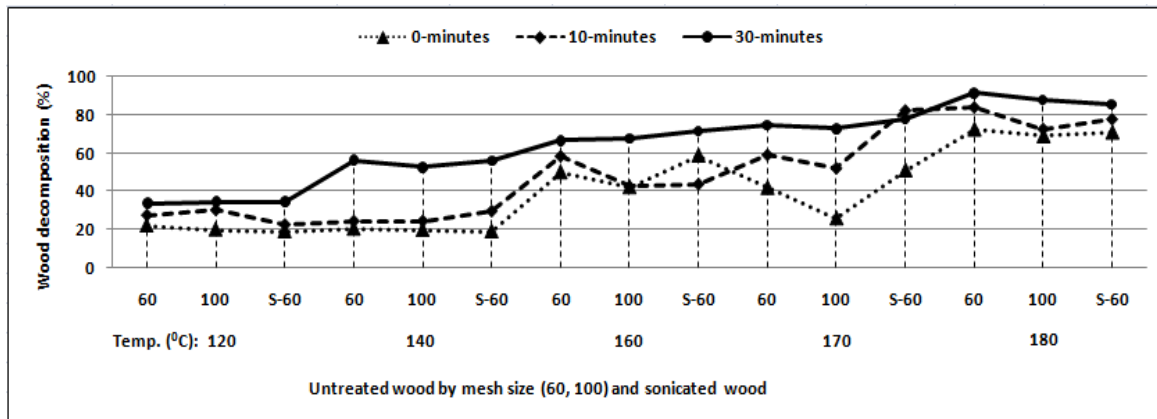


Figure 4.4 Wood residue decomposition as a function of cook temperature, time, ultra-sound and mechanical pretreatment.

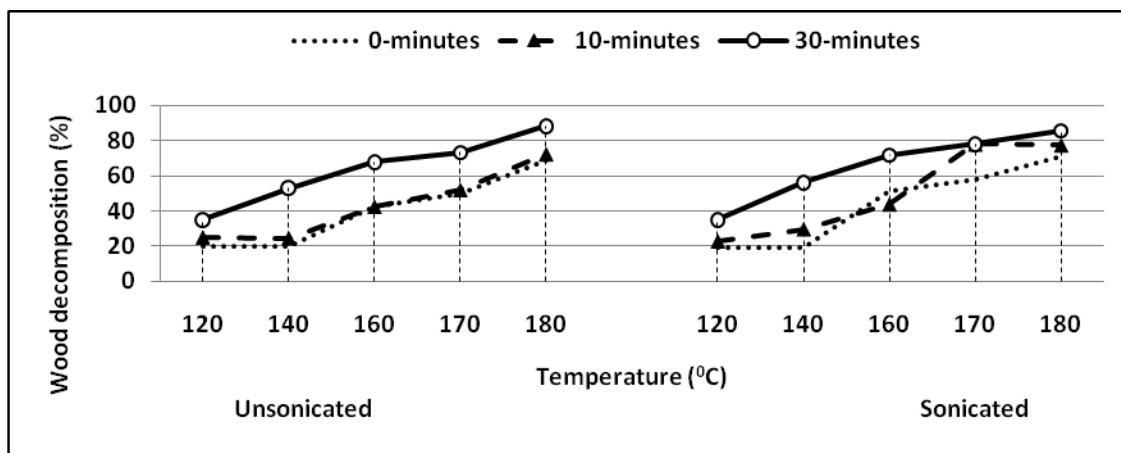


Figure 4.5 Comparison between ultrasonic and mechanically treated wood particles with changes in cook temperature and time.

