Biochemical and thermochemical conversions of short rotation coppice poplar for carbohydrate and fuel production in lignocellulosic biorefineries

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Abstract

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The economic success of a potential biorefinery is directly related to the use of economic feasible biomass. Due to high cost, it is unlikely that a wood-based biorefinery would use only whitewood material from conventional forestry plantation as feedstock. Instead, short rotation coppice can be a potential feasible substitute because of its low cost. Yet, the efficacy of conversion using short rotation coppice, a more heterogeneous feedstock, has not been investigated. This work studied the influence of using 2-year-old poplar coppice on the overall sugar yield via biochemical conversion and the bio-oil production via thermochemical conversion.

By harvesting all aboveground parts of 2-year-old poplar coppice, the biomass was comprised of four different fractions – leaves, bark, branches, and whitewood chips. From this, three samples from coppice plantation – whole tree coppice (WTC), noleaf coppice (NLC), and leaf coppice (LC) were prepared and studied. A sample of mature wood from forestry plantation – whitewood

forestry (WWF) was used as standard for reference. In biochemical conversion, all samples were processed via steam pretreatment and enzymatic hydrolysis for sugar production. In thermochemical conversion, samples were fast pyrolyzed in a fluidized bed reactor and the final bio-oil yield and composition were measured. Results show that converting the 2-year-old poplar coppice is promising in biorefinery. Leaf removal is essential for biochemical conversion, as it improved the sugar yield by 150 kg/tonne and the sugar recovery by 40%. The NLC achieved over 350 kg/tonne overall sugar yield and up to 70% sugar recovery. Given these results, we evaluated the economics of converting 2-year-old poplar coppice in biochemical conversion. For thermochemical conversion, leaf removal did not impact the bio-oil yield, as both WTC and NLC achieved similar bio-oil yield of 55%. Energy recovery rate was used to evaluate the energy efficiency of thermochemical conversion. By lowering the energy recovery rate by 2.8%, leaf removal showed only small impact on the energy efficiency of fast pyrolysis.

Further investigation was conducted to assess the biochemical conversion performance by using two leafless poplar coppice hybrids from two plantation sites. Processed in the same condition, poplar coppice showed different overall sugar yields between low-productive hybrid and high-productive hybrid for both sites. Correspondingly, the difference illustrates a remarkable fluctuation in the potential product yield, leading to a pronounced variability in economics for feedstock user. Economic modelling showed that the biorefinery will achieve a 7% to 12% revenue increase by using the low-productive hybrid compared to high-productive hybrid. In contrast, the feedstock productivity dramatically influences the economics of the feedstock grower. Growing the high-productive hybrid means either 11% - 25% reduction in land use or considerable extra revenue from the surplus feedstock. A new business model integrating both

plantation and biorefinery could help to fully accommodate the needs of both sides and enhance the overall efficiency in feedstock production and biofuel production.

One reason of low biochemical conversion yield from short rotation coppice poplar is its high content of non-structural components (NSCs), which include inorganic ash and organic extractives. To study the impact of NSCs and improve the biochemical conversion yield, we preprocessed the coppice poplar by neutral and/or acidic wash. Preprocess significantly reduced ash and extractives content as much as 70% and 50%, respectively. It appears that preprocess changed the buffering capacity of the biomass via ash removal and thereby facilitate the hemicellulose solubilization and enzymatic hydrolysis. The overall sugar yield after pretreatment and hydrolysis was 18-22% higher when the biomass had been preprocessed. Meanwhile, removal of extractives during preprocessing reduced the formation of inhibitor and improved the fermentation yield. The ethanol yield was 36-50% higher for the preprocessed biomass during fermentation of liquid fraction. The economic assessment shows that introduction of one preprocessing unit (acidic-neutral wash) in the biorefinery could increase $43.1 MM/year in gross revenue and greatly benefit the economics.

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For my wife, Qingxiu Gao, whose love I can always rely on

Chapter 1. **Introduction and literature review**

1.1 Biofuels development and current status

Limitations of fossil fuels, growing rates of greenhouse gas emissions, and excessive environmental pollution are forcing people to think about constructing the world future based on the renewable and sustainable energy.

To date, first generation biofuel, mostly ethanol, is mainly produced using starch and sucrose from food crops like corn and sugarcane [1]. Yet, there has been intensive debate over energy balance, economics, and environmental impacts of these first-generation biofuels [2–4]. Studies revealed the negative energy balance of food crop based biofuel [3]; namely, it may require more energy input to produce ethanol than the energy output of ethanol. In addition, researchers found out that first generation biofuel offers only modest savings in greenhouse-gas emissions compared to fossil fuel [4]. First generation ethanol production may in fact release more greenhouse-gas than conventional fuels once the direct land use changes are considered [5]. Starch/sucrose based biofuel may not solve the energy and environment problems; in addition, it diverts human food resources and threatens the world's food security, giving rise to more economic and social problems [6].

As one of most abundant renewable sources on earth, lignocellulosic biomass has the potential to become a major source to produce renewable fuels and chemicals. It is reported that every year there are more than 1 billion tons of biomass potentially available from forestland and agricultural land in the U.S. [7], enough to produce sufficient biofuels to displace one third of the national fossil fuel consumption. Fuel production from lignocellulosic biomass has a more

favorable energy efficiency and a smaller environmental impact than the first generation biofuel [8]. In addition, using lignocellulosic biomass does not directly compete with global food supply and raises fewer ethical concerns. Given that, lignocellulosic biomass is regarded as one of the most promising alternative replacements of fossil fuels. Development of biorefinery technologies is underway to convert lignocellulosic biomass into fuels and chemicals which are now mainly generated from petrochemical feedstocks.

1.2 Lignocellulosic biomass

Lignocellulosic biomass composition and structure

Primarily, lignocellulosic biomass is made up of three major components: cellulose, hemicellulose, and lignin, together with some minor components, such as extractives (small organics) and ash (inorganic minerals) [9,10]. Cellulose fibers link together to form microfibrils and build the skeleton of lignocellulose. Hemicellulose and lignin tightly bind around the clusters of microfibrils, forming nanoscale composites that make up the rigid structure of the plant cell wall [10]. As shown in Table 1.1, the proportion of each component depends on the feedstock type and differentiates across species. For example, the lignin content of softwood is relatively higher than hardwood and agricultural residues [10], while the cellulose content of herbaceous plants is lower than woody biomass.

Table 1.1 Composition of various lignocellulosic feedstocks [11]

	Composition (%)		
	Lignin	**Cellulose**	**Hemicellulose**
Softwood	25-35	45-50	25-35
Hardwood	18-25	40-55	24-40
Herbaceous biomass	10-30	25-45	10-40

Cellulose

As the major component of lignocellulosic material, cellulose is the primary target of bioconversion. Linked by β-(1-4) glycosidic bonds, D-glucose units comprise the cellulose chain as a linear polymer backbone [10]. The number of glucose units in each chain of cellulose is known as the degree of polymerization (DP) [12], which varies across different biomass types. The hydrogen bonds connect between cellulose molecules build up the elementary structures of fibrils which include stable crystalline region and disordered amorphous region [10]. Generally, the cellulose structure determines its resistance to heat, chemicals and enzymes; and the crystalline region is more recalcitrant than amorphous region [12,13].

Hemicellulose

Unlike cellulose, hemicellulose has a branched structure and is more heterogeneous in chemical composition [12]. As the second major polysaccharide, hemicellulose consists of different C5 and C6 sugars, including hexoses (D-glucose, D-mannose, and D-galactose) and pentoses (D-xylose and L-arabinose) [12]. Hardwood and herbaceous hemicelluloses are mostly composed of highly acetylated glucuronoxylan with small amount of glucomannans, while softwoods have higher proportion of galactoglucomannans and partly acetylated arabinoglucuronoxylan [10,12]. Owing to the higher acetate group content which can form organic acids during pretreatment, the hardwood and herbaceous hemicelluloses are more labile in pretreatment, undergoing acid autohydrolysis more readily, than softwood hemicellulose [14].

Lignin and other phenolic compounds

As the second most abundant organic substrate within plant biomass, lignin plays the roles in decreasing the permeability of water across the cell wall and protecting the cell wall against the penetration of destructive enzymes [15,16].

Lignin has three main phenolic precursors. As shown in Figure 1.1, they are guaiacyl (G, derived from coniferyl alcohol), syringyl (S, derived from sinapyl alcohol), and ρ-hydroxyphenyl (H, derived from p-coumaryl alcohol). In plant tissue, lignin is generally combined with hemicellulose and its composition and structure varies among species [17]. Regardless of source, softwood lignin consists almost exclusively of G-type lignin. This refers to a so-called "guaiacyl lignin", which is known to have much stronger restriction in fiber swelling and enzyme permeability than syringyl-type lignin [18]. In contrast to softwood lignin, hardwood lignin differs by species. Generally, it has considerably more S derived units, and the difference among the species primarily reflects in the ratio of S to G units [10]. The hardwood lignin (the copolymer of G and S units) has a greater methoxylation than softwood lignin; as such, it has a lower degree of polymerization and more free phenolic hydroxyls, providing less condensation and less recalcitrance than softwood lignin [19]. In herbaceous biomass, lignin is composed of all three phenolic precursors and presents the lowest mechanical strength [20].

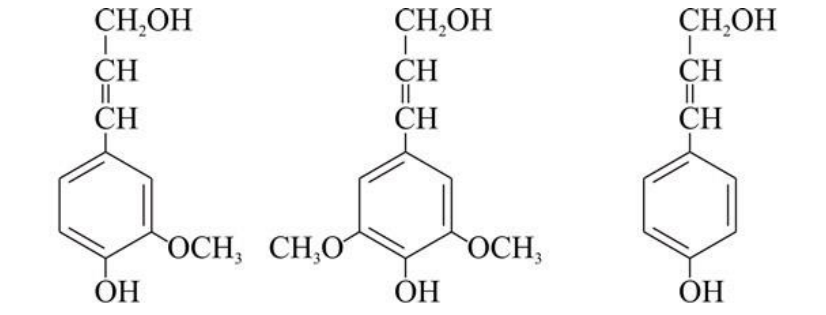

Figure 1.1 Chemical structures of the phenolic precursors used to construct the lignin [21]

It is noteworthy that in some parts of the plant like foliage, bark, branch, and juvenile wood, phenolic compounds are not necessarily fully lignified. Instead, some phenolic compounds are present in the form of low-molecular-weight phenols and polyphenols [22]. In particular, foliage consists higher amount of phenolic compounds like tannins, some flavonoids, and different types of phenolic glycosides [22,23].

Extractives

In addition to carbohydrates and lignin, lignocellulosic biomass contains a variety of organic compounds which can be extracted with water or organic solvents. They are called extractives (also mentioned as organic non-structural component in Chapter 6). Different types of extractives serve different functions: aliphatic compounds play as energy storage (fats) and evaporation control (waxes); terpenes and terpenoids are the major components of the resin and protect the plant after becoming wounded; phenolic extractives contribute to the protection against microbiological and insect attacks [10].

Ash

The ash contained in the lignocellulosic feedstock originates from two sources: introduced soil, rock, and inorganic contamination during processing, and physiological minerals embedded in the plant structure [24]. The former is also mentioned as inorganic non-structural component in Chapter 6. Generally, ash consists of silica, alkaline/alkali metals such as Ca, K, and Na, and other inorganics. Ash composition is distributed non-uniformly within different plants and varies across parts of a plant [10,24].

As a minor constitute of the biomass, ash has not attracted sufficient attention, especially in biochemical conversion, until recently when pioneer commerical-scale biorefinery facilities are deployed for operation. Although it is not fully understood, high ash content biomass is reported to raise a series of considerable problems in both biochemical conversion and thermochemical conversion [24]. For biochemical conversion, high levels of ash directly correlate to lower carbohydrate content of raw biomass [25]. Because of its high neutralization capacity, ash was reported to negatively affect the acid based pretreatment performance [26]. Additionally, ash has shown inhibitive effects on enzyme activity by lowering the conversion efficiency of enzymatic hydrolysis [27]. For thermochemical conversion, the presence of ash reduces the feedstock overall energy density and promotes undesirable secondary reactions [24]. For fast pyrolysis in particular, ash fouls the catalysts and contributes to subsequent instability and corrosivity of the bio-oil [24,28]. For both pathways, ash agglomeration raises operational issues such as slag formation, surface deposition and corrosion, jeopardizing the stablity and continuity of the processes [29]. Furthermore, high ash level will inevitably increase the cost in wastewater treatment and solid waste disposal, and may potentially increase the risks of creating hazardous air pollutants [24].

1.2.1 From lignocellulosic biomass to biorefinery feedstock

Many types of lignocellulosic biomass can be treated as feedstocks for fuels and chemicals production [7]. Those feedstocks include: 1) agricultural residues (e.g., corn stover, wheat straw, and sugarcane bagasse); 2) energy crops including woody biomass (e.g., hybrid poplar and willow) and herbaceous biomass (e.g., switchgrass, miscanthus, sorghum, and energy cane); 3)

forest resources (e.g. logging byproducts and forest residues); and 4) industrial and municipal organic wastes (e.g., yard wastes, recycled paper, and constructional wood waste) [30].

Biomass is estimated to have a potential to meet up to 60% of the world's primary energy consumption [31]. However, this massive quantity of biomass does not necessarily represent a viable feedstock for lignocellulosic biorefineries. In fact, it is not possible to transform all biomass into tangible conversion feedstock. Three major factors – quantity, quality, and cost – characterize the feedstock and determine the total amount of feedstock commercial-scale biorefineries could obtain.

Quantity

Feedstock quantity plays a key role in establishing the lignocellulosic biorefinery. Given the considerably low bulk density of raw materials, a commercial-scale biofuel facility relies upon large volume of feedstock to meet its capacity [32]. Compared with the existing agricultural, logging, and food supply chains, preparing lignocellulosic feedstocks appears to be more challenging. To ensure an adequate biomass supply across seasons, it requires larger land area, longer transportation distance, and more complicated handling processes [31]. To address these challenges, researchers have approached the problem from several directions. For example, development of fast growing energy crops using breeding and/or genetic tools helps to increase the land productivity, thus maximizing the biomass yield for a given land basis [33]. Investigation in using a broad range of feedstocks across regions and mixing different types of biomass ensure the local feedstock supply [34]. Deployment of new harvesting and collection techniques improves the feedstock handling efficiency [35]. Design of improved, regionally distributed supply systems allow for greater economical logistic control [36].

Quality

Feedstock quality is also critical to the conversion process. The definition of high quality feedstock is dependent on the type of conversion process. Feedstock quality is highly correlated with the physical (e.g. particle size, moisture, and density) and chemical (e.g. cellulose content, lignin structure and composition, and ash level) properties. For biochemical conversion, high sugar content is more favorable and usually attributes to higher conversion yield [24]. For thermochemical conversion, high sugar content is not the only criteria. Instead, feedstock with high organic content but low inorganic content is preferable in the thermochemical conversion processes. Sourcing a single type of feedstock with guaranteed physical and chemical properties year-round is ideal to ensure the stable conversion process. Unfortunately, given the necessity of large feedstock quantities, uniform feedstocks are a rarity. A commercial-scale biorefinery must have the capacity to accept a variety of biomass to meet its feedstock input requirement. The feedstock quality is affected by various factors, including inherent species variability, plantation conditions, seasonal variations, and harvest, collection and storage practices [35]. Over the decades, intensive research has been done in improving the conversion yield of specific feedstock by optimizing the processing conditions [37–39]. However, depending on where and how the crops are grown, inconsistent characteristics often result in fluctuation of the conversion yield [40]. Even for the same type of feedstock, heterogeneity of the biomass quality was prevalently observed, reflecting in uncertain convertibility [41].

Cost

With both quantity and quality concerns, feedstock cost is the major determinant in the economic success of a biorefinery. Even with the rapid progression of agricultural and transportation

technologies, there is no way to ensure both high-volume and high-quality biomass supply without significantly increase of the feedstock cost. The current reality is that feedstock cost is used to adapt the supply chain requirements by balancing the quantity and quality for each specific conversion technology [42]. This reality allows the access of the largest volume of biomass with acceptable quality specifications [7]. Yet, feedstock cost still contributes to more than 40% of the total operating costs in the current biorefinery schemes [43–45].

