Bioconversion of heterogeneous lignocellulosic biomass for sugar production

Rodrigo Morales Vera

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2015

Reading Committee:

Renata Bura, Chair

Richard Roy Gustafson

Fernando Resende

Program Authorized to Offer Degree:

Environmental and Forest Sciences

University of Washington

Abstract

Bioconversion of heterogeneous lignocellulosic biomass for sugar production

Rodrigo Morales Vera

Chair of the Supervisory Committee:

Dr. Renata Bura
School of Environmental and Forest Sciences

In order to accomplish large scale utilization of lignocellulosic feedstocks to produce fuels and chemicals, a consistent, inexpensive and stable supply of biomass from a variety of sources will be required. These biomass will be heterogeneous and will change as a function of time and price, and will most likely be available to the biorefinery in a mixed input stream with diverse physico chemical properties. Consequently a potential biomass processing facility must be able to convert these diverse feedstock without significantly altering the overall performance, sugar yields and fuel production. Currently, most bioconversion research has been carried out with high quality raw material such as clean wood chips and agricultural residues and little attention has been paid to the efficiency of converting diverse feedstocks into fermentable sugars and fuels. Since particle size reduction is expensive and energy intensive but a critical operation for preparing the biomass for pretreatment, initial particle size heterogeneity was studied during the bioconversion of hybrid poplar.

Different particles sizes, ranging from 0.2x0.2 cm to 2.0x1.5 cm, plus an equal mixture of all the particles, were used to determine the influence of initial particle size heterogeneity during sugar production of hybrid poplar (HP) via bioconversion. It was found that there is essentially no effect of particle size heterogeneity on saccharification after steam pretreatment. The overall sugar recovery from all the samples ranged from 87-90% and 61-64% for glucose and xylose respectively and was not influenced by particle size. Alongside showing that bioconversion of HP managed different particles size, and considering the availability of wheat straw (WS) as potential feedstock for a biorefinery. The influence of mixing these two feedstock during the bioconversion for sugar production were investigated. Despite that raw HP and WS have different physico-chemical properties, mixing both types of biomass positively affects the bioconversion process. In fact, mixed biomass exhibited on average 20% more sugar production than either single biomass. Since it was showed that is technically feasible the utilization of different combinations HP and WS as a feedstock for sugar production, a techno-economics analysis was performed to determine and compare the economic feasibility of processing simultaneously mixed biomass (HP and WS) vs. using single HP and WS in a campaign processing plan for ethanol production. The ethanol yields from process simulations estimated that mixed biomass yield 10 more gallons of ethanol per ton of biomass than using single HP and WS in a campaign system. Mixed biomass generate almost twice as much income per year than using single biomass in campaign plan processing, which is equivalent to extra 13.5 million per year. Techno-economic analysis indicated that production of ethanol using mixed biomass is more competitive vs. using single HP and WS in campaign design processing to ethanol production.

Table of Contents

List of figures

List of tables

Preface

This PhD thesis is submitted as the partial fulfillment of the requirements for the PhD degree at the University of Washington. The work included in this thesis was carried out at the School of Environmental and Forest Sciences, Bioresource Science and Engineering Department (Biofuels and Biochemicals Laboratory Group) at the University of Washington from Oct 2010 to Feb 2015 under the supervision of Professor Renata Bura and Professor Rick Gustafson.

Chapter 1. Consists of a general introduction which describes the theoretical background of this PhD research.

Chapter 2. A version of this material has been published as Morales R, Bura R, Gustafson R: Handling heterogeneous particles sizes of hybrid poplar for sugar production in Biomass and Bioenergy journal. I performed all experiments. Renata Bura and I conceived the experiments, Rick Gustafson supervised the heat transfer modeling and I wrote the manuscript for the published paper. In addition, I performed a steam pretreatment data reproducibility study to test the data reliability generated by the steam explosion equipment (Appendix 1).

Chapter 3A. A version of this material will be published as Morales R, Bura R: Improving the overall sugar yield by mixing hybrid poplar and wheat straw biomass prior to steam explosion and enzymatic hydrolysis in Biotechnology for Biofuels journal. I performed all experiments. Renata Bura and I conceived the experiments and I wrote the manuscript for the published paper. In addition, I performed a study to optimize steam pretreatment conditions for wheat straw (Appendix 2).

Chapter 3B. A version of this material will be published as Morales R, Bura R, Gustafson R: Techno-economic analysis and environmental impacts of mixing hybrid poplar and wheat straw for ethanol production. I experimentally generated the data for the model. I performed the Aspen modeling for the techno-economic analysis. Renata Bura and Rick Gustafson supervised the final results. Additionally, I will perform the life cycle analysis (LCA) of using mixed biomass for ethanol production.

Chapter 4. Consists of overall conclusions, future work and references of this PhD research project.

Acknowledgements

I would like to express my special appreciation and thanks to my advisor and chair Dr. Renata Bura, who has been a tremendous mentor for me. I would like to thank her for encouraging my research and for help me to grow as a research scientist. Her advice on both research as well as on my career have been invaluable. I give my sincere thanks to Rick Gustafson who always was there when I needed some extra advice in modeling matters. I give my sincere thanks to the rest of my committee members Fernando Resende and Bill McKean which were very helpful throughout my research.

I would like to thank to Shannon Ewanick for all her invaluable insights, constant support and encouragement throughout my research work. Also I would like to thanks to my current and former fellows graduate students at the biofuels and bioproducts laboratory (BBL), Azra Vajzovic, Elliott Schmitt, Erik Budsberg, Jordan Crawford, Chang Dou and Mandana Ehsanipour.

I would like to thank to all my friends in Seattle, especially to The Cumbieros, who allow me to bring the South American music flavor to the Seattle music scene.

I would like to thank express my gratitude to my parents Antonio Morales, Gloria Vera and my brother Sergio Morales for their love and support from Chile. Finally, I would like to thank to my wife, Sol for her patience and love, and obviously for being the mother of our beloved daughter Amara Inti.

CHAPTER 1: INTRODUCTION

1.1 <u>Conversion of biomass to fuels and chemicals</u>

Biomass has a significant potential to provide liquid, solid and gaseous fuels in conjunction with the co-production of food, fiber and new bioproducts (Chum & Overend, 2004). Biomass derived liquid transportation fuels and energy products have been proposed as part of the solution to climate change and human's heavy dependence on fossil fuels, because the biomass can be produced renewably from a variety of domestic sources, and the production and use of bioenergy/biofuel products can potentially lower environmental impacts compared to their petroleum counterparts (Yue et al., 2014).

The United States has diverse and abundant biomass resources that can potentially be used as biofuels and chemicals feedstocks. The U.S. agriculture and forest resources have the capability to sustainably produce one billion dry tons of biomass annually enough to displace approximately 30% of the country's 2003 petroleum consumption (Turhollow et al., 2014). However, biomass in its raw, "as-harvested", form is not necessarily a good feedstock for fuels and chemicals production (Perlack & Stokes, 2011). Lignocellulosic biomass is low in energy density and bulk density and has great variability of physical and chemical attributes, all of which can reduce the feedstock's energy value and make supply system logistics and conversion process more complex and expensive (DOE, 2012).

1.2 __Physico – chemical biomass characteristics__

1.2.1 Biomass physical characteristics

Moisture content, particle size, and bulk density are among several physical properties that affects directly the feedstock supply system and bioconversion processes. Moisture content affects negatively biomass storage increasing microbial contamination (Shinners et al., 2007) but impacts positively ethanol yields via bioconversion (Ewanick & Bura, 2011). Size reduction increases the bulk density and flowability properties of biomass (Miao et al., 2011) making desirable to handle small particles to improve the overall efficiency of the supply chain. However, feedstock particle sizing impacts the economics of cellulosic ethanol through its effects on conversion yield and energy cost (Vidal et al., 2010). Bulk density influences the transportation and storage cost, as well as the subsequent thermo-chemical/biological processing (McKendry, 2002).

1.2.2 Biomass chemical characteristics

Biomass mainly is composed of varying amounts of cellulose, hemicellulose, lignin and minor components as extractives and ash. Generally, woody biomass is more abundant in cellulose and lignin, whereas grass biomass has higher contents of hemicellulose (mainly xylan), extractives, and ashes (Zhao at al., 2012). Moreover, softwood has higher lignin composition than hardwood and agricultural residues. The proportion of cellulose, hemicellulose, and lignin in a biomass feedstock is a very important criterion in determining its suitability as an economically viable feedstock for the bioconversion (Sannigrahi et al., 2010).

Cellulose

Cellulose is a homopolysaccharide linear polymer that consists of glucose units linked by β (1→4) glycosidic bonds (Sjöström, 1993). The cellulose chain has a strong tendency to form intra- and inter-molecular hydrogen bonds by the hydroxyl groups on these linear cellulose chains, which stiffens the chains and promotes aggregation into a crystal line structure (Pu et al., 2008). The degree of polymerization of cellulose, number of glucose units in polymer, in natural materials can range from ~10000 in cotton fibers and bacterial cellulose to 250–500 in regenerated cellulose fibers (Klemm et al., 2005). The degree of polymerization (DP) and crystallinity of cellulose can be a limiting factor in its enzymatic conversion to glucose (Puri, 1984).

Hemicellulose

Hemicelluloses are heteropolysaccharides polymers formed by a wide variety of building blocks including pentoses (e.g., xylose, rhamnose and arabinose), hexoses (e.g., glucose, mannose and galactose) and uronic acids (e.g., 4-O-methyl-glucuronic and galacturonic acids) (Fengel & Wegener, 1989). The hemicellulose content of softwoods and hardwood differ significantly. Hardwood hemicelluloses are mostly composed of highly acetylated heteroxylans, generally classified as 4-O-methyl glucuronoxylans. Hexosans are also present but in very low amounts as glucomannans (Ramos, 2003).

Lignin

Lignin is an amorphous polymer consisting of phenylpropane units, and their precursors are three aromatic alcohols (monolignols) namely p-coumaryl, coniferyl and sinapyl alcohols. The respective aromatic constituents of these alcohols in the polymer are called p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) (Lewis & Yamamoto, 1990). Hardwoods lignin mainly contain guaiacyl and syringyl units (Lapierre et al., 1995). Softwood lignin is composed of primarily guaiacyl subunits, which is known to restrict fibre swelling and enzyme permeability more than syringyl lignin due to reduced methoxylation (Ramos et al., 1992). Whereas the lignins of herbaceous plants contain all three units (H, G, S) in significant amounts with different ratios (Billa et al., 1998)

Extractives

Extractives are the organic substances which have low molecular weight and are soluble in neutral solvents. Extractives include terpenes, tall oil and the fatty acids, esters, and triglycerides. Resin which is a combination of terpenes, lignans and other aromatics, fats, waxes, fatty acids and alcohols, terpentines, tannins and flavonoids are categorized as extractives as well (Demirbas, 2009). The compounds present in the extractives fraction are a function of the solvent, which is usually hot water, ethanol, acetone, dichloromethane, or a mixture of ethanol/benzene. For large scale lignocellulosic biorefinery operations, however, extractives can be a potential source of value-added coproducts (Sannigrahi et al., 2010).

Ash

The inorganic elements present in biomass constitute its ash content (Sannigrahi et al., 2010). The composition and quantity of ash depends on type of biomass (softwood, hardwood, grasses), plant tissue (bark, branch, stemwood, etc), soil type and climate among others (Pitman, 2006). Woody ash is dominated by Ca, Si, Al, K and Mg. In general, hardwood ash contains more K and P than softwoods but less Ca and Si

(Olanders & Syeenari, 1995). Herbaceous ashes contain mostly Si, K, Ca, S and Cl Biomass ash content will impact the quality of the non-fermentable ligneous residue that is made available for thermal conversion (Bakker & Elbersen, 2005).

1.3 Bioconversion process of lignocellulosic

The bioconversion of lignocellulosic biomass to ethanol consists of three main steps. Pretreatment, to improve accessibility of the substrate to subsequent hydrolysis by cellulases and finally fermentation of the resulting sugars to fuels and chemicals (Bura et al., 2009).

1.3.1 Pretreatment

Since lignocellulosic biomass has been naturally engineered to be recalcitrant to the action of microorganisms, it is crucial to apply a pretreatment process to alter the lignocellulosic matrix to make cellulose more accessible to enzymes by altering the surrounding hemicellulose and lignin. Several methods have been proposed to pretreat biomass and are classified as biological, chemical and physical, or combinations of these categories.

Biological pretreatments typically utilize wood degrading fungi to modify the chemical composition of the lignocellulosic feedstock. Specifically white rot fungi, which can actively access and degrade the lignin component. Nevertheless, this type of pretreatment is time consuming, and require careful growth conditions as well as large amounts of space. Consequently, is considered to be less attractive commercially (Chandra et al., 2007).

Chemical pretreatments include ammonia, solvent, wet oxidation, alkali, acid and others. Chemicals can also be used to catalyze physical pretreatments, as in the case of pH-controlled hydrothermolysis and acid-catalyzed steam explosion (Ewanick, 2012).

Physical pretreatment methods include comminution (mechanical reduction in biomass particulate size), uncatalyzed steam explosion, and hydrothermolysis. Comminution, including dry, wet, and vibratory ball milling and compression milling is sometimes needed to make material handling easier through subsequent processing steps (Mosier et al., 2005).

1.3.2 Steam Pretreatment

Steam pretreatment has been proposed as an efficient pretreatment method for lignocellulosic materials because of its limited use of chemicals, low energy consumption, and short reaction time (Galbe & Zacchi, 2007). Steam pretreatment (explosion) combines both physical and chemical elements, causing the rupture of the wood cell wall structure, hydrolysis, and solubilization of the biomass components (Ramos & Saddler, 1994). An acid catalyst as H_2SO_4 or SO_2 is required for effective steam explosion of many feedstocks. Acetylated hemicellulose groups release acetic acid during pretreatment, leading to autohydrolysis, but addition of acid enables shorter residence times and lower temperatures to be employed (Ramos, 2003). During steam pretreatment the biomass is put in a large vessel and steam with a high temperature (temperatures up to 240°C) and pressure, is applied for a few minutes. After a set time, the steam is released and the biomass is quickly cooled down (Hendriks & Zeeman, 2009).

1.3.3 Enzymatic hydrolysis

Enzymatic hydrolysis of cellulose to glucose is carried out by cellulase enzymes. Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo-1,4-d-glucanohydrolase) which attacks regions of low crystallinity in the cellulose fiber, creating free chain-ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4-β-d-glucan cellobiohydrolase) which degrades the molecule further by removing cellobiose units from the free chain-ends; (3) β-glucosidase which hydrolyzes cellobiose to produce glucose (Coughlin & Ljungdahl, 1988).

The assignment of specific substrate factors that render a substrate recalcitrant to cellulase hydrolysis is a controversial subject (Chandra et al., 2007). Crystallinity, DP and specific surface area (Mansfield et al., 1999) as well as lignin (Kumar et al., 2012), hemicellulose (Bura et. al 2009) and acetyl group content are among substrate factors affecting saccharification (Selig et al., 2009).

Generally, hydrolysis is performed under mild conditions (e.g. pH 4.5-5.0 and temperature 40-50°C). Therefore, it is expected low corrosion problems, low utility consumption, and low toxicity of the hydrolyzates as the main advantages of this process (Taherzadeh & Keikhosro, 2007).

1.3.4 Fermentation

The fermentation uses microorganisms to convert a fermentable substrate into recoverable products (usually alcohols or organic acids). Ethanol is currently the most required fermentation product, but the production of many other chemical compounds as hydrogen, methanol and succinic acid, among others; are nowadays object of many research and development activities (Cherubini, 2010).

Hexoses, mainly glucose, are the most frequent fermentation substrates, while pentoses (sugars from hemicellulose), glycerol and other hydrocarbons required the development of customized fermentation organisms to enable their conversion to ethanol (Hamelinck et al., 2005). An important factor preventing industrial utilization of lignocelluloses for bioethanol production is the lack of microorganisms able to efficiently ferment (with high yield and high rate) all sugars (both pentoses and hexoses) released during pretreatment and hydrolysis (Talebnia et al., 2010)

Fermentation Inhibitors

During hydrolysis of lignocellulosic materials a wide range of compounds which are inhibitory to microorganisms are formed or released. Based on their origin the inhibitors are usually divided in three major groups: weak acids, furan derivatives, and phenolic compounds (Palmqvist & Hahn-Hägerdal, 2000). These compounds limit efficient utilization of the hydrolysates for fuels and chemicals production by fermentation.

Aromatic degradation products from sugar degradation, predominantly furans as furfural and 5-Hydroxymethyl furfural (HMF), phenolics formed by solubilization and hydrolytic or oxidative cleavage of lignin (Klinke et al., 2004) and acetic acid and other organic acids released from hemicelluloses (Dai & McDonald, 2014) are important fermentation inhibitors for different ethanologenic microorganism as *S. cerevisiae*, *Z. mobilis and E. coli* (Franden et al.,2013).

1.4 ***Economics of a lignocellulosic based biorefinery***

In order to determine a commercial viability of a lignocellulosic based biorefinery a techno-economic assessment is necessary to establish capital and operating cost profile of the process (Grierson & Strezov, 2012). Significant research and funding have focused on understanding the relationship between biomass productivity, supply systems costs, conversion technology, operation cost and yield of bioenergy and biofuels products (Daystar et al., 2013).

Current barriers to make economically feasible cellulosic ethanol production include biomass cost and availability (Wu et al., 2010 and Gonzalez et al., 2011) pretreatment costs, high capital expenditure (CAPEX), equipment scale up and overall production costs (Wyman, 1994; Aden et al., 2002; Mosier et al.,2005; Tao & Aden, 2009; Wyman, 2007; Gonzalez et al., 2011). However, the contribution of biomass cost to the overall production cost of lignocellulosic bioethanol proves to be one of the most significant (Gnansounou & Dauriat, 2010). Consequently, to ensure that future lignocellulosic biomass biorefineries are sustainable and economically feasible, it is necessary to reduce the cost of raw materials and to improve the process yields (Gonzalez, 2012). A low cost feedstock will be diverse, changing as a function of seasonality and price and will most likely be available from multiple sources to the biorefinery.

1.5 ***Objectives***

The overall objective of this doctoral research is to improve the sugar production from heterogeneous lignocellulosic biomass via bioconversion, consequently increasing the production of potential biofuels and other biochemicals.

Specific research objectives:

- Determine the influence of particle size heterogeneity on sugar production during bioconversion of hybrid poplar via steam explosion and enzymatic hydrolysis and to evaluate the robustness of steam explosion as a pretreatment for simultaneously processing different particle sizes (chapter 2).
- Assess the influence of mixing wheat straw and hybrid poplar biomass on the overall sugar production via steam pretreatment and enzymatic saccharification (chapter 3A).
- Evaluate the economic feasibility of mixing wheat straw and hybrid poplar biomass in a lignocellulosic based ethanol biorefinery (chapter 3B).

CHAPTER 2: HANDLING HETEROGENEOUS PARTICLE SIZES OF HYBRID POPLAR FOR SUGAR PRODUCTION

Abstract

Five chip sizes of freshly harvested hybrid poplar, ranging from 0.2x0.2 cm to 2.0x1.5 cm, plus an equal mixture of all the particles, were used to determine the influence of initial particle size heterogeneity on sugar recovery after steam explosion pretreatment and enzymatic hydrolysis and the robustness of steam explosion as a pretreatment for simultaneously processing different particle sizes. It was found that there is essentially no effect of particle size heterogeneity on sugar recovery. Enzymatic digestibility of solids from all the particles showed similar yields ranging from 78 to 82% glucan conversion after 72 hours. The overall sugar recovery from all the samples ranged from 87-90% and 61-64% for glucose and xylose respectively and was not influenced by particle size. An unsteady heat transfer model was used to assess the intra-particle temperature profile of the wood during pretreatment. It was found that all the wood chips, regardless of size, are essentially isothermal. This supports the experimental result of no significant difference in sugar yields between different particle sizes. Steam pretreatment appears to be a robust method that can accommodate a wide range of particle sizes.

2.1 Introduction

The use of lignocellulosic biomass for the production of fuels and chemicals will be imperative if petroleum is to be replaced by renewable and sustainable alternatives. Production of fuels from lignocellulosic material has attracted worldwide interest because they do not compete with food, and have significantly lower global warming potential (Gonzalez-Garcia et al., 2010). The United States has diverse and abundant biomass resources that can potentially be used as fuels and chemicals feedstocks. Biomass in its raw, “as-harvested”, form may not, however, be a good feedstock for fuels and chemicals production (Perlack & Stokes, 2011). It is low in energy and bulk density, and has variable physical attributes, all of which can reduce the feedstock’s energy value and make supply system logistics complex and expensive (Kenney & Ovard, 2012).

Feedstock cost is a major economic factor in commercial scale biofuels production, contributing 40-50% of the operating costs in a lignocellulosic biomass based biorefinery (Juneja et al., 2013). The low selling price of the products generated by a biorefinery do not permit a biomass conversion facility to afford ‘pristine’ feedstock composed of clean and homogeneous high quality raw material (Kenney et al., 2013). Low cost feedstocks will have heterogeneous chemical compositions (Shi et al., 2013) and physical characteristics. This variability results from plant physiology, agronomic and environmental conditions, as well as differences in the way biomass is harvested, stored and processed prior to conversion (Kenney & Ovard, 2013). Heterogeneous biomass, including characteristics such as particle size, moisture content, bark content, leaf/needle content and general chemical attributes of the biomass, will affect the bioconversion process (Ewanick, 2012). Nonetheless, the capability for a biorefinery to handle heterogeneous feedstock – including particle size - is an important criteria for economic viability.

Size reduction has been proposed as a critical operation for preparing physically heterogeneous feedstock for use in a biorefinery (Yancey et al., 2009). However, size reduction of biomass along with transportation and storage costs make up between 13 and 28% of total feedstock costs (Miao et al., 2011). Thus, to reduce feedstock costs, particle size heterogeneity in the feedstock may need to be tolerated. In order to make the biomass to biofuels and biochemicals process economically feasible and sustainable, a robust bioconversion system should handle different particles sizes without altering the overall sugar yields.