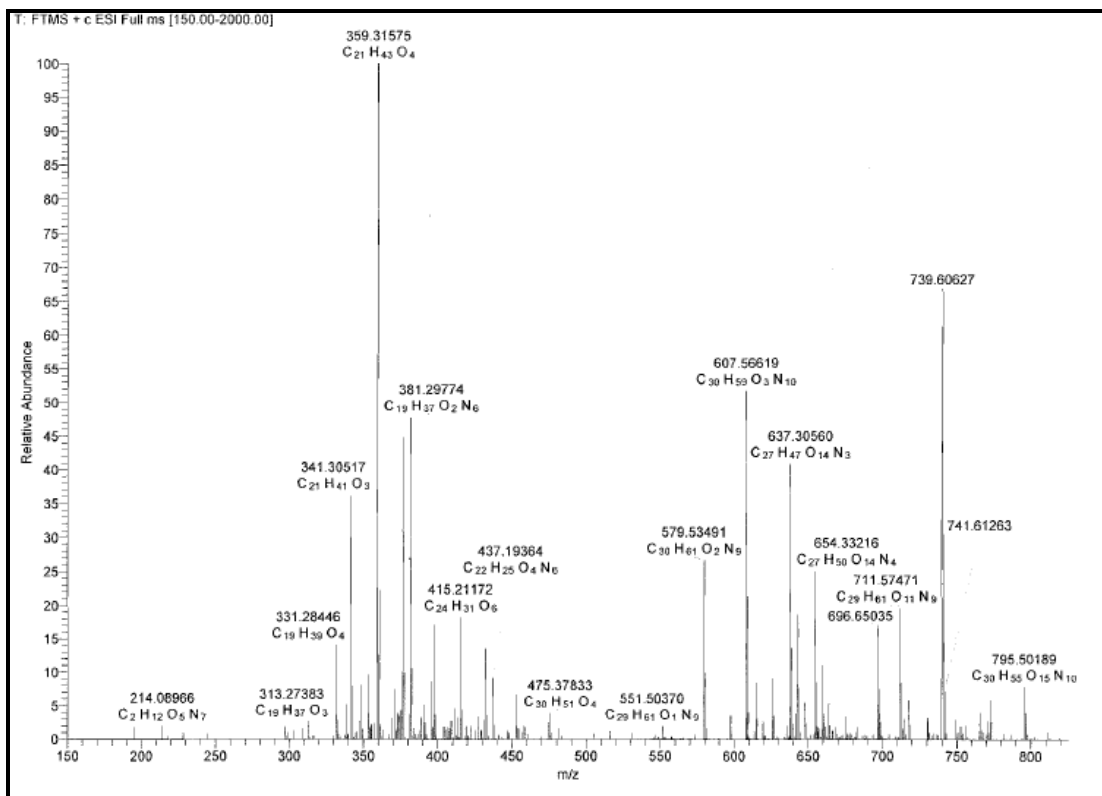


Figure 4.6 FT-Mass spectrometric analysis of yellow-poplar residue after hydrogen peroxide treatment inside microwave accelerated reaction system (MARS).

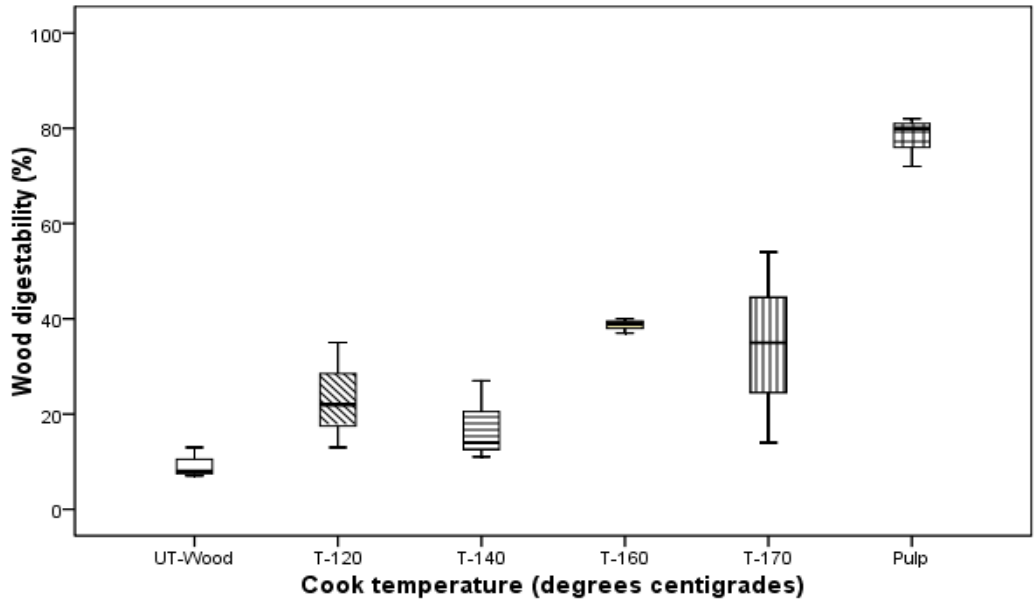


Figure 4.7 Percentage digestibility of untreated (UT-wood)/treated (T-120 to T-170) yellow-poplar residue and cellulose using ACCELLERACETM 1000 at 0.2 ml product/g cellulose, 50°C and pH 4.0-4.5.

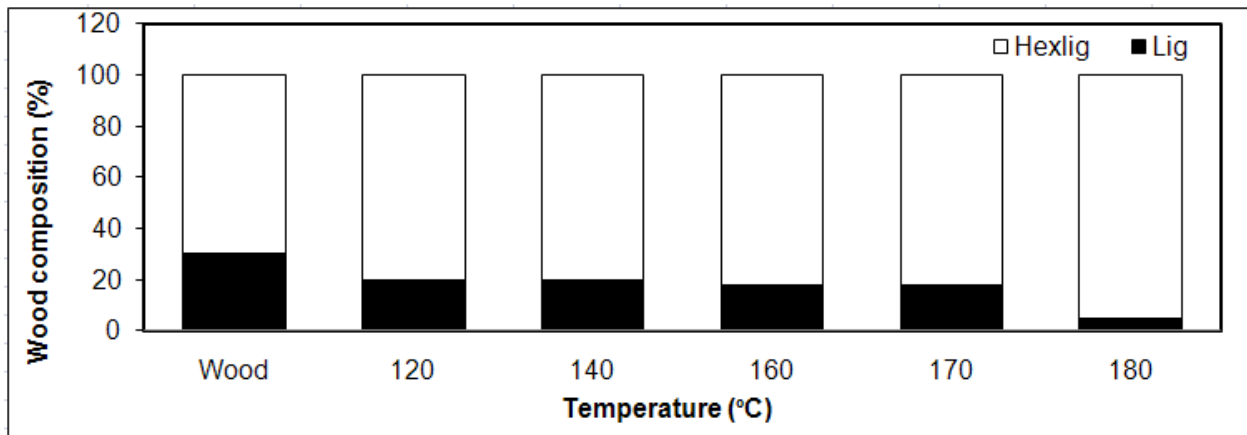


Figure 4.8 Simultaneous reduction in residual lignin (Klason soluble lignin) and hemicelluloses, extractives and soluble lignin (Hexlig) with increasing cook temperature.