Taken together, there is obviously a trade-off among feedstock quantity, quality, and cost. Pristine feedstocks used in most of conversion research composed of clean, homogeneous biomass are certainly easy to convert and can achieve higher product yields, but they are expensive and can hardly meet the volume for commercial-scale biofuel production. Others like commodity scale field-run feedstocks are more realistic for biorefinery facilities as they are cost competitive and easy to obtain, whereas the correlated conversion yields are lower given their heterogeneous and low quality [35].

1.2.2 **Poplar as biorefinery feedstock**

Poplar, along with its hybrids within the genus *Populus*, is well recognized as an excellent bioenergy feedstock [46]. As highlighted in the literature, poplar is one of the most productive temperate wood species with rapid growth rate and abundant biomass accumulation [46]. Besides growing on forest lands or farm lands, poplar can thrive on marginal lands with little fertilization and irrigation. Given the diverse genetic base and available genome sequence, it is easy to tailor traits of poplar through crossbreeding and/or genetic modification [47]. As a perennial woody biomass, poplar can be harvested year-round to ensure the constant feedstock input of a biorefinery. Such on-demand harvest reduces feedstock handling costs by eliminating

the need for feedstock storage infrastructure and avoiding degradation during long-term storage [35,48]. In addition to these economic advantages, poplar has important traits for sustainability and has been proved to be beneficial in some environmental applications [49,50]. Poplar tree farms could provide many environmental services including carbon sequestration, erosion control, soil remediation, wastewater treatment, and wildlife habitat [49].

Poplar, depending on the final application, is cultivated in two types of plantation system - short rotation coppice (SRC) and short rotation forestry (SRF). Both systems share the similar assets of fast growing, ease of cloning, marginal land usage, and environmental service. Poplars from the two systems are different in terms of stand density, growing cycle, harvest strategy, and biomass yield [50].

1.2.3 **Short rotation coppice vs short rotation forestry**

Short rotation coppice (SRC) system was initially developed for energy oriented woody crop production, especially as solid fuel for heat and power generation [51]. In order to achieve higher biomass yields per unit land area, woody crops (e.g. poplar) are cultivated at very high densities in SRC system [33,50,52]. Managed by intensive agricultural practices, trees grow in shorter cycles for every two to five years and are harvested through coppicing. With the help of fully mechanized agricultural machinery, coppicing can ideally harvest 100% of the aboveground components of the trees [53]. As Figure 1.2 shows, SRC poplars are harvested where the aboveground parts of trees are cut and chipped as the harvester moves down the row. Also, coppicing has been proven to trigger intrinsic juvenile growth such that trees will regenerate from the developed root system, allowing multiple tree harvests without replanting for up to 20

years [54,55]. Regarding the higher productivity and efficiency, the feedstock supply from SRC plantation system is likely to be less expensive.

Figure 1.2 Harvest practice of SRC poplar plantation (Jefferson, OR, 2013) [56]

Short rotation forestry (SRF) is another plantation system widely applied to provide wood products (e.g. pulpwood, sawlog, and lumber) to various markets. In SRF system, woody crops are managed with relatively more conventional tree farming practices for 8 to 20 years until harvest [50,57]. Trees like poplars are grown to obtain large, single-trunk stems at lower densities [50]. The reported productivity of poplar from SRF system is lower (6.1 – 16.3 oven dry tonne (odt) ha^{-1} $year^{-1}$) than that of SRC system (up to 25 odt ha^{-1} $year^{-1}$) [58]. During SRF harvest, mature trees are felled, delimbed, and debarked using traditional logging equipment and methods. Typically, less than 30% of the original tree biomass is capable to be processed into commercial wood [59]. The residues, such as bark, branch, and leaf are retained in the field or sold as hog fuel at breakeven price. The limited whitewood yield, long term managing, complex

handling, and energy-intensive harvesting process attribute to the high cost of SRF wood. Indeed, regarding the low value of biorefinery products and current low fossil fuel price, the market price of SRF wood is prohibitively high for use in making fuels or chemicals [60].

Feedstock cost and availability have always been bottlenecks in biorefinery commercialization. SRC plantation system demonstrates an attractive alternative to provide sufficient feedstock at reasonable prices. However, so far, the majority of tree-to-fuel research has been centered on using mature wood from conventional SRF tree farms [61–64].

1.3 Biochemical conversion

The biochemical conversion of lignocellulosic biomass into fuels and chemicals consists of three main processes: pretreatment, altering the biomass structure to make cellulose more accessible to enzymes and partially release the sugars; enzymatic hydrolysis, applying enzymes to catalyze the degradation of polysaccharides into monomeric sugars; and fermentation, converting sugars into desired products using microorganisms. Challenges exist in each step of the bioconversion scheme. First, pretreatment requires a balanced severity for both good biomass fractionation and limited degradation product formation. Second, enzymatic hydrolysis is still one of the most costly processes of biofuel production; it represents a major barrier to the economic feasibility of commercial biofuel. Finally, an ideal microorganism and a well-developed process are important to high fermentation yield. In general, to achieve the highest product output, a good overall sugar yield from lignocellulosic feedstock is the primary objective.

Pretreatment

To survive in nature, plants has evolved a recalcitrant structure that resists attack from outside. In order to obtain fermentable sugars, the lignocellulosic structure must be broken down to remove the impediments and better expose the polysaccharides to enzymes for hydrolysis. As shown in Figure 1.3, pretreatment opens up the lignocellulosic matrix by breaking the lignin seal, releasing the hemicellulose, and reducing the cellulose crystallinity [65]. As the initial step of the biochemical conversion process, the efficiency of pretreatment determines the overall sugar recovery and subsequent fuel yield. Indeed, the success of biochemical conversion process relies on an effective pretreatment.

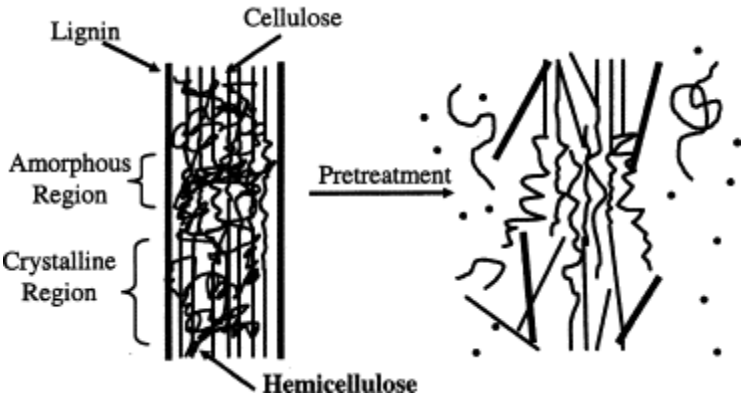

Figure 1.3 Effects of pretreatment on lignocellulosic biomass [39]

Nowadays, intensive efforts have been made in developing pretreatment technologies to convert lignocellulosic materials. Pretreatment technologies include dilute sulfuric acid [66], liquid hot water [67], ammonia fiber expansion (AFEX) [68], sulfur dioxide (SO_2) steam pretreatment [69], lime [70], aqueous ammonia [71], and organosolv [72]. Among them, sulfur dioxide steam pretreatment is reported to achieve highest sugar recovery – up to 90% total monomeric sugars of the original sugars after enzymatic hydrolysis [73].

Steam pretreatment, sometimes called “steam explosion”, is one of the most widely used pretreatment methods in laboratory research and pilot/demonstration plants [74,75]. It is chosen as the pretreatment method in this research because it is adaptable for many reaction conditions and capable of pretreating a wild range of feedstocks [34,40]. During steam pretreatment, lignocellulosic biomass is rapidly heated by high-pressure steam. After being held for a specified period of time, the pretreated material is rapidly released from the reactor by a pressure vent [12,39]. Acetylated hemicellulose groups release acetic acid during pretreatment, leading to autohydrolysis. In most cases, the addition of acid catalysts helps to increase the effectiveness of the steam pretreatment [12,62,76,77]. Introducing gaseous sulfur dioxide (SO_2) as the acid catalyst enables a more complete fractionation with shorter residence time and lower reaction temperature [62,78].

Enzymatic Hydrolysis

As stated above, enzymatic hydrolysis is the key step to obtain fermentable sugars. To hydrolyze polysaccharides into monomeric units, a host of different glycolytic enzymes are applied to pretreated biomass. These enzymes, collectively named cellulases, are synthesized by several microorganisms. One of the most studied strain is the fungi *Trichoderma reesei*. Currently, it is the most prominently industrialized microorganism in cellulase production [13,79].

There are primarily three groups of cellulases applied in enzymatic hydrolysis [13,80]: endoglucanases randomly attack β-(1-4) glycosidic bonds within amorphous cellulose and generate cellulose chains with free ends; exoglucanases travel along the cellulose chains from either reducing or non-reducing ends, peeling off cellobiose units; β-glucosidases hydrolyze the cellobiose into glucose. End-product inhibition is prevalent in all three processes, and limits the

hydrolysis speed especially when each enzyme is used separately. Therefore, saccharification is carried out by a mixture of different enzymes [81].

Structurally, the recalcitrance of lignocellulosic biomass impedes the accessibility of enzymes to cellulose. Many factors contribute to this problem: the crystalline structure and the high degree of polymerization of cellulose; cellulose sheathing by hemicellulose and lignin; the limited accessible surface area; and the heterogeneous character of particle size [39,62,82–84].

Additionally, chemicals released during pretreatment are reported to inhibit the cellulase activity and limit the extent of enzymatic hydrolysis [85,86]. Those compounds known as inhibitors include furfural and 5-hydroxymethylfurfural (5-HMF); aliphatic acids, such as acetic, formic, and levulinic acid; and phenolic compounds, most of which are formed during degradation or transformation of hemicellulose-derived sugars and solubilized lignin fragments [86]. Depending on the feedstock characteristics and pretreatment methods, the types and concentrations of inhibitor compounds vary significantly [85].

Fermentation

Fermentation typically refers to the conversion of sugar to chemicals using microorganisms. The goal is to convert all the fermentable sugars from lignocellulosic biomass into products (*e.g.* ethanol, xylitol). In nature, a variety of bacteria and yeast are able to ferment sugars, mainly glucose, to target products. Co-fermentation of pentoses and hexoses used to be a challenge in industrial utilization of all the sugars obtained from lignocelluloses. For example, there was essentially no commercially suitable bacteria or yeast in using xylose and arabinose in ethanol

fermentation [87]. Following the advancement in molecular biology, currently many microorganisms have been genetically modified to metabolize both pentose and hexose [88].

1.4 Economics of biochemical conversion

Techno-economic analysis (TEA) provides a useful methodology that is used to quantify the technical and economic performance of biofuel conversion pathways [89]. As the simplified representation of a commercial-scale facility, TEA is regarded as a cost-efficient method for selecting the most promising conversion pathways. It helps to direct research over unit operation that requires further development [90]. Toward the industrialization of laboratory and pilot technology, TEA has played a key role in providing important insights into diverse sets of feedstocks, process inputs, conversion reactions, and fuel outputs [89].

A large number of studies have been done to evaluate the economics of various biochemical conversion pathways [43,90]. Among them, the process design and economics report by DOE's National Renewable Energy Laboratory (NREL) establishes a TEA benchmark of cellulosic ethanol manufacturing based upon applicable best practices in engineering, construction, and operation [43]. Along with a series of publications, the report serves as a conceptual platform where researchers can input experimental results and make economic comparison between different conversion parameters [43,91–94].

From the perspective of TEA, barriers to economically feasible cellulosic biofuel production include feedstock cost, other operation and management expenses, and capital expenditure [95]. Feedstock cost is identified as a major operating cost component, and accounts for approximately one-third of the final minimum ethanol selling price (MSEP) [43–45]. As discussed above, to

ensure the success of a commercial-scale lignocellulosic biorefinery, it is essential to reduce the feedstock cost through the introduction of fast growing crops into an optimized supply system.

1.5 Thermochemical conversion

Currently, fuel-oriented thermochemical conversion technologies tend to be grouped into two categories: 1) fast pyrolysis focuses on liquid bio-oil products and 2) gasification focuses on the production of gaseous products. Different from biochemical conversion, thermochemical conversion relies on heat and/or physical catalyst to convert biomass into products [96].

The typical reaction temperature of thermochemical conversion ranges from 300 °C to 1,000 °C [97]. Fast pyrolysis is the thermal decomposition of biomass into liquid products (bio-oil) in the absence of oxygen at temperature ranges from 400 °C to 650 °C [96]. Gasification, on the other hand, is an exothermic partial oxidation with complete depolymerization of biomass at temperature over 800 °C to gaseous intermediates (syngas) rich in CO, H_2, CH_4, and CO_2 [38].

1.5.1 **Fast pyrolysis**

Subject to the residence time, pyrolysis is categorized into groups: slow pyrolysis and fast pyrolysis. Slow (or conventional) pyrolysis has been applied for charcoal preparation from woody biomass since ancient times. Historically, biomass is pyrolyzed at the reaction temperature of around 400 °C with residence times of minutes to even days [98]. In slow pyrolysis, char and syngas are the major products with liquid tars produced in small quantities. Fast pyrolysis is more productive toward liquid fuels from lignocellulosic biomass and has been introduced as a promising thermochemical conversion pathway in future biorefineries.

Unlike slow pyrolysis, fast pyrolysis is a thermal decomposition process where the biomass is rapidly (usually 1-2 seconds) heated to temperature around 500 °C to produce mostly liquid product (bio-oil), smaller amount of solid, and gaseous products, known as bio-oil, char, and non-condensable gases, respectively. The direct liquid product, bio-oil, has been tested as a substitute for fuel oil or diesel in boilers, furnaces, engines, and turbines for heat and power generation. Following deoxygenation upgrading, bio-oil can potentially be used as a precursor for transportation fuels and chemicals [99].

Bio-oil is a viscous mixture of oxygenated compounds, which include small acids, ketones, phenols, anhydrosugars, aldehydes, guaiacols, esters, and furans. Besides, as the most abundant component in bio-oil, water represents 15-35% of its mass [100]. The water mainly comes from feedstock moisture and dehydration reactions during the pyrolysis. Because of the higher oxygen content (35-40 wt.%), raw pyrolysis bio-oil usually has a much lower energy density than petroleum based oil [99]. The higher heating value (HHV) of bio-oil ranges from 14–18 MJ/kg, which is less than half of that for conventional fuel oils, 42–44 MJ/kg [101].

Char is an inevitable byproduct of fast pyrolysis. The general understanding about the mechanism for char formation is that it occurs during depolymerization of the bulk aromatic structure [102]. As a macromolecular assembly of aromatic rings, lignin is reported to facilitate higher char yield than polysaccharides during fast pyrolysis [103–105]. Char could be considered as a solid fuel like charcoal, and is combusted to provide heat for the fast pyrolysis process [106]. In addition, alternative usage of char has been studied, including activated carbon, soil amendment, and even catalyst support [106–108].

Non-condensable gas, as the final pyrolysis product, is rich in CO_2 and CO and usually consists of a small amount of light hydrocarbons like CH_4 and C_2H_6 [109]. Although the non-condensable gas has a low energy density, research has shown that it could provide heat and power via combustion [98] or act as a feedstock in production of valuable fuels and chemicals via either chemical catalysis process (e.g. Fischer-Tropsch) [110] or syngas fermentation [111].

The reaction conditions and feedstock characteristics are two major factors affecting the yields and distribution of products in fast pyrolysis [112]. Significant efforts have been made to optimize the reaction temperature, residence time, and heating rate of fast pyrolysis using a variety of feedstocks in different reactor configurations [99,101].

1.6 Research objectives

The overarching goal of this Ph.D. project is to convert short rotation coppice (SRC) poplar into fuels and chemicals by implementing both biochemical and thermochemical conversions. For biochemical conversion, the objective is to improve the sugar yield and recovery from SRC poplar, thereby increasing the potential ethanol yields. For thermochemical conversion, the objective is to improve the bio-oil yield from SRC poplar fast pyrolysis.