Most bioconversion research has been carried out using high quality, homogeneous biomass. This biomass, such as screened white wood chips or milled agricultural residues, is typically uniformly comminuted. Little attention has been paid to the efficacy of converting variable particle sizes biomass into fermentable sugars and fuels. The influence of particle size on bioconversion had been studied by a few researchers. Brownell et al. (1986), analyzed the effect of chip size on bioconversion of aspen wood to better understand the influence of the particle size on lignocellulosic bioconversion. They concluded that smaller chip size (3.2 mm fiber direction) resulted in more rapid chip heating and more extensive decomposition during pretreatment, leading to high enzymatic hydrolysis yields. Brownell et al. (1986), also concluded that larger aspen wood chips produced more of the fermentation inhibitors 5-hydroxymethylfurfural (HMF) and furfural, due to slow particle heating. Negro et al. (2003), steam pretreated two poplar particle sizes, 2 to 5 mm and 12 to 15 mm, and found no effect on either enzyme digestibility of pretreated solids or the downstream ethanol fermentation yield. The effect of using mixed particle sizes on overall sugar production via steam pretreatment and bioconversion has not been investigated.

As mentioned previously, the economic success of a potential biorefinery is directly related to the use of low cost biomass. While a more heterogeneous raw material is less expensive, the efficiency of the whole bioconversion process using mixtures of different particles sizes has not yet been investigated. In this research, the effect of feedstock particle size heterogeneity was evaluated using a single raw material, and one set of pretreatment conditions along with diverse chip sizes and robust statistical analysis with replicates of all performed experiments. The objectives of this research were twofold. Firstly, to determine the influence of particle size heterogeneity on sugar production during bioconversion of hybrid poplar via steam explosion and enzymatic hydrolysis. Secondly, to evaluate the robustness of steam explosion as a pretreatment for simultaneously processing different particle sizes ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm. A heat transfer analysis was performed to provide a theoretical underpinning for the experimental results.

2.2 ***Methods***

Five particle sizes ranging from 0.2 x 0.2 cm to 2.0 x 1.5 cm, plus an equal weighted mixture of all the particles, were impregnated with SO_2 (3% w/w) and steam pretreated at 195°C for 5 minutes to determine the influence of particle size on bioconversion of hybrid poplar for sugar production. The conditions for steam pretreatment for hybrid poplar were chosen for previous work done by Ewanick (2012). The steam pretreated particles were then chemically characterized and saccharified at

5% (w/v) solids consistency, 5 FPU/g cellulase. A complete mass balance of carbohydrates was determined to assess overall yield. Figure 2-1 shows process flow for the bioconversion experiments.

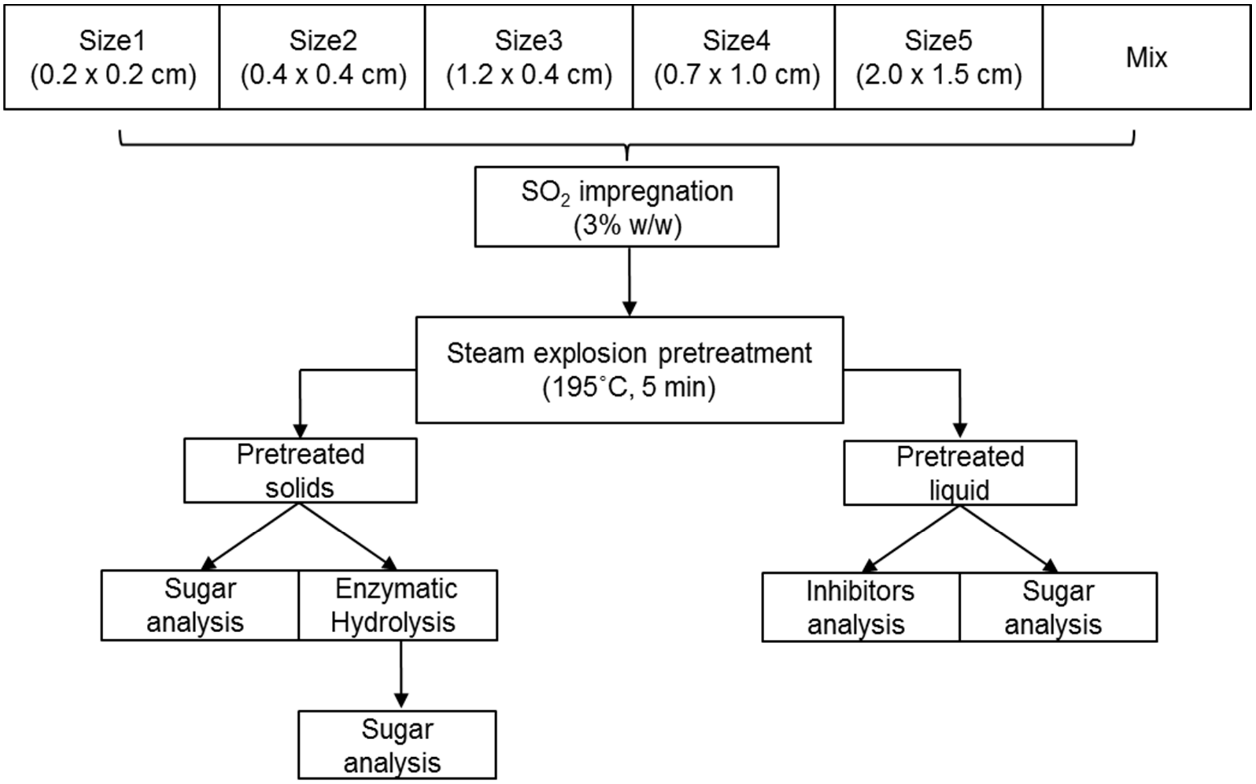


Figure 2-1. Process flow diagram for bioconversion of hybrid poplar into carbohydrates.

2.2.1. Material

Freshly harvested 18-year-old hybrid poplar, *Populus deltoides x Populus nigra*, from Puyallup, WA was used for this study. The biomass was comminuted using two different processes to obtain five different size fractions: size S1 (length: 0.2 x width: 0.2 x thickness: 0.1 cm), S2 (0.4 x 0.4 x 0.4 cm) and S3 (1.2 x 0.4 x 0.4 cm) (referred to as Crumbles™) were made using a patented technology developed by Forest Concepts, LLC and previously described by Dooley et al. (2013). In the Forest Concepts process, Crumbles™ particles are produced by feeding wood veneer in a direction normal to the grain through a counter rotating pair of intermeshing cutting discs aligned axially perpendicular to the direction of veneer travel. Size 4 (0.7 x 1.0 x 0.2 cm) and 5 (2.0 x 1.5 x 0.4 cm) (chips), were made using a slant disc chipper (Acrowood, Everett, WA). In addition, a mixture was prepared of all the particles in equal proportions. The particles had a moisture content of 60-65% and were stored at -20°C until use.

2.2.2 Pretreatment and processing conditions

The bioconversion process flow for this research is shown in Fig. 2-1. Prior to pretreatment, 800 g of dry biomass of each of the six feedstocks (sizes) was impregnated with gaseous sulfur dioxide (3% w/w) and sealed in airtight plastic bags. Specifically, 24 g of SO_2 was added to the 800 g of dry biomass from a gas cylinder in to a plastic bag containing the biomass. The weight of the biomass was monitored overnight to determine the gas retention for each chips size. Samples were then subdivided into 400 g samples and pretreated using a 2.7l batch steam gun manufactured by Aurora Technical (Savona, BC, Canada) at 195°C for 5 min. At the end of the reaction time, a pneumatic valve was opened between the pressurized reaction tank and the collection tank, causing the explosion of the biomass, which was discharged into the collection tank. The resulting slurry was vacuum filtered to separate the liquid fraction from the pretreated solids. Both fractions were analyzed as described below and used to construct a mass balance of carbohydrates and lignin. Solids were washed with deionized water equal to 20 times the mass of solids prior to analysis and hydrolysis. Each steam explosion was performed 3 times for each different size fraction and the mixture, resulting in a total of 18 substrates, all of which were analyzed as described below.

2.2.3 Instrumental analysis

High Pressure Liquid Chromatography (HPLC)

Carbohydrates were measured on a Dionex (Sunnyvale, CA) HPLC (ICS-3000) system equipped with an AS autosampler, ED electrochemical detector, dual pumps, and anion exchange column (Dionex, CarboPac PA1). Deionized water at 1 ml/min was used as an eluent, and post-column addition of 0.2 M NaOH at a flow rate of 0.5 ml/min ensured optimization of baseline stability and detector sensitivity. After each analysis, the column was reconditioned with 0.25 M NaOH. Twenty microliters of each sample were injected after filtration through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Samples were measured against standards consisting of arabinose, galactose, glucose, xylose, and mannose. In addition, fucose was used as an internal standard.

Acetic acid, furfural and 5-hydromethylfurfural were measured using refractive index detection on a Shimadzu Prominence LC. Separation of these compounds was achieved by an anion exchange column (REZEX RHM-Mono-saccharide H^+ (8%), Phenomenex, Inc., Torrance, CA) with an isocratic mobile phase that of 5 mM H_2SO_4 at a flow rate of 0.6 ml/min. The column oven temperature was maintained at 63°C. Twenty microliters of each sample were injected after being appropriately diluted in deionized water and filtered through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Standards were prepared and used to quantify the unknown samples.

2.2.4 Compositional analysis

Insoluble carbohydrates, acetate groups and lignin

Raw hybrid poplar and solids were analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate and acetates content using TAPPI method T-222 om-98 (TAPPI, 1998) and NREL procedure LAP 017 (Ehrman & Ruiz, 1998), respectively. Briefly, 0.2 g of finely ground oven dried sample was treated with 3 ml of H_2SO_4 at 72% for 120 minutes at room temperature, then diluted into 120 ml total volume, then autoclaved at 121˚C for 60 minutes. The autoclaved samples were then filtered using a glass crucible to separate the acid insoluble lignin (AIL) from the acid soluble lignin (ASL) and carbohydrates. The AIL was determined by weighting the oven dried crucibles, and the ASL in the filtrate is analyzed by UV at 205 nm. The filtrate was analyzed by HPLC for carbohydrate composition and acetates content.

Soluble carbohydrates

Monomeric and oligomeric soluble carbohydrates were determined using NREL LAP TP-510-42623 (Sluiter et al., 2004). Briefly, 0.7 ml of 70% H_2SO_4 was added to 15 ml of the liquid samples, and the volume made up to 20 ml with water. Samples were autoclaved at 121˚C for 60 minutes and analyzed by HPLC as described previously. Monomeric sugars were determined by analyzing the original samples without acid hydrolysis. Oligomeric sugar was calculated by subtracting monomeric sugar content from total sugar content determined after acid hydrolysis.

2.2.5 Enzymatic hydrolysis

Enzymatic hydrolysis of washed solids was done at 5% w/v solids in a total volume of 50 ml in 125 ml Erlenmeyer flasks. The solution was buffered at pH 4.8 with 0.05 M citric acid buffer and the hydrolysis was carried out at 50˚C and 150 rpm shaking on an orbital shaking incubator (New Brunswick). Cellulase (Celluclast 1.5 L, 26 FPU/ml, Sigma) was added at 5 FPU/g cellulose and supplemental beta-glucosidase (Novozym 188, 492 CBU/ml, Sigma) was added at 10 CBU/g cellulose. 1 ml samples were periodically removed and analyzed for glucose content.

2.2.6 Bulk density analysis

Bulk density of different particles of hybrid poplar was determined according to the ASAE Standard S269.4 DEC 91 for cubes, pellets, and crumbles (ASABE, 2007). One cylindrical container with a specific inside diameter was used for determination of bulk density of different particle sizes and the mix. Each measurement was repeated for five times. Biomass was poured into the container from a certain height to facilitate the free flowing of the samples until the container was overflowed. The excess material was removed by striking a straight edge across the top. The loosely filled container was tapped on the laboratory bench five times by dropping the container from a height of 10 cm. Filling and tapping was repeated until the container overflowed. The weight of the material with the container was recorded. The net weight of the sample was obtained by subtracting the weight of the empty container. The filled container was weighed to 0.01g precision. Bulk density was calculated by dividing the mass by the container volume.

2.2.7 Statistical analysis

One way ANOVA at 5% alpha was performed for each dependent variable to assess statistical differences as a function of particle size. In the cases where statistically significant differences were found (p value<0.05), a “Tukey test” (multiple comparison of means) was performed to find the specific group or groups of particles that were causing those differences. Data analysis was done using the statistical software R version 2.12.1.

2.3 Heat transfer model

An unsteady state heat conduction in a finite slab with a 4 mm thickness – the thickest chips - was used to investigate the pretreatment reaction uniformity. A heat balance on the chip gives the following differential equation:

$$\frac{\partial T}{\partial t} = \alpha \left(\frac{\partial^2 T}{\partial x^2}\right), \alpha = \frac{k}{\rho\, Cp} \tag{1}$$

Where T is the temperature, t is the reaction time, x: position in the slab, α is the thermal diffusivity, k is the thermal conductivity, ρ is the density, and Cp is the heat capacity. With initial and boundary conditions of:

$$t = 0, T = T_i, -b < x < b \tag{2}$$

$$x = -b, \qquad T = T_s\,, \forall\, t > 0 \tag{3}$$

$$x = b, \qquad T = T_s\,, \forall\, t > 0 \tag{4}$$

Where, T_i is the particle initial temperature (25° C), b is the particle thickness, x is the position in the slab (x=0 chip center) and T_s is the particle surface temperature.

The analytical solution to equations (1)-(4) is obtained in the dimensionless form as follows:

$$\frac{T\ (x,t) - Ts}{Ti - Ts} = 2\sum_{n=0}^{\infty} \frac{(-1)^n}{(n+0.5)\pi}\ e^{\left[-(n+0.5)^2\pi^2\frac{\alpha t}{b^2}\right]} \cos\left[(n+0.5)\ \pi\ \frac{x}{b}\right] \tag{5}$$

Where, $T\ (x,t)$ is the temperature in position x at time t.

The thermal diffusivity (α) was calculated using the density of hybrid poplar and the thermo physical properties of wood which were estimated from relations published by Simpson and TenWolde (1999) (Table 2-1). The model was run and solved using the numerical computing software Matlab version 8.1.0.604 (R2013a).

Table 2-1. Hybrid poplar density and thermophysical properties.

Property	Value	Unit
Thermal conductivity (k)	0.23	W m^{-1} K^{-1}
Hybrid poplar density (ρ)	355	Kg m^{-3}
Heat capacity (Cp)	1255.47	J Kg^{-1} K^{-1}

2.4 <u>Results and discussion</u>

2.4.1 Raw material composition and bulk density

Glucan, xylan, acetate and lignin content, and bulk density of the original untreated biomass corresponding to the six different groups of particles are presented in Table 2-2. 59-67% of the total dry matter consisted of glucan (47 to 52%) and xylan (12 to 15%), representing the two main fermentable sugars in the raw biomass. The acetate group content varied from 3.5 to 3.8% and the presence of lignin, which includes the acid soluble and acid insoluble lignin, was similar among the particle sizes; ranging from 25 to 26%. No statistical differences were found among the sizes in terms of glucan, xylan, acetates and lignin content. The chemical composition of raw poplar was found to be similar to results obtained by Negro et al. (2003); and Bura et al. (2012). The bulk density of all of the different particle sizes was low and not substantially different among the different particles sizes, ranging from 0.15 to 0.19 g/cm^3.

Table 2-2. Composition analysis of raw poplar expressed as % dry matter and bulk density (BD) for the different groups of particles prior to pretreatment. Error intervals are for standard deviations from triplicate measurements.

Sample	Glucan (%)	Xylan (%)	Acetate (%)	Lignin (%)	BD* (g/cm^3)
S1	48.6 ± 0.8	13.5 ± 1.6	3.7 ± 0.1	25.6 ± 0.3	0.16 ± 0.01
S2	49.0 ± 1.0	13.0 ± 1.0	3.8 ± 0.1	26.1 ± 0.4	0.15 ± 0.01
S3	51.9 ± 1.5	12.6 ± 1.5	3.5 ± 0.1	24.7 ± 0.3	0.17 ± 0.01
S4	49.1 ± 0.6	14.6 ± 1.6	3.8 ± 0.4	26.4 ± 0.3	0.17 ± 0.01
S5	46.9 ± 0.8	12.3 ± 0.8	3.5 ± 0.3	25.1 ± 0.3	0.19 ± 0.01
Mix	49.1 ± 0.6	13.2 ± 1.0	3.6 ± 0.1	25.6 ± 0.8	0.18 ± 0.01

BD*: Bulk density.

2.4.2 Solids composition after pretreatment

The influence of particle size on the chemical composition of solids after steam pretreatment was measured. The chemical composition of solids after steam pretreatment, expressed as % dry matter for different particle sizes and the mix are shown in Fig. 2-2. The glucan and lignin content ranged from 61 to 65% and from 32 to 36%, respectively. No statistically significant differences were found for either glucan or lignin content for all the particles tested, including the mix of particles. As expected, only glucan and lignin were found in solids, since all the hemicellulose was solubilized into the liquid fraction. Similar values were obtained by Bura et al. (2012), who pretreated poplar using steam pretreatment under similar conditions, reporting values for glucan and lignin of 68 and 31% respectively. In another study done by Negro et al. (2003), testing two sets of poplar particle size, 2 to 5 mm and 12 to 15 mm, using uncatalyzed steam pretreatment, the cellulose, hemicellulose and lignin content of the washed solid fraction ranged from 56-59%, 4-9% and 30-38% respectively. The high levels of hemicellulose observed in the solid fractions were likely due to the lack of acid catalyst during pretreatment. Negro et al. (2003), also reported no significant influence of the chip size on the chemical composition of solids after pretreatment.

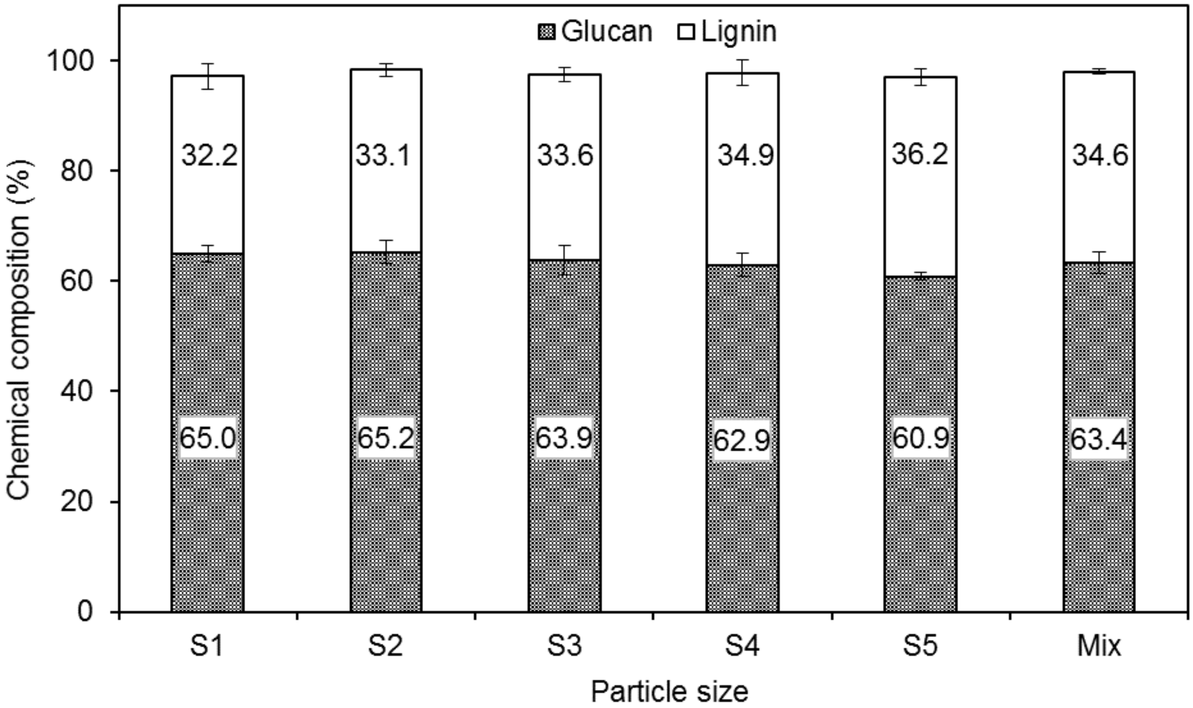


Figure 2-2. Composition of solid fractions of pretreated hybrid poplar expressed as % dry matter for different particle sizes. Error bars indicate standard deviations from triplicate measurements.

In order to test steam pretreatment as a suitable fractionation method for simultaneously processing a heterogeneous mixture of different particle sizes, the chemical composition of the mixed particle sizes after steam explosion was determined. It was found that the glucan and lignin content of the pretreated mixed samples was identical in comparison with the uniform particle size samples; and no statistical differences were found between the mixed sample and other particles sizes (S1-S5). Steam pretreatment was therefore able to produce a consistent slurry regardless of particle size heterogeneity, indicating the robustness of this method for biomass fractionation.

2.4.3 Liquid stream composition after pretreatment

The amount of sugar, acetic acid, furfural and 5-hydroxymethylfurfural (HMF) in the liquid fractions after pretreatment were measured and the results are shown in Table 2-3. Table 2-3 shows that the glucose and xylose content, expressed as a % of the total starting weight of raw biomass, ranged from 6 to 9% and from 8 to 10%, respectively. The majority of the sugars were in monomeric form, ranging from 84 to 88% and 90 to 94% for glucose and xylose respectively, showing the efficiency of SO_2 in hydrolyzing carbohydrates to monomeric sugars (Clark et al., 1989). A statistically significant difference was found in the glucose content in the liquid streams, where the smallest size (S1) showed the lowest glucose recovery (6.4%). Similar trends were found by Ballesteros et al. (2002), who steam pretreated herbaceous agricultural waste (*Brassica carinata*) using three particle sizes (2-5, 5-8 and 8-12 mm), where the smallest size showed the lowest cellulose recovery. In the present study, no statistically significant differences were observed in xylose content of the liquid stream among the different particle sizes, including the mix of particles. Furthermore, the particle size did not influence the presence of sugars in monomeric or oligomer form in the liquid stream. As expected, more xylose than glucose was found in the liquid streams, because hemicellulose is more labile the cellulose and becomes solubilized during pretreatment.