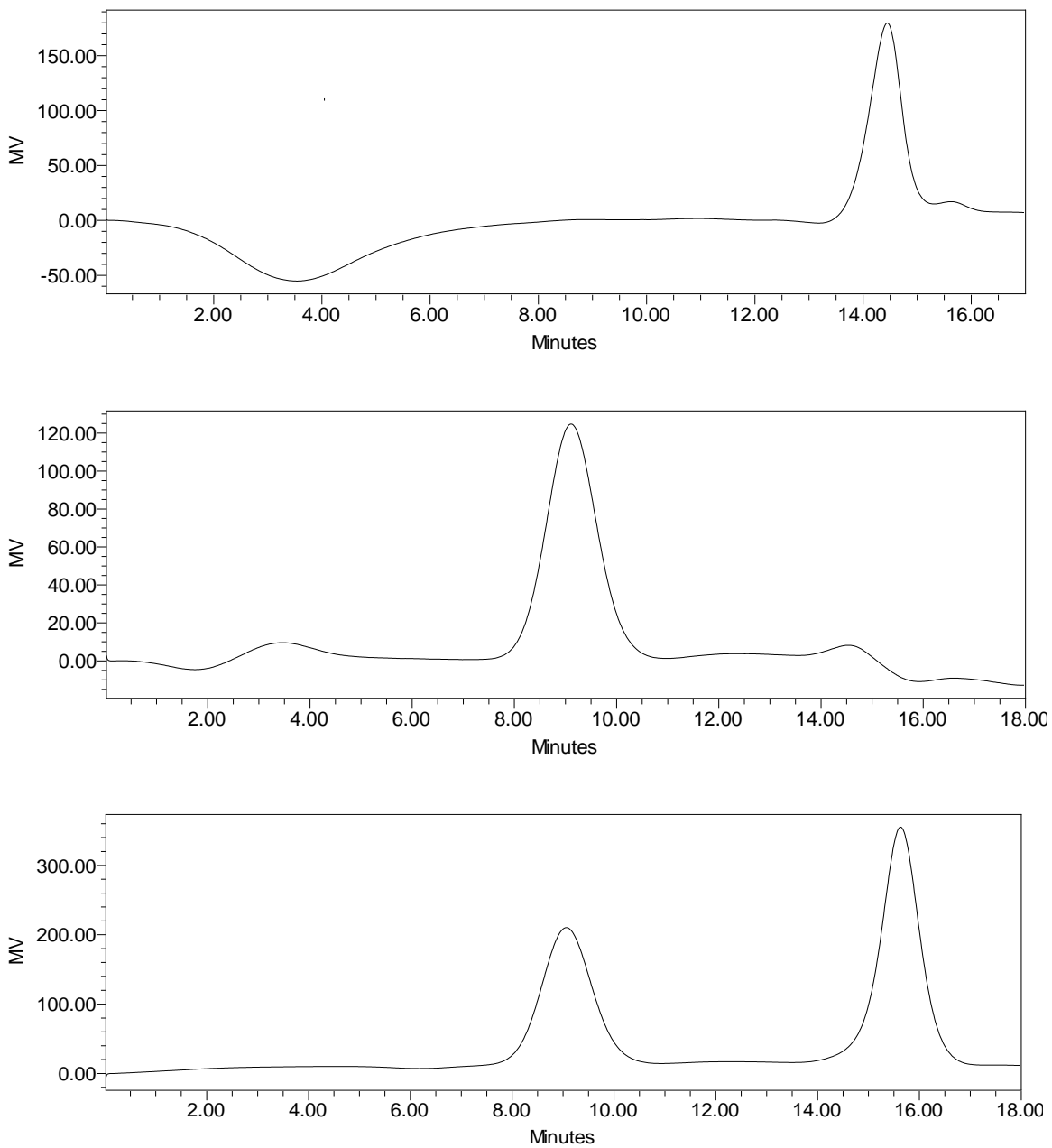


Figure 4.9 HPLC chromatograph of wood residue extracts after chemical pretreatment in microwave (top), standard glucose (middle), and glucose from ACCELLERACETM 1000 treated wood residue (bottom).