The specific research objectives include:

i) Understand the physical and chemical characteristics of first rotation 2-year-old SRC poplar by comparing with whitewood from SRF (WWF) (Chapter 2);
ii) Assess the influence of leaf removal in sugar production from SRC poplar using steam pretreatment and enzymatic hydrolysis (Chapter 2);
iii) Analyze the economics of a SRC poplar based ethanol biorefinery (Chapter 2);

iv) Determine the product distribution in fast pyrolysis of SRC poplar (Chapter 3);

v) Investigate the impact of leaf removal in bio-oil yield and composition during fast pyrolysis (Chapter 3);

vi) Evaluate the energy recovery rate in fast pyrolysis of SRC poplar samples (Chapter 3);

vii) Compare the biochemical conversion yield by using different SRC poplar clones from different sites (Chapter 4);

viii) Discuss the feedstock as commodity from grower and end-user perspectives, and propose a new business model integrating both plantation and biorefinery (Chapter 4);

ix) Study the effects of non-structural components (including inorganic ash and organic extractives) in biochemical conversion of SRC poplar using preprocessing methods (Chapter 5);

x) Assess the economics of having preprocess as an additional unit process in biofuel plant (Chapter 5);

xi) Summarize the work presented and address future research (Chapter 6).

Chapter 2. Can we use short rotation coppice poplar for sugar based **biorefinery feedstock? Bioconversion of 2-year-old poplar grown as short rotation coppice** [1]

Abstract

Background: Feedstock cost is a substantial barrier to the commercialization of lignocellulosic biorefineries. Poplar grown using a short rotation coppice (SRC) system has the potential to provide a low-cost feedstock and economically viable sugar yields for fuels and chemicals production. In the coppice management regime, poplars are harvested after two years' growth to develop the root system and establish the trees. The biomass from these two-year-old trees is very heterogeneous, and includes components of leaf, bark, branch, and wood chip. This material is quite different than the samples that have been used in most poplar bioconversion research, which come from mature trees of short rotation forestry (SRF) plantations. If the coppice management regime is to be used, it is important that feedstock growers maximize their revenue from this initial harvest, but the heterogeneous nature of the biomass may be challenging for bioconversion. This work evaluates bioconversion of 2-year-old poplar coppice and compares its performance to whitewood chips from 12-year-old poplar.

Results: The 2-year-old whole tree coppice (WTC) comprises 37% leaf, 9% bark, 12% branch, and 42% wood chip. As expected, the chemical compositions of each component were markedly

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different. The leaf has a low sugar content but is high in phenolics, ash, and extractives. By removing the leaves, the sugar content of the biomass increased significantly, while the phenolic, ash, and extractives contents decreased. Leaf removal improved monomeric sugar yield by 147 kg/tonne of biomass following steam pretreatment and enzymatic hydrolysis. Bioconversion of the no-leaf coppice (NLC) achieved a 67% overall sugar recovery, showing no significant difference to mature whitewood from forestry plantation (WWF, 71%). The overall sugar yield of NLC was 135 kg/tonne less than that of WWF, due to the low inherent sugar content in original biomass. An economic analysis shows the minimum ethanol selling price required to cover the operating cost of NLC bioconversion was $1.69/gallon.

Conclusions: Leaf removal resulted in significant improvement in overall monomeric sugar production from SRC biomass. Leaf removal is essential to achieve good yields in bioconversion of poplar. Economic analysis suggests the NLC could be a reasonable feedstock provided it can be obtained at a discounted price.

Keywords: Poplar, short rotation coppice, steam explosion, sugar yield, saccharification, leaf removal, whole tree harvest, economic analysis

2.1 Background

Biorefineries require low cost feedstock and cost-competitive conversion processes to be economically viable. Significant progress has been made to improve biomass to fuel conversion, but feedstock cost is still a major factor impeding biorefinery commercialization [43]. For example, raw feedstocks are reported to contribute over 40% of the operating costs in lignocellulosic biomass based ethanol production [43,113].

Poplar, woody biomass from different species (or hybrids) within the genus *Populus*, is well recognized as an excellent bioenergy feedstock [46]. Poplar is one of the most productive temperate wood species with rapid growth rate and abundant biomass accumulation [46]. Besides growing on forest lands or farm lands, poplar can thrive on marginal lands with little fertilization or irrigation [114]. Given the diverse genetic base and available genome sequence [47], it is easy to tailor characteristics of poplar through crossbreeding and/or genetic modification. Progresses have been made to achieve higher saccharification or fermentation yields from poplar by increasing cellulose content, altering the content and S/G ratio of lignin, and suppressing the synthesis of certain hemicellulose [46]. As a perennial woody biomass, poplar can be harvested any time to fulfill the feedstock requirements of a biorefinery. The on-demand harvest reduces feedstock handling costs by eliminating the need for feedstock storage infrastructure and avoiding degradation during long-term storage [35,48]. In addition to these economic advantages, poplar has important traits for sustainability and possesses proven environmental benefits. Poplar tree farms provide many environmental services including carbon sequestration, erosion control, soil remediation, wastewater treatment, and wildlife habitat [49].

Poplar, depending on the final application, is cultivated in two types of plantation systems - short rotation coppice (SRC) and short rotation forestry (SRF) (Figure 2.1). Both systems share traits of fast growing trees, ease of cloning, marginal land usage, and environmental services, but are different in terms of stand density, growing rotation, and harvest strategy (Table 2.1).

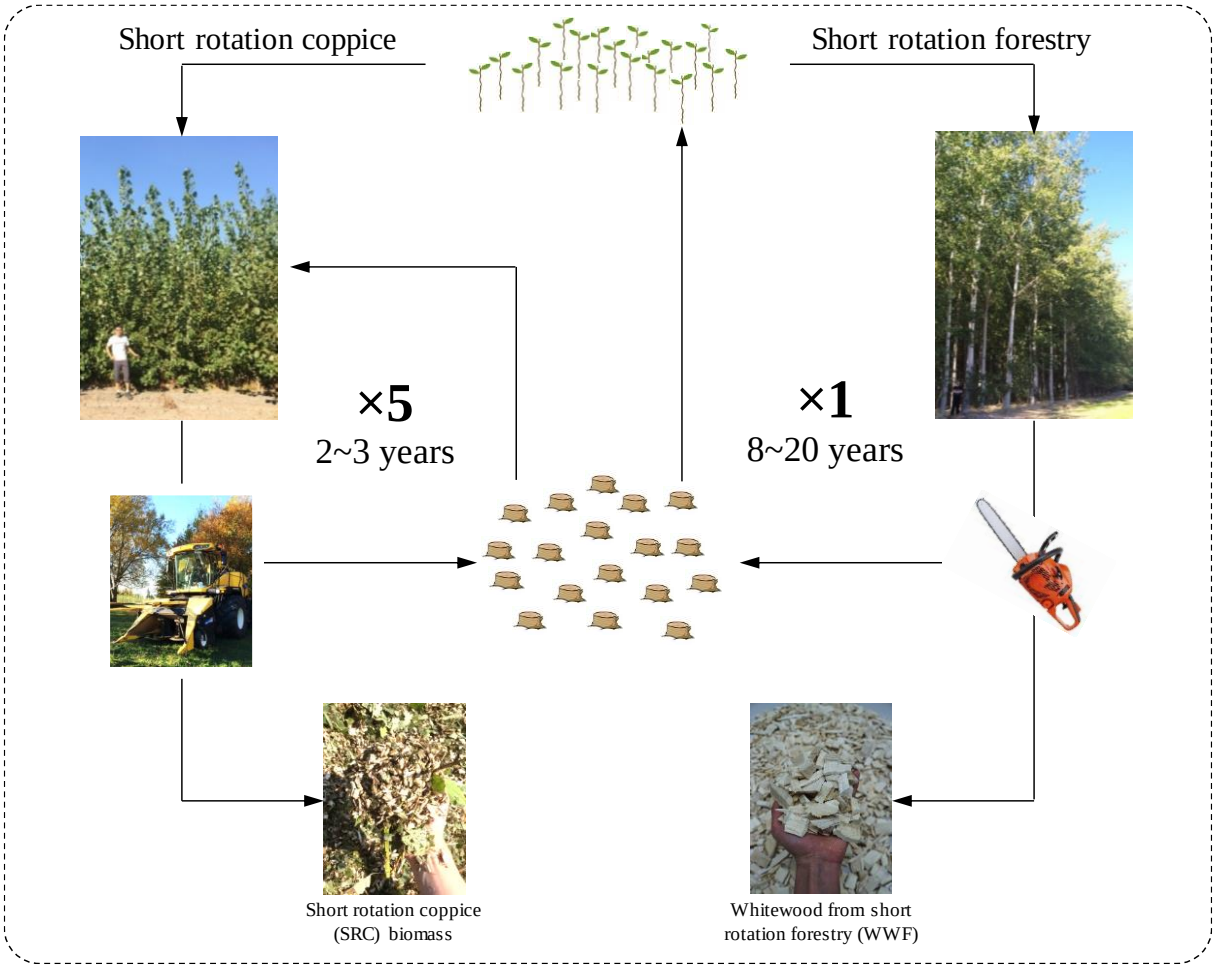

Figure 2.1 Plantation management strategies of SRF and SRC system

The short rotation coppice (SRC) system was initially developed for energy crop production, especially solid fuel for heat and power generation [51]. Poplars are planted at densities higher than 1,500 trees ha^{-1} in the SRC system and maintained using intensive agricultural-type crop

management (Table 2.1) to achieve maximal biomass yields - up to 25 odt ha^{-1} $year^{-1}$ (research plot records) [33,50,115]. The high yield and whole tree harvesting result in a lower feedstock cost.

Table 2.1 Management strategies, current applications of poplar from short rotation forestry system (SRF) and short rotation coppice system (SRC)

	Short rotation coppice (SRC)	Short rotation forestry (SRF)
Management strategies		
Tree density (stand/ha)	≥ 1,500 [33]	500 – 1,500 [33,50]
Plantation rotation (years)	2 – 5 [116]	8 – 20 [50,57]
Productivity (odt* ha^{-1} $year^{-1}$)	Up to 25 [115] [a]	6.1 – 16.3 [58] [b]
Harvest biomass (%)	~100	30 [59]
Applications	Heat and power	Pulpwood, sawlog, and lumber
Price ($/odt)	N/A [c]	50 ~ 120 [117]

* "odt" stands for oven dry tonne.
[a] leafless total coppice data
[b] saw log component data
[c] feedstock market price of SRC not available.

Short rotation forestry (SRF) is another tree plantation system, which has been commercialized to provide woody commodities such as pulpwood, sawlogs, and lumber to various markets. In the SRF system, poplars are managed with more conventional tree farming practices using 8 to 20 year rotations [50,57] (Figure 2.1). Poplars are grown as large, single-trunk trees at densities of 500 to 1,500 trees ha^{-1} [50]. The reported productivity of SRF system is lower (6.1 – 16.3 odt ha^{-1} $year^{-1}$) [58], but the products like sawlog, lumber, and pulpwood are more valuable compared to the coppice products from the SRC system (Table 2.1). Poplars managed in SRC tree farms are attractive alternatives to conventional SRF practice to provide less expensive biomass for biorefineries. SRC management accelerates plant growth and maximizes land

productivity, and provides revenue in as short as two years. Coppicing eliminates the costs of land preparation and replantation for up to 20 years (Figure 2.1). Initially planted from cuttings, poplars are grown for two years before the first coppicing to properly establish the root system. The already developed root system stimulates the growth of multiple stems per stump, supporting greater biomass accumulation in a shorter time commitment [54,55]. In the succeeding rotations, coppicing triggers vigorous juvenile growth, accelerates plant intrinsic regeneration, and ultimately maximizes the biomass yield [51,118]. When establishing the SRC plantation, the first cycle (a two-year cycle) grows from the cuttings and yields less biomass per hectare compared to the subsequent rotations. However, as mentioned above, the first coppice is essential for the following rotations and is unavoidable in an approximate 20-year lifespan of SRC tree farms. The SRC poplar used in this study is originated from the first cycle after two-year growth.

Harvesting represents one of the most energy intensive operations in the tree farm practice and adds a significant cost in the overall feedstock supply chain [119]. Small-dimensioned trees in SRC system (2 to 5 years depending on the growth) can be harvested efficiently using fully mechanized agricultural machinery which integrates cutting and chipping. Schweier et al. [120] evaluated a “trees to chips” harvester and described the system as reliable and cost-efficient.

Bioconversion of debarked poplar wood from mature trees has been extensively studied [73]. In addition, there has been some research on bioconversion of poplar with bark to assess how heterogeneous woody biomass might perform. Those investigations gave mixed results with DeMartini [121] reporting that bark was inhibitory to enzymatic actions, while Schütt [77] found no significant difference between debarked and non-debarked poplar during enzymatic

hydrolysis of steam pretreated solids. No one has investigated bioconversion of whole tree poplar, which contains (whitewood) chips, bark, branches, and even leaves if harvest is done in the growing season; neither to mention the material that will be produced in the initial two-year harvest is very heterogeneous. Poplar for biofuels will be grown using the SRC system because of its earlier growth culmination and shorter harvest frequency. It is essential, therefore, that the performance of this material for bioconversion be thoroughly assessed. The purpose of our research is to make this assessment. This paper presents research on the bioconversion of SRC poplar from the initial two-year harvest. It aims to 1) characterize the SRC poplar in terms of physical and chemical properties, 2) investigate and improve the sugar production of SRC poplar in bioconversion, and 3) evaluate the economics of SRC poplar to biofuel process. Subsequent papers will present work on bioconversion of poplar coppice harvested in ensuing rotations.

2.2 Methods

SRC poplars were harvested and chipped using a whole tree harvester after two-year growth following planting. The harvested biomass, the whole tree coppice (WTC), was characterized by separating and analyzing its four main components: leaf, bark, branch, and (whitewood) chip. By sorting out leaves, we obtained leaf coppice (LC, including only leaf) and no-leaf coppice (NLC, including bark, branch, and chip) (Figure 2.2). The chemical composition of WTC, NLC, and LC were analyzed and compared with whitewood from forestry (WWF). The four poplar feedstocks were then processed in the same way through pretreatment and enzymatic hydrolysis. The WTC, NLC, LC, and WWF were steam pretreated at 195°C for 5 minutes with SO_2 (3% w/w) impregnation. After separation, the chemical compositions of the water insoluble fractions (WIF) and water soluble fractions (WSF) were analyzed. Water insoluble fractions were then

enzymatically hydrolyzed at 5% (w/v) consistency and 5 FPU/g cellulose enzymes loading (Figure 2.2). The overall monomeric sugar yields (kg monomeric sugars/tonne biomass) and recoveries (kg monomeric sugars/kg original sugars × 100%) were used to assess the impact of leaf removal following steam pretreatment and enzymatic hydrolysis, and to evaluate the efficiency of using coppice in bioconversion. The experimental results were input into a modified NREL biochemical conversion model for economic analysis [43].