Similar results were found by Negro et al. (2003), but they found that particle size did affect the liquid stream xylose content, with a higher xylose concentration resulting from the smaller particles.

Acetic acid is formed by hydrolysis of acetyl groups in the hemicellulose when using hydrothermal pretreatments to fractionate hardwood and agricultural residues. Table 2-3 shows that the acetic acid content in the water soluble fraction ranged from 3.8 to 4.6% w/w of initial raw material (15-18 g/L) and did not show any significant statistical difference among the different particle size, including the mix of particles. Similar results were found by Vajzovic (2012), who steam preatreated hybrid poplar with 3% SO_2 at 195º C for 5 minutes, and reported an acetic acid content of 3.7%. Schutt et al. (2013), steam pretreated poplar using more severe pretreatment conditions, and reported lower values of acetic acid ranging from 2.8-3 %. The reduced acetic acid content can be explained by the use of dry biomass, which can result in ineffective SO_2 catalysis and incomplete hemicellulose dissolution (Ewanick & Bura, 2011).

Furans such as furfural from pentoses and 5-hydroxymethylfurfural (HMF) from hexoses, which are the products of dehydration reactions during pretreatment under severe acidic conditions (Klinke et al., 2004), were also found in the liquid fractions (Table 2-3). The concentration of furfural was lower than 1.6% (6.5 g/L) and HMF was less than 0.3% (1.2 g/L) for all the six groups of particles. Similar results were obtained by Vajzovic (2012), who pretreated hybrid poplar using steam pretreatment under the same conditions used in this present study, and reported values for of 1.3% for furfural and 0.3% for HMF.

As shown in Table 2-3, the chemical composition of the liquid stream coming from the mix of particles was very similar to the rest of the different sizes and no statistical differences were found for acetic acid and xylose content between the mixes and the other particles sizes.

Table 2-3. Composition of liquid fraction of pretreated hybrid poplar expressed as % of the total starting weight of raw biomass for the different sizes. Error intervals are for standard deviations from triplicate measurements.

	Glucose (%)		Xylose (%)		Acetic acid (%)	Furfural (%)	HMF[a] (%)
	Total	Mon[b]	Total	Mon[b]			
S1	6.4 ± 0.2	87	8.9 ± 0.8	93	3.80 ± 0.23	1.08 ± 0.05	0.21 ± 0.01
S2	7.3 ± 0.7	87	8.2 ± 1.3	94	3.87 ± 0.56	1.20 ± 0.06	0.26 ± 0.03
S3	8.5 ± 0.4	88	8.2 ± 0.7	95	4.24 ± 0.39	1.34 ± 0.17	0.29 ± 0.02
S4	7.5 ± 0.6	88	10.1 ± 0.3	93	4.61 ± 0.32	1.54 ± 0.06	0.28 ± 0.03
S5	8.0 ± 0.1	84	9.3 ± 0.6	90	4.30 ± 0.31	1.59 ± 0.12	0.29 ± 0.01
Mix	8.1 ± 0.4	84	8.9 ± 0.1	93	4.12 ± 0.12	1.44 ± 0.15	0.25 ± 0.01

[a] 5-hydroxymethylfurfural, [b] Monomeric sugar.

2.4.4 Enzymatic hydrolysis

The influence of raw material particle size on enzymatic digestibility of the washed pretreated solids was evaluated. Figure 2-3 shows the cellulose conversion of six particle size investigated. It can be seen that the enzymatic hydrolysis of the all steam pretreated particles showed similar glucan to glucose conversion. A conversion range from 78 to 82% was observed during 72 hours of enzymatic hydrolysis for all the particle sizes, and no statistically significant differences were found among the different pretreated substrates, including the mix of particles. This trend has been previously observed by Negro et al. (2003), who enzymatically hydrolyzed uncatalyzed steam pretreated poplar coming from two particle size fractions, 2 to 5 mm and 12 to 15 mm; Negro found no significant effect of particle size on enzymatic digestibility. Similarly, Monavari et al. (2009), steam pretreated 3 different spruce chip sizes (1-2, 3-4 and 5-6 mm in thickness with an average length of 30-45 mm) and concluded that particle size did not significantly influence the enzymatic hydrolysis of the substrates, suggesting that feedstock initial particle size of is not an important factor for enzymatic saccharification of biomass.

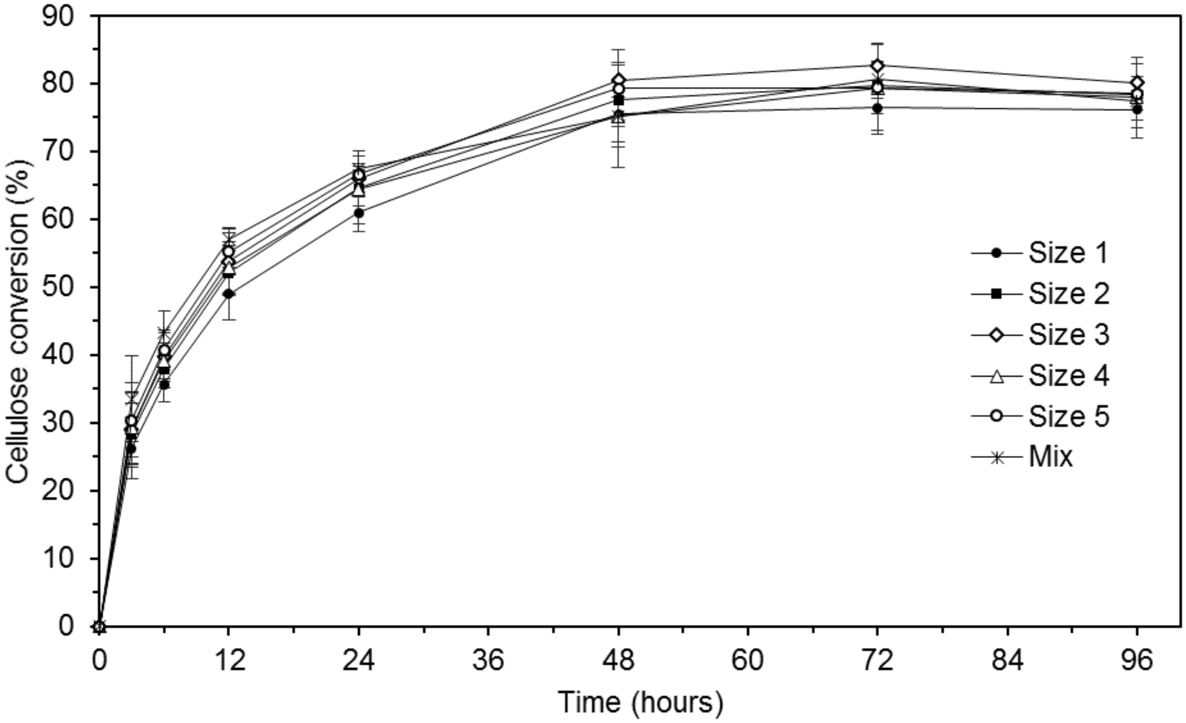


Figure 2-3. Cellulose to glucose conversion for steam pretreated hybrid poplar during enzymatic hydrolysis at 5% (w/v) solids consistency and 5 FPU/g cellulose and 10 CBU/g cellulose enzymes loading. Error intervals indicate standard deviations from triplicate measurements.

Chandra et al. (2007), have examined the properties of various lignocellulosic substrates that have been modified during pretreatment and subsequent hydrolysis. They found that hemicellulose and lignin content, crystallinity, degree of polymerization, and available surface area, are important characteristics for subsequent enzyme hydrolysis. In the current study, the similar chemical composition of all solids after pretreatment, in which no xylan and acetic acid were present, might have contributed to the similar saccharification for all sizes. When xylan is present on the surface of cellulose the action of cellulases can be hindered (Kabel et al., 2007). According to Chang and Holtzapple (2000), lignin and acetyl groups function as parallel barriers for enzymatic hydrolysis. The absence of acetyl groups as well the similar lignin content in solids for all the particle sizes resulted in similar saccharification curves for all the sizes, including the mix of particles. It appears that a wide range of particle sizes can be effectively processed during steam pretreatment, resulting in pretreated substrates with similar chemical properties. Therefore the initial particle size heterogeneity of hybrid poplar is not an important factor to consider if the fractionation process is sufficiently robust to complete hemicellulose and acetyl groups hydrolysis.

2.4.5 Total sugar recovery

The overall sugar recovery after pretreatment and enzymatic hydrolysis was calculated and is shown in Table 2-4. Table 2-4 shows the combined overall recovery of glucose and xylose of solid and liquid fraction, expressed as % of the theoretical amount available in the raw material. It can be seen that 87 to 90% of glucose was recovered in all samples. 61-64% of the xylose was recovered, all of it after steam pretreatment. No statistical differences were found in the total sugar recovery among the particle sizes, including the mix of particles. These results parallel the equivalent chemical composition of solid and liquid streams among the different pretreated substrates after steam explosion, as well as the similar enzymatic hydrolysis conversion for all the particles tested including the mixes of particles. The initial size heterogeneity of the different particles did not significantly affect the overall sugar yields.

Table 2-4. Combined overall recovery of monomeric glucose and xylose of solid and liquid fraction, after pretreatment and enzymatic hydrolysis, expressed as % of the theoretical available in the raw material. Error intervals are for standard deviations from triplicate measurements.

Sample	Glucose (%)	Xylose (%)
S1	87.3 ± 2.0	62.1 ± 1.1
S2	87.7 ± 1.5	62.0 ± 1.8
S3	90.2 ± 1.3	61.6 ± 1.4
S4	89.8 ± 2.4	63.7 ± 1.8
S5	88.6 ± 2.7	64.4 ± 1.7
Mix	88.5 ± 1.6	61.1 ± 0.9

Previous research has shown mixed effects of particle size on sugar recovery. Negro et al. (2003), observed higher cellulose recovery for larger sizes and higher xylose recovery for smaller particles. Monavari et al. (2009), established that the glucose recovery decreased with increasing chip size after steam explosion and enzymatic hydrolysis. Conversely, Cullis et al. (2004), reported an improvement in glucose and hemicellulose derived sugar recovery by increasing chip size and moisture content. Identical effects were found by Ballesteros et al. (2000), where higher cellulose and hemicellulose recovery were obtained for larger particles (8-12 mm). Similar results were found by Liu et al. (2013), who pretreated five different particles sizes of corn stover ranging from 0.5 to 2.5 cm. They established that high pressure steam penetrated the interior of the bed of corn stover easier with larger particle size due to the high porosity or low bulk density. The improved steam penetration with larger particles resulted in higher conversion yields compared to those obtained with smaller particles and higher bulk density. In the current study, the bulk density was low for all the particle sizes and not substantially different. High pressure steam should equally penetrate the interior of the different bed of particles, resulting in the efficient biomass steam explosion for all the particles tested. While previous studies reported an effect of chip size on sugar recovery, the statistical significance on the sugar recovery and the effect of simultaneously processing different particle sizes were never established.

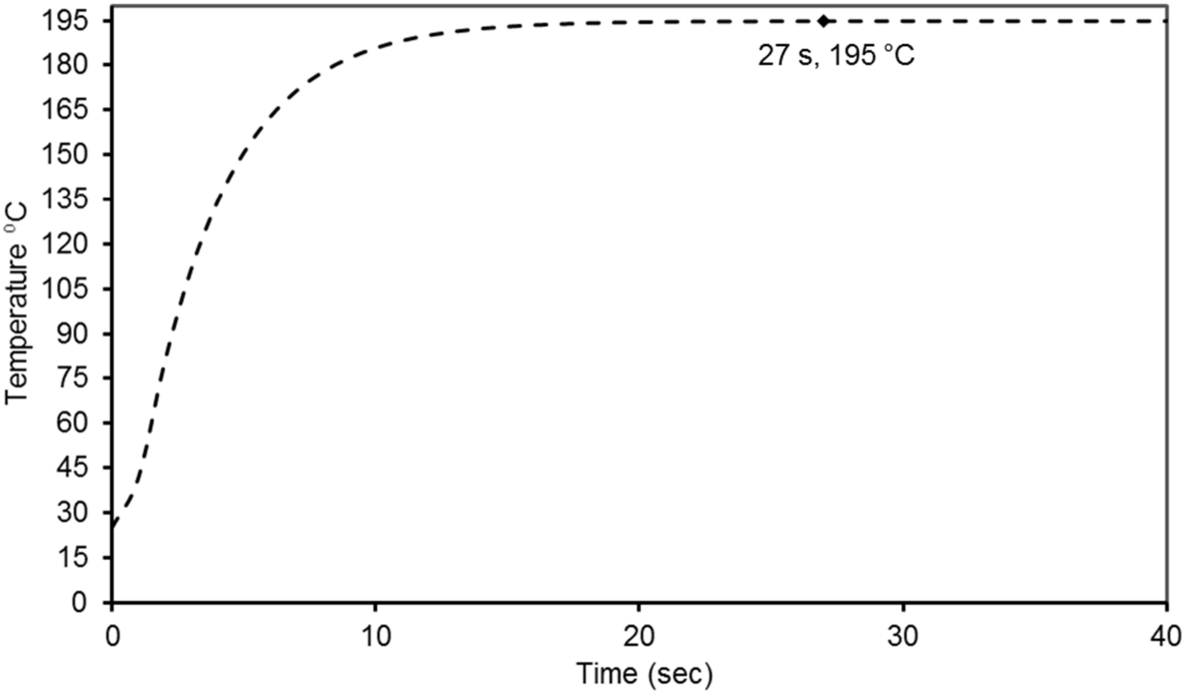


Figure 2-4. Calculated temperature profile at the center of a 4 mm thick poplar particle.

The sugar recovery results show that the efficacy of pretreatment and saccharification was independent of particle size. An unsteady state heat transfer model was developed to better understand the insensitivity of steam explosion pretreatment to particle size. Figure 2-4 shows the calculated temperature trajectory at the center of the thickest chip (4 mm) when the temperature in the reactor is held at 195°C. It can be seen that the center of the thickest chips reaches the target temperature in approximately 27 seconds. Thinner chips, of course, will reach the target temperature quicker. The total reaction time in the steam gun was 5 minutes so it can be safely assumed that all the particles are isothermal during pretreatment. The absence of significant temperature gradients, regardless of particle size, is consistent with the uniformity of sugar recovery for all the particles investigated.

2.5 __Conclusions__

The influence of initial particle size heterogeneity of hybrid poplar on sugar recovery and enzymatic hydrolysis after steam explosion pretreatment and the robustness of steam explosion as a pretreatment for simultaneously processing different particle sizes were investigated. The solid fractions after pretreatment for all the particle sizes had equivalent chemical compositions, including an absence of acetyl groups and xylans. Saccharification of the solids resulted in similar cellulose to glucose yields ranging from 78% to 82%. All the particle sizes investigated also had similar sugar recoveries in the liquid stream after pretreatment. The overall sugar recovery after pretreatment and saccharification ranged from 87-90% and 61-64% for glucose and xylose, respectively, and there were no significant differences among the different particle size samples. An unsteady heat transfer analysis shows that all the particle sizes investigated are essentially isothermal during pretreatment, providing theoretical support of the experimental results. Consequently, the initial chip size does not significantly affect the bioconversion process, from steam explosion to overall sugar recovery after saccharification. Steam pretreatment is therefore a robust method that can accommodate different particles dimensions (0.2 x 0.2 cm to 2.0 x 1.5 cm) in the same reactor, without significantly altering downstream bioprocess conversion processes.

*2.6 **Acknowledgements***

The authors would like to thank U.S. Department of Energy, Bioenergy Technologies Office, Small Business Innovation Research Program for the financial support through the Grant Contract No. DE-SC0002291, and Fulbright Program and the Administrative Department of Science and Technology of Chile (CONICYT) for the financial support through the Fulbright-CONICYT Scholarship (2010) of Rodrigo Morales Ph.D research. In addition, we would like to thank Kendall Kreft at Acrowood for assistance with their slant chipper and Forest Concepts for providing the raw biomass. Thanks also to Dr. Shannon Ewanick for all her invaluable insights.

CHAPTER 3: IMPROVING THE OVERALL SUGAR YIELD BY MIXING HYBRID POPLAR AND WHEAT STRAW BIOMASS PRIOR TO STEAM EXPLOSION AND ENZYMATIC HYDROLYSIS: SUGAR PRODUCTION AND ECONOMIC PERFORMANCE

Abstract

To ensure that future lignocellulosic biomass biorefineries are sustainable and economically feasible, it is necessary to reduce the cost of raw materials and to improve the process yields. A low cost feedstock will be diverse, changing as a function of seasonality and price and will most likely be available from multiple sources to the biorefinery. Unfortunately, little attention has been shown to determining the influence of processing mixed biomass during steam pretreatment (SP) and enzymatic hydrolysis (EH) and how mixed biomass (MB) will influence the economic performance of a biomass to ethanol facility. In this study, hybrid poplar (HP) and wheat straw (WS) in three different proportions were impregnated with 3% SO_2 and steam pretreated at 195˚C for 5 minutes. Surprisingly, after fractionation, on average 13% more sugar was recovered using mixed feedstock than either single biomass, showing the robustness of SP for processing different mixtures of HP and WS. After SP and EH, MB showed on average 20% higher sugar yields than single HP and WS. Since feedstock cost and sugar yields are key factors determining the economic performance of a biorefinery, a techno-economic model was performed to investigate the economic feasibility of using HP and WS during bioconversion to ethanol under two scenarios. The first scenario used MB (75% HP and 25% WS) which is processed simultaneously and the second scenario used a 'campaign' production plan (CP). MB generate almost twice as much income per year ($13.5 MM) than using single biomass in CP. Consequently, production of ethanol using mixed biomass is more competitive to campaign design processing to ethanol production.

*3.1 **Introduction***

Based on current technologies, the cost of production of fuels and chemicals from lignocellulosic material is relatively high (Sun and Cheng, 2002). To make a future lignocellulosic biomass biorefinery economically feasible, it is necessary to improve the process yields. Considerable research efforts have been exerted to improve sugar yields from biomass feedstocks, chemically modify raw materials using genetic engineering techniques (Dinus, 2001 & Chabannes et al., 2001), develop novel pretreatment methods and reactor designs (Degenstein, et al., 2011; Brodeur, 2013 & Hou et al., 2012), and design reactors for saccharification of high consistency solids (Jørgensen et al., 2007). However, there has been less attention to pre-processing of lignocellulosic biomass as an alternative way to improve the overall biomass to sugar conversion.

Currently the feedstock cost is the major determinant in the viability of commercial scale production of fuels and chemicals, contributing to 40-45% of the operating costs in a lignocellulosic biomass based biorefinery (Juneja et al., 2013). In addition, the low selling price of the products generated by a biorefinery does not allow the biomass conversion facility to purchase 'pristine' feedstocks composed of clean, homogeneous and high quality biomass (Kenney et al., 2013). Moreover, a consistent and stable supply of feedstocks will be required by the biorefinaries to accomplish large scale utilization of lignocellulosic biomass. Therefore, in order to develop economically viable and sustainable processes of converting biomass to fuels and chemicals, it would be imperative to process the cheapest raw material available in a specific region. These feedstocks will be diverse and will change as a function of time and price, and will most likely be available in a mixed input stream to the biorefinery (Shi et al., 2013).

Consequently, a potential biomass processing facility must be able to convert these diverse feedstock without significantly altering the overall performance, sugar yields and fuel production. Currently, most bioconversion research has been carried out with high quality raw materials such as screened wood chips and little attention has been paid to the efficiency of converting heterogeneous mixtures of feedstocks into fermentable sugars and fuels.

In the Pacific Northwest of the United States there is year round availability of 24,000 ha hybrid poplar (WSU., 2000), with yields ranging from 6.9 to 19.1 tonne ha^{-1} yr^{-1} (Berguson et al., 2010) and seasonal availability of approximately 1.2 million ha of planted wheat (USDA, 2013) producing more than 3 million dry tons per year of wheat straw (Frear et al., 2005 and Graf & Koehler., 2001). Wheat straw (WS) costs much less than hybrid poplar (HP) ($27-50 vs. $77-105/dry tonne) (Qureshi et al, 2013 and Juneja et al, 2013) (Berguson et al., 2010 and Huang et al., 2009). In addition, since HP is available through the year and WS is harvested in late spring, these two type of feedstocks are seasonally complementary. These characteristics make HP and WS excellent potential raw materials for a future lignocellulosic based biorefinery in this region.

Despite the fact that several studies have successfully showed the advantages of processing HP and WS for the production of fuels and chemicals (Bura et al., 2009; Wyman et al, 2009; Sannigrahi et al., 2009; Ballesteros et al., 2006; Georgieva et al., 2008) there are no studies focused on the bioconversion of combined woody biomass and agricultural residues. Thus, the combining of wheat straw and poplar for the production of sugars is an unexplored opportunity for maintaining the productivity and profitability of a biorefinery. In this regard, for first time, this research investigated the effect of using mixed feedstocks on bioconversion for sugar production (chapter 3A) and the economic performance of using mixed biomass during bioconversion to ethanol (chapter 3B).

CHAPTER 3A: IMPROVING THE OVERALL SUGAR YIELD BY MIXING HYBRID POPLAR AND WHEAT STRAW BIOMASS PRIOR TO STEAM EXPLOSION AND ENZYMATIC HYDROLYSIS

3A.1 Objectives

The overall goal of this study is to determine the influence of mixing wheat straw and hybrid poplar biomass on the overall sugar production via steam pretreatment and enzymatic saccharification. Specifically, the objectives of this investigation were to assess the influence of mixing hybrid poplar (HP) and wheat straw (WS) on the following: (1) chemical properties of solids and sugar recovery after steam pretreatment, (2) enzymatic digestibility of solids, (3) sugar yields after steam explosion pretreatment and enzymatic hydrolysis. In addition, we assessed the robustness of steam explosion as a pretreatment for concurrently processing different combinations of HP and WS.