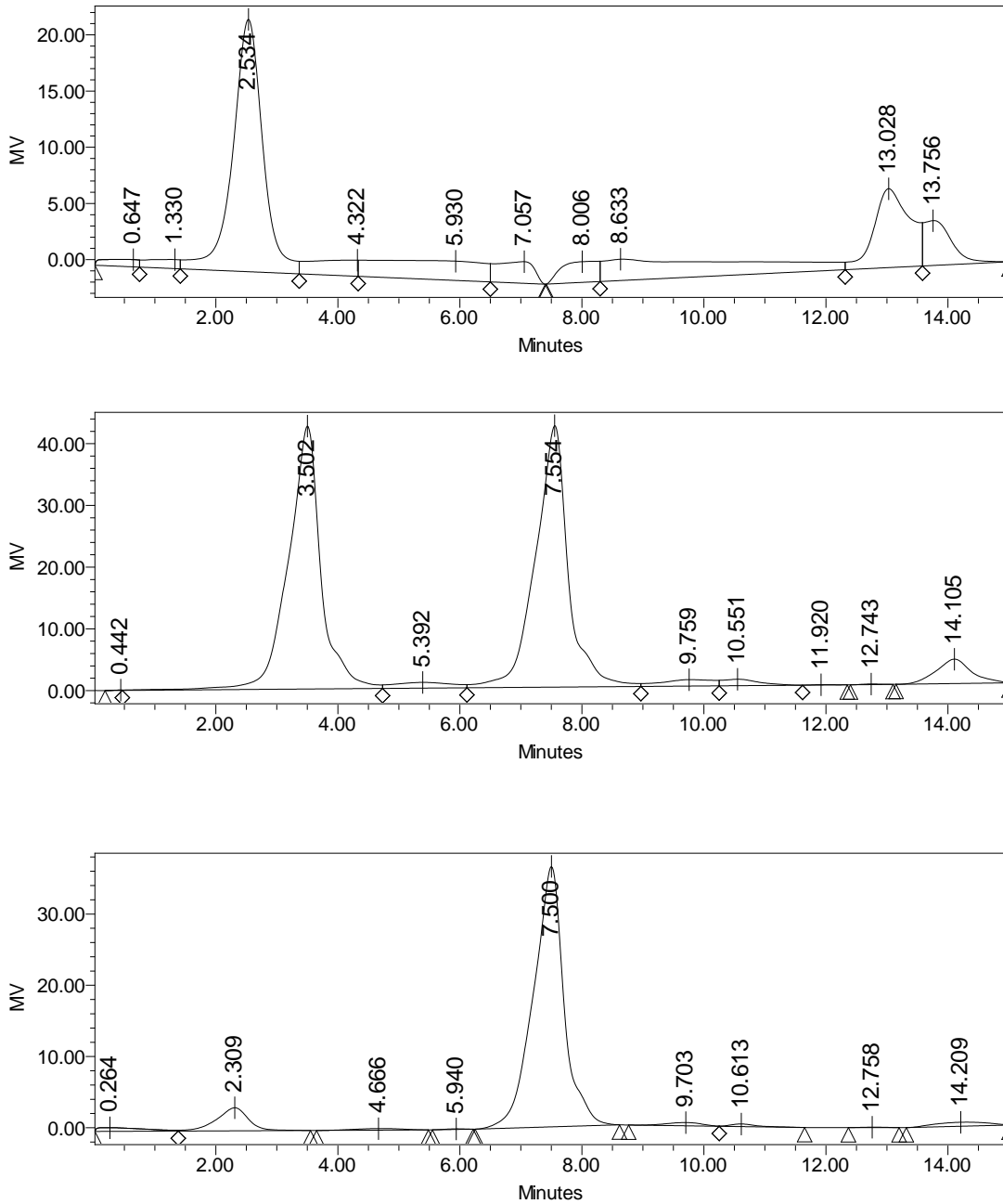


Figure 4.10 Aerobic fermentation of wood glucose to ethanol in BioFlo 110 reactor at 37°C for 48 hours. (top) standard ethanol, (middle) substrate Y120, and (bottom) pulp.