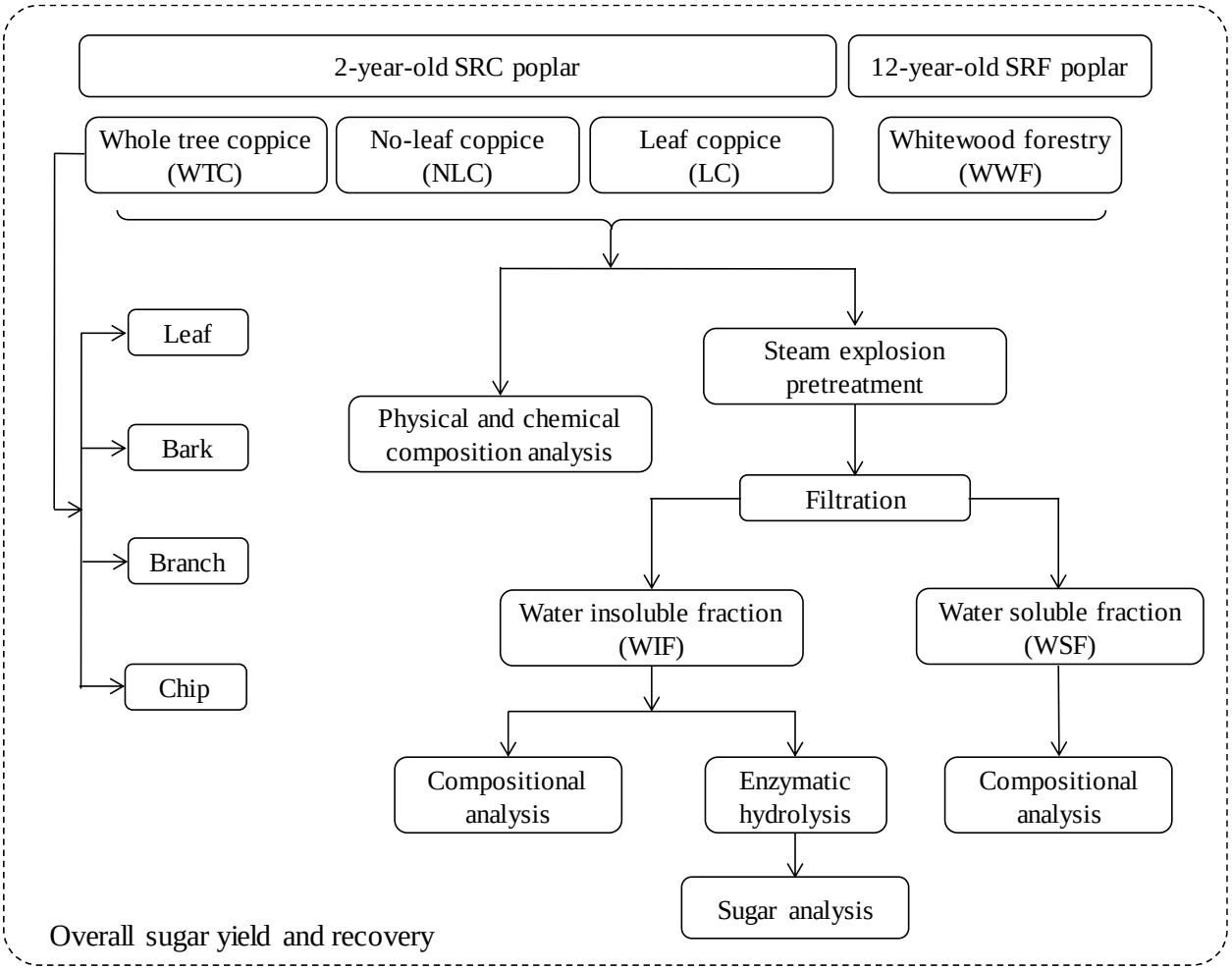

Figure 2.2 Process flow diagram for bioconversion of different poplar feedstocks (WTC, NLC, LC, and WWF) into sugars

2.2.1 **Raw material**

2-year-old SRC poplar used in this research is a hybrid of *Populus trichocarpa* and *Populus deltoides*, obtained from a plantation near Jefferson, OR managed by GreenWood Resources (Portland, OR). In spring 2012, poplars were established with 10" to 22" long cuttings in rows with at high density of 1452 stems per acre (3630 stems per hectare). In September 2013, the harvest of stock like crops occurred before defoliation. The 2-year-old poplar was harvested and chipped using a fully mechanized harvester – a modified forage harvester New Holland FR9080 (New Turin, Italy) equipped with 130FB biomass header [120] (Figure 2.1). As Figure 2 shows, NLC and LC were prepared by separating the leaf component from 5 kg original coppice feedstock. 1 kg (OD) of the WTC was manually sorted into four components: leaf, bark, branch, and (whitewood) chip. After sorting, the individual components were oven dried to determine the physical composition of the feedstock. In the current study, all the feedstocks obtained from 1st cycle are identified as coppice sample. Chips from mature 12-year-old poplars (*Populus deltoides* × *Populus nigra*, clone OP-367), also provided by GreenWood Resources, were included in this study as reference biomass. Fresh poplar was debarked and chipped into 1.0 cm × 2.0 cm at 5 mm thickness chips. These clean poplar chips are termed whitewood forestry (WWF). All samples were stored and kept frozen at -20 °C until use.

2.2.2 **Pretreatment and processing conditions**

Four types of poplar feedstocks, WTC, NLC, LC, and WWF, were processed in the same condition. Each feedstock of 600 g oven-dried (OD) weight was pre-impregnated overnight with anhydrous SO_2 in plastic bags at atmospheric pressure. The amount of SO_2 added to the bag

corresponded to 3% (w/w) loading, and was determined by weighing the bag before and after the addition of gas.

Steam explosion pretreatment was performed in a 2.7-liter batch reactor (Aurora Technical, Savona, BC, Canada). Briefly, samples were loaded and heated at temperature 195 °C for 5 min. Following the reaction time, the pneumatic valve was opened to explode and discharge the biomass into a collection container.

After steam explosion, the pretreated biomass slurry was separated into WSF and WIF using vacuum filtration. The WIF was then washed with a volume of deionized water equivalent to 20 times the dry weight of the sample to remove the free sugars.

2.2.3 **Enzymatic hydrolysis**

Enzymatic hydrolysis was carried out using cellulase (Celluclast 1.5 L, Sigma) at 5 Filter Paper Units (FPU)/g cellulose and β-glucosidase (Novozyme 188, Sigma) at 10 cellobiase units (CBU)/g cellulose. The WIF was hydrolyzed at 5% (w/v) consistency in a total volume of 50 ml in 125 ml Erlenmeyer flasks. The flasks were incubated at 50°C and 175 rpm in a New Brunswick shaker. Additionally, 50 mM citrate buffer was added to maintain the pH at 4.8, and tetracycline (40 µg/ml) and cycloheximide (30 µg/ml) were used to inhibit microbial contamination. 1 ml samples were taken periodically, boiled for 10 min to denature enzymes, filtered through a 0.22 µm syringe filter, and stored at -20 °C until analysis.

2.2.4 **Compositional analysis**

Ash and extractives

Ash content of raw biomass samples was measured gravimetrically by heating 20-mesh-milled dry biomass to 575 ± 25°C for 18 ± 6 hours [122]. Water and ethanol extractives of raw biomass were determined according to National Renewable Energy Laboratory (NREL) methods [123].

Soluble fraction carbohydrates and degradation products

Monomeric/oligomeric soluble carbohydrates and degradation products were determined using NREL LAP TP-510-42623 [124]. Briefly, 0.7 ml of 72% 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. The samples were then analyzed by a Dionex (Sunnyvale, CA) HPLC (ICS-3000) system equipped with an anion exchange column (Dionex, CarboPac PA1) and an ED electrochemical detector using deionized water at a flow rate of 1 ml/min as an eluent [62]. Oligomeric sugar was calculated by subtracting monomeric sugar content from total sugar content determined after acid hydrolysis.

Degradation products, such as acetic acid, furfural and 5-HMF were determined using a Shimadzu Prominence LC equipped with an anion exchange column (Rezex RHM Monosaccharide H^+ (8%) Phenomenex, Inc., Torrance, CA) using 5 mM H_2SO_4 at 0.6 ml/min [62]. Phenolic concentration in the WSF was assayed by the Folin Ciocalteu method [125] using a spectrophotometer (Shimadzu, Tokyo, Japan) at 765 nm. Gallic acid was used as calibration standard.

Insoluble fraction carbohydrates, acetate groups and phenolics

The chemical composition of raw biomass and WIF were determined according to a modified method derived from TAPPI Standard Method [126] and NREL protocols [127]. Briefly, 0.2 g of

finely ground oven dried sample is treated with 3 ml 72% H_2SO_4 for 120 min at room temperature, then diluted into 120 ml total volume and autoclaved at 121°C for 60 min. Klason lignin contents were determined by gravimetric methods to estimate phenolic content. It should be noted that in components like leaf, branch, and bark, phenolic compounds are not necessarily fully lignified and therefore are defined as phenolics other than lignin [23]. After filtration through tared sintered-glass crucibles, the carbohydrate and acetyl composition of the filtrate is analyzed by HPLC and the acid soluble lignin (phenolics) in the filtrate is analyzed by UV at 205 nm [62].

2.2.5 **Sugar yield and recovery calculation**

A complete mass balance was calculated using the composition and total mass of each WSF and WIF leaving pretreatment and enzymatic hydrolysis [73]. Yields and recoveries were calculated based on the input feedstock mass and original sugars available in the raw feed, respectively. In this manuscript, "yield" was defined as the amount of sugars converted from unit weight of feedstock. It is determined based on the total mass of sugars in the solid and liquid phases normalized by the initial oven dry mass of biomass (kg sugars/tonne biomass). Recovery is defined as the percentage of theoretical sugar yield by calculating the total mass of sugars in the solid and liquid phases normalized by the initial mass of sugars in the biomass (kg sugars/kg original sugars × 100%). Similarly, monomeric sugar yield is defined as total mass of monomeric sugars in the hydrolyzed solid and liquid phases normalized by the initial oven dry mass of biomass (kg monomeric sugars/tonne biomass) and monomeric sugar recovery is defined as the total mass of monomeric sugars in the hydrolyzed solid and liquid phases normalized by the initial mass of sugars in the biomass (kg monomeric sugars/kg original sugar × 100%).

2.2.6 **Buffering capacity test**

The buffering capacity of SRC biomass was investigated by titration as described by [128]. Briefly, 50 g OD weight of raw biomass was soaked in 1 liter deionized water at a temperature of 80°C for 30 min. Biomass was then removed by filtration and 800ml of liquid was titrated by 0.1 M H_2SO_4. deionized water was used as blank for reference.

2.2.7 **Economic assessment**

The economic potential for using the two-year 1st cycle coppice as a biorefinery feedstock was assessed. It was assumed that this material is an intermittent feedstock and the biorefinery would be designed to handle the feedstock harvested from subsequent rotations. We determined the ethanol selling price the biorefinery would need to cover the operating cost of producing alcohol from the two-year-old crops. Any selling price less than this would result in the biorefinery losing money and would not be an acceptable. The minimum ethanol price was calculated following the method outlined in the 2011 biochemical conversion of lignocellulosic ethanol report from NREL [43], modified for the poplar feedstock. An Aspen Plus model (Aspen Plus™, V8.6) of the biorefinery was developed using the yields and recoveries determined in this research. Process yields are the most critical variable driving process economics and the process yields used in the Aspen model are given in the additional file. The feedstock price was determined from the heating value of the poplar with the assumption that the most realistic market for the two-year-old trees would be hog fuel. A price of $2.8/MMBtu ($0.00265/MJ) was used for the calculation [129].

2.2.8 **Data analysis**

The results were subjected to one-way analysis of variance (ANOVA) analysis followed by a Tukey's test. Triplicate samples were separate runs conducted for each type of feedstock. All data are represented as the mean of triplicates with standard deviation. The presentation of percent differences in data comparison is absolute values, unless otherwise indicated. Chemical composition, sugar conversion of enzymatic hydrolysis, sugar yield and recovery following steam pretreatment, and monomeric sugar yield and recovery after steam pretreatment and enzymatic hydrolysis were analyzed based on 5% alpha level (95% confidence interval). Statistical differences in chemical composition, physical characteristics and sugar yield were determined from p-values ($p < 0.05$). Data were analyzed using R (version 3.0.1) software. In this manuscript, any data analysis mentioned as "significant" represents statistically significant ($p<0.05$).

2.3 Results and discussion

2.3.1 **Physical and chemical composition of SRC biomass**

As Table 2.2 shows, WTC consisted of 37% leaf, 9% bark, 12% branch, and 42% chip. Chips comprised less than half of the dry WTC, while leaves comprised over one third of the total dry biomass. The relatively small chip content was mainly due to the tree age and management practice [130]. In a conventional poplar plantation, leaf, bark, and branch components are considered low value and may be discarded after harvest.

The chemical composition of the four main components are listed in Table 2.2. It was found that the chemical compositions across the four main components were significantly different. Among

all components, chip represented the best general chemical composition for biofuel production, with the highest sugar content (55.8%), the lowest total phenolic content (24.2%), and just 1.3% ash. In contrast, leaf had the lowest total sugar (22.7%) but the highest total phenolic and ash contents of 39.8% and 10.5%, respectively. In bark and branch, the total sugar, total phenolic, and ash contents were in between those of chip and leaf.

As Table 2.2 reveals, the WWF exhibited significantly higher total sugar content, and lower acetic acid, ash, and extractives contents than all the components from WTC. Although the chip was most suitable for bioconversion among four WTC components, it was still less desirable than WWF. In particular, WWF had a 7.2% higher total sugar content, 0.7% lower ash content, and 7.1% less extractives compared to the chip from WTC. Previous studies demonstrated that the radius, length, and cell wall thickness of fibers increase as tree ages, and as a result the chemical composition differs between juvenile and mature wood [10]. Our observations confirmed the fact that juvenile wood is lower in cellulose, but higher in lignin, ash, and extractives than mature wood [121,131].

The low-sugar-content leaf consisted of 37% of the total dry WTC biomass (Table 2.2). Consequently, the presence of leaf increased the noncarbohydrate constituent and reduced the proportion of convertible sugar in WTC. Additionally, high phenolic content, ash, and extractives in the leaves will have negative impacts on the bioconversion process [27,132–134].

The leaves appeared to be problematic so we conducted a leaf separation to change the raw biomass chemical composition and examine the impacts of leaf removal in bioconversion.

Table 2.2 Physical and chemical composition of four components in SRC poplar and SRF poplar

		Physical composition (%)	Chemical composition (%)									
			Arabinan	Galactan	Glucan	Xylan	Mannan	Total sugar	Total phenolics	Acetic Acid	Ash	Extractives
WTC	Leaf	37	2.9 ± 0.1	2.3 ± 0.1	13.1 ± 0.6	3.9 ± 0.2	0.6 ± 0.1	22.7 ± 0.1	39.8 ± 0.1	2.3 ± 0.3	10.5 ± 0.1	27.6 ± 1.3
	Bark	9	4.0 ± 0.1	2.1 ± 0.1	24.4 ± 0.1	4.1 ± 0.1	0.6 ± 0.1	35.2 ± 0.1	32.9 ± 0.3	2.6 ± 0.2	6.9 ± 0.2	27.7 ± 0.5
	Branch	12	2.9 ± 0.1	1.8 ± 0.1	23.9 ± 0.1	7.2 ± 0.4	0.9 ± 0.1	36.8 ± 0.1	29.2 ± 0.4	3.4 ± 0.2	5.7 ± 0.1	21.9 ± 0.9
	Chip	42	0.5 ± 0.1	0.7 ± 0.1	38.5 ± 0.3	14.3 ± 0.1	1.8 ± 0.2	55.8 ± 0.2	24.2 ± 0.2	4.4 ± 0.3	1.3 ± 0.1	12.3 ± 0.4
WWF		NA	0.4 ± 0.0	0.4 ± 0.1	46.5 ± 0.9	13.1 ± 0.2	2.6 ± 0.1	63.0 ± 0.4	25.3 ± 0.2	1.2 ± 0.1	0.7 ± 0.1	5.2 ± 0.4

± All data are represented as the mean of triplicates with standard deviation.

Table 2.3 presents the compositional analysis of NLC, WTC, LC, and WWF. Leaf removal significantly changed the physical composition of SRC biomass, increasing chip composition to 67%. The original biomass - WTC was composed of 41.3% total sugar, 32.1% total phenolics, 3.7% acetic acid, 5.5% ash and 21.0% extractives (Table 2.3). Besides glucan and xylan, the WTC consisted of 5.0% of minor sugars, which were mainly attributed to the presence of bark, branch, and leaf, as these sugars are rarely observed in mature poplar wood [10]. Following leaf removal, the sugar content increased by 8.2%, while total phenolic, ash, and extractives contents decreased by 5.3%, 2.1%, and 4.3%, respectively. Leaf removal generated a biomass (NLC) with a 49.5% total sugar content.

The total sugar content of the NLC was still 13.6% lower, and the ash and extractives content was 4 and 2 times higher, respectively, compared to WWF.