3A.2 Methods and materials

Hybrid poplar (HP), wheat straw (WS) and three mixtures with different combination of both types of biomass (M1: 75% HP, 25% WS; M2: 50% HP, 50% WS and M3: 25% HP, 75% WS), were impregnated with SO_2 (3% w/w), steam pretreated at 195°C for 5 minutes and enzymatically saccharified at 5% (w/v) solids consistency, 5 FPU/g cellulase to determine the influence of using mixed biomass on overall sugar yields via bioconversion. The conditions for steam pretreatment for HP were chosen for previous work done by Ewanick (2012), WS parameters were optimized based on H_2SO_4 WS steam pretreatment published by Ballesteros et al. (2006) (Appendix 2). A complete mass balance of carbohydrates was determined to assess overall sugars yield. Figure 3A-1 shows process flow for the bioconversion experiments.

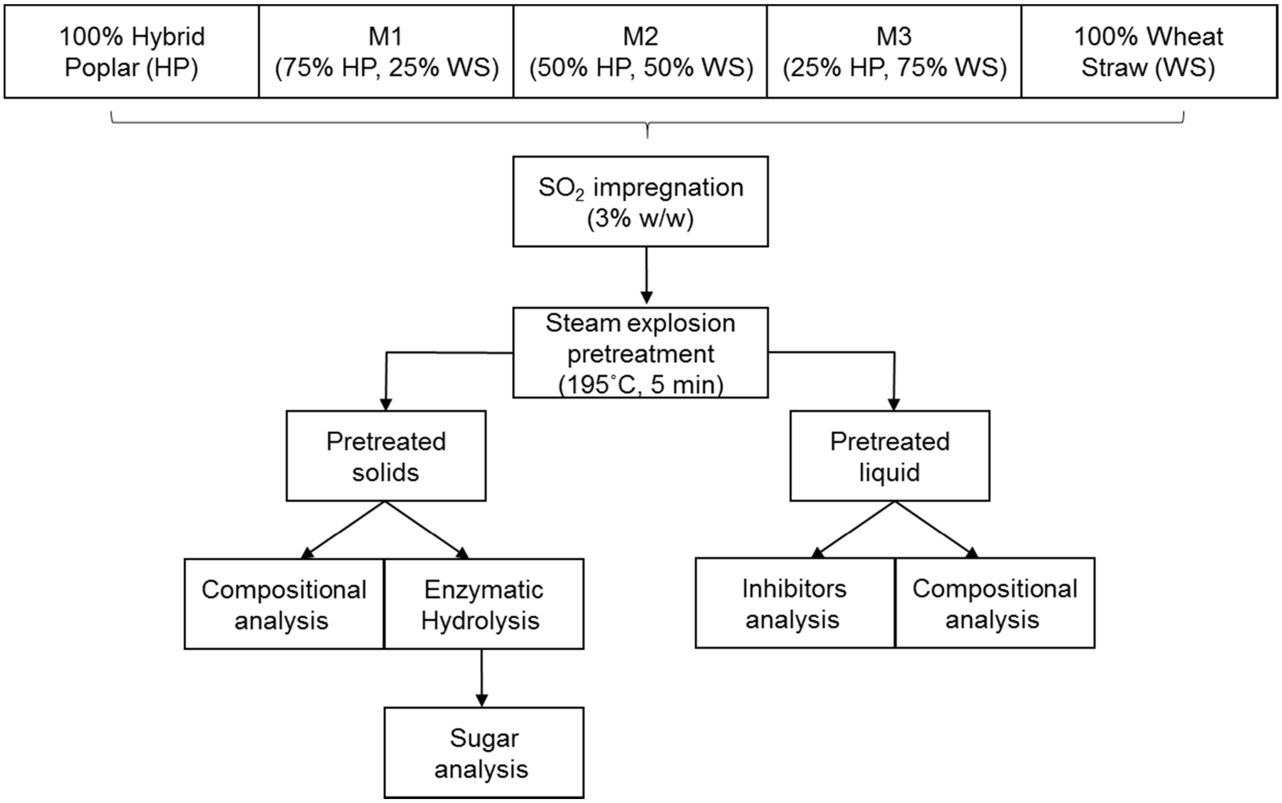


Figure 3A-1. Process flow diagram for bioconversion of hybrid poplar (HP), wheat straw (WS) and the different mixtures into carbohydrates.

3A.2.1 Material

Two types of biomass were used for this study. Freshly harvested 18-year-old hybrid poplar (HP), *Populus deltoides x Populus nigra*, from Puyallup, WA, was kindly provided by Washington State University (WSU) and wheat straw (WS), *Tritricum* spp. from Eastern Washington, was kindly provided by the Paper Science Center of the University of Washington. HP was comminuted with a slant disc chipper (Acrowood, Everett, WA) and then screened to particles approximately 2cm x 2cm x 0.4 cm with a moisture content of 60%. WS was baled and received as full length straw. Straw was then chipped with an ECHO Bear Cat Chipper/Shredder to straws less than 2-3 cm with a moisture content of 10%. Prior to pretreatment, each type of biomass was submerged in water for 24 h. Each was then centrifuge to remove the excess water to obtain a moisture content approximately of 60%. HP and WS were used to prepare three different mixtures containing different proportions of each type of biomass (M1: 75% HP, 25% WS; AM2: 50% HP, 50% WS and M3: 25% HP, 75% WS).

3A.2.2 Pretreatment and processing conditions

The bioconversion process flow for this research is shown in Figure 3A-1. Prior to pretreatment, 300 g of dry biomass of each of the five feedstocks (HP, M1, M2, M3 and WS) was impregnated with gaseous sulfur dioxide (3% w/w) and sealed in airtight plastic bags. Specifically, 9 g of SO_2 was added to the 300 g of dry biomass from a gas cylinder in to a plastic bag containing the biomass. The weight of the biomass was monitored overnight to determine the gas retention for each feedstock. Samples were then subdivided into 150 g samples and pretreated using a 2.7l batch steam gun manufactured by Aurora Technical (Savona, BC, Canada) at 195°C for 5 min. At the end of the reaction time, a pneumatic valve was opened between the pressurized reaction tank and the collection tank, causing the explosion of the biomass, which was discharged into the collection tank. The resulting slurry was vacuum filtered to separate the liquid fraction from the pretreated solids. Both fractions were analyzed as described below and used to construct a mass balance of carbohydrates and lignin. Solids were washed with deionized water equal to 20 times the mass of solids prior to analysis and hydrolysis. Each steam explosion was performed 3 times for each sample, resulting in a total of 15 substrates, all of which were analyzed as described below.

3A.2.3 Instrumental analysis

High Pressure Liquid Chromatography (HPLC)

Carbohydrates were measured on a Dionex (Sunnyvale, CA) HPLC (ICS-3000) system equipped with an AS autosampler, ED electrochemical detector, dual pumps, and anion exchange column (Dionex, CarboPac PA1). Deionized water at 1 ml/min was used as an eluent, and post-column addition of 0.2 M NaOH at a flow rate of 0.5 ml/min ensured optimization of baseline stability and detector sensitivity. After each analysis, the column was reconditioned with 0.25 M NaOH. Twenty microliters of each sample were injected after filtration through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Samples were measured against standards consisting of arabinose, galactose, glucose, xylose, and mannose. In addition, fucose was used as an internal standard.

Acetic acid, furfural and 5-hydromethylfurfural were measured using refractive index detection on a Shimadzu Prominence LC. Separation of these compounds was achieved by an anion exchange column (REZEX RHM-Mono-saccharide H^+ (8%), Phenomenex, Inc., Torrance, CA) with an isocratic mobile phase that of 5 mM H_2SO_4 at a flow rate of 0.6 ml/min. The column oven temperature was maintained at 63°C. Twenty microliters of each sample were injected after being appropriately diluted in deionized water and filtered through a 0.22 µm syringe filter (Restek Corp., Bellefonte, PA). Standards were prepared and used to quantify the unknown samples.

3A.2.4 Compositional analysis

Insoluble carbohydrates, acetate groups and lignin

Raw materials as HP, WS and the mixtures (M1, M2 and M3) and respective pretreated solids were analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate and acetates content using TAPPI method T-222 om-98 (Tappi, 1998) and NREL procedure LAP 017 (Ehrman and Ruiz, 1998) respectively. Briefly, 0.2 g of finely ground oven dried sample was treated with 3 ml of H_2SO_4 at 72% for 120 minutes at room temperature, then diluted into 120 ml total volume, then autoclaved at 121˚C for 60 minutes. The autoclaved samples were then filtered using a glass crucible to separate the acid insoluble lignin (AIL) from the acid soluble lignin (ASL) and carbohydrates. The AIL was determined by weighting the oven dried crucibles, and the ASL in the filtrate was analyzed by UV at 205 nm. The filtrate was analyzed by HPLC for carbohydrate composition and acetates content.

Soluble carbohydrates

Monomeric and oligomeric soluble carbohydrates were determined using NREL LAP TP-510-42623 (Sluiter et al., 2004). Briefly, 0.7 mL of 70% H_2SO_4 was added to 15 mL of the liquid samples, and the volume made up to 20 mL with water. Samples were autoclaved at 121˚C for 60 minutes and analyzed by HPLC as described previously. Monomeric sugars were determined by analyzing the original samples without acid hydrolysis. Oligomeric sugar was calculated by subtracting monomeric sugar content from total sugar content determined after acid hydrolysis.

Total phenolics

Total phenolic approximate concentration in the hydrolysates was assayed spectrophotometrically at 765 nm by Folin Ciocalteu method (Singleton et al., 1999) using a FTIR spectrophotometer (Shimadzu, Tokyo, Japan). Gallic acid was used as calibration standard.

3A.2.5 Enzymatic Hydrolysis

Enzymatic hydrolysis of washed solids was done at 5% w/v solids in a total volume of 50 ml in 125 ml Erlenmeyer flasks. The solution was buffered at pH 4.8 with 0.05 M citric acid buffer and the hydrolysis was carried out at 50˚C and 150 rpm shaking on an orbital shaking incubator (New Brunswick). Cellulase (Celluclast 1.5 L, 26 FPU/ml, Sigma) was added at 5 FPU/g cellulose and supplemental beta-glucosidase (Novozym 188, 492 CBU/ml, Sigma) was added at 10 CBU/g cellulose. 1 ml samples were periodically removed and analyzed for glucose and xylose content.

3A.2.6 Statistical Analysis

One way ANOVA at 5% alpha was performed for each dependent variable to assess statistical differences as a function of the feedstock. In the cases where statistically significant differences were found (p value<0.05), a “Tukey test” (multiple comparison of means) was performed to find the specific group or groups of feedstock that were causing those differences. Data analysis was done using the statistical software R version 2.12.1.

3A.3 ***Results and discussion***

In this study, hybrid poplar (HP), wheat straw (WS) and three mixtures with different combination of both types of biomass (M1: 75% HP, 25% WS; M2: 50% HP, 50% WS and M3: 25% HP, 75% WS) were used to determine the influence of using mixed biomass on overall sugar yields following steam pretreatment and subsequent enzymatic hydrolysis. It is well know that single HP and WS can be successfully convert to sugars via steam pretreatment and saccharification, however is lesser know is whether mixing both type of biomass could maintain or increase the overall sugar yields. In order to facilitate a future techno-economic comparison among different raw materials used in this research, the unit used for the characterization of liquid and solids after pretreatment as well as overall sugar yield was kg/tonne of raw biomass.

3A.3.1 Raw material composition

Hybrid poplar (HP) and wheat straw (WS) were chosen for their availability in the Pacific Northwest and for their seasonal feedstock complementarity (HP is available throughout the year and WS is harvested in spring and autumn). The composition of the original untreated HP, WS and the different mixtures are presented in Table 3A-1. The total polysaccharide content of all the biomass ranged from 53% to 56%. The compositional analysis showed significant differences for most of the components found in HP and WS except galactan and total lignin. Arabinan, xylan, extractives and ash content increased from 0.2 to 2%, from 12 to 17.5%, from 6.1 to 9.4% and from 0.5 to 4.3% respectively, when WS was added to the samples. The presence of glucan, mannan and acetic acid increased from 32.6 to 41.5, from 0 to 1.7% and from 2.6 to 3.7% respectively, when HP was supplemented to the mixtures. The presence of lignin, including acid soluble and insoluble, was similar among the different samples, ranging from 24 to 25%. Significant differences were found among the mixtures in terms of extractives and ash content. The sugar composition of the HP and WS was similar to compositions observed by other investigators. However, some variability was observed for WS lignin which was 7% higher, and extractives content which was 6% lower than values found by Ballesteros et al. (2006), showing the compositional variability among different WS samples.

Table 3A-1. Compositional analysis of raw biomass expressed as % dry matter.

	HP	M1	M2	M3	WS
Arabinan	0.2 ± 0.0	0.8 ± 0.2	1.2 ± 0.1	1.8 ± 0.1	2.0 ± 0.1
Galactan	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Glucan	41.5 ± 1.5	36.6 ± 1.0	37.7 ± 1.4	36.4 ± 0.7	32.6 ± 1.4
Xylan	12.0 ± 1.0	15.2 ± 0.4	14.8 ± 0.2	15.3 ± 1.0	17.5 ± 0.2
Mannan	1.7 ± 0.1	1.5 ± 0.1	1.2 ± 0.1	0.7 ± 0.1	0.0 ± 0.0
Total sugars	55.8 ± 2.6	54.5 ± 1.7	55.3 ± 1.9	54.6 ± 2.0	52.5 ± 1.8
Acetate	3.7 ± 0.0	3.2 ± 0.1	3.0 ± 0.1	2.9 ± 0.3	2.6 ± 0.2
Total lignin	24.2 ± 0.4	23.8 ± 0.2	23.9 ± 0.4	24.5 ± 0.4	23.5 ± 0.9
Extractives	6.1 ± 0.2	6.1 ± 0.1	7.3 ± 0.4	8.3 ± 0.5	9.4 ± 0.5
Ash	0.5 ± 0.1	1.5 ± 0.0	2.5 ± 0.0	3.4 ± 0.0	4.3 ± 0.0

HP: Hybrid poplar, M1, M2 and M3: Mixed biomass, WS: Wheat straw, Total lignin: acid soluble lignin + acid insoluble lignin.

HP and WS are made up of different types of hemicellulose. Glucuronoxylan has been identified as the main hemicellulose in poplar, whereas the most abundant hemicellulose constituents in WS as well as corn stover, rye, barley, oat, rice, and sorghum are arabinoxylans (Ebringerová & Heinze, 2000; Polizeli et al., 2005). Therefore, when WS is added to the mixtures the arabinan content increased (Table 3A-1). As glucose and xylose made up the majority of carbohydrates in the raw material, arabinose, galactose and mannose were reported as minor sugars in the subsequent analysis.

3A.3.2 Solids composition after pretreatment

The chemical composition of solids after steam pretreatment, expressed as % dry matter for the different pretreated samples are shown in Figure 3A-2. Only glucan, xylan, lignin and ash were found in the resulting solids of the pretreated samples, ranging from 62 to 66%, from 1 to 4%, from 28 to 35%, and from 1 to 4% respectively, except in HP were xylan and ash were not found. At least 96% of the hemicellulosic sugars were solubilized into the liquid fraction, except for some minor quantities of WS xylan which was less labile than HP xylan. Thus, more xylose remained in solids of the mixtures containing more WS. Since raw WS contained the highest ash content, after pretreatment, the presence of ash increased when more WS was supplemented to the samples. Glucan content along with lignin comprised at least 90% of the pretreated solids. No statistically significant differences were found for glucan content for all the samples tested. Nevertheless, despite the fact that total lignin content was similar among all the unpretreated samples, the lignin content in pretreated solids increased when the proportion of HP increased. Considering the similar chemical composition after pretreatment of the different mixtures of biomass (M1-M3), it is possible to conclude that steam pretreatment with SO_2 is a robust fractionation method able to produce homogeneous slurry from a diverse mixture of biomass containing different combinations of HP and WS.

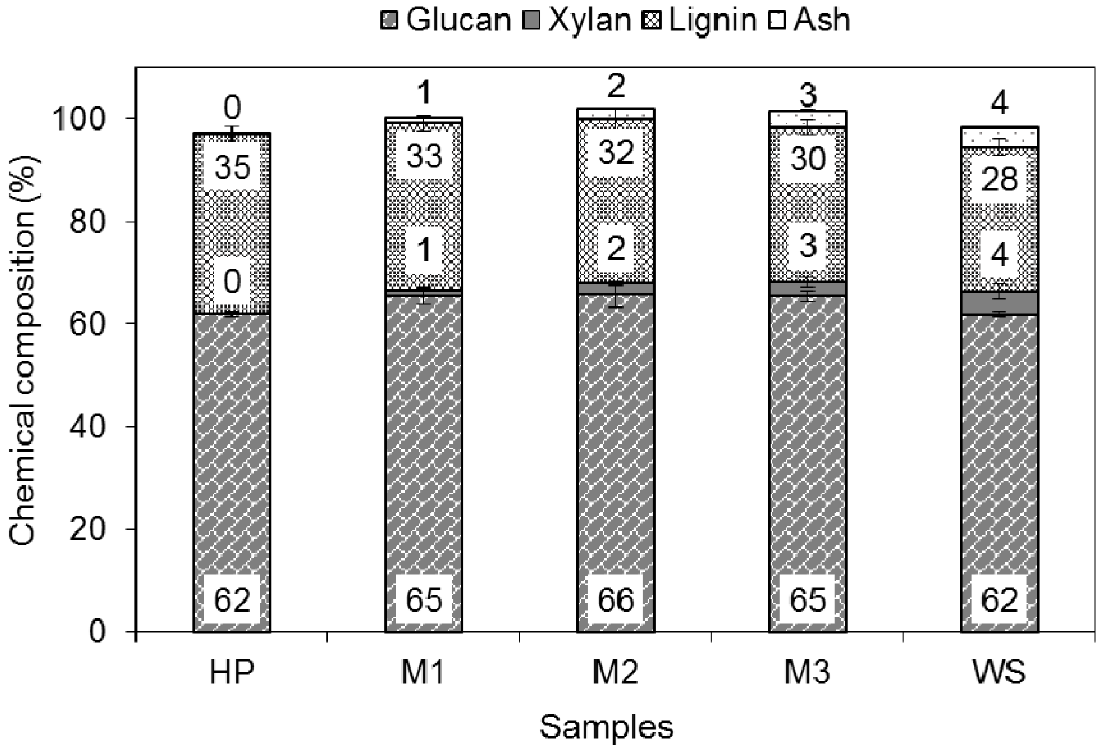


Figure 3A-2. Composition of solid fractions of pretreated hybrid poplar (HP), mixtures (M1, M2, M3) and wheat straw (WS) expressed as % dry matter.

3A.3.3 Liquid stream composition after pretreatment

The amount of sugars, acetic acid, furfural, 5-hydroxymethylfurfural, total phenolics and pH in the pretreated liquid fractions were measured. Table 3A-2 shows that glucose, xylose and minor sugar content expressed as kg per tonne of raw biomass ranged from 5 to 63 kg/tonne, from 75 to 146 kg/tonne, and from 6 to 13 kg/tonne respectively. The majority of the sugars, excluding WS, were in monomeric form, ranging from 88 to 98%, 87 to 99% and 72 to 87% for glucose, xylose and minor sugars respectively. The amount of glucose increased with decreased WS addition, which agrees with the chemical composition of the raw material, where more glucan content was observed for the samples containing more HP. The amount of xylose solubilized in the liquid fraction was highest in the experiment with high fraction of WS, as expected, due to higher content of this type of hemicellulose in WS. Examining the glucose, xylose and minor sugar contribution to the total sugar concentration, the mixed biomass (M1-M3) showed the highest total sugar concentration, ranging from 175 to 179 kg/tonne, indicating its potential for use as a monomeric sugar raw material for a lignocellulosic based biorefinery.

Table 3A-2. Sugar composition of liquid fraction of pretreated samples expressed as kg/tonne of raw biomass.

	Glucose		Xylose		Minor sugars		Total sugars	
	Total	% mon [a]	Total	% mon [a]	Total	% mon [a]	Total	% mon [a]
HP	63 ± 3	93	75 ± 2	90	6 ± 1	72	144 ± 6	85
M1	59 ± 3	95	114 ± 4	99	6 ± 1	78	179 ± 8	91
M2	43 ± 3	98	119 ± 4	99	13 ± 2	87	175 ± 9	95
M3	20 ± 3	88	146 ± 6	99	11 ± 1	83	177 ± 9	90
WS	5 ± 1	67	116 ± 5	77	8 ± 1	58	129 ± 7	67

HP: Hybrid poplar, WS: Wheat straw, M1: 75% HP, 25% WS, M2: 50% HP, 50% WS and M3: 25% HP, 75% WS, [a] monomeric sugar.

Acetic acid, furans such as furfural from pentoses and 5-hydroxymethylfurfural (HMF) from hexoses, and total phenolics from lignin were also found in the liquid stream (Table 3A-3). When more wheat straw was added to the samples less acetic acid, total phenolics and a higher pH in the liquid fraction were found, ranging from 12 to 36 kg/tonne, from 30 to 34 kg/tonne and from 1.4 to 1.8 pH respectively. The concentration of furfural and HMF for all the samples was less than 13 kg/tonne and 4 kg/tonne respectively. These relatively low furan concentrations are likely due to the optimal steam pretreatment conditions used which minimized sugar degradation.

Table 3A-3. Sugar composition of liquid fraction of pretreated samples expressed as kg/tonne of raw biomass.