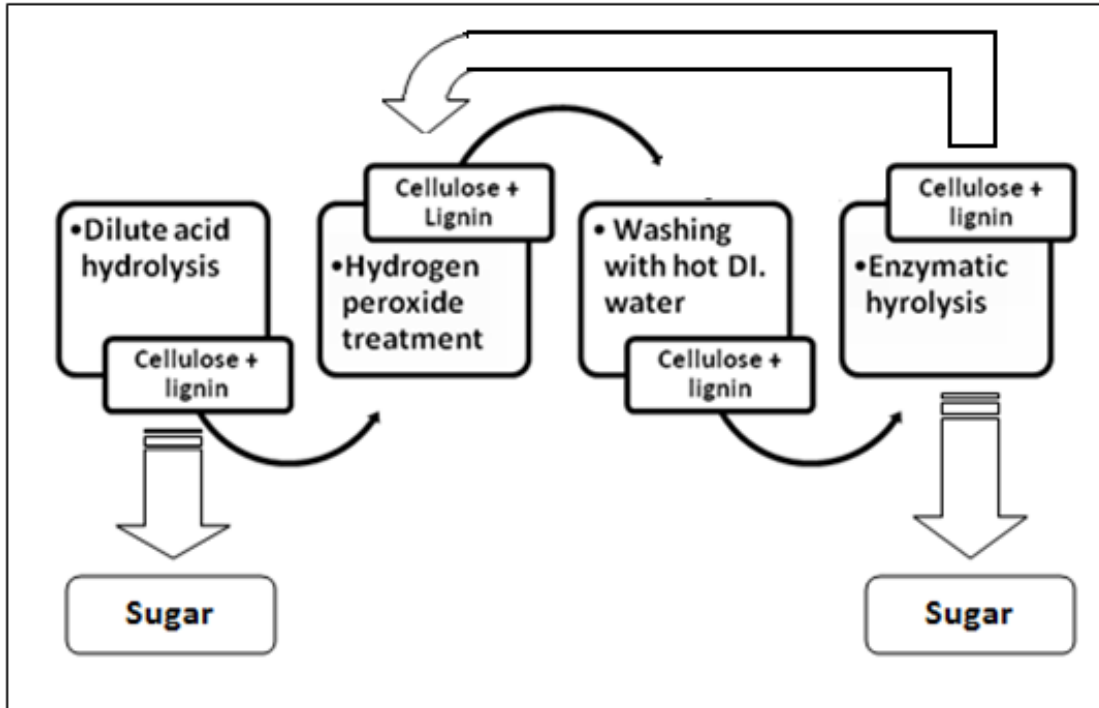


Figure 4.11 Improved sugar recovery model (HeCyPE) from pretreated yellow-poplar residue via enzymatic hydrolysis.

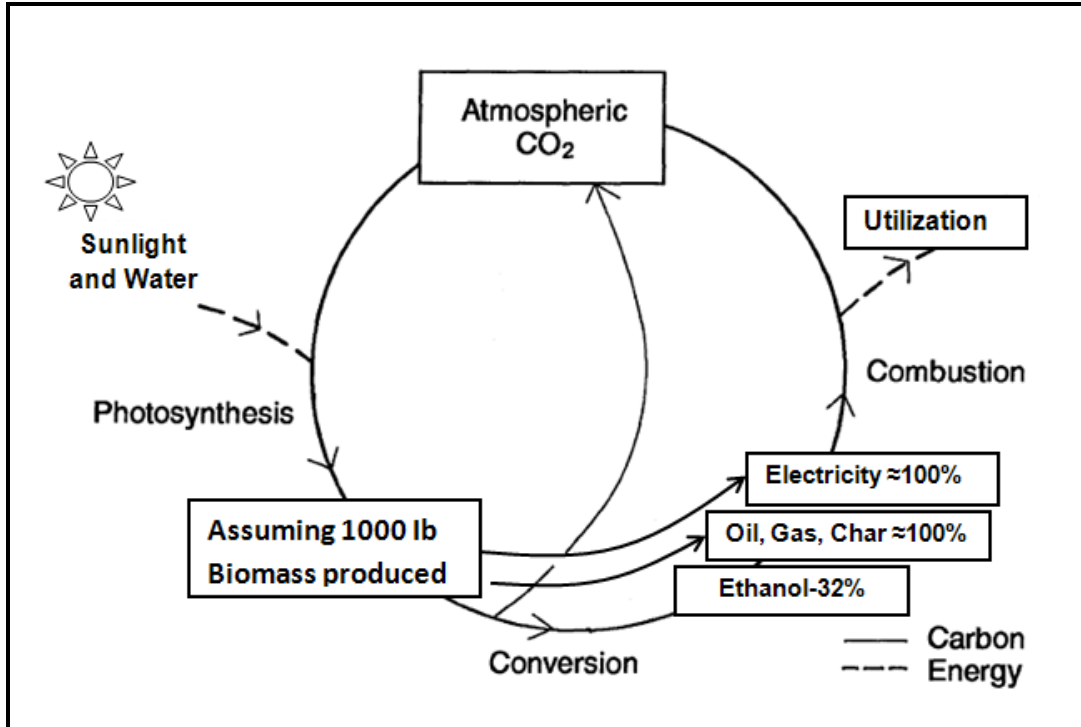


Figure 4.12 Carbon and energy flows of production and utilization of woody biomass. (Modified from Lynd et al. 2006).

CHAPTER 5: SUMMARY

Our results indicated that logging residues of yellow-poplar (*Liriodendron tulipifera*) and red oak (*Quercus rubra*) were dried on site to a moisture range of 7.4% to 39%. Chemical properties of yellow-poplar and red oak wood residues were less variable between heartwood and sapwood and across year of harvest, except in samples which experienced increased fungal decay due to length of exposure. Measured chemical properties of wood residues generally showed differences among species, sound, and decayed wood residues of yellow-poplar and red oak. Percentage lignin of decayed residues was higher than in sound undecayed wood residues. Higher extractive and lignin content at the expense of lower holocellulose content were observed in decayed (2005 harvest) sapwood residues of yellow-poplar and red oak woods.

Observed volatile content was about 80%; 20% for carbon and about 1% for ash content in both species, regardless of their decay condition. The heating values showed no significant differences between sapwood and heartwood of decayed and undecayed residues. The residues of the two hardwood species used in this study can be used as feedstock or serve as substitute species for the production of fermentable sugars, lignin, and synthetic gases (a raw material for the Fisher Tropsch process) for the production biofuels, bio-chemical products, and electricity generation through direct combustion of wood owing due to their comparable physical and heat related properties.