Table 2.3 Chemical and physical composition of NLC, WTC, LC, and WWF

	Physical composition (%)				Chemical composition (%)									
	Leaf	**Bark**	**Branch**	**Chip**	**Arabinan**	**Galactan**	**Glucan**	**Xylan**	**Mannan**	**Total sugar**	**Total phenolics**	**Acetic acid**	**Ash**	**Extractives**
NLC	0	15	18	67	1.5 ± 0.1	1.3 ± 0.1	33.2 ± 0.5	11.9 ± 0.3	1.6 ± 0.1	49.5 ± 0.2	26.8 ± 1.0	4.3 ± 0.3	3.4 ± 0.0	16.7 ± 0.0
WTC	37	9	12	42	2.1 ± 0.1	1.7 ± 0.1	26.9 ± 0.5	9.4 ± 0.3	1.2 ± 0.1	41.3 ± 0.2	32.1 ± 1.0	3.7 ± 0.1	5.5 ± 0.1	21.0 ± 0.6
LC	100	0	0	0	2.9 ± 0.1	2.3 ± 0.1	13.1 ± 0.6	3.9 ± 0.2	0.6 ± 0.0	22.7 ± 0.1	39.8 ± 0.1	2.6 ± 0.5	10.5 ± 0.1	27.6 ± 1.3
WWF	0	0	0	100	0.4 ± 0.0	0.4 ± 0.1	46.5 ± 0.9	13.1 ± 0.2	2.6 ± 0.1	63.1 ± 0.4	25.3 ± 0.2	1.2 ± 0.1	0.7 ± 0.1	5.2 ± 0.4

± All data are represented as the mean of triplicates with standard deviation.

2.3.2 Chemical composition of water insoluble fraction (WIF) after pretreatment

Following pretreatment and liquid-solid separation of all samples, the compositions of the solid, water insoluble fraction (WIF), and the liquid, water soluble fraction (WSF), were analyzed. Expressed as percent of dry matter, Table 2.4 shows the trends of the WIF chemical constituents were generally consistent with the compositional data in the raw biomass. The sugar and phenolic content of three coppice samples ranged from 17.9% to 55.2% and from 37.4% to 50.5%, respectively. Comparing WTC and NLC shows that leaf removal increased the WIF sugar content by 13.8% and lowered the phenolic and ash contents by 6.2% and 3.8%, respectively.

Typically, WIF of LC contained the lowest sugar content, but the highest phenolic and ash contents. Although leaf removal significantly enhanced the sugar content and reduced the phenolic and ash contents of WIF, the NLC sugar content was still 8.7% lower, and phenolics and ash were 6.2% and 1.9%, respectively, higher than WWF.

Table 2.4 Chemical composition of WIF after steam pretreatment of poplar samples (as percentages of the solid weight)

	Chemical composition (%)								
	Arabinan	**Galactan**	**Glucan**	**Xylan**	**Mannan**	**Total sugar**	**Total phenolics**	**Acetic acid**	**Ash**
NLC	0.1 ± 0.1	0.1 ± 0.1	52.2 ± 0.3	2.4 ± 0.3	0.5 ± 0.1	55.2 ± 0.1	37.4 ± 0.1	1.6 ± 0.2	2.0 ± 0.1
WTC	0.1 ± 0.1	0.4 ± 0.1	36.6 ± 0.1	3.6 ± 0.3	0.7 ± 0.1	41.4 ± 0.1	43.6 ± 0.1	1.9 ± 0.1	5.8 ± 0.3
LC	0.4 ± 0.1	0.9 ± 0.2	13.1 ± 2.9	3.2 ± 0.6	0.4 ± 0.1	17.9 ± 0.6	50.5 ± 0.2	2.5 ± 0.9	9.8 ± 0.4
WWF	0.0 ± 0.0	0.0 ± 0.0	62.4 ± 2.4	1.1 ± 0.1	0.4 ± 0.1	63.9 ± 0.3	31.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.1

± All data are represented as the mean of triplicates with standard deviation.

2.3.3 Chemical composition of water soluble fraction (WSF) after pretreatment

The amount of sugars, degradation products, and the pH in the WSF after pretreatment were measured. Table 2.5 shows that sugar yields in the WSF varied across different poplar samples. Consistent with the results in Table 2.4, the majority of minor sugars resided in the WSF, as hemicellulose was mostly dissolved during pretreatment for all poplar samples. Sugar yields were presented in the unit of kg/tonne, implying the total soluble sugars released during pretreatment. For the coppice samples, the glucose and xylose yields ranged between 15.8 to 63.5 kg/tonne and 6.9 to 96.0 kg/tonne, respectively. Minor sugars, including arabinose, galactose and

mannose, represented a non-negligible composition in the WSF, and their yields ranged between 7.6 to 9.7 kg/tonne, 5.6 to 8.5 kg/tonne, and 1.1 to 14.3 kg/tonne, respectively. The trends of total sugars in WSF matched the sugar content in original biomass. As the amount of the individual sugars decreased from NLC to LC (Table 2.5), the total sugars decreased from 192.1 to 37.0 kg/tonne in the WSF. The monomeric sugar percentage in the WSF is critical because it indicates the amount of direct fermentable sugars. Most sugars in the WSF were recovered in monomeric form except for the LC biomass. As shown in Table 2.5, leaf removal significantly changed the total amount of dissolved sugar in WSF – from 108 kg/tonne of WTC to 192 kg/tonne of NLC – a increase of 83.8 kg/tonne. Meanwhile, leaf removal increased the proportion of monomeric sugars by 12%. The fermentable sugar yield in WSF was markedly increased by removing leaves. The sugar yield in WSF of WWF, however, was 18.2 kg/tonne higher than NLC. Interestingly, the monomeric sugar content of WWF and NLC were about the same at 82% and 83%, respectively.

Table 2.5 Sugar yield in WSF after steam explosion (expressed as kg/tonne raw biomass)

	Arabinose		**Galactose**		**Glucose**		**Xylose**		**Mannose**		**Total sugars**	
	Total	**% mon[a]**	**Total**	**% mon**	**Total**	**% mon**	**Total**	**% mon**	**Total**	**% mon**	**Total**	**% mon**
NLC	9.7 ± 0.3	100	8.5 ± 0.2	90	63.5 ± 2.5	78	96.0 ± 1.2	81	14.3 ± 1.1	79	192.1 ± 0.1	82
WTC	9.1 ± 1.2	100	7.5 ± 0.7	71	39.2 ± 4.1	64	45.5 ± 2.4	71	7.0 ± 0.3	65	108.3 ± 0.3	70
LC	7.6 ± 0.2	60	5.6 ± 0.8	23	15.8 ± 2.1	41	6.9 ± 1.2	10	1.1 ± 0.1	33	37.0 ± 0.2	36
WWF	2.9 ± 0.2	93	5.4 ± 0.4	79	62.9 ± 0.7	76	122.2 ± 8.9	87	16.8 ± 1.0	85	210.3 ± 0.5	83

[a] "% mono" describes the percentage of soluble sugar presented in monomeric form.
± All data are represented as the mean of triplicate measurement.

It is shown in Table 2.6 that the degradation products, including acetic acid, furfurals, 5-hydroxymethylfurfural (HMF), and phenolics, were found at different amounts in the WSFs. Leaf removal increased the amount of acetic acid, furfural, and HMF in the WSF by 10.0 kg/tonne, 8.3 kg/tonne, and 0.4 kg/tonne, respectively (Table 2.6). It is known that with an increase of pretreatment severity, more sugars will be solubilized as monosaccharides and potentially more will be degraded into furans [62]. All samples were steam pretreated at the same reaction temperature and residence time with the same SO_2 loading, however, the pH of WSF decreased from 3.1 to 1.9 as a result of leaf removal (Table 2.6). The lower pH in the WSF from the NLC is partially a result of the higher acetic acid concentration compared to that from WTC. In addition, the ash in the leaf appears to provide some buffering capacity to the WSF [34]. Figure 2.3 shows the titration curve of three coppice samples in comparison with the blank (deionized water). It appears that for any level of acid addition, the pH of extractant from the leafy material is higher than the blank or any of the other biomass type, demonstrating the larger buffering capacity of the leaves.

Table 2.6 Acetic acid, furans, phenolics yields (expressed as kg/tonne raw biomass), and pH of WSF after steam explosion

	pH	Acetic acid	Furfural	HMF [a]	Phenolics
NLC	1.9	48.8 ± 1.5	14.8 ± 1.5	2.2 ± 0.1	23.2 ± 2.0
WTC	3.1	38.8 ± 1.3	6.5 ± 0.9	1.8 ± 0.2	21.9 ± 1.8
LC	3.7	25.2 ± 0.9	0.5 ± 0.1	0.6 ± 0.1	28.1 ± 0.2
WWF	1.6	33.5 ± 0.6	5.2 ± 0.4	1.6 ± 0.1	8.0 ± 0.4

[a] 5-hydroxymethyl furfural

± All data are represented as the mean of triplicate measurement.

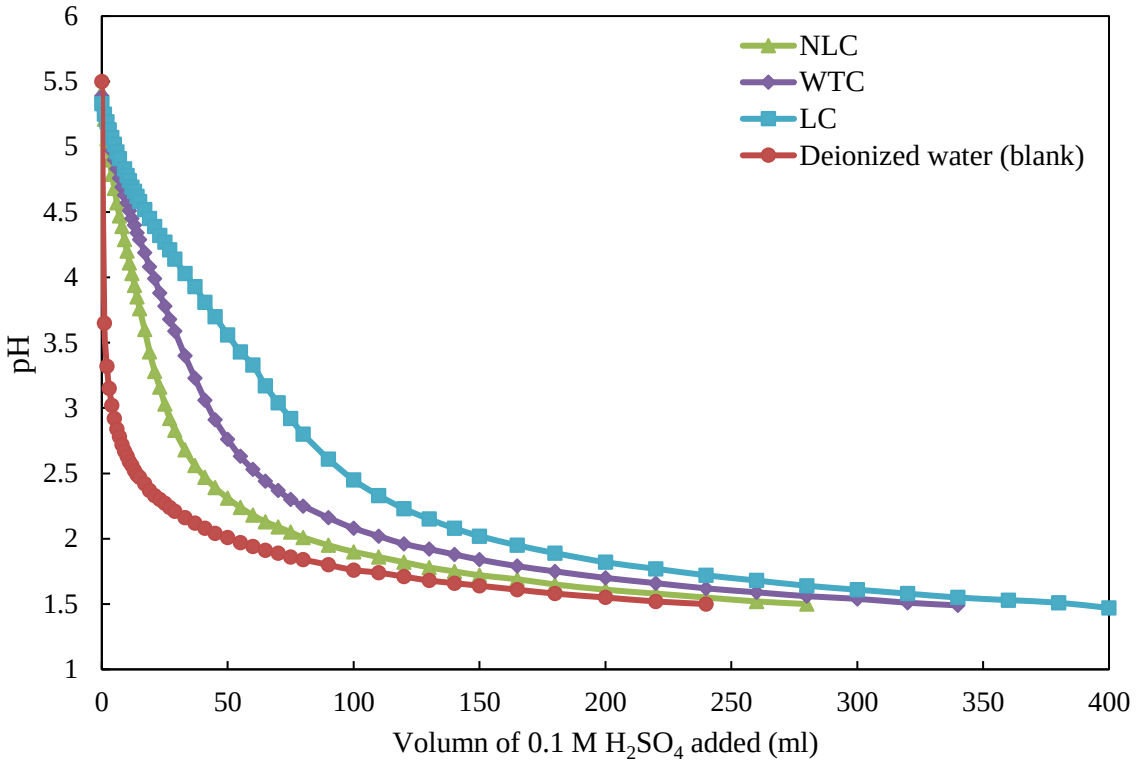

Figure 2.3 Titration curves with 0.1 M H_2SO_4 for water extracts of NLC, WTC, LC, and the deionized water (blank)

2.3.4 Sugar yield and recovery after steam pretreatment

Following steam pretreatment, total sugar yield was calculated for each of the poplar samples by combining the sugars in WIF (Table 2.4) and WSF (Table 2.5). Figure 2.4 presents the total sugar yield for each poplar sample. The yields ranged from 200 kg/tonne to 656 kg/tonne. Corresponding to the compositional characteristics of raw biomass (Table 2.3), NLC achieved the highest sugar yield of 483 kg/tonne among the three coppice samples, WTC had an intermediate sugar recovery of 390 kg/tonne, whereas LC had the lowest sugar recovery of 200 kg/tonne. WWF recovered 656 kg/tonne sugar in total because of its high sugar content in the original biomass. Leaf removal improved the total sugar yield by 93 kg/tonne after steam

pretreatment. Interestingly, the sugar recovery was similar across all poplar samples, ranging from 85% to 92%. Although the total sugar yield was much lower in NLC, its sugar recovery showed no significant difference from WWF (Figure 2.4), indicating that the large difference in sugar recoveries was mainly due to the different original sugar contents (Table 2.3).

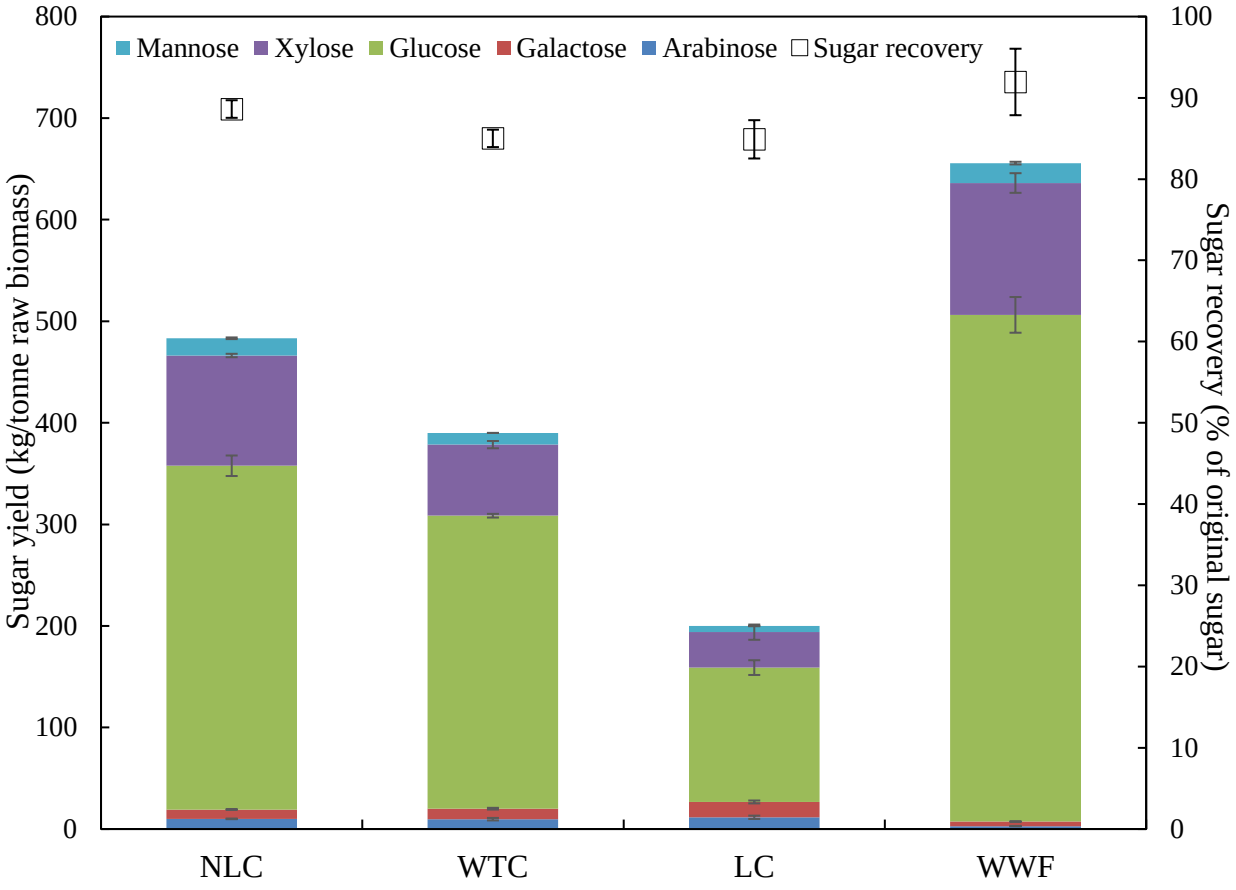

Figure 2.4 Sugar yield (kg/tonne), expressed as total mass of sugar per unit raw biomass, and sugar recovery (%), expressed as total mass of sugar per unit original sugar after pretreatment, of NLC, WTC, LC, and WWF. Error bars indicate standard deviation from triplicate measurements

2.3.5 **Enzymatic hydrolysis of water insoluble fraction (WIF)**

Following steam pretreatment, the enzymatic digestibility of WIF samples was evaluated at 5% (w/v) consistency with 5 FPU/g cellulose enzyme loading. Figure 2.5 highlights the differences in digestibility between poplar samples after 72 hours of saccharification. Results in Figure 2.5 reveal that of NLC had the highest overall sugar conversion; a 72.7% cellulose to glucose conversion and a 54.7% xylan to xylose conversion. The WTC had lower hydrolysis conversions of 52.6% glucose conversion and 36.3% xylose conversion. Notably, leaf removal improved the enzymatic hydrolysis, resulting in 20% higher glucose conversion and 18% higher xylose conversion. LC had the lowest glucose conversion (23.5%) and xylose conversion (19.5%). By comparison, the glucose conversion of WWF (73.5%) was similar to NLC, while the xylose conversion was lower (34.1%).