	Acetic acid	Furfural	HMF[b]	Total phenolics	pH
Hybrid poplar (HP)	36 ± 4	13 ± 1	4 ± 1	34 ± 3	1.4
M1 (75% HP, 25% WS)	31 ± 1	11 ± 1	2 ± 0	33 ± 4	1.5
M2 (50% HP, 50% WS)	29 ± 3	9 ± 1	2 ± 1	32 ± 1	1.6
M3 (25% HP, 75% WS)	21 ± 5	6 ± 2	1 ± 1	31 ± 4	1.7
Wheat straw (WS)	12 ± 4	4 ± 2	0 ± 0	30 ± 2	1.8

[b] 5 hydroxymethylfurfural.

The different mixtures of biomass did influence the presence of sugars in monomeric or oligomer form in the liquid stream. This trend could be explained by the presence of acetates and ash in the mixed biomass samples (Table 3A-1), as well as the pH in the liquid hydrolysates obtained after pretreatment (Table 3A-3). Acetates was increased by adding more HP, while ash content was added by increasing the amount of WS in the mixtures. It is well known that during steam pretreatment organic acids such as acetic acid, in conjunction with sulfur dioxide, generate acidic conditions to solubilize hemicellulosic sugar in the liquid stream. In this more acidic environment, more sugars are released in monomeric form but at the same time more sugar degradation products are generated (Chen & Heiningen, 2010). The presence of ash, present at higher levels by adding WS to the mixtures, could 'buffer' extreme acidic conditions, avoiding the generation of sugar degradation products including furans. This synergistic effect, caused by mixing HP and WS is supported by observing pure HP and WS trends. HP with the lowest pH (1.4) and the highest acetic acid content (3.7%) shows less monomeric sugar (85%) than the average of the mixtures (92%). However at the same time it shows the highest furan content, indicating more sugar degradation products. On the other hand, WS with the highest pH (1.8) and the highest ash content (4.2%) contains only 58% of the sugars in monomeric form and has minimal furan formation.

3A.3.4 Sugar recovery after steam pretreatment

After steam pretreatment the combined sugar recovery from pretreated solids and liquid hydrolysates was calculated for each of the sulfur dioxide impregnated samples. Table 3A-4 shows the sugar recovery expressed as % of the theoretical amount available in the raw material. For all samples, recovery was high, with at least 89% of the sugars recovered. Nevertheless, significant differences in recovery were found between mixed (M1, M2 and M3) and single biomasses (HP and WS), ranging from 104 to 107% and from 89 to 95% respectively. The higher sugar recoveries observed for the HP and WS mixtures are likely due to two factors. First, the similar sugar composition of the solids after steam pretreatment, resulted in a glucan plus xylan content ranging from 32 to 35 kg/tonne of raw biomass for all the pretreated samples. Second, the higher sugar recovery obtained in the liquid stream of mixed biomass, as a consequence of the synergistic effect of mixing HP and WS. As a result, the mixed biomass (M1, M2 and M3) showed a total sugar content on average 14% higher than single biomass (HP and WS). Thus, processing of mixtures containing different combination of HP and WS increased the sugar recovery after steam pretreatment.

Table 3A-4. Combined sugar recovery of solid and liquid fraction, after steam pretreatment of hybrid poplar, mixed biomass and wheat straw expressed as % of the theoretical sugars available in the raw material.

Hybrid poplar (HP)	88.6 ± 3.9
M1 (75% HP, 25% WS)	105.5 ± 5.6
M2 (50% HP, 50% WS)	104.2 ± 4.4
M3 (25% HP, 75% WS)	106.6 ± 7.3
Wheat straw (WS)	94.5 ± 2.1

3A.3.5 Enzymatic Hydrolysis

Following pretreatment, the enzymatic digestibility of the washed pretreated solids was evaluated. All the samples were enzymatically hydrolyzed at 5% consistency and 5 FPU/g cellulose enzyme loading. Figure 3A-3 shows the cellulose to glucose conversion after 48 hours of saccharification. The extent of cellulose conversion highlighted the differences in digestibility between HP and WS, as well as the mixed biomass (M1, M2 and M3). For all samples, a conversion range from 73 to 81 % was observed after 48 hours of enzymatic hydrolysis. A statistically significance difference was found between saccharification of HP and WS with a 73% and 81% cellulose to glucose conversion respectively. Among the mixtures, conversions ranged from 74 to 77% for M1 to M3 respectively with no statistical differences among HP and WS mixtures (M1-M3).

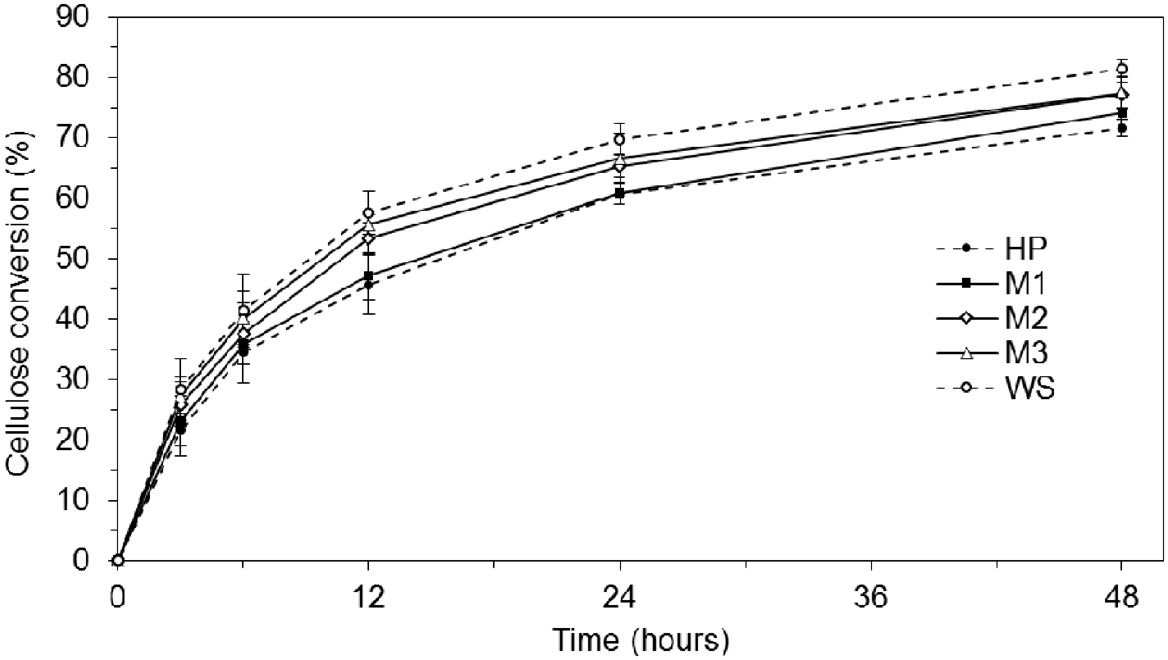


Figure 3A-3. Cellulose to glucose conversion for steam pretreated hybrid poplar (HP), mixtures (M1, M2, M3), and wheat straw (WS) during enzymatic hydrolysis at 5% (w/v) solids consistency and 5 FPU/g cellulose and 10 CBU/g cellulose enzymes loading.

It has been shown previously that agricultural biomass is more responsive to steam pretreatment, resulting in a solid substrate that is easier to hydrolyze than hardwood-derived substrates (Eklund et al., 1995 and Sassner et al., 2005). Therefore, as expected, WS showed better saccharification yields than HP. In addition, according to Santos et al. (2012), lignin acts as a physical barrier blocking cellulose from enzyme binding thereby reducing cellulose accessibility and conversion to glucose. Consequently lignin content and lignin composition are among the key substrate components that control enzymatic hydrolysis efficiency. It is well known that woody and grasses lignocellulosics have different type of lignin. According to their relative proportions of hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units, grass lignin have been classified as HGS-lignin, which are known to be different from those of softwoods (G-lignin) or hardwoods (GS-lignin)

(Del Rio et al., 2007). Specifically, poplar contains GS lignin, with a G/S ratio ranging from 1.0-3.0 (Studer et al., 2011). Wheat straw lignin comprises HGS-lignin associated with p-coumarates and ferulates with H, G and S proportions ranging from 5-6, 49-64, 30-46% respectively (Del Rio et al., 2012 and Buranov and Mazza, 2008). Therefore, the lower lignin content and different type of lignin could contribute to the improved saccharification of WS solids compared to HP. Consequently, in terms of mixed biomass, saccharification yields were improved when WS was present in higher amounts. Xylan removal had been previously reported as an enhancer of cellulose digestibility (Bura et. al 2009). However, in the present study, where M1, M2, M3 and WS pretreated solids contained only 1, 2, 3 and 4% xylan respectively, other factors such as lignin content and type likely had a greater contribution to differences in saccharification for all of the steam pretreated substrates.

3A.3.6 Total sugar yields

After steam pretreatment and enzymatic hydrolysis, the overall monomeric sugar yield was calculated for each single biomass (HP and WS) and the three different HP and WS mixtures (M1, M2 and M3). Figure 3A-4 shows the overall sugar yields of glucose, xylose and minor sugars in the solid and liquid fraction, after pretreatment and saccharification, expressed as kg of monomeric sugar per tonne of raw biomass. Glucose, xylose and minor sugars content ranged from 309 to 393 kg/tonne, from 75 to 146 kg/tonne, and from 6 to 13 kg/tonne respectively. Since no prior research has been done using similar mixtures of HP and WS, only single biomass data found in the literature was used for comparison. Wyman et al. (2009), steam pretreated and saccharified poplar wood using similar fractionation conditions but used 3 times higher enzyme loading than the present study, obtaining 100% and 64% of glucose and xylose recovery respectively after pretreatment and saccharification. In the current study, due to the lower enzyme loading, 24% and 8% less glucose and xylose respectively were recovered using single HP after pretreatment and enzymatic hydrolysis. Nevertheless, after pretreatment, similar sugar recoveries were observed, resulting in 100% and 57% of glucose and xylose recovery respectively (data not shown). For WS, Linde et al. (2008), steam pretreated 0.2% H_2SO_4 impregnated WS at 190° C for 5 min, resulting in 102 and 103% of glucose and xylose recovery after fractionation. In the present study, similar glucose recovery was observed (105%), but 10% less xylose was recovered (90%) after fractionation (data not shown), this trend is probably due to the higher temperature used during pretreatment (195° C), resulting in more xylose to furfural degradation. The overall sugar recovery after fractionation and enzymatic hydrolysis showed by Linde et al (2008) was higher than the present study (99.6 compared with 74%), due to higher enzyme loading (15 vs 5 FPU), longer saccharification times (96 compared with 48 hours) and Linde et al (2008), considered both monomeric and oligomers sugars.

Similar overall monomeric sugar yields were obtained from both HP and WS. To understand these similar results, it is necessary to recognize how the glucose and xylose content of the raw HP and WS influenced the different processes during the bioconversion. Considering that raw HP exhibited 9% more glucan content but 5% less xylan content in comparison with raw WS, after pretreatment the HP hydrolysate exhibited 58 kg/tonne more glucose than single WS liquid stream. Additionally, the better enzymatic hydrolysability of the pretreated WS solids was not enough to compensate for the lower initial glucose content in the raw material, resulting in an overall HP glucose yield 11%

higher than single WS. Since no xylose was recovered during saccharification of the steam pretreated solids, only the xylose solubilized in the liquid was considered in the overall sugar recovery. Therefore, as expected from the chemical composition of the raw biomass, WS yielded 35% more xylose than HP. Similarly, although less than 42% of the minor sugars were recovered after pretreatment, WS exhibited slightly higher minor sugar yields than HP. As a result of the increased glucose content in raw HP along with higher xylose content and better saccharification of WS, equal overall sugar yields were obtained using HP and WS; 429 and 432 kg of monomeric sugars per tonne of raw biomass respectively.

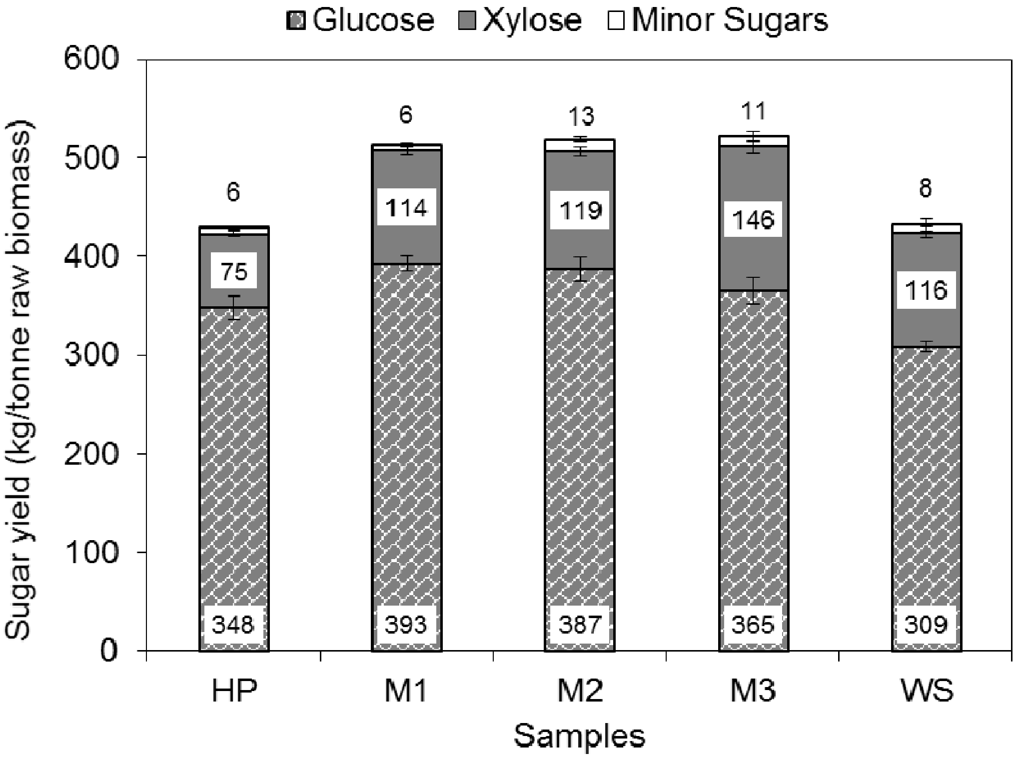


Figure 3A-4. The overall sugar yields of glucose, xylose and minor sugars after pretreatment and enzymatic hydrolysis in the solid and liquid fractions, expressed as kg of monomer sugar per tonne of raw biomass. HP: Hybrid poplar, M1, M2 and M3: Mixed biomass, WS: Wheat straw.

Regarding HP and WS mixtures, in comparison with single biomass, higher sugar yields ranging from 513-521 kg per tonne of biomass were exhibited for M1, M2 and M3 samples. This is a statistically significant increase of 20% compared to single HP and WS. In general, higher glucose, xylose and minor sugar yields after pretreatment and saccharification were found by mixing HP and WS. This trend likely is due to a synergistic effect during pretreatment, caused by interaction of acetic acid content observed in HP and ash content exhibited by WS as explained before. The glucose yield for the different HP and WS mixtures varied mostly according the glucose recovery obtained in the hydrolysates after pretreatment, since the glucan content was similar in the unpretreated mixtures and the solids saccharification yields were similar among the mixed samples. Therefore the glucose yield was highest when more HP was present in the mixtures, resulting in the higher glucose yield in M1. In terms of xylose yields, only the xylose solubilized in the liquid was considered for the overall sugar recovery since no xylose was recovered during saccharification. Thus, as expected from the raw material compositional analysis, higher xylose yields were observed for the mixtures containing more WS biomass.

It is therefore possible to conclude that processing mixed feedstocks containing different combination of HP and WS will improve the sugar yields after steam pretreatment and enzymatic hydrolysis. Mixing lignocellulosic biomass is an excellent opportunity for potentially increasing the productivity and profitability of a future biorefinery located in the Pacific Northwest. Aside from changing the overall thinking about negative impacts of the heterogeneous nature of biomass during bioprocessing, this approach minimizes disruptive process swings due to biomass availability, and enables the processing of biomass with higher ash content such as WS. In addition, using mixed HP and WS as a feedstock could generate greater yields and additional revenue in HP/WS to ethanol bioconversion facility.

Future work, a techno-economic analysis will be performed to determine the economic feasibility of using different combinations of HP and WS as feedstock in a lignocellulosic based ethanol biorefinery. In addition, considering that the long-term viability of using any feedstock for fuel production depends not only on its yield but also on the sustained production capacity, reduction in greenhouse gas emissions and net energy output. A life cycle assessment (LCA), will be performed to determine the environmental impacts of mixing HP and WS on bioconversion for ethanol production.

3A.4 ***<u>Conclusions</u>***

In this study hybrid poplar (HP) and wheat straw (WS) were used to determine the influence of using mixed biomass (MB) during steam pretreatment (SP) and subsequent enzymatic hydrolysis on overall sugar yields. Despite raw HP and WS having different physico-chemical properties, mixing both types of biomass positively affects the bioconversion process. Based on the overall sugar yields, mixed biomass exhibited on average 20% more sugar production than either single biomass. It was postulated that there is a synergistic effect caused by interaction of acetic acid content in HP and ash content in WS, resulting in more monomeric sugar recovery and less sugar degradation in the biomass mixtures. In addition, considering the similar chemical composition and saccharification of pretreated MB, as well as the increased sugar yields, it is possible to conclude that SP with SO_2 is a robust and efficient fractionation process that can concurrently process mixed feedstock containing different combinations of HP and WS.

CHAPTER 3B: TECHNO-ECONOMIC FEASIBILITY OF MIXING HYBRID POPLAR AND WHEAT STRAW ON BIOCONVERSION FOR ETHANOL PRODUCTION

3B.1 Objectives

The overall objective of this research is to evaluate the economic feasibility of mixing hybrid poplar (HP) and wheat straw (WS) during bioconversion to ethanol. Specifically, the goals of economic analysis were to estimate both capital and operating costs of the biorefinery as well as payback period of the investment. In this paper, based on two different annual production plans, two scenarios were analyzed. The first scenario used mixed biomass (75% HP and 25% WS) (MB) which is processed simultaneously and the second scenario used a 'campaign' production plan (CP), where a campaign is the production of ethanol using one specific single biomass (HP or WS) in consecutive months.

3B.2 Technical modeling

A comprehensive technical analysis was performed for the conversion of poplar, wheat straw and mixed feedstock into ethanol using modified process model developed by NREL (Humbird et al. 2011). The process is illustrated in Figure 3B-1. It consists of eight areas: feedstock storage and handling, pretreatment and hydrolysate conditioning, enzymatic hydrolysis, fermentation, product separation, distillation, wastewater treatment, lignin combustion for production of electricity and steam, and other utilities. The detailed experimental data in the bioconversion of poplar, wheat straw and mixed feedstock to ethanol model was based on the lab-scale results as described in Chapter 3A "Improving the overall sugar yield by mixing hybrid poplar and wheat straw biomass prior to steam explosion and enzymatic hydrolysis”. The model was developed using Aspen for an ethanol plant with a processing capacity of 2,205 tons of biomass per day (Humbird et al. 2011). The model was used to determine material and energy balances, to estimate capital cost, operating cost, payback period, chemicals and utilities used in the plant as well as the unit production cost of ethanol for MB and CP.

3B.3 Economic modeling

3B.3.1 Assumptions for economic analysis

Biomass price including transportation cost were assumed to be $70 and $45 per ton for hybrid poplar (HP) and wheat straw (WS) respectively (Berguson et al., 2010 and Juneja et al., 2013). The price of the biomass for the mixture and campaign processing was calculated using the weighted average (75% HP, 25% WS) resulting in $64 per ton. Costs of specific equipment (pretreatment reactor, fermenters, pumps, turbine/generator, among others), raw materials (sulfur dioxide, ammonia, corn steep liquor, boiler chemicals) (Appendix 3), for ethanol production process were calculated based on the corn stover to ethanol NREL model (Humbird et al., 2011). Assumptions used in the process models to calculate direct cost, indirect cost, and direct fixed cost are provided elsewhere (Humbird et al., 2011). The ethanol selling price was calculated using the last five years average price (NASDAQ, 2015) (Appendix 4). The plant size in the present design is 2,205 dry tons/day, with 350 expected operating days per year. The annual feedstock requirement is 773,000 dry ton/year. The feedstock composition and sugar yields are based on investigation of Chapter 3A. The moisture content of the biomass was assumed to be 60%.