Results on thermochemical conversion of wood residues during ASO/PASO pretreatment indicated that PASO treatment degrade wood residue more than ASO treatment. Hydrolysis of wood during ASO/PASO pretreatment increased with increases in sodium hydroxide concentration over cook time. Of all treatment combinations, the highest hydrolysis rate

observed was with PASO treatment at 0.3g sodium hydroxide concentration and 6 hours cook time. All observed alkali treatments showed little or no effect on decomposition of wood lignin with low sugar yield. Sugar yield resulting from enzymatic hydrolysis of ASO, PASO treatments failed to show any significant difference between species and the condition of wood residue (sound versus decayed wood). 62% of the original ASO/PASO treated wood residue was converted to fermentable sugar, while over 80% of hardwood pulp fibers and paper wastes were hydrolyzed.

The effects of ultrasonication, wood particle size, and green chemical at various temperature and time have been evaluated in this study. Increase in WRV with time indicated increased microfibrillation of yellow-poplar residues. However, this outcome fails to show any significant effect on wood pretreatment and subsequent hydrolysis at higher temperatures. It was generally observed that during chemical pretreatment wood constituents were progressively hydrolyzed with increases in temperature. The observed hydrolysis ranged from 20-90% for all categories of wood within 0-10 minutes of cook time. Hydrolysis of wood particles in the pretreatment chemical was directly proportional to temperature and time, regardless of particle size. HPLC analysis of the extracts indicated no glucose molecules, while mass spectrometric analysis showed a spectrum of high molecular weight sugars.

Pretreatment of yellow-poplar residue had significant impact on glucose sugar release during enzymatic hydrolysis because glucose produced from chemically treated residue at 120°C in MARS showed comparable yield with a lignin free commercial pulp. Production of glucose was predominantly influenced by the nature of substrate, substrate morphology, pH, cook time and enzyme loading. Application of microwave technology is faster than most conventional heating sources and capable of ensuring deeper penetration of pretreatment chemicals at wood

sorption sites. The new conversion model (HeCyPE) is proposed with the potential to increase wood sugar recovery to the maximum theoretical yield.

APPENDIX A: FIELD DATA COLLECTION

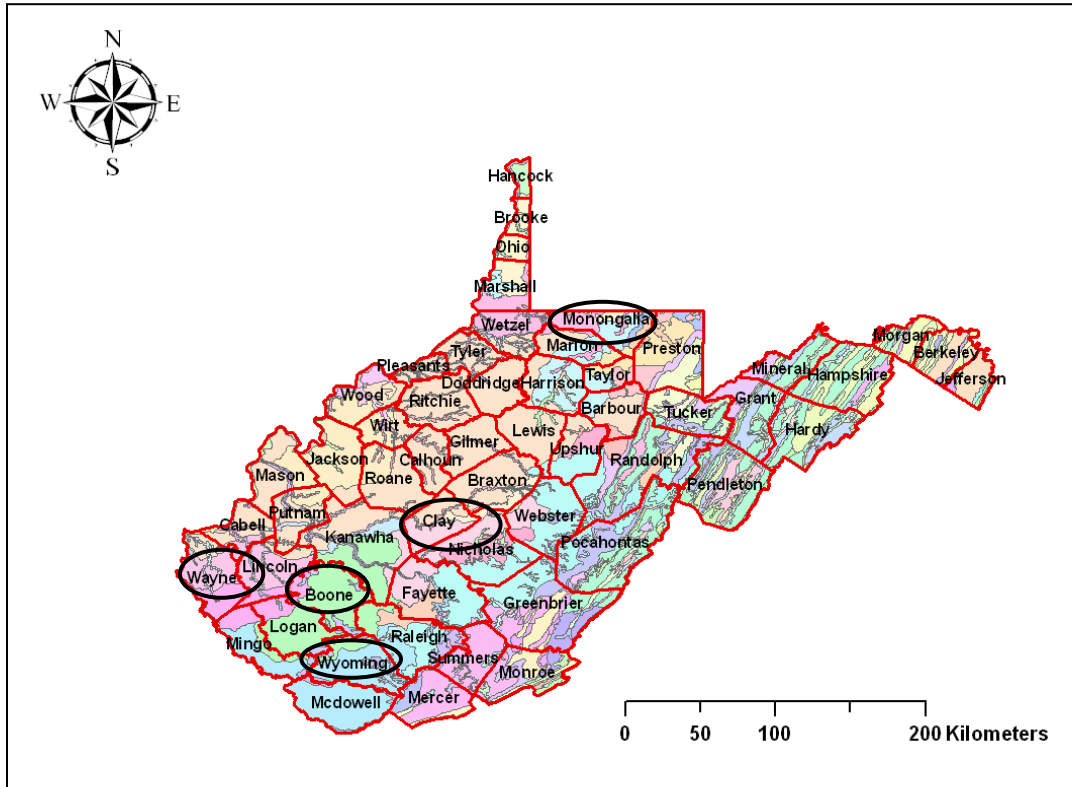


Figure A.1. West Virginia counties showing residue collection sites.