The digestibility of pretreated coppice samples demonstrated an improvement in sugar conversion with leaf removal and underscored the low digestibility of steam pretreated LC. The low yield and digestibility of the LC is attributed to the enzyme inhibition of phenolic compounds and ash. Phenolic compounds have been reported to inhibit and/or deactivate cellulase and β-glucosidases [133,134]. The steam pretreated coppice samples, especially WTC and LC, had relatively high phenolic content of 43.6% and 50.5%, respectively (Table 2.4). Contrary to whitewood chips where more phenolic compounds are lignified, the foliage phenolic compounds are present in the form of low molecular weight polyphenols [23] such as carotenoid, flavonoid, and tannin. These low molecule polyphenols can be easily broken into monomeric phenolics during pretreatment, releasing more mono phenolics, resulting in stronger inhibition [134]. It has also been suggested that ash in steam pretreated biomass, especially the metal ions,

hindered the action of cellulase and β-glucosidases once it exceeds certain content thresholds [27]. Compared to NLC, the two-fold greater WIF ash content of WTC could partially explain the 20% lower cellulose conversion and 18% lower xylan conversion. Leafy material with its high phenolic and ash content, strongly inhibits enzymatic hydrolysis. Prior leaf removal will be necessary to achieve high saccharification yields at modest enzyme loadings.

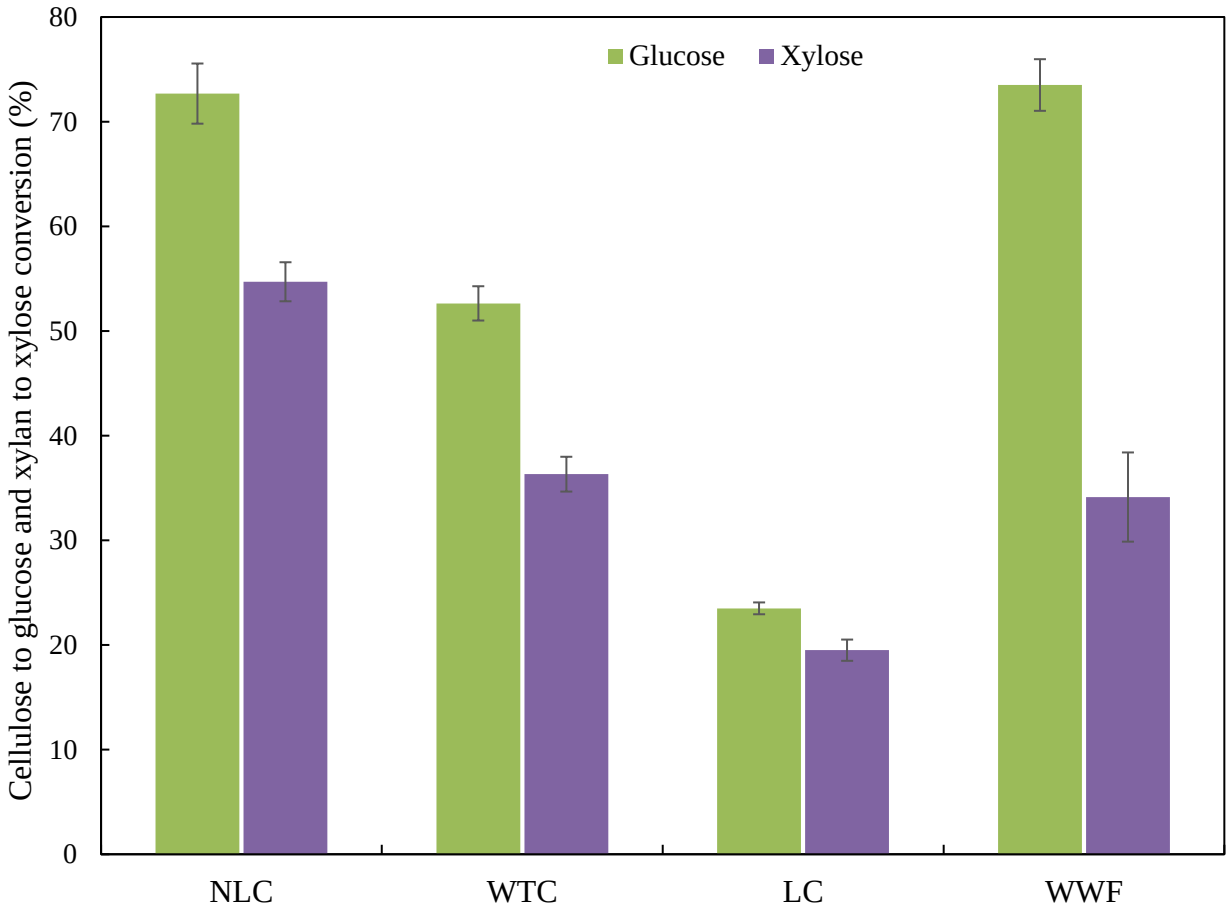

Figure 2.5 72 h cellulose to glucose and xylan to xylose conversion of steam pretreated NLC, WTC, LC and WWF at 5% (w/v) solids consistency with 5 FPU/g cellulose and 10 CBU/g cellulose enzyme loading. Error bars indicate standard deviation from triplicate measurements

2.3.6 Overall sugar yield and recovery after steam pretreatment and enzymatic hydrolysis

The overall monomeric sugar available after pretreatment and enzymatic hydrolysis determines the total amount of fermentable sugars from bioconversion, and is calculated by adding monomeric sugars in WSF and hydrolyzed WIF (Figure 2.6). For each biomass, bioconversion efficiency is also expressed in two formats - the overall monomeric sugar yield at kg/tonne demonstrates the monomeric sugar from per unit biomass; and overall monomeric sugar recovery (in percentage) represents the monomeric sugar from original sugar. Within all three coppice samples, NLC had the highest overall sugar yield of 363 kg/tonne and LC had the lowest of 66 kg/tonne. The highest overall sugar yield of NLC is a direct result of the high sugar content in original biomass (Table 2.3), high sugar recovery in WSF and WIF, and the most efficient WIF digestibility. Leaf removal increased the overall sugar yield by 147 kg/tonne, which accounts for 40% relative increase from the WTC (Figure 2.6). Not surprisingly, only 27% original sugars were recovered from LC. NLC recovered 67% sugar after pretreatment and enzymatic hydrolysis, showing no significant difference with WWF (71%). It appears that the branch and bark in the NCL does not impair the bioconversion sugar recovery efficiency. The inherent sugar content was lower in original NLC biomass, however, resulting in 136 kg/tonne less total sugar yield of NLC compared to WWF (Figure 2.6).

The differences in sugar yields for NLC and WTC show that leafy material hinders the bioconversion sugar recovery. Moreover, since leaf had an extremely high moisture content of around 70% (data not shown), the mass proportion of leaf will be even higher in fresh biomass. From a logistic point of view, the high moisture content will accelerate microbial driven deterioration of the biomass during transportation and storage [35]. Considering the difficulty in

removing leaf from the harvested poplar mixture (as we experienced), we suggest a leaf separation operation during harvest. Thompson et al. [28] has developed an air classification method to separate the heterogeneous biomass mixture into anatomical or visually unique fractions. Ideally, a similar air separation mechanism can be integrated into the coppice harvester to efficiently classify and collect both leaf and no-leaf components during harvest.

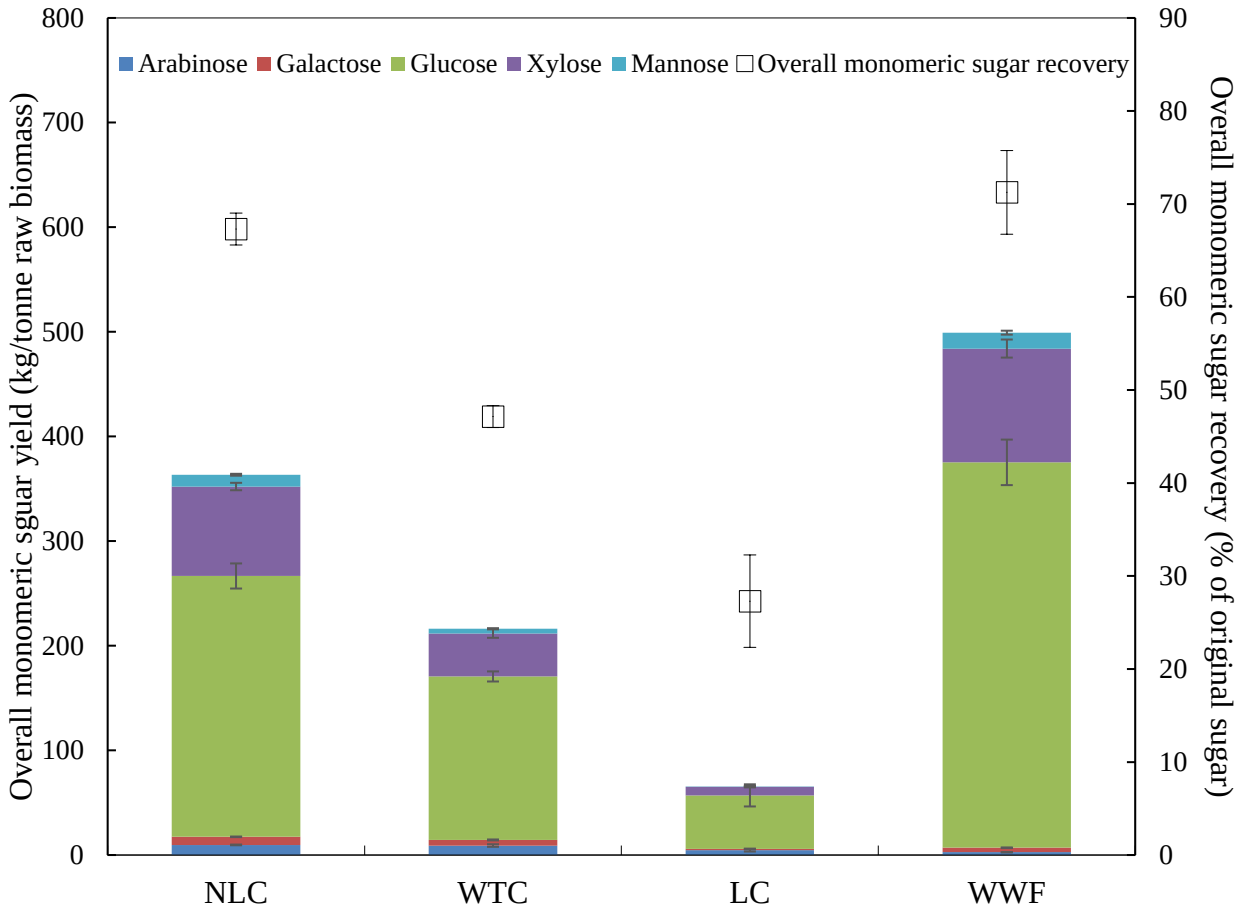

Figure 2.6 Overall monomeric sugar yield (kg/tonne), expressed as monomeric sugar per unit raw biomass, and overall monomeric sugar recovery (%), expressed as monomeric sugar per unit original sugar after pretreatment and enzymatic hydrolysis, of NLC, WTC, LC and WWF. Error bars indicate standard deviation from triplicate measurements

2.3.7 **Economic assessment**

With the assumptions that NLC poplar could be purchased for its heating value - $53/tonne - we calculated the ethanol selling price required to cover the operating cost using the yield data in the current study. The simulated biorefinery used 700,000 tonne of feedstock per year and produced 144 million liter/year (38 million gallon/year) at an ethanol conversion of 206 liter/dry tonne feedstock (49 gallon/dry US ton feedstock). The minimum ethanol selling price required to cover the operating cost was found to be $1.69/gallon, which is approximately the same as the current ethanol selling price ($1.65/gallon, 2017). Despite the low bioconversion yield, it appears that the NLC biomass could be a reasonable feedstock provided it can be obtained at a discounted price reflecting its lower quality.

The economics of using whole tree chips may be improved if higher value materials can be produced from leaves separated before bioconversion. In a recent review study, Devappa et al. [22] summarized a number of value added natural chemicals which can be extracted and purified from tree residues including leaf. Tree foliage is also recognized as a high quality supplement for animal feed [135]. Traditionally, poplar leaf has been used as a crude protein fodder resource for livestock, particularly for ruminant animals [57,136]. In this current study, we were able to obtain about 12% of crude protein from the LC following a sonication protein extraction method (data not shown).

Returning leafy material to the tree farm could also have environmental benefits. Ecologically, foliage serves several important functions in plantation soil [137]. It improves soil structure and increases solid porosity for better aeration and moisture holding capacity. In addition, upon decomposition, foliage litter returns the minerals and organics to the site, and becomes a source

of plant nutrients [137]. Complete leaf removal during whole tree harvest might increase the potential of soil erosion, site degradation, and accelerate nutrient withdrawals [138]. Harvesting trees during the dormant season or using a defoliant would ensure the tree farm realizes the environmental benefits of the leafy material.

2.4 Conclusions

Growing poplar using a SRC system requires the trees to be harvested after two-year growth such that the root system is well established. Bioconversion of WTC composed of (whitewood) chip, bark, branch, and leaf from this initial harvest were evaluated and compared to bioconversion of WWF. It was found that leafy material makes up more than 1/3 of WTC but has a low sugar content, and high phenolic, ash, and extractives contents. Leaf removal significantly changed the chemical composition of coppice sample, and improved monomeric sugar yield by 147 kg/tonne after steam pretreatment and enzymatic hydrolysis. With the presence of bark and branch, the NLC achieved 67% monomeric sugar recovery, showing no significant difference compared to that of WWF (71%). The overall sugar yield of NLC was 135 kg/tonne less than that of WWF, however, due the low inherent sugar content of the NLC. These findings demonstrate that it is essential to remove the leaves prior to pretreatment to ensure a better overall sugar yield. A minimum ethanol selling price to cover operating expenses of $1.69/gallon was established from the economic analysis, assuming the NLC feedstock is available for its fuel value.

The growth and maturity of the wood is limited in the first cycle. We would anticipate that the following rotations will have more wood chips, less bark/branch (the second cycle is showing 60% whitewood content in recent harvest), and therefore a higher overall sugar content. In

addition, several practices could potentially enhance the compositional characteristics of short rotation poplar and fortify the sugar content, such as choosing higher sugar composition hybrid/clone, modifying the management strategy, and/or mixing with other sugar rich feedstock.

Abbreviations

SRC: short rotation coppice; SRF: short rotation forestry; WTC: whole tree coppice; NLC: no-leaf coppice; LC: leaf coppice; WWF: whitewood forestry; WSF: water soluble fraction; WIF: water insoluble fraction; OD: oven dry; HMF: 5-hydroxymethylfurfural; FPU: filter paper unit; CBU: cellobiase unit; HPLC: high pressure liquid chromatography; NREL: National Renewable Energy Laboratory; ANOVA: analysis of variance.