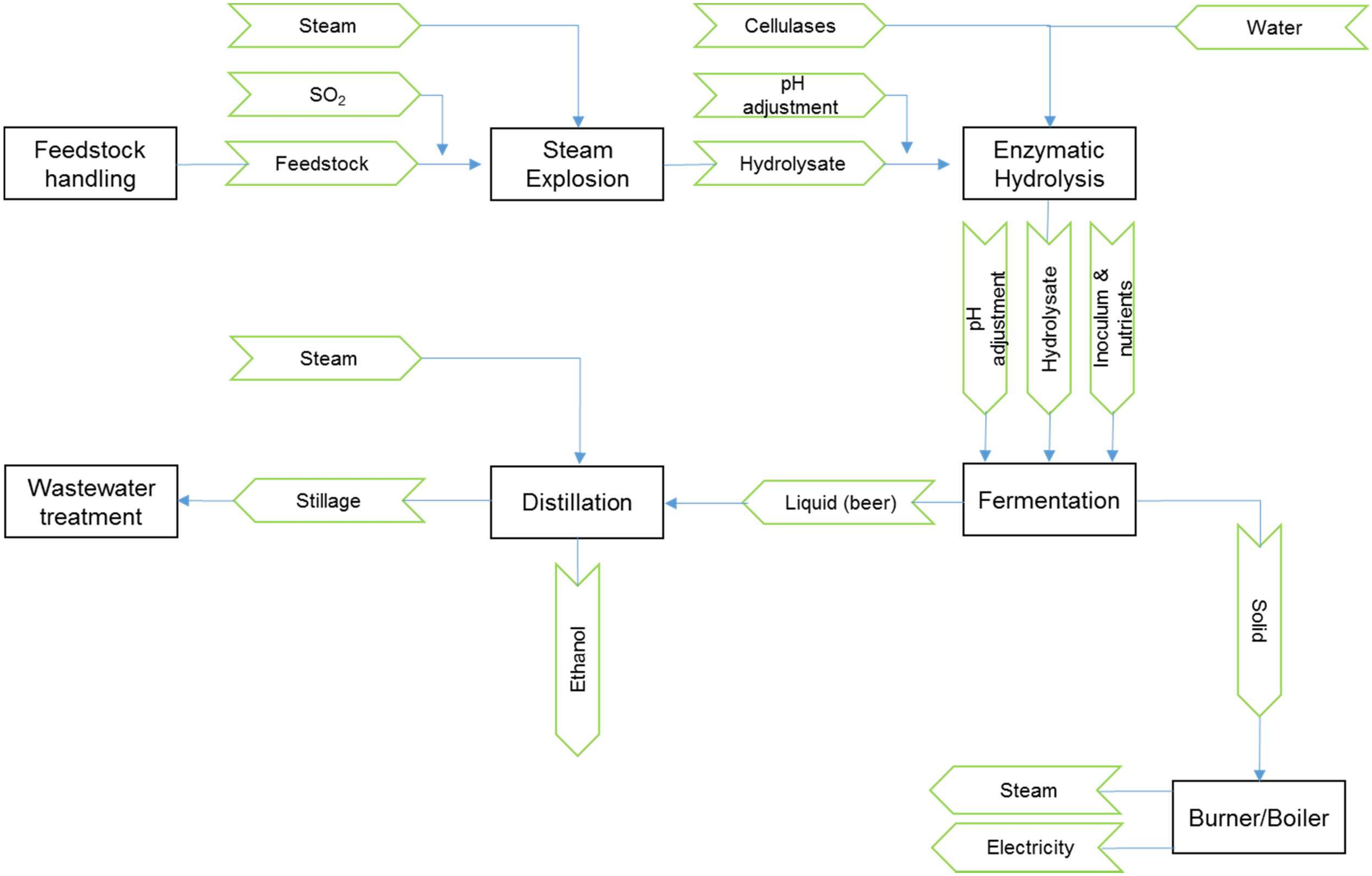


Figure 3B-1. Simplified flow diagram of the overall bioconversion process to ethanol production.

3B.3.2 Capital expenses (CAPEX)

The capital cost was estimated using a well-known method of factored estimation. The method used starts with costs of major pieces of equipment based on corn stover to ethanol NREL model (Humbird et al.,2011). Using the flow data generated during process simulation, the cost of major pieces of equipment is scaled based on Equation 3B-1. The flow components, $Flow_a$ and $Flow_b$, are simply mass, heat, or work data generated from simulation. The scaling exponent, n, is found in literature and is different for various types of equipment, but usually is in the range of 0.5 to 0.8.

$$Cost_a = Cost_b \times \left(\frac{Flow_a}{Flow_b}\right)^n \text{[Eq 3B-1]}$$

The scaled cost is then multiplied by an installation factor. Similar total capital expenses were assumed for all the scenarios, since a unique plant was used for the different cases.

3B.3.3 Operating expenses (OPEX)

Operating expense estimation is done as follows. A list of inputs and outputs of the biorefinery model is compiled. This includes raw materials entering the biorefinery, and waste and byproducts exiting the facility. Costs are associated with each item, based on literature sources or publicly available industry quotes (Humbird et al., 2011; Juneja et al., 2013 and Berguson et al., 2010). Fixed costs comprise around 20% of the cash cost to produce the biofuel, and include labor, maintenance, overhead, administration, among others. Labor costs are estimated by number of employees and salary, while other fixed costs are estimated by factors of labor or capital. Any excess electricity is to be sold to a nearby grid at $0.06 per kWh. Based on simulation results, a cost per hour, year, or gallon of ethanol fuel may be estimated. The operating cost by running the facility under campaign processing plan (CP), were calculated using the weighted average (75% HP, 25% WS) between running the facility using single HP and WS (Appendix 5).

3B.4 ***<u>Results and Discussion</u>***

The main discussion in this paper focuses on the influence of mixing hybrid poplar and wheat straw biomass on ethanol production cost. So far, most of the studies targeted the development of viable technologies for ethanol production using single biomass. There are not studies have considered the impact of mixing different biomass species which is expected to be significant in terms of production cost. In this work a broad understanding of the impact of mixing HP and WS on ethanol production cost was intended. Three different combinations of HP and WS were subjected to SO_2 steam pretreatment followed by enzymatic hydrolysis. Since similar sugar yields were obtained by using different mixtures, the mixture containing 75% HP and 25% WS was chosen for economic analysis. The data were then entered in process modeling software to generate material and energy balance calculations to be used for economical evaluation. Three models of ethanol production plant with annual biomass processing capacity of 773,000 tons were simulated in Aspen Plus.

3B.4.1 Ethanol yields and ethanol unit cost

One of the most important aspects when evaluating conversion of biomass to liquid biofuels is the yield (quantity of liquid fuel that can be obtained from the raw material used). As shown in Table 3B-1, the number of gallons per ton of feedstock varies widely between using mixed feedstock (MB) and single feedstock during campaign processing (CP). The ethanol yields from process simulations were estimated 71 and 61 gal/dry ton of MB and CP respectively. As similar sugar content (glucan and xylan) were found in HP and WS ranging from 50% to 53% respectively, parallel ethanol production yield were estimated for running the biorefinery using only single biomass and campaign processing. The ethanol yield from MB was comparatively higher than CP, mainly due to the synergist effect caused by mixing HP with WS as discussed Chapter 3A. Unit cost of ethanol production from MB and CP was estimated by diving the total annual production cost by amount of the ethanol produced, resulting in \$1.5 and \$1.7 per gallon of ethanol respectively (Table 3B-1). The difference in yield and unit cost of ethanol for the different production plans shows the importance of using mixed feedstock simultaneously when considering industrial production of ethanol.

Table 3B-1. Ethanol production rate, yields and unit cost for the different scenarios for ethanol production.

Item	Unit	MB	CP
Ethanol production rate	MM gal/year	54.6	47.5
Ethanol yields	gal/ton	70.7	61.4
Ethanol unit cost	$/gal	1.5	1.7

MB: Mixed biomass, CP: Campaign processing

3B.4.2 Capital cost and operating cost

The amount of capital expenditures (CAPEX) and the operating cost (OPEX) for the different biomass and processing plans are shown in Table 3B-2. The capital cost of the ethanol production were estimated 8.9, 8.7, 7.7 and 8.9 $/gal of hybrid poplar (HP), wheat straw (WS), mixed biomass (MB) and campaign processing (CP) respectively. Again, the calculated capital cost per gallon of ethanol was lower using MB than CP, mainly due to the higher ethanol yields caused by synergist effect of mixing HP with WS. The operating cost (OPEX) of the ethanol production were estimated 83 and 80 MM$/year for MB and CP respectively. The feedstock cost contribution to total operating cost ranged from 59 to 61% for MB and CP respectively. Similar feedstock cost contribution (60%) were found by Humbird et al., (2011) using corn stover.

Table 3B-2. Total capital investment (TCI) and operational cost (OPEX) for the different scenarios for ethanol production.

Item	Unit	MB	CP
TCI	MM$	419.7	419.7
TCI	$/gal	7.7	8.9
OPEX	MM$/year	83.3	80.3

MB: Mixed biomass, CP: Campaign processing

3B.4.2 Revenues, income and payback period

Considering the average ethanol selling price of 2.3 $/gal for the last five years, revenues, income and payback period were calculated ranging from 109 to 126 MM$/year, 29 to 42 MM$/year and 10 to 15 years respectively (Table 3B-3). The lowest revenue was obtained when the biorefinery was running under campaign processing (CP) (109 MM$/year). The highest revenue of 126 MM$/year was achieved when mixed feedstock was use in the ethanol production plant. In terms of income, if the biorefinery runs using MB or CP plan, processing simultaneously mixed biomass generate 1.5 times more income than campaign production design. This is mainly due to the higher sugar/ethanol yields obtained by mixing HP and WS. The payback period was 9.9 and 14.6 years for MB and CP respectively. Since higher ethanol yields were achieved processing simultaneously HP and WS, a shorter payback period was calculated for processing mixed biomass. Consequently, processing mixed biomass concurrently is more economically attractive than running the system by campaign processing.

Table 3B-3. Revenues, income and payback period (PBP) for the different scenarios for ethanol production.

Item	Unit	MB	CP
Revenues	MM$/year	125.6	109.1
Income	MM$/year	42.3	28.8
PBP	year	9.9	14.6

MB: Mixed biomass, CP: Campaign processing

3B.5 ***Conclusions***

A comprehensive techno-economic analysis was performed to evaluate the economic feasibility of mixing hybrid poplar (HP) and wheat straw (WS) on bioconversion for ethanol production. Based on different annually production plans, two scenarios were analyzed. The first scenario used mixed biomass (75% HP and 25% WS) (MB) which is processed simultaneously, the second scenario used single biomass as HP and WS as a feedstock for a campaign' production plan (CP). MB production plan yield 10 gallons more ethanol per ton of biomass than single HP and WS. The ethanol production costs from MB and CP were estimated \$1.5 and \$1.7 per gallon respectively. Major fractions of the operating cost were found to be associated with biomass cost ranging from 59% to 61%. Energy produced from lignin residue during ethanol production was found sufficient to provide the process heat in the plant. Mixed biomass generate almost twice as much income per year than using single biomass in campaign plan processing, which is equivalent to extra 13.5 million per year. Consequently, it will take 4.7 more years to recover the investment by running the biorefinery with a campaign methodology in comparison by using mixed biomass. Techno-economic analysis indicated that production of ethanol using mixed biomass is more competitive to campaign design processing to ethanol production.

4.1 Conclusions

- The initial particle size heterogeneity of hybrid poplar ranging from 0.2x0.2 cm to 2.0x1.5 cm do not affect significantly the sugar recovery during bioconversion. Similar overall sugar recovery after steam pretreatment and saccharification from all the samples were observed, ranging from 87-90% and 61-64% for glucose and xylose respectively.
- Mixing hybrid poplar and wheat straw was an effective preprocessing method for improving the overall sugar yields during the bioconversion. Mixed biomass exhibited on average 20% higher sugar yields after steam pretreatment and enzymatic hydrolysis, in comparison with single biomass.
- Steam pretreatment with sulfur dioxide is a robust method that can concurrently process different particle sizes of hybrid poplar ranging from 0.2x0.2 cm to 2.0x1.5 cm. In addition, steam pretreatment showed to process simultaneously different combinations of hybrid poplar and wheat straw, without significant alteration of processing conditions.
- Techno-economic analysis indicated that production of ethanol using mixed biomass is more competitive to campaign modality. Mixed biomass generated almost twice much income per year than using single biomass in a campaign plan processing, which is equivalent to extra 13.5 million dollars per year.

4.2 ***Future work***

In this thesis, the possibility of using heterogeneous lignocellulosic biomass to demonstrate the technical and economical bioconversion of this type of biomass to sugars has been the main focus. However, there are still questions waiting for being answered, consequently a future work should expand on this research in the following ways:

- Since it was showed that different particle sizes and a heterogeneous mixture do not affect the sugar production, it would be interested to test a wider range of particle sizes and mixtures. The idea is to check when the particle size will affect the sugar recovery. Finally, it would be interested to model the energy required to decrease the size of the particles, which would be helpful to determine the additional revenue generated by using bigger particles.
- The effect of mixing hybrid poplar (HP) and wheat straw (WS) on sugar yields was studied. Since it is well known that softwoods are more recalcitrant to steam pretreatment then hardwoods, it would interested to find if by mixing biomass we could improve the pretreatability/digestibility of softwoods.
- In the techno-economics analysis, would be useful to adjust the Aspen model for specific handling and preprocessing wheat straw biomass, since wheat straw will possibly arrive to facility in bales with 10% moisture content. Extra equipment will be needed for comminution, and more steam might be required for pre-steaming dry biomass. A sensibility analysis could be performed to analyze the tolerance of the system to moisture content and particle size variability.

- A life cycle assessment (LCA), would be performed to determine environmental impacts of mixing hybrid poplar and wheat straw on bioconversion for ethanol production. In this regard, a LCA of a comparable system using a single raw material and mixed feedstock will be simulated using a traditional ethanologen fermentation pathway. The three LCAs will allow to compare environmental outputs between the traditional single raw material lignocellulosic bioconversion process and the proposed mixed feedstocks bioconversion process.

4.3 References

Aden, A., Ruth, M., Ibsen, K., Jechura, J. (2002). Lignocellulosic biomass to ethanol process design and economics utilizing co-current dilute acid prehydrolysis and enzymatic hydrolysis for corn stover. NREL, (June). Retrieved from http://oai.dtic.mil/oai/oai?verb=getRecord&metadataPrefix=html&identifier=ADA436469

ASABE (2007). ASABE Standards. S269.4. Cubes, pellets and crumbles-definitions and methods for determining density, durability and moisture content. St. Joseph, Mich. ASABE.

Bakker, R., & Elbersen, H. (2005). Managing ash content and quality in herbaceous biomass: an analysis from plant to product. 14th European Biomass Conference. Paris (October), 210–213.

Ballesteros I, Oliva J, Navarro A, Gonzales A, Carrasco J, Ballesteros M. (2000). Effect of chip size on steam explosion pretreatment of softwood. Appl Biochem Biotechnol; 84-86: 97-110.

Ballesteros I, Oliva JM, Negro MJ, Manzanares P, Ballesteros, M. (2002). Enzymatic hydrolysis of steam exploded herbaceous agricultural waste (*Brassica carinata*) at different particle sizes. Process Biochem; 38: 187-92.

Ballesteros, I., Negro, M., Oliva, J. (2006). Ethanol production from steam-explosion pretreated wheat straw. Applied biochemistry and biotechnology, 129, 496–508

Berguson, W.E., J. Eaton, B. Stanton. (2010). Development of hybrid poplar for commercial production in the United States: The Pacific Northwest and Minnesota experience. In: Sustainable Alternative Fuel Feedstock Opportunities, Challenges and Roadmaps for Six U.S. Regions. R. Braun, D. Karlen and D. Johnson, eds. Soil and Conservation Society, Ankeny, IA.

Billa, E., Koukios, E.G., Monties, B. (1998). Investigation of lignins structure in cereal crops by chemical degradation methods. Polym. Degrad. Stab. 59, 71–75.

Brodeur, G. (2013). Developing a Novel Two-Stage Pretreatment of Lignocellulosic Biomass for Enhanced Bioprocessing. Electronic Theses, Treatises and Dissertations. Paper 7312.

Brownell H, Yu E, Saddler, J. (1986). Steam explosion pretreatment of wood: effect of chip size, acid, moisture content and pressure drop. Biotechnol Bioeng; 28: 792-801.

Bura R, Ewanick S, Gustafson R. (2012). Assessment of *Arundo donax* (giant reed) as feedstock for conversion to ethanol. Tappi 2012; 11: 59-66.

Bura, R., Chandra, R., Saddler, J. (2009). Influence of xylan on the enzymatic hydrolysis of steam pretreated corn stover and hybrid poplar. Biotechnology Progress, 315–322. doi:10.1021/bp.98

Buranov, A. U., & Mazza, G. (2008). Lignin in straw of herbaceous crops. Industrial Crops and Products, 28(3), 237–259. doi:10.1016/j.indcrop.2008.03.008

Chabannes, M., Barakate, A., Lapierre, C., Marita, J. M., Ralph, J., Pean, M., Boudet, A. M. (2001). Strong decrease in lignin content without significant alteration of plant development is induced by simultaneous down-regulation of cinnamoyl CoA reductase (CCR) and cinnamyl alcohol dehydrogenase (CAD) in tobacco plants. The Plant Journal, 28(3), 257–270. doi:10.1046/j.1365-313X.2001.01140.x

Chandra R, Bura R, Mabee W, Berlin A (2007). Substrate pretreatment: The key to effective enzymatic hydrolysis of lignocellulosics?. Adv Biochem Eng Biotechnol;108: 67-93.

Chang V, & Holtzapple M. (2000). Fundamental factors affecting biomass enzymatic reactivity. Appl Biochem Biotechnol; 84: 5-37.

Chen, X., Lawoko, M., Heiningen, A. Van. (2010). Kinetics and mechanism of autohydrolysis of hardwoods. Bioresource Technology, 101(20), 7812–9. doi:10.1016/j.biortech.2010.05.006

Cherubini, F. (2010). The biorefinery concept: Using biomass instead of oil for producing energy and chemicals. Energy Conversion and Management, 51(7), 1412–1421. doi:10.1016/j.enconman.2010.01.015

Chum, H., & Overend, R. (2004). Biomass Conversion to Fuels and Chemicals. Prepr. Pap.-Am. Chem. Soc., Div. Fuel Chem, 49. Retrieved from http://web.anl.gov/PCS/acsfuel/preprint archive/Files/Merge/Vol-49_2-0006.pdf

Clark T, Mackie K, Dare P, McDonals A. (1989). Steam explosion of the softwood *Pinus radiata* with sulphur dioxide addition. J Wood Chem Technol 1989; 9: 135-166.

Coughlin, M. P., & Ljungdahl, L. G. (1988). Comparative Biochemistry of Fungal and Bacterial Cellulolytic Enzyme Systems. In J.-P. Aubert, P. Beguin, & J. Millet (Eds.), Biochemistry and Genetics of Cellulose Degradation (pp. 11–30).

Cullis I, Saddler J, Mansfield S. (2004). Effect of initial moisture content and chip size on the bioconversion efficiency of softwood lignocellulosics. Biotechnol Bioeng; 85: 413-421.

Dai, J., & McDonald, A. G. (2014). Production of fermentable sugars and polyhydroxybutyrate from hybrid poplar: Response surface model optimization of a hot-water pretreatment and subsequent enzymatic hydrolysis. Biomass and Bioenergy, 71, 275–284. doi:10.1016/j.biombioe.2014.09.030

Daystar, J., Gonzalez, R., Reeb, C. (2013). Economics, environmental impacts, and supply chain analysis of cellulosic biomass for biofuels in the southern US: Pine, eucalyptus, unmanaged hardwoods, forest. BioResources, 9(1), 393–444. Retrieved from http://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_09_1_393_Daystar_Economics_Supply_Chain_Biofuel

Degenstein, J. C., Kamireddy, S., Tucker, M. P., Ji, Y. (2011). Novel Batch Reactor for the Dilute Acid Pretreatment of Lignocellulosic Feedstocks with Improved Heating and Cooling Kinetics. International Journal of Chemical Reactor Engineering, 9(1). doi:10.2202/1542-6580.2737

Del Río, J. C., Gutiérrez, A., Rodríguez, I. M., Ibarra, D., Martínez, Á. T. (2007). Composition of non-woody plant lignins and cinnamic acids by Py-GC/MS, Py/TMAH and FT-IR. Journal of Analytical and Applied Pyrolysis, 79(1-2), 39–46. doi:10.1016/j.jaap.2006.09.003

Demirbas, A. (2009). Fuels from Biomass. In Biohydrogen (pp. 43–60). Springer. doi:10.1007/978-1-84882-511-6_2

Dinus, R. J. (2001). Genetic Improvement of Poplar Feedstock Quality for Ethanol Production. Applied Biochemistry and Biotechnology, 91-93(1-9), 23–34. doi:10.1385/ABAB:91-93:1-9:23

DOE. Department of Energy of Unites States. (2012). Biomass Densification Workshop: Transforming Raw Biomass to Feedstock – Summary Report. Idaho National Laboratory, Idaho Falls, ID.

Dooley J, Lanning D, Lanning C. (2013). Woody biomass size reduction with selective material orientation. Biofuels 2013; 4: 35-43.

Ebringerová, A., & Heinze, T. (2000). Xylan and xylan derivatives - biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. Macromolecular Rapid Communications, 21(9), 542–556. doi:10.1002/1521-3927(20000601)21:9<542::AID-MARC542>3.0.CO;2-7

Ehrman T, & Ruiz R. (1998). Determination of O-Acyl groups in biomass by high performance liquid chromatography. Technical Report NREL/LAP-017.

Eklund, R., Galbe, M., Zacchi, G. (1995). The influence of SO2 and H2SO4 impregnation of willow prior to steam pretreatment. Bioresource Technology, 52(3), 225–229. doi:10.1016/0960-8524(95)00042-D

Ewanick, S. (2012) Improving the bioconversion yield of carbohydrates and ethanol from lignocellulosic biomass. Doctoral dissertation, University of Washington. AAT 3552776

Ewanick, S., & Bura, R. (2011). The effect of biomass moisture content on bioethanol yields from steam pretreated switchgrass and sugarcane bagasse. Bioresource Technology, 102(3), 2651–2658. doi:10.1016/j.biortech.2010.10.117

Fengel, D. & Wegener G. (1989). Wood: chemistry, ultrastructure, reactions, Walter de Gruyter: Berlin.

Franden, M. A., Pilath, H. M., Mohagheghi, A., Pienkos, P. T., Zhang, M. (2013). Inhibition of growth of Zymomonas mobilis by model compounds found in lignocellulosic hydrolysates. Biotechnology for Biofuels, 6(1), 99. doi:10.1186/1754-6834-6-99

Frear, C., Zhao, B., Fu, G., Richardson, M., Chen, S., Fuchs, M. (2005). Biomass Inventory and Bioenergy Assessment: An Evaluation of Organic Material Resources for Bioenergy Production in Washington State. BioCycle, (05).