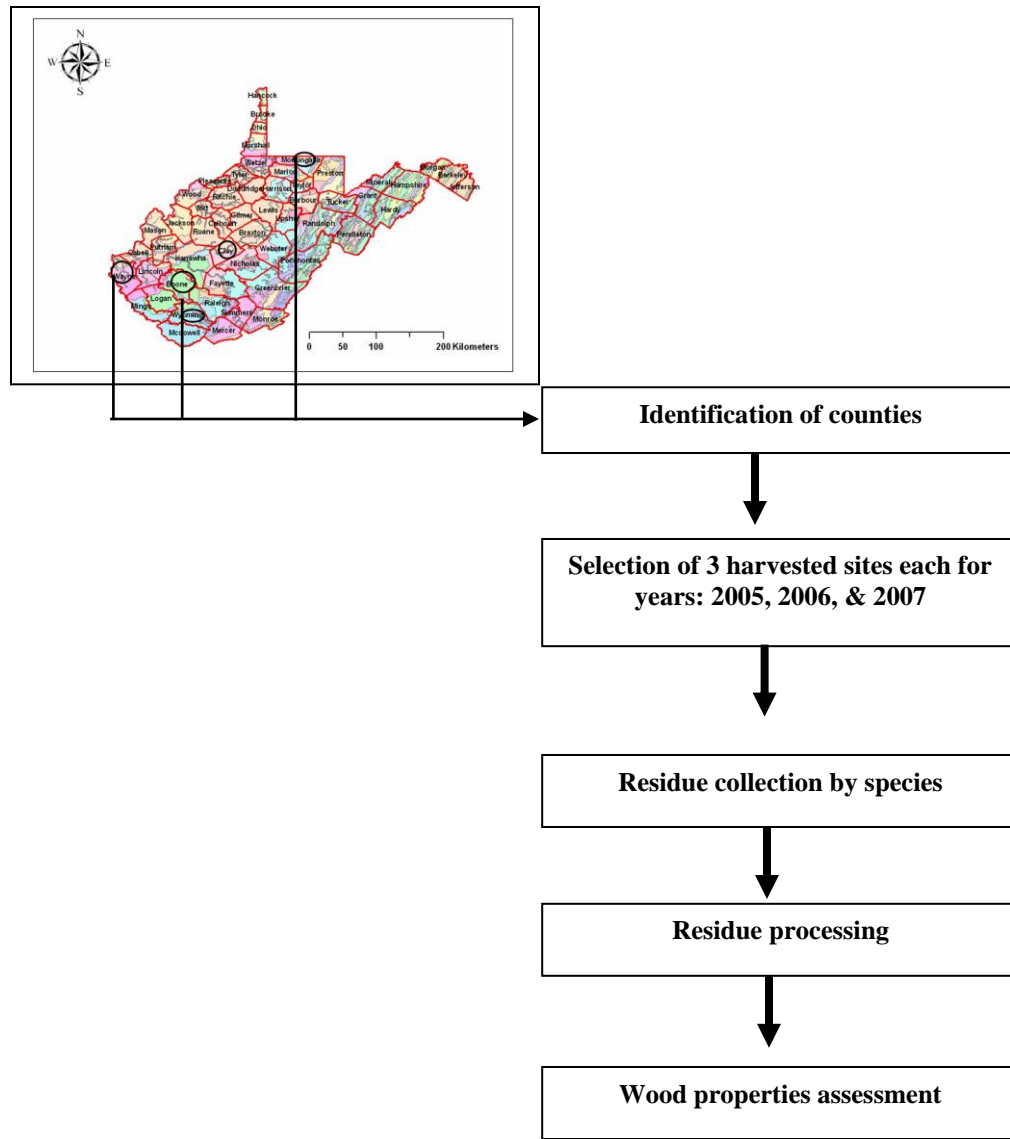


Figure A.2. Schematic diagram of logging residue collection procedures.

APPENDIX B: EXPERIMENT PROCEDURES



Figure B.1. Bomb calorimeter with accessories.

6300 Parr Bomb Calorimeter-For Heat value determination. (Source: http://www.parrinst.com/default.cfm?Page_ID=182. 2010).

B.1.1 Sample Preparation

1. Pulverize wood using a wood miller to produce particles with sizes between 80- 100mm mesh sizes and dry in the oven for 24 hours
2. Pellet dried samples using a manually operated pellet machine into 1-2 mm diameter tablets

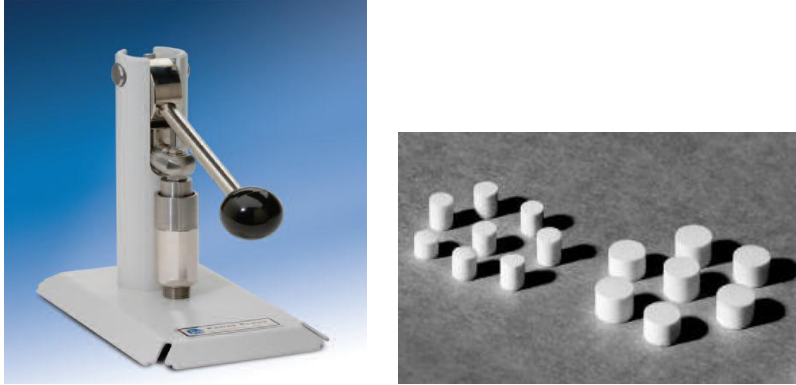


Figure B.1. Pellet press and prepared wood pellets for calorific value determination. (Source: http://www.parrinst.com/doc_library/members/2811.pdf. 2010).