Additional Table S1. Relevant process yields and assumptions for economic assessment

Process	Parameter	
Feedstock supply	Feedstock price	$53/tonne [1]
Pretreatment (steam explosion)	Solid recovery	34% [2]
Enzymatic hydrolysis	Cellulose to glucose	72.7% [2]
	Xylan to xylose	54.7% [2]
Fermentation	Glucose to ethanol	95% [3]
	Xylose to ethanol	85% [3]
	Arabinose to ethanol	85% [3]
	Galactose to ethanol	0% [3]
	Mannose to ethanol	0% [3]
	Beer ethanol concentration	4.0% [4]
Utilities	Electricity price	$0.07/kWh
Sale	Ethanol price	$1.65/gallon [5]

[1] The feedstock price was determined based on the heating value of $2.8/MMBtu ($0.00265/MJ)

[2] Based on current research (experimental data)

[3] Based on NREL report (2011 biochemical conversion of lignocellulosic ethanol report)

[4] Based on process simulation (Aspen model)

[5] Ethanol price based on market price of denatured fuel ethanol (Trading economics, April 17, 2017)

* The operating cost is updated to 2015 USD using the Inorganic Chemical Index and the Labor Index

Chapter 3. **Fast pyrolysis of short rotation coppice poplar: an investigation in thermochemical conversion of a realistic feedstock for the biorefinery** [2]

Abstract

Short rotation coppice (SRC) is a promising plantation system because it provides inexpensive feedstock for biorefineries. This study investigated the conversion of 2-year-old first rotation SRC poplar into bio-oil via fast pyrolysis. The impact of leaf removal was studied by comparing the yields and compositions of bio-oil from noleaf coppice (NLC) and whole tree coppice (WTC). Leaf removal did not affect the bio-oil yield of SRC poplar samples (~55%), but lowered the char yield from 19.7% to 13.6% and increased the gas yield from 14.3% to 17.7%. The chemical compositions of bio-oils, char, and non-condensable gases obtained from WTC and NLC were different. Leaf removal changed the elemental composition of the bio-oil. The bio-oil from WTC had higher H (9.1%) and O (64.8%), but lower C (25.3%) than that from NLC. Consequently, leaf removal increased the higher heating value (HHV) of bio-oil by 2.1 MJ/kg (16.0%). Based on the results of the current research and our previous study, the energy recovery rate for producing liquid fuel via fast pyrolysis of SRC poplar samples (29.4~34.1%) is markedly higher than that obtained by biochemical conversion (15.6~25.5%).

[2] Published: Dou, C., Chandler, D. S., Resende, F. L., & Bura, R. Fast Pyrolysis of Short Rotation Coppice Poplar: An Investigation in Thermochemical Conversion of a Realistic Feedstock for the Biorefinery. ACS Sustainable Chemistry & Engineering, 2017, 5(8), 6746-6755.[182]

3.1 Introduction

The price and availability of feedstocks are serious obstacles in biofuel commercialization. It is difficult to ensure the quality and simultaneously control the variability of the field run feedstock. In the current lignocellulosic biofuel designs, the feedstocks contribute to more than 40% of the total operating cost [43–45]. The success of the future biorefinery relies on using feedstocks which are low in cost, high in volume, and with low variability.

Poplar is one of the most productive temperate wood species and has been well recognized as a competitive biorefinery feedstock [33]. So far, the majority of poplar-to-fuel studies have been centered on using mature wood from conventional tree farms, namely the short rotation forestry (SRF) [46,61–63]. However, due to the long-term cultivation (as it usually takes 10-20 years to grow the SRF poplar) and the low-efficiency harvesting practice (as no more than 30% of the SRF poplar biomass is eventually used), the market price of SRF wood is prohibitively high for use in biorefineries [59,60].

Different from SRF system, the short rotation coppice (SRC) system is established at a much higher tree density and cultivated by intensive agronomic practices. To achieve maximal land productivity, SRC trees are grown in shorter cycles of two to five years and are harvested through coppicing. Ideally, coppicing can harvest 100% of the aboveground components of trees. Also, coppicing triggers plants' intrinsic growth such that trees will regenerate from the developed root system, allowing multiple harvests without replanting for up to 20 years [54]. Instead of waiting for 10+ years for feedstock from conventional SRF system, the biorefinery could rely on the SRC system and obtain feedstock in a much shorter time interval.

Previous research in our group showed that using SRC poplar for fuel production through biochemical conversion could be challenging [139]. The chemical composition of SRC samples,

especially the high phenolic content of leaves, negatively affects the product yield. Interestingly, we found that leaf removal increased the sugar and decreased the phenolic content of SRC poplar, resulting in higher sugar yield. However, leaf removal means additional biomass processing, not to mention that a large fraction of harvested SRC will not be utilized. Leaf removal might greatly increase the feedstock cost and raise the total cost of fuel production. This raises a question: can we obtain better fuel yield from SRC poplar with leaves by other conversion methods?

Fast pyrolysis is a thermochemical conversion process in the absence of oxygen, where the biomass is rapidly decomposed into bio-oil, char, and non-condensable gases. Unlike biochemical conversion, fast pyrolysis is characterized by a very short reaction time – usually 1-2 seconds – and the direct liquid product, bio-oil, is regarded as a potential substitute for petroleum fuels after upgrading [99]. Contrary to the biochemical conversion, the effect of high phenolic content is less pronounced in thermochemical conversion processes.

Fast pyrolysis of poplar has been discussed in many reports. The majority of the fast pyrolysis research was conducted using wood samples from mature trees (like WWF) [61,140–143]. To the best of our knowledge, not much research has been done on fast pyrolysis of SRC poplar either with (WTC) or without leaves (NLC). To improve the indigenous biomass utilization and avoid leaf separation, herein, we propose fast pyrolysis as a potential thermochemical method to convert SRC poplar into bio-oil. In the present study, we investigated the bio-oil yields of SRC samples and compared the pyrolysis products composition between WTC and NLC. Similar to our previous work in biochemical conversion, whitewood from SRF (WWF) was included as a standard for reference.

In the current study, we investigated the conversion of SRC poplar into bio-oil via fast pyrolysis. This work aims to 1) determine the temperature for maximum yield of volatiles in micropyrolysis; 2) study the fast pyrolysis product yield, especially bio-oil yield, from SRC poplar in a fluidized bed reactor; 3) assess the energy recovery rate of using SRC poplar in thermochemical conversion (fast pyrolysis) and compare it with biochemical conversion.

3.2 Materials and methods

3.2.1 Feedstock and characterization

Figure S1 briefly demonstrates the harvest, collection, and preparation processes of the feedstocks. The aboveground parts of the 2-year-old SRC poplar (including leaves) were coppiced and chipped into WTC by the harvester. Upon harvesting, NLC was prepared by removing the leaf material from the WTC. The reference sample, WWF was provided in the form of commercial wood chips from 12-year-old poplar grown in SRF system. All feedstocks were ground into particle size of 0.30 mm. All the ground biomass was oven dried before sample preparation. However, strong electrostatic effects of completely dried samples impede the feeding and transferring system of the fluidized bed reactor. To ensure the biomass fed constantly throughout the experiments, the oven dried samples were then kept in room condition until moisture was in equilibrium.

1 kg (OD) of the WTC was manually sorted into four fractions, including leaf, bark, branch, and (whitewood) chip. Physical composition of SRC samples was calculated based on the dry weight of each fraction. The chemical composition was determined following the NREL protocols [144]. The composition of major mineral elements were determined using induced coupled plasma optical emission spectrometry (ICP-OES, Thermo-Scientific, iCAP 6300) [145].

3.2.2 **Fast pyrolysis in micropyrolyzer**

Micropyrolysis experiments were carried out in a commercial micropyrolyzer (Pyroprobe model 5200, CDS Analytical Inc.) connected to an online Gas Chromatography/Mass Spectrometry equipped with a Flame Ionization Detector (Py-GC/MS-FID). The pyrolysis was performed at the filament temperatures ranging from 475°C to 675°C at a heating ramp of 1000°C/s. Upon reaching the set point temperature, the probe temperature was kept constant for 60 s. Vapors produced were swept into a trap at 50°C to allow collection of condensable products. After the time elapsed, the trap was then heated up to 300°C to desorb the condensed vapors (more instrumental settings are described in the Supporting Information). A previous study has shown that for the micropyrolyzer (Pyroprobe model 5200), the actual temperature of the biomass was approximately 75°C lower than the filament temperature [146]. Herein, the pyrolysis temperature reported in micropyrolysis is the filament temperature.

3.2.3 **Fluidized bed reactor and its operation**

As shown in Figure 3.1, the reactor includes three main components: the feeding unit (hopper, vibrator, auger equipped with motor, and funnel), the reacting unit (fluidized bed and furnace), and the separation/collection unit (cyclone with char collector, impinger, condensers, oil collectors, and filter). Pyrolysis in the fluidized bed reactor was conducted at the temperature of 500°C measured by four thermocouples. 500 g of aluminum oxide sand with a mean diameter of 0.33 mm (54 mesh) and a specific gravity of 3.94 (Kramer Industries, Inc., Piscataway, NJ) was used as bed material. For each type of feedstock, biomass was continuously fed into the preheated fluidized bed at a rate of 10 g/min. N_2 with a flow rate of 30 standard liters per minute (SLPM) was introduced into the system as carrier gas and fluidizing gas. Pyrolysis products were

sent out into a cyclone separator to collect the char. The pyrolysis vapors were then collected as bio-oil through a multi-stage condensation system, which includes an impinger, three counter-current double pipe condensers, and a coalescing filter. Yields of bio-oil and char from the fluidized bed reactor were measured gravimetrically. The bio-oil was collected from the impinger and the oil collectors. All condensing components were weighed before and after pyrolysis to ensure accurate mass balance. A RESTEK (Bellefonte, PA) multilayer sampling bag was used to continuously sample the non-condensable gas after the filter.

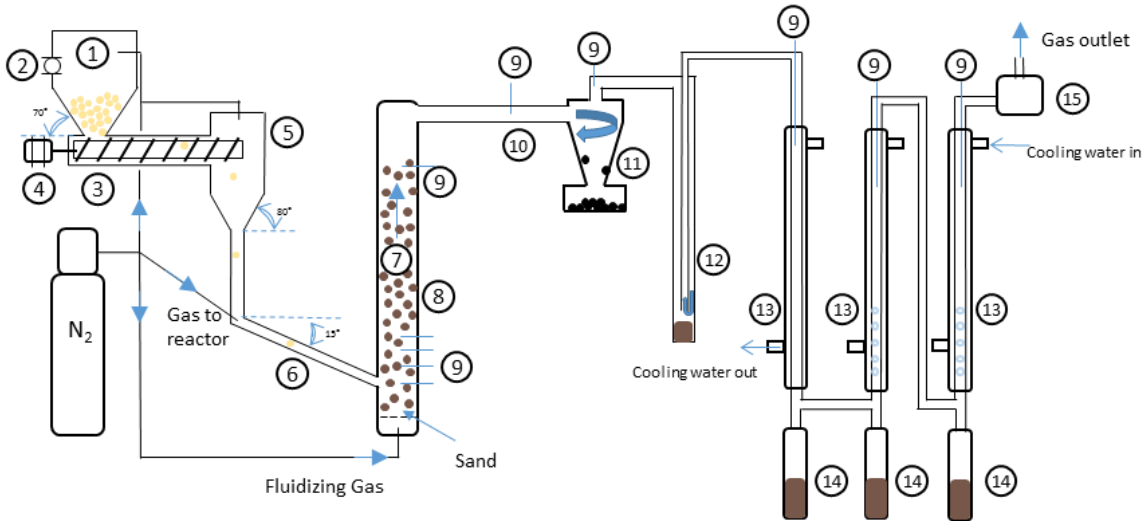

Figure 3.1 Schematic diagram of the bench scale fluidized bed pyrolysis unit: (1) hopper, (2) vibrator, (3) auger (screw conveyor), (4) motor, (5) funnel, (6) feeding line, (7) fluidized bed reactor, (8) furnace (not shown), (9) thermocouple, (10) transfer line, (11) cyclone and char collector, (12) impinger, (13) condenser, (14) oil collector, (15) filter

3.2.4 Fast pyrolysis product analysis

Yields of bio-oil and char from the fluidized bed reactor were measured gravimetrically. The bio-oil was collected from the impinger and the oil collectors. Bio-oil water content was

quantified by Karl Fisher Titration (Titroline KF, Schott Instruments) in accordance with ASTM E203-08 [147]. The chemical composition of the volatiles from the micropyrolyzer and the bio-oil from fluidized bed reactor were determined by GC/MS-FID (QP2010, Ultra, Shimadzu) equipped with a SHRXI-5MS capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness, Shimadzu) using helium as carrier gas. The GC oven temperature was initially held at 40°C for 4 min, followed by a ramp to 300°C at a rate of 10°C/min, which was then held for 5 min. The analyzed components were identified by comparing mass spectra with NIST 2010 library, and then quantified by using the parallel FID with external standards. For micropyrolysis, the on-line detection was performed in the Py-GC/MS-FID system. For fast pyrolysis in the fluidized bed reactor, a weighed amount of bio-oil sample was diluted with methanol before injection into the GC. 24 standards were used to quantify up to 31 compounds.

The total mass of char was measured by burning the solids collected in reactor bed, transfer line, and char collector at 600°C overnight and calculating the mass difference. To characterize the functional groups, char from WTC, NLC and their raw biomass were analyzed by FTIR spectrophotometer (IRPrestige-21, Shimadzu) with wave numbers of 500-4000 cm^{-1} at a resolution of 4 cm^{-1} and 48 scans. The non-condensable gas sample was collected throughout the experiment and analyzed in a Gas Chromatography/Thermal Conductivity Detectors (GC/TCD, GC-2014, Shimadzu), where CO, CH_4, CO_2, C_2H_4, and C_2H_6 were quantified.

3.2.5 Elemental (CHNO) analysis, higher heating value calculation, and energy recovery rate determination

The elemental composition of the raw biomass and crude bio-oil were measured using an elemental analyzer (EA 2400 Series II, PerkinElmer). For all samples, the O content was

determined by difference. The higher heating value (HHV) of the raw biomass and bio-oil samples were estimated using Equation 1 [148]:

$$HHV\ (MJ/kg) = 0.3491C + 1.1783H + 0.1005S - 0.1034O - 0.0151N - 0.0211Ash \quad \text{(Equation 1)}$$

* the elements are expressed as mass percentage

The energy recovery rate was introduced to compare the energy efficiency of converting SRC poplar into liquid fuels between thermochemical conversion (fast pyrolysis) and biochemical conversion. For both processes, the liquid fuel was considered as the target product. As defined by Equation 2, the energy recovery rate was calculated based on the total HHV of fuel outputs versus total HHV of feedstock inputs in each process [149]:

$$\text{Energy recovery rate } (\%) = \frac{\text{HHV of fuel} \times \text{Total mass of fuel}^*}{\text{HHV of biomass} \times \text{Total mass of input biomass}} \times 100\% \quad \text{(Equation 2)}$$

* "fuel" denotes bio-oil from fast pyrolysis or ethanol from biochemical conversion

The crude bio-oil from fast pyrolysis contains a high amount of oxygenates and water, and is not appropriate for direct use as fuel. Therefore, it needs further upgrading. According to a conceptual energy balance proposed by Bridgwater [150], 78% of the energy from the crude bio-oil could be recovered through hydrotreating and refining. From that, the final energy content of upgraded fuel product was determined for all bio-oil samples. The upgraded fuel energy content was used for the energy recovery rate calculation and was reported in the results and discussion session. For biochemical conversion, ethanol was considered as the liquid fuel product. The ethanol yield was determined based on the results in previous studies [43,139]. The HHV of ethanol (29.8 MJ/kg) was obtained from Hydrogen Analysis Resource Center of the Department of Energy [151].

It is important to keep in mind that, as defined here, the energy recovery rate is a comparison of the energy content of the product to the energy content of the feedstock. This is different than a complete energy balance, which considers the energy inputs needed to produce the fuel. Though these considerations are important, they are complex and beyond the scope of the present manuscript. Process energy flows, such as distillation in biochemical conversion, and biomass drying and grinding in fast pyrolysis, were not considered here.