Galbe M, & Zacchi G. (2007). Pretreatment of lignocellulosic materials for efficient bioethanol production. In Olsson L, editor. Advances in Biochemical Engineering/Biotechnology, Biofuels, Vol. 108. Berlin: Springer; 2007:41–65

Georgieva, T., Hou, X., Hilstrøm, T., Ahring, B. (2008). Enzymatic hydrolysis and ethanol fermentation of high dry matter wet-exploded wheat straw at low enzyme loading. Biotechnology for Fuels, 148(1-3), 35–44. doi:10.1007/s12010-007-8085-z

Gnansounou, E., & Dauriat, A. (2010). Techno-economic analysis of lignocellulosic ethanol: A review. Bioresource Technology, 101(13), 4980–91. doi:10.1016/j.biortech.2010.02.009

Gonzalez, R., Daystar, J., Jett, M., Treasure, T., Jameel, H., Venditti, R., Phillips, R. (2012). Economics of cellulosic ethanol production in a thermochemical pathway for softwood, hardwood, corn stover and switchgrass. Fuel Processing Technology, 94(1), 113–122. doi:10.1016/j.fuproc.2011.10.003

Gonzalez, R., Jameel, H., Chang, H. (2011). Thermo-mechanical pulping as a pretreatment for agricultural biomass for biochemical conversion. BioResources, 6(Rfa 2010), 1599–1614. Retrieved from https://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_06_2_1599_Gonzalez_JCTPS_TMP_Pretreat_Biomass

Gonzalez, R., Treasure, T., Wright, J., Saloni, D., Phillips, R., Abt, R., Jameel, H. (2011). Exploring the potential of Eucalyptus for energy production in the Southern United States: Financial analysis of delivered biomass. Part I. Biomass and Bioenergy, 35(2), 755–766. doi:10.1016/j.biombioe.2010.10.011

González-García S, Gasol CM, Gabarrell X, Rieradevall J, Moreira MT, Feijoo G.(2010). Environmental profile of ethanol from poplar biomass as transport fuel in Southern Europe. Renew Energy; 35(5):1014-1023.

Graf, A., & T. Koehler. (2000). Oregon cellulose ethanol study: an evaluation of the potential for ethanol production in Oregon using cellulose based feedstocks. Prepared for Oregon Office of Energy. Available at: http://www.oregon.gov/ENERGY/RENEW/Biomass/study.shtml

Grierson, S., & Strezov, V. (2012). Life cycle assessment of the microalgae biofuel value chain: A critical review of existing studies. In: Bionature: The Third International Conference on Bioenvironment, Biodiversity and Renewable Energies, (c), 1–6. Retrieved from http://www.thinkmind.org/index.php?view=article&articleid=bionature_2012_1_10_60064

Hamelinck, C. N., Hooijdonk, G. Van, Faaij, A. P. (2005). Ethanol from lignocellulosic biomass: techno-economic performance in short-, middle- and long-term. Biomass and Bioenergy, 28(4), 384–410. doi:10.1016/j.biombioe.2004.09.002

Hendriks, T., & Zeeman, G. (2009). Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100(1), 10–8. doi:10.1016/j.biortech.2008.05.027

Hou, X.D., Smith, T. J., Li, N., Zong, M.H. (2012). Novel renewable ionic liquids as highly effective solvents for pretreatment of rice straw biomass by selective removal of lignin. Biotechnology and Bioengineering, 109(10), 2484–93. doi:10.1002/bit.24522

Huang, HJ., Ramaswamy, S., Al-Dajani, W., Tschirner, U., Cairncross, R. (2009). Effect of biomass species and plant size on cellulosic ethanol: A comparative process and economic analysis. Biomass and Bioenergy, 33(2), 234–246. doi:10.1016/j.biombioe.2008.05.007

Humbird, D., Davis, R., Tao, L., Kinchin, C., Hsu, D., Aden, A. (2011). Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol. Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. NREL, (May).

Jørgensen, H., Vibe-Pedersen, J., Larsen, J., Felby, C. (2007). Liquefaction of lignocellulose at high-solids concentrations. Biotechnology and Bioengineering, 96(5), 862–70. doi:10.1002/bit.21115

Juneja A, Kumar D, Murthy G. (2013). Economic feasibility and environmental life cycle assessment of ethanol production from lignocellulosic feedstock in Pacific Northwest US. J Renew Sustain Energy; 023142: 1-18.

Kabel M, Bos G, Zeevalking J, Voragen A, Schols H. (2007). Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. Bioresour Technol 2007; 98: 2034-2042.

Kenney K & Ovard L. (2013). Advanced feedstocks for advanced biofuels: transforming biomass to feedstocks. Biofuels; 4(1): 1-3.

Kenney K, Smith W, Gresham G, Westover T. (2013). Understanding biomass feedstock variability. Biofuels 2013; 4: 111-127.

Kenney KL, & Ovard LP. (2012). Biomass densification workshop: transforming raw biomass to feedstock – summary report. Idaho Falls (ID): Idaho National Laboratory 2012. [Sponsored by the U.S. Department of Energy].

Klemm D, Heublein B, Fink HP, Bohn A. (2005).Cellulose: Fascinating biopolymer and sustainable raw material. Angew Chem-Int Edit 44:3358–3393

Klinke H, Thomsen A, Ahring B. (2004). Inhibition of ethanol producing yeast and bacteria by degradation products produced during pretreatment of biomass. Appl Microbiol Biotechnol; 66: 10-26.

Kumar, L., Arantes, V., Chandra, R., Saddler, J. (2012). The lignin present in steam pretreated softwood binds enzymes and limits cellulose accessibility. Bioresource Technology, 103(1), 201–8. doi:10.1016/j.biortech.2011.09.091

Lapierre, C., Pollet, B., Rolando, C., (1995). New insights into the molecular architecture of hardwood lignins by chemical degradative methods. Res. Chem. Intermed. 21 (3–5), 397–412.

Lewis, N.G., & Yamamoto, E., (1990). Lignin: occurrence, biogenesis and biodegradation. Annu. Rev. Plant Mol. Biol. 41, 455–496.

Linde, M., Jakobsson, E., Galbe, M., Zacchi, G. (2008). Steam pretreatment of dilute H2SO4-impregnated wheat straw and SSF with low yeast and enzyme loadings for bioethanol production. Biomass and Bioenergy, 32(4), 326–332. doi:10.1016/j.biombioe.2007.09.013

Liu ZH, Qin L, Pang F, Jin MJ, Li BZ, Kang Y, Yuan YJ. (2013). Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover. Ind Crops Prod; 44: 176-184.

Mansfield, S. D., Mooney, C., Saddler, J. N. (1999). Substrate and Enzyme Characteristics that Limit Cellulose Hydrolysis. Biotechnology Progress, 15(5), 804–816.

McKendry, P. (2002). Energy production from biomass (part 1): overview of biomass. Bioresource Technology, 83(1), 37–46. doi:10.1016/S0960-8524(01)00118-3

Miao, Z., Grift, T.E., Hansen, A.C., Ting, K.C. (2011). Energy requirement for comminution of biomass in relation to particle physical properties. Ind. Crops Prod. 96, 673–686

Monavari S, Galbe M, Zacchi G. (2009). Impact of impregnation time and chip size on sugar yield in pretreatment of softwood for ethanol production. Bioresour Technol; 100: 6312-6316.

Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., Ladisch, M. (2005). Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresource Technology, 96, 673–686. doi:10.1016/j.biortech.2004.06.025

NASDAQ. (2015).Commodities: Latest Ethanol Futures Price & Chart. Retrieved January 15, 2015, from http://www.nasdaq.com/markets/ethanol.aspx?timeframe=5y

Negro M, Manzanares P, Ballesteros I, Oliva J, Cabañas A, Ballesteros M. (2003). Hydrothermal pretreatment conditions to enhance ethanol production from poplar biomass. Appl Biochem Biotechnol; 105-108: 87-100.

Olanders, B., & Steenari, B. (1995). Characterization of ashes from wood and straw. Biomass and Bioenergy, 8(2), 105–115. Retrieved from http://www.sciencedirect.com/science/article/pii/096195349500004Q

Palmqvist, E., & Hahn-Hägerdal, B. (2000). Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition. Bioresource Technology, 74, 25–33. Retrieved from http://www.sciencedirect.com/science/article/pii/S0960852499001613

Perlack, R. D., & Stokes, B. J. (2011). U.S. Billion-Ton Update: biomass supply for a bioenergy and bioproducts industry. Tech. Rep. ORNL/TM-2011/224, Oak Ridge National Laboratory.

Pitman, R. M. (2006). Wood ash use in forestry - a review of the environmental impacts. Forestry, 79(5), 563–588. doi:10.1093/forestry/cpl041

Polizeli, M. L. T. M., Rizzatti, a C. S., Monti, R., Terenzi, H. F., Jorge, J. a, Amorim, D. S. (2005). Xylanases from fungi: properties and industrial applications. Applied Microbiology and Biotechnology, 67(5), 577–91. doi:10.1007/s00253-005-1904-7

Pu Y, Zhang D, Singh PM, Ragauskas AJ. (2008). The new forestry biofuels sector. Biofuels Bioprod Bioref 2:58–73.

Puri VP. (1984). Effect of crystallinity and degree of polymerization of cellulose on enzymatic saccharification. Biotechnol Bioeng 26:1219–1222

Qureshi, N., Saha, B. C., Cotta, M. a., Singh, V. (2013). An economic evaluation of biological conversion of wheat straw to butanol: A biofuel. Energy Conversion and Management, 65, 456–462. doi:10.1016/j.enconman.2012.09.015

Ramos LP, & Saddler JN. (1994). Enzyme recycling during fed-batch hydrolysis of cellulose derived from steam-exploded *Eucalyptus viminalis*. Appl Biochem. Biotechnol. 1994;45/46:193–207

Ramos LP, Breuil C, Saddler JN. (1992) Comparison of steam pretreatment of eucalyptus, aspen, and spruce wood chips and their enzymatic hydrolysis. Applied biochemistry and biotechnology, 34:37–48

Ramos, L. P. (2003). The chemistry involved in the steam pretreatment of lignocellulosic materials. Quim.Nova, 26(6), 863–871.

Sannigrahi, P., Ragauskas, A. J., Tuskan, G. A. (2010). Poplar as a feedstock for biofuels: A review of compositional characteristics. Biofuels, Bioproducts and Biorefining, 4(2), 209–226. doi:10.1002/bbb.206

Santos, R. B., Lee, J. M., Jameel, H., Chang, H.-M., Lucia, L. a. (2012). Effects of hardwood structural and chemical characteristics on enzymatic hydrolysis for biofuel production. Bioresource Technology, 110, 232–8. doi:10.1016/j.biortech.2012.01.085

Sassner, P., Galbe, M., Zacchi, G. (2005). Steam Pretreatment of Salix with and without SO2 Impregnation for Production of Bioethanol. Applied Biochemistry and Biotechnology, 124(1-3), 1101–1118. doi:10.1385/ABAB:124:1-3:1101

Schutt F, Haas N, Dehne L, Koch GJ, Saake B. (2013). Steam pretreatment for enzymatic hydrolysis of poplar wood: comparison of optimal conditions with and without SO2 impregnation. Holzforsch 2013; 67: 9-17.

Selig, M. J., Adney, W. S., Himmel, M. E., Decker, S. R. (2009). The impact of cell wall acetylation on corn stover hydrolysis by cellulolytic and xylanolytic enzymes. Cellulose, 16(4), 711–722. doi:10.1007/s10570-009-9322-0

Shi J, Thompson V, Yancey N, Stavila V, Simmons B, Singh S. (2013). Impact of mixed feedstocks and feedstock densification on ionic liquid pretreatment efficiency. Biofuels; 4(1): 63-72.

Shinners, K., Binversie, B., Muck, R., Weimer, P. (2007). Comparison of wet and dry corn stover harvest and storage. Biomass and Bioenergy, 31(4), 211–221. doi:10.1016/j.biombioe.2006.04.007

Simpson W, TenWolde, A. (1999) Physical properties and moisture relations of wood. In: Forest products laboratory. 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 p.

Sjöström, E. (1993). Wood chemistry. San Diego,CA: Academic Press.

Sluiter A, Hames B, Ruiz R, Scarlata C, Sluiter J, Templeton D, Crocker D. (2004). Determination of sugar, byproducts, and degradation products in liquid fraction process samples. Golden (CO). Technical Report NREL/TP-510-42623.

Studer, M. H., DeMartini, J. D., Davis, M. F., Sykes, R. W., Davison, B., Keller, M., Wyman, C. E. (2011). Lignin content in natural Populus variants affects sugar release. Proceedings of the National Academy of Sciences of the United States of America, 108(15), 6300–5. doi:10.1073/pnas.1009252108

Sun, Y., & Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. Bioresource Technology, 83(1), 1–11. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12058826.

Taherzadeh, M., & Keikhosro, K. (2007). Enzyme based hydrolysis processes for ethanol from lignocellulosic materials: a review. BioResources, 2, 707–738. Retrieved from http://ojs.cnr.ncsu.edu/index.php/BioRes/article/view/BioRes_2_4_707_738_Taherzadeh_Karimi_EnzymeBased_Hydrol_Ethanol_Review/81

Talebnia, F., Karakashev, D., Angelidaki, I. (2010). Production of bioethanol from wheat straw: An overview on pretreatment, hydrolysis and fermentation. Bioresource Technology, 101(13), 4744–53. doi:10.1016/j.biortech.2009.11.080

Tao, L., & Aden, A. (2009). The economics of current and future biofuels, In Vitro Cellular & Developmental Biology-Plant 45.199–217

TAPPI. TAPPI standard methods, T-222 om-98. Tappi Journal. TAPPI Press; 1998.

Turhollow, A., Perlack, R., Eaton, L., Langholtz, M., Brandt, C., Downing, M., Lebow, P. (2014). The updated billion-ton resource assessment. Biomass and Bioenergy, 70, 149–164. doi:10.1016/j.biombioe.2014.09.007

USDA National Agricultural Statistical Service http://www.nass.usda.gov/#topSNovember 2007> November 2014

Vajzovic, A. (2012). Production of xylitol and ethanol from lignocellulosics. Doctoral dissertation, University of Washington; 2012. AAT 3552867.

Vidal, B. C., Dien, B. S., Ting, K. C., Singh, V. (2011). Influence of feedstock particle size on lignocellulose conversion--a review. Applied Biochemistry and Biotechnology, 164(8), 1405–21. doi:10.1007/s12010-011-9221-3

Wu, X., McLaren, J., Madl, R., Wang., D. (2010) Biofuels from lignocellulosic biomass. In Sustainable Biotechnology. 19–41

Wyman, C. (1994). Ethanol from lignocellulosic biomass: technology, economics, and opportunities. Bioresource Technology, 50. Retrieved from http://www.sciencedirect.com/science/article/pii/0960852494902143

Wyman, C. E. (2007). What is (and is not) vital to advancing cellulosic ethanol. Trends in Biotechnology, 25(4), 153–7. doi:10.1016/j.tibtech.2007.02.009

Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., Lee, Y. Y., Saddler, J. N. (2009). Comparative sugar recovery and fermentation data following pretreatment of poplar wood by leading technologies. Biotechnology Progress, 25(2), 333–9. doi:10.1002/btpr.142

Yancey N, Wright C, Connor C. (2009). Preprocessing moist lignocellulosic biomass for biorefinery feedstocks. In ASABE Annual International Meeting. Reno, NV: 2009:1-17.

Yue, D., You, F., Snyder, S. W. (2014). Biomass-to-bioenergy and biofuel supply chain optimization: Overview, key issues and challenges. Computers & Chemical Engineering, 66, 36–56. doi:10.1016/j.compchemeng.2013.11.016

Zhao, X., Zhang, L., Liu, D. (2012). Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels, Bioproducts and Biorefining, 6, 465–482. doi:10.1002/bbb

APPENDIX 1: STEAM GUN REPRODUCIBILITY

Objective

Determine the steam explosion data reproducibility using hybrid poplar chips during four different trials at 212° C for 10 minutes.

Methodology

Four sets of 400 g of hybrid poplar chips were steam pretreated using the same conditions, 212° C for 10 minutes, in four different trials. The solid and liquid fractions of steam pretreated particles were then chemically characterized as is shown in Fig A1. Solids fractions were analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate and acetates content. Liquid fractions were analyzed for monomeric and oligomeric soluble carbohydrates as well as 5-hydroxymethyl furfurals and furfurals. Average and standard deviation were use as dispersion characteristics to determine the reproducibility of results. A carbohydrate mass balance was performed to determine the reproducibility of sugar recovery results of the different hybrid poplar samples.

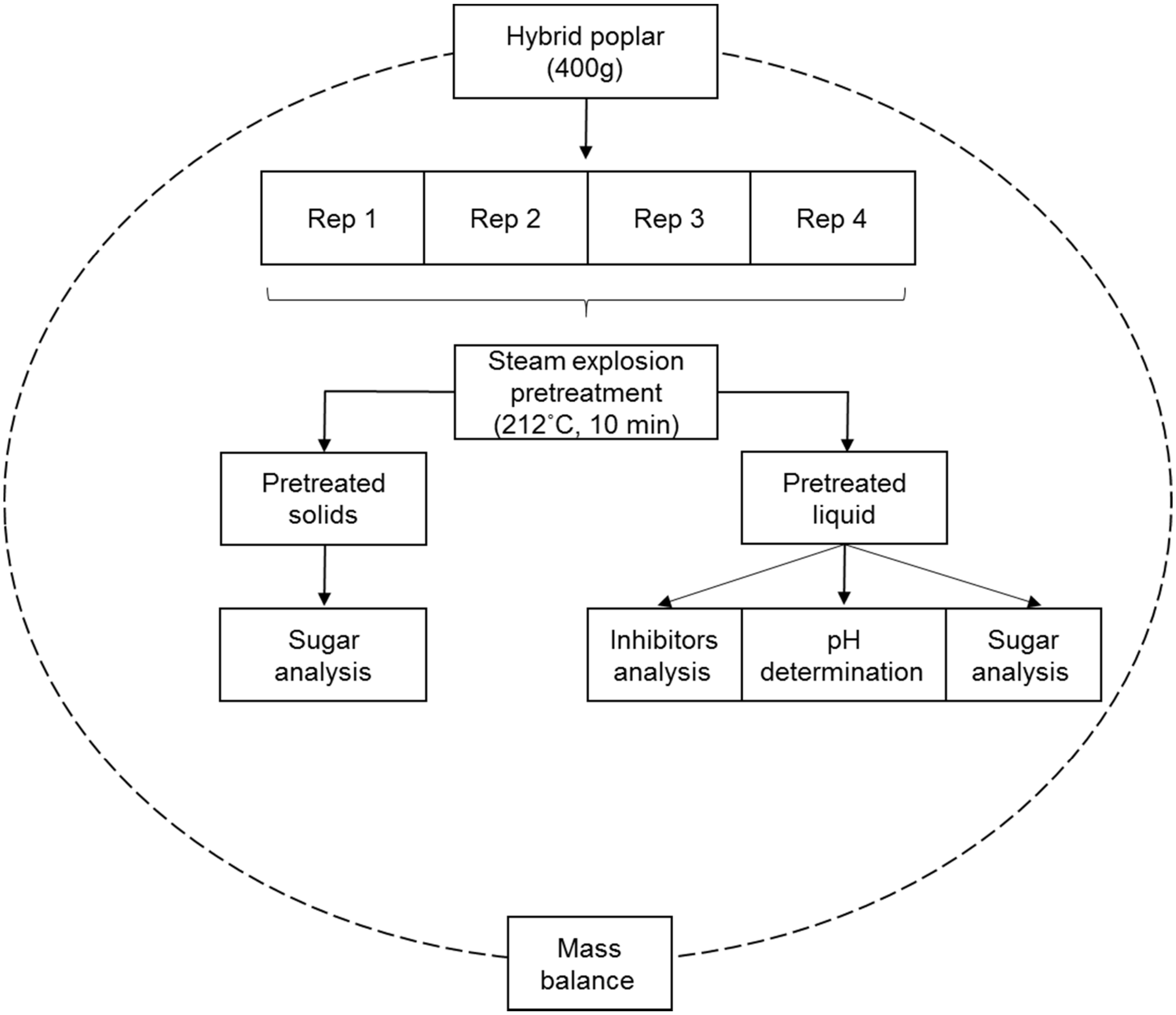


Fig A1. Flow diagram steam gun reproducibility study.

Results

Solid fraction after pretreatment

The chemical composition of solids after steam pretreatment, expressed as % dry matter for the different pretreated samples are shown in Table A1. Arabinan (Ara), galactan (Gal), glucan (Glu), xylan (Xyl), mannan (Man), acid insoluble lignin (AIL) and acid soluble lignin (ASL) content were identical among the four samples, with small standard deviations ranging from 0.0 (arabinan and galactan) to 0.8 (glucan).

Table A1. Composition of solid fractions of pretreated hybrid poplar expressed as % dry matter for different particle sizes. Ara: arabinan, Gal: galactan, Glu: glucan, Xyl: xylan, Man: mannan, AA: acetates, AIL: acid insoluble lignin, ASL: acid soluble lignin.

Sample	Ara	Gal	Glu	Xyl	Man	AA	AIL	ASL	Total
Rep 1	0.0	0.2	54.3	3.5	0.5	0.8	30.3	2.8	92.4
Rep 2	0.0	0.2	56.0	3.5	0.4	0.8	30.2	3.0	94.0
Rep 3	0.0	0.2	54.9	3.9	0.5	0.9	29.9	2.8	93.1
Rep 4	0.0	0.2	54.2	3.6	0.5	0.8	29.3	2.7	91.4
Ave	0.0	0.2	54.8	3.6	0.5	0.8	29.9	2.8	92.7
SD	0.0	0.0	0.8	0.2	0.1	0.0	0.4	0.1	1.1

Liquid fraction after pretreatment

The chemical composition of liquid after steam pretreatment, expressed as g of compound per g of biomass for the different pretreated samples are shown in Table 2A. Arabinan (Ara), galactan (Gal), glucan (Glu), xylan (Xyl), mannan (Man), acetic acid (AA), Hydromethylfurfural (HMF) and furfural (FF) content were identical among the four samples, with small standard deviations ranging from 0.0 (arabinan, galactan and hydrometylfurfural) to 0.3 (xylan). The pH was identical among the four replicas with a standard deviation of 0.

Table 2A. Composition of liquid fraction and pH of pretreated hybrid poplar expressed as % of the total starting weight of raw biomass. Ara: arabinan, Gal: galactan, Glu: glucan, Xyl: xylan, Man: mannan, AA: acetates, AIL: acid insoluble lignin, ASL: acid soluble lignin.