3. Place wood pellets into the capsule and lower into the oxygen combustion chamber of the par bomb calorimeter and follow the under listed procedures on machine operation.

B.1.2 Par 6300 Operation

Par bomb calorimeter basic operation procedure is outlined below. Each step is accompanied with detailed instructions as outlined in the instruction manual of the machine for accurate operation of the machine.

1. Power up the machine by switch located at the rare end of the machine.
2. Allow the calorimeter computer to boot completely and switch the water pump of the machine directly on the display screen (touch screen interphone).

3. Allow the machine to warm up and the water bucket of the oxygen bomb filled with water. This may take between 30-1 hour. After this, the machine will prompt you to start operation through the activated start menu on its computer screen.
4. Run a pretest by clicking the pretest button to ensure that the machine is fully operational. (This will take about 15 minutes).
5. Run standard samples (about 20-25 benzoic acid tables) to get a good energy equivalent (EE) average value that will be used for wood heat value computations.
6. At the completion of the standards, insert the wood pellet (should be about 1g in weight).



Figure B.2. 500 Watt ultrasonic processor (VCX 750, Sonics & Materials, Inc., Newtown, CT).

For wood particle reduction to nano scale sizes. (Source: <http://www.sonics.biz/liquid-newsheet/VCX500-750.pdf>).

B.2.1. Operation

1. Soak powdered wood sample in water for 24 hours
2. Using the adjustable mount of the probe, set the tip of the ultrasonicator probe at the center of the beaker containing wood and water mixture.
3. On the instrument control/ monitor box, set amplitude at 80, time: (from one to several minutes).
4. Close the sound proof chamber of the ultrasonicator where the sample (wood and water) is located.
5. Press the start button to initiate machine operation for the set time



Figure B.3 Microwave assisted rapid system (MARS)-For variable heat application on wood and chemical mixture during pretreatment process MARS, product of CEM operations (Source: <http://www.cem.com/page86.html>).

B.3.1. MARS operation

MARS operation is similar to home based microwaves, with the exception that it come with a wide range of temperature from 0-300⁰C, Watts, (600-1600) and pressure modification for different applications.

B.3.2. Operation

1. Mix about 2-10g of wood with 20 ml of pretreatment chemical, shake to get a good mix.
2. Pure the mixture into the XP-1500 Plus™ or ESP-1500 Plus vessel, close, and insert vessel into the circular turn table inside the microwave chamber.

3. Set the process conditions; temperature, time, and watt using the buttons on the front panel of MARS machine.
4. Close the microwave chamber together with the samples and press the start button to activate MARS operation.
5. At the end of the experiment, allow microwave (MARS) to cool down, then remove samples for separation and HPLC analysis.



Figure B.4 Bioreactor (BioFlo 110, Fisher Scientific)-For enzyme and yeast cultivation during wood conversion to sugar and ethanol.

B.4.1. Operation and procedures

1. Consult the machine manual before and during the initial running attempt on the machine
2. Basic Procedures: Wash the fermentation chamber with water and sterilize in an autoclave at over 100⁰C for about an hour, after sterilization allow to cool down inside the autoclave before removal.
3. Power up the system and set the running condition (temperature, agitation, pH, and time) using the computer screen of the machine.
4. Pure pretreated wood samples or pulp or sugar (in case of fermentation) mixed with deionized water, enzyme or yeast (for fermentation), buffer (pH 4-5.5), and cycloexamide (only for enzymatic hydrolysis process). Bubble nitrogen gas through the mixture during anaerobic fermentation process for max ethanol yield.

5. Let the sample run in the bioreactor for the desired length of time (24-72 hours), collect liquid samples at different intervals for sugar or ethanol analysis.



Figure B.5 High Performance Liquid Chromatography (HPLC)-For Sugar and ethanol analysis.

(Source: www.water.com).

B.5.1. Operation and procedures

1. Purchase an appropriate sugar column (sugar pack, from waters corporation or other officer vendors and insert into the column chamber.
2. Fill up the HPLC solvent bottles with the appropriate solvent (e.g. HPLC grade water or filtered/ deionized tap water), then power up the HPLC machine and the detector. Set column temperature at the appropriate temperature based on previous studies and column specification for good base peaks.

3. Purge the HPLC system by following the direction on the display screen. The steps involved are: 1) dry prime, wet prime, equilibrate etc.
4. Sample preparation: Filter sugar samples (from wood) and standards that will be used for calibrations.
5. Inject filtered sugar samples into vials using a pipette or sterilized syringe. Place sugar vials into the HPLC sample manager turn table.
6. Set the HPLC running condition; like vial numbers, elution time, etc with the computer, save setting and click start to running sugar standard samples (at different concentration with replicates) there after the unknown samples.
7. For more detailed information, consult HPLC manual and trained personnel before and during machine operation.