Sample	Ara	Gal	Glu	Xyl	Man	AA	HMF	FF	pH
Rep 1	0.1	0.7	1.6	5.4	0.9	2.4	0.3	0.9	2.9
Rep 2	0.1	0.6	1.7	5.8	0.9	2.7	0.4	1.0	2.9
Rep 3	0.1	0.6	1.5	5.0	0.9	2.6	0.4	1.0	2.9
Rep 4	0.1	0.6	1.6	5.2	0.9	2.7	0.4	1.1	2.8
Ave	0.1	0.6	1.6	5.4	0.9	2.6	0.4	1.0	2.9
SD	0.0	0.0	0.1	0.3	0.0	0.2	0.0	0.1	0.0

Sugar recovery

After steam pretreatment the combined sugar recovery from pretreated solids and liquid hydrolysates was calculated for each of the steam pretreated samples. Table 3A shows the combined overall recovery of sugars of solid and liquid fraction, expressed as % of the theoretical amount available in the raw material. Arabinan (Ara), galactan (Gal), glucan (Glu), xylan (Xyl), and mannan (Man) content were very similar among the four samples, with standard deviations ranging from 1.2 (xylan) to 1.6 (arabinose and galactan).

Table 3A. Combined sugar recovery of solid and liquid fraction after pretreatment expressed as % of the theoretical available in the raw material. Ara: arabinan, Gal: galactan, Glu: glucan, Xyl: xylan, Man: mannan.

Sample	Ara	Gal	Glu	Xyl	Man
Rep 1	36.9	84.4	91.6	44.1	98.8
Rep 2	33.9	83.0	93.4	46.4	97.1
Rep 3	36.5	80.6	91.1	44.2	99.7
Rep 4	34.1	82.8	94.4	43.6	96.4
Ave	35.4	82.7	92.6	44.6	98.0
SD	1.6	1.6	1.5	1.2	1.5

Conclusions

The data reproducibility of the steam gun using hybrid poplar chips under same treatment conditions (212° C for 10 min) during four different trials were tested. Solid and liquid fractions of the different steam pretreated samples showed identical sugar and inhibitors content. The combined sugar recovery of solid and liquid fractions after pretreatment showed were similar for the four set of samples. Consequently, the data generated by steam gun of the bioproducts and biofuels laboratory (BBL) of the University of Washington is reliable and reproducible with an expected error of 1-2% in the sugar recovery.

APPENDIX 2: WHEAT STRAW STEAM PRETREATMENT OPTIMIZATION CONDITIONS

Objective

Investigate the influence of four different steam explosion pretreatment parameters on the subsequent sugar recovery and potential saccharification of wheat straw.

Methodology

Four different steam pretreatment conditions were chosen to determine the optimal parameters to recover the highest amount of sugars contained in original wheat straw (WS). The conditions were based on H_2SO_4 WS steam pretreatment published by Ballesteros et al. (2006). Severity was controlled by incrementing the temperature in 5°C from 180 to 195°C. The solid and liquid fraction of steam pretreated particles were then chemically characterized as is shown in Fig 2A. Solids fractions were analyzed gravimetrically for lignin content, photometrically for soluble lignin, and by HPLC for carbohydrate and acetates content. Liquid fractions were analyzed for monomeric and oligomeric soluble carbohydrates as well as 5-hydroxymethyl furfurals and furfurals. The effects of varying the temperature were assessed by sugars recovery after pretreatment as well as visual inspection of the washed solids. After pretreatment, a carbohydrate mass balance was performed to determine the sugar recovery for the different steam pretreated WS samples.

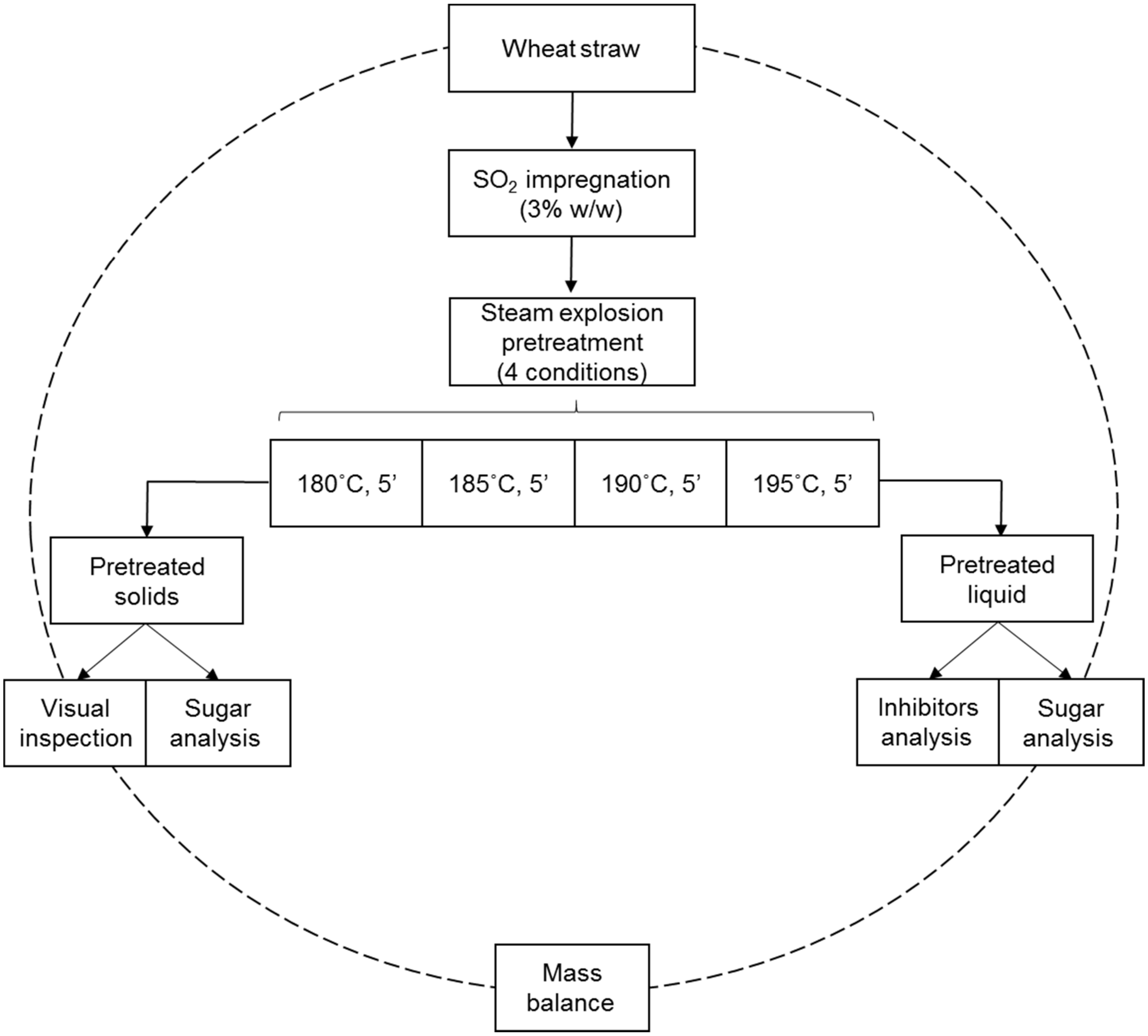


Fig 2A. Flow diagram wheat straw optimization steam pretreatment conditions.

Results

Solid fraction after pretreatment

The chemical composition of solids after steam pretreatment, expressed as % dry matter for the different pretreated samples are shown in Table 2A. Only glucan, xylan, lignin were found in the resulting solids of the pretreated samples, ranging from 67 to 68%, from 3 to 7%, and from 27 to 29% respectively. During the bioconversion process, enzymatic hydrolysis of solids is affected by the presence of long fiber and rejects, as well as xylan and lignin content (Bura et al., 2009, Mansfield et al., 1999). However, since similar lignin content was observed in the samples, xylan content might be a good indicator of solids hydrolysability. Higher temperatures were able to solubilize more xylan from the solids, consequently, a suitable temperature for being used during WS pretreatment might be 195°C.

Table 2A. Composition of solid fractions of pretreated wheat straw expressed as % dry matter for different samples. SD: standard deviation.

Sample	Glucan	Xylan	Lignin	Total
180°C, 5 min, 3% SO_2	66.6	6.0	27.4	100.1
SD	2.1	0.1	0.5	2.7
185°C, 5 min, 3% SO_2	67.7	4.9	28.9	101.5
SD	2.1	0.4	0.3	2.8
190°C, 5 min, 3% SO_2	67.0	4.0	28.5	100.4
SD	2.6	0.5	0.5	3.5
195°C, 5 min, 3% SO_2	67.9	3.3	28.5	99.7
SD	1.5	0.1	0.5	2.1

Fig. 2B shows pictures of the pretreated WS solids using 4 different conditions. The solids produced during steam explosion of 180 and 185°C (Fig A and B respectively) showed an import amount of fibers bundles and rejects, resulting in a more heterogeneous paste in comparison with the solids obtained at 190 and 195 °C (Fig C and D respectively). In fact, there is a noticeable relation between temperature and solids heterogeneity, higher temperatures during the pretreatment produces more homogeneous slurry which is preferred for complete enzymatic hydrolysis. However, higher temperatures generate more fermentation inhibitors compounds by as 5-hydroxymethyl furfural and furfurals.

A: 180°C, 5 min, 3% SO_2 B: 185°C, 5 min, 3% SO_2

C: 190°C, 5 min, 3% SO_2 D: 195°C, 5 min, 3% SO_2

Fig. 2B. Pictures of washed solid fractions after different steam pretreatment conditions of wheat straw.

Liquid fraction after pretreatment

The amount of glucose, xylose, 5-hydroxymethylfurfurals (HMFs) and furfural (FF), in the pretreated liquid fractions were measured. Table 2B shows that glucose, xylose and HMF content expressed as % of g compound per g of raw biomass ranged from 0.6 to 1.5 %, from 11.4 to 17%, and from 0 to 0.24% respectively. No 5-HMFs were found in the samples. Since more glucose and xylose were found in monomeric form and low levels of fermentation inhibitors were detected in the liquid stream generate by the more severe steam pretreatment conditions, the pretreatment of WS using 195°C, 5 min, 3% SO_2 is the optimum pretreatment condition among all the treatments tested.

Table 2B. Composition of liquid fraction of pretreated wheat straw expressed as % of g compound per g of raw biomass. SD: standard deviation.

Sample	Glucose	Xylose	HMF	FF
180°C, 5 min, 3% SO_2	0.6	10.4	0.0	0.0
SD	0.02	0.1	0.0	0.0
185°C, 5 min, 3% SO_2	1.0	15.2	0.0	0.17
SD	0.01	0.1	0.0	0.0
190°C, 5 min, 3% SO_2	1.2	16.1	0.0	0.24
SD	0.04	0.5	0.0	0.0
195°C, 5 min, 3% SO_2	2.5	17.0	0.0	0.24
SD	0.01	0.0	0.0	0.0

Sugar recovery

After steam pretreatment the combined sugar recovery from pretreated solids and liquid hydrolysates was calculated for each of the steam pretreatment conditions tested. Table 2C shows the combined overall recovery of sugars of solid and liquid fraction, expressed as % of the theoretical amount available in the raw material. After pretreatment 100% of oligomeric glucose was recovery for all the samples tested. Xylose recovery, ranged from 65 to 77% for the different steam pretreatment conditions applied. From 2C is possible to determine that higher temperatures allowed higher xylose recovery.

Table 2C. Combined sugar recovery of solid and liquid fraction after pretreatment expressed as % of the theoretical available in the raw material.

Sample	Glucose	Xylose
180°C, 5 min, 3% SO_2	100	65
SD	2.1	1.5
185°C, 5 min, 3% SO_2	100	70
SD	3.4	1.4
190°C, 5 min, 3% SO_2	100	73
SD	2.7	1.2
195°C, 5 min, 3% SO_2	100	77
SD	2.9	0.9

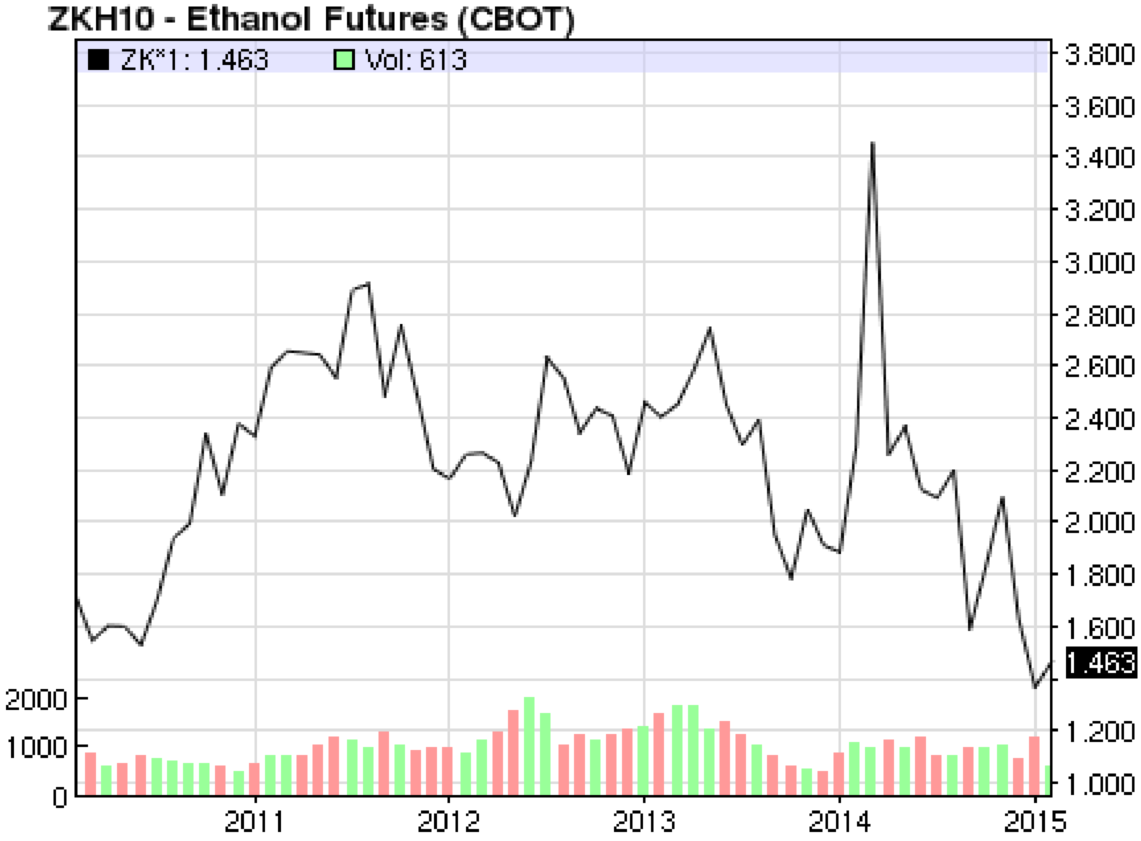
Conclusions

Four different steam pretreatment conditions were tested to determine the optimal parameters to recover the highest amount of sugars contained in original wheat straw. Chemical composition and aspect characteristics of solids, showed that 195°C for 5 minutes after exposure to 3% SO_2, are suitable steam pretreatment parameters to recover maximum amount of glucose and xylose in comparison with the other conditions tested. Consequently, the steam pretreatment conditions of 195°C, 5 minutes reaction time and 3% SO_2 impregnation were chosen for fractionation of WS in future experiments.

APPENDIX 3: DETAILED OPERATING COST FOR THE DIFFERENT SCENARIOS FOR ETHANOL PRODUCTION.

Variable Operating Costs			**HYBRID POPLAR (HP)**			**WHEAT STRAW (WS)**			**MIXED BIOMASS (MB)**			**CAMPAIGN PROCESSING (CP)**		
	Raw Material	**Stream No.**	**Kg/hr**	**MM$/yr (2007)**	**Cents/Gal EtOH**	**Kg/hr**	**MM$/yr (2007)**	**Cents/Gal EtOH**	**Kg/hr**	**MM$/yr (2007)**	**Cents/Gal EtOH**	**Kg/hr**	**MM$/yr (2007)**	**Cents/Gal EtOH**
Raw Materials														
NONE	Feedstock	STRM.101	208333	53.96	114.36	208333	34.77	72.08	208333	49.16	90.06	208333	49.16	103.79
A200	Sulfur Dioxide	STRM.710	2500	5.32	11.28	2500	5.32	11.04	2500	5.32	9.75	2500	5.32	11.22
	Ammonia	STRM.A200.273	1047	3.95	8.37	1047	3.95	8.19	1047	3.95	7.24	1047	3.95	8.33
A300	Corn Steep Liquor	STRM.A700.CSL-A300	1155	0.55	1.17	1155	0.55	1.14	1155	0.55	1.01	1155	0.55	1.16
	Diammonium Phosph	STRM.755	142	1.18	2.49	142	1.18	2.44	142	1.18	2.16	142	1.18	2.48
	Sorbitol	SORBIT	44	0.42	0.89	44	0.42	0.87	44	0.42	0.77	44	0.42	0.88
A400	Purchased Enzyme	CCIS.ENZYME.750	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
	Glucose	CMIX.GLUCOSE.A400.401	2396	11.70	24.79	2396	11.70	24.25	2396	11.70	21.43	2396	11.70	24.66
	Corn Steep Liquor	STRM.A700.CSL-A400	163	0.08	0.17	163	0.08	0.16	163	0.08	0.14	163	0.08	0.16
	Ammonia	STRM.A700.NH3-A400	114	0.43	0.91	114	0.43	0.89	114	0.43	0.79	114	0.43	0.91
	Host nutrients	STRM.A400.440	67	0.46	0.98	67	0.46	0.96	67	0.46	0.84	67	0.46	0.97
	Sulfur Dioxide	STRM.A400.442	16	0.04	0.09	16	0.04	0.09	16	0.04	0.08	16	0.04	0.09
A600	Caustic (as pure)	CMIX.NAOH.A600.632	2243	2.82	5.98	2243	2.82	5.85	2243	2.82	5.17	2243	2.82	5.95
A800	Boiler Chems	STRM.A900.921	0	0.01	0.02	0	0.01	0.02	0	0.01	0.02	0	0.01	0.02
	FGD Lime	STRM.A800.851	893	1.50	3.18	893	1.50	3.11	893	1.50	2.74	893	1.50	3.16
	Feedstock	STRM.A800.840	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00	0	0.00	0.00
A900	Cooling Tower Chems	STRM.A900.922	2	0.06	0.13	2	0.06	0.13	2	0.06	0.11	2	0.06	0.13
	Makeup Water	STRM.903	148980	0.32	0.69	148980	0.32	0.67	148980	0.32	0.59	148980	0.32	0.68
	Subtotal			**82.81**	**175.49**		**63.62**	**131.88**		**78.01**	**142.90**		**78.01**	**164.59**
Waste Streams														
A800	Disposal of Ash	STRM.A800.809	4449	1.19	2.52	4459	1.19	2.47	4399	1.18	2.16	4451	1.19	2.51
	Subtotal			**1.19**	**2.52**		**1.19**	**2.47**		**1.18**	**2.16**		**1.19**	**2.51**
By-Products and Credits														
	Grid Electricity	WORK.A900.WKNET	18071	8.69	18.41	21358	10.27	21.29	12694	6.10	11.18	18893	9.08	19.13
	Area 100 Electricity	WORK.A900.WKA100	859	0.41	0.88	859	0.41	0.86	859	0.41	0.76	859	0.41	0.87
	Subtotal			**9.10**	**19.29**		**10.68**	**22.14**		**6.52**	**11.94**	**0.00**	**9.50**	**20.00**
	Total Variable Operating Costs			**74.90**	**159.60**		**54.13**	**113.07**		**72.67**	**133.88**	**0.00**	**69.71**	**147.97**
Fixed Operating Costs												0	0	0
	Position	**Salary**		**MM$/yr (2007)**	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**
Labor & Supervision														
	Plant Manager	147000												
	Plant Engineer	70000												
	Maintenance Supr	57000												
	Maintenance Tech	40000												
	Lab Manager	56000												
	Lab Technician	40000												
	Lab Tech-Enzyme	40000												
	Shift Supervisor	48000												
	Shift Operators	40000												
	Shift Oper-Enzyme	40000												
	Yard Employees	28000												
	Clerks & Secretaries	36000												
	Total Salaries			2.48	5.25		2.48	5.13		2.48	4.54		2.48	5.22
	Labor Burden (90%)			2.23	4.72		2.23	4.62		2.23	4.08		2.23	4.70
Other Overhead														
	Maintenance	3.0%		3.11	6.58		3.11	6.44		3.11	5.69		3.11	6.55
	Property Insur. & Tax	0.7%		2.79	5.90		2.79	5.77		2.79	5.10		2.79	5.87
	Total Fixed Operating Costs			**10.60**	**22.46**		**10.60**	**21.96**		**10.60**	**19.42**		**10.60**	**22.34**
				MM$/yr (2007)	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**		**MM$/yr (2007)**	**Cents/Gal EtOH**
Total Operating Costs				**85.50**	**182.06**		**64.73**	**135.03**		**83.27**	**153.30**	**0.00**	**80.30**	**170.30**

APPENDIX 4: LAST FIVE YEARS ETHANOL SELLING PRICE (NASDAQ)



APPENDIX 5: HYBRID POPLAR (HP) AND WHEAT STRAW (WS) ECONOMICS PARAMETERS USED FOR CALCULATION OF BIOMASS CAMPAIGN PROCESSING ECONOMICS PARAMETERS FOR ETHANOL PRODUCTION

Item	Unit	HP	WS
Ethanol production rate	MM gal/year	47.2	48.2
Ethanol yields	gal/ton	61.1	62.4
Ethanol unit cost	$/gal	1.8	1.3
TCI	MM$	419.7	419.7
TCI	$/ton	8.9	8.7
OPEX	MM$/year	85.5	64.7
Revenues	MM$/year	108.5	110.9
Income	MM$/year	23.0	46.2
PBP	year	18.2	9.1

HP: Hybrid poplar, WS: Wheat straw