3.2.6 **Data analysis**

All trials in the micropyrolyzer and fluidized bed reactor were performed in triplicate, except for WWF in the fluidized bed reactor which was carried out in duplicate (as the reproducibility was established by the triplicates of the first two feedstocks). The figures reported with error bars represent the average with standard deviations. In this manuscript, any data analysis mentioned as "significant" represents statistically significant (p value < 0.05).

3.3 Results and discussions

In this study, two feedstocks of 2-year-old SRC poplar – whole tree coppice (WTC) and no-leaf coppice (NLC) were fast pyrolyzed to determine the product yields and to evaluate the fast pyrolysis efficacy for SRC poplar. In addition, a sample from 12-year-old SRF poplar – whitewood forestry (WWF) was used as a standard for reference. First, the physical and chemical properties of the three poplar feedstocks were investigated. Then, we used a micropyrolyzer to determine the temperature for maximum volatile yield from different poplar feedstocks. Due to the constraints of the micropyrolyzer, it is difficult to collect all pyrolysis products and to achieve the mass balance closure. To further investigate the bio-oil yield from fast pyrolysis, the three poplar feedstocks were then pyrolyzed at the temperature selected in the

micropyrolysis experiments using a bench-scale fluidized bed reactor. Yield and composition of pyrolysis products (bio-oil, char, and gases) were analyzed and compared across WTC, NLC, and WWF. Energy recovery rate was applied to evaluate the process efficiency using the HHV and yield of fuel products from thermochemical and biochemical conversions.

3.3.1 **Feedstock characterization**

The physical and chemical composition of WTC, NLC, and WWF are given in Table 3.1. The moisture content of the three feedstocks ranged from 4.3% to 7.0%. The WTC was composed of 37% leaf, 9% bark, 12% branch, and 42% chip. Following leaf removal, the newly prepared biomass, NLC, contains 15% bark, 18% branch, and 67% chip. The WWF is 100% mature wood chips. Compared to WWF, the SRC samples were found to have higher content of phenolics/lignin, ash, acetate, and extractives, but less carbohydrates. It is notable that leaf removal significantly altered the chemical composition of the SRC samples. The total carbohydrate content increased by 8%, while the total phenolics/lignin, ash, and extractives content decreased by 5%, 2%, and 4%, respectively. This is due to the leaf materials having low carbohydrate (22.7%), high phenolics/lignin (39.8%), ash (10.5%), and extractives (27.6%) content.

Table 3.1 also gives the elemental composition of NLC, WTC, and WWF. Generally, all poplar samples show similar compositions for the organic fraction, with the exception of a relatively higher N content in WTC, which is possibly due to the leaf proteins. Based on that, HHV of the three poplar samples was determined, ranging from 18.5 to 18.8 MJ/kg. A higher concentration of mineral elements was found in WTC than NLC. In contrast, WWF has much lower content of inorganics, in agreement with its lower ash content.

Table 3.1 Physical and chemical properties of WTC, NLC, and WWF (as percentage of dry weight)

	WTC	NLC	WWF
Moisture content (%)	7.0 ± 0.1	6.2 ± 0.4	4.3 ± 0.5
Physical components (%)			
Leaf	37	0	0
Bark	9	15	0
Branch	12	18	0
Chip	42	67	100
Chemical composition (%)			
Total carbohydrates	41.3 ± 0.2	49.5 ± 0.2	63.1 ± 0.4
Total phenolics/lignin	32.1 ± 1.0	26.8 ± 1.0	25.3 ± 0.2
Ash	5.5 ± 0.1	3.4 ± 0.0	0.7 ± 0.1
Acetate	3.7 ± 0.1	4.3 ± 0.3	1.2 ± 0.1
Extractives	21.0 ± 0.6	16.7 ± 0.0	5.2 ± 0.4
Organic fraction element (%)			
C	45.8	46.6	46.7
H	5.9	6.0	6.2
N	1.4	0.6	0.2
O	41.5	43.5	46.5
HHV (MJ/kg)	18.5	18.8	18.8
Major mineral element (%)			
Ca	1.30	0.63	0.11
K	0.49	0.40	0.14
Mg	0.23	0.14	0.03
Na	0.03	0.02	0.01

3.3.2 Effect of temperature on fast pyrolysis in micropyrolyzer

Given the SRC poplar samples as new feedstocks for fast pyrolysis, it is necessary to investigate the effect of reaction conditions through screening trials. Temperature is one of the most important operating parameters affecting the pyrolysis product yields. Therefore, micropyrolysis experiments were performed to study the role of temperature on the formation of volatiles in fast pyrolysis and to determine the temperature for maximum yield of volatiles. The volatiles are defined as the organic products of pyrolysis with a boiling point lower than 300°C

(detectable by GC-MS). This definition excludes the oligomeric products from pyrolysis. Therefore, there is no guarantee that the condition studied maximized the yield of bio-oil, but it maximized the yield of volatiles. Temperatures ranging from 475°C to 625°C were studied for the three poplar feedstocks using an analytical Py-GC/MS-FID as described above.

Figure 3.2 depicts the total volatiles yields for WTC, NLC, and WWF at 475°C, 525°C, 575°C, 625°C, and 675°C. As the pyrolysis temperature increased, the volatile yields from both WTC and WWF initially increased, and then decreased. This trend has been reported previously: the increasing temperature facilitates the primary decomposition reactions of the biomass into volatiles, whereas, further temperature increase leads to secondary cracking reactions of the volatiles to produce permanent gases [112,152]. For WTC and WWF, the maximal yield in micropyrolysis was observed at 575°C and was in agreement with previous research [153]. Conversely, pyrolysis temperatures exhibited no significant influence on the volatile yield from NLC. At all temperatures, WTC presented the lowest total volatile yields from 8.8% to 11.1%, while WWF achieved the highest yields from 13.0% to 18.5%. With a few exceptions, most of the differences between NLC and WTC were not statistically significant. The volatiles were classified in categories. As Figure S3 shows, the product selectivity at 575°C is similar among the three poplar samples. Overall, there is no statistical significant difference in the selectivity for acids, ketones, furans, phenols, esters, and guaiacols, while the selectivity for anhydrosugars was significantly lower for SRC samples (WTC and NLC) than WWF.

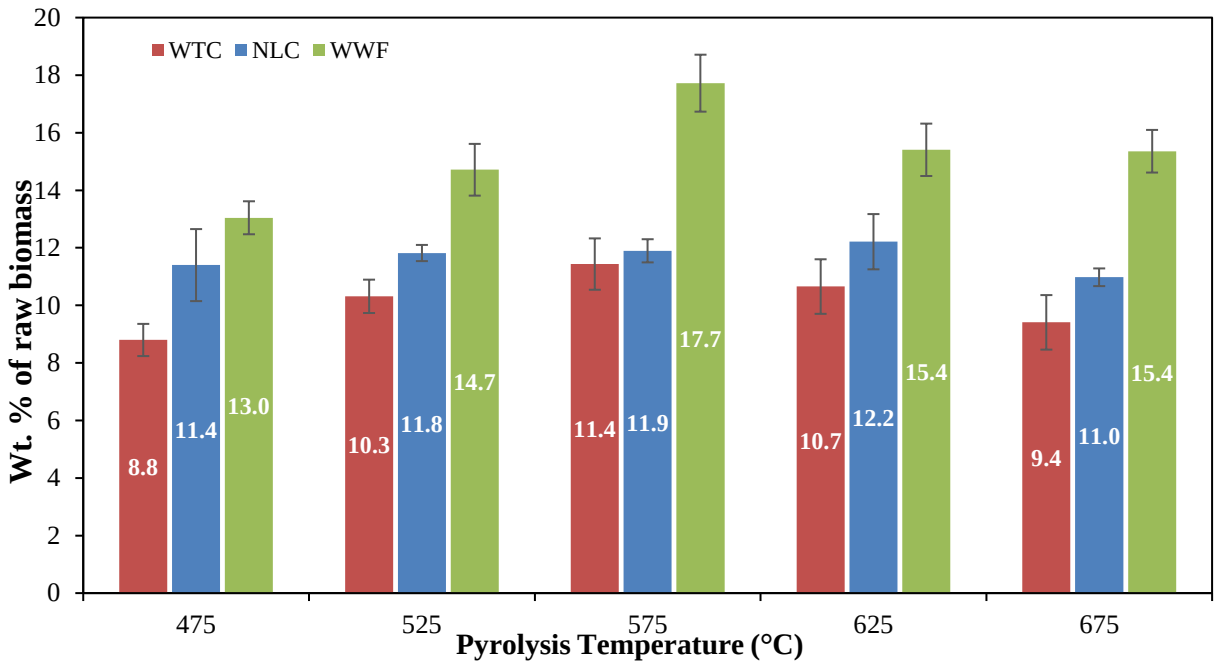

Figure 3.2 Yield of volatiles for WTC, NLC, and WWF as a function of temperature in the micropyrolyzer

3.3.3 Fast pyrolysis in the fluidized bed reactor

When lignocellulosic biomass undergoes fast pyrolysis, it produces liquid bio-oil, solid char, and non-condensable gas. The micropyrolyzer connected with GC/MS-FID performed on-line analysis of the pyrolysis vapors. However, one of the drawbacks of the analytical Py-GC/MS-FID is that it does not allow products collection, thus only the volatile constituents of bio-oil can be quantitatively analyzed. The total yield of bio-oils, char, and gases cannot be determined. As such, a fluidized bed reactor was used to further examine the total mass of bio-oil, along with the char and gas yields of the three poplar samples during fast pyrolysis. As Thangalazhy-Gopakumar [146] studied, the temperature setting of the micropyrolyzer (Pyroprobe model 5200) is 75°C higher than the actual temperature of the biomass. Therefore, the reaction temperature in

the fluidized bed reactor was adjusted to 500°C, which is equivalent to the temperature of 575°C obtained in the micropyrolyzer as the temperature for maximum volatiles production.

Figure 3.3 shows the fast pyrolysis yields obtained in the fluidized bed reactor. For WTC, NLC and WWF, the yields of bio-oil were 54.6%, 55.4%, and 60.9%, respectively; the yields of char were 19.7%, 13.6%, and 8.8%, respectively; the yields of non-condensable gases were 14.3%, 17.7%, and 17.5%, respectively. Overall, we achieved 88.6% total mass recovery for WTC, which was similar to 86.7% for NLC and 87.2% for WWF.

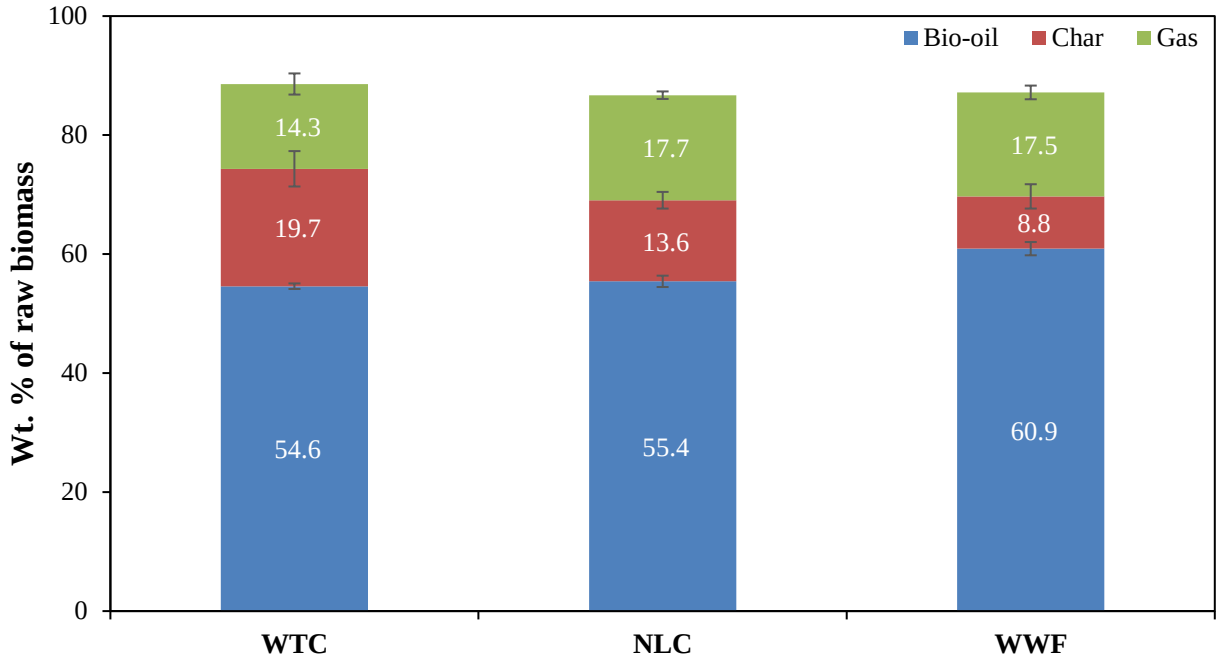

Figure 3.3 Product yields from pyrolysis of WTC, NLC, and WWF in the fluidized bed reactor

Bio-oil yield

Comparing these results, no significant difference in bio-oil yield was observed between WTC and NLC, whereas the bio-oil yield of WWF was significantly higher than SRC samples. Generally, bio-oil is generated from carbohydrates and phenolics/lignin. The similar combined

content of carbohydrates and phenolics/lignin for WTC (73.4%) and NLC (76.3%) explains the equivalent bio-oil yields. This is a significant result because it indicates that leaf removal does not affect the bio-oil yield in fast pyrolysis of SRC poplar, in contrast to what has been reported for biochemical conversion [139]. On the other hand, due to the higher total content of carbohydrates and phenolics/lignin, considerably more bio-oil was collected from WWF.

The water content of the bio-oil from WTC, NLC, and WWF were 55.2%, 41.4%, and 29.4%, respectively. This water came from both the original moisture in the feedstock and the dehydration reactions occurring during pyrolysis [99]. After discounting the feed moisture, the reaction derived water was 48.2%, 35.2%, and 25.1% from WTC, NLC, and WWF, respectively. The difference of bio-oil water content among poplar samples is a consequence of their different raw chemical composition. For example, more inorganics in the SRC samples (especially WTC) could promote secondary dehydration reactions, leading to the production of additional water [101].

Bio-oil characterization

The bio-oil was composed of a mixture of oxygenates. By classifying organic compounds into groups, Figure 3.4 indicates the product selectivity of the bio-oil across the three poplar samples. Both bio-oils from WTC and NLC had similar composition of, in descending order of product selectivity, ketones (~22%), phenols (~10%), anhydrosugars (~10%), guaiacols (~6%), esters (~6%), and furans (~6%). Herein, it must be noted that the GC-MS is only able to detect the volatile components of the bio-oil. The heavier, oligomeric components cannot be detected and are therefore excluded from the selectivity reported. Table S1 specifies the chemical compounds in each organic group. When compared to WTC, bio-oil from NLC showed lower selectivity to acids (i.e. acetic acid). Similar to water, acetic acid is one of the pyrolysis degradation products

and indicates the level of secondary reactions during fast pyrolysis [103]. The leaf removal process reduced the ash content, limiting the extent of secondary reactions which could produce acids. Although the anhydrosugars (levoglucosan) content between WTC and NLC is not significantly different, the standard deviations are large. Because of its high boiling point, it is difficult to quantify levoglucosan in GC/MS, which leads to large variations. It is possible that more levoglucosan dehydrated with higher ash content, but we could not conclude it here because of the large standard deviations. In addition, higher selectivity to aldehydes (e.g. glycolaldehyde) was observed in bio-oil from NLC. That could result from higher carbohydrate content in raw biomass, as glycolaldehyde is mainly obtained from polysaccharides. Surprisingly, despite the large difference in total phenolics/lignin between WTC and NLC feedstocks (5.3%), no significant difference of bio-oil selectivity was observed in phenols, which entails that more phenolic compounds from WTC may end up in the char or break into non-condensable gases. The findings suggest that leaf removal only slightly changed the bio-oil product selectivity of SRC samples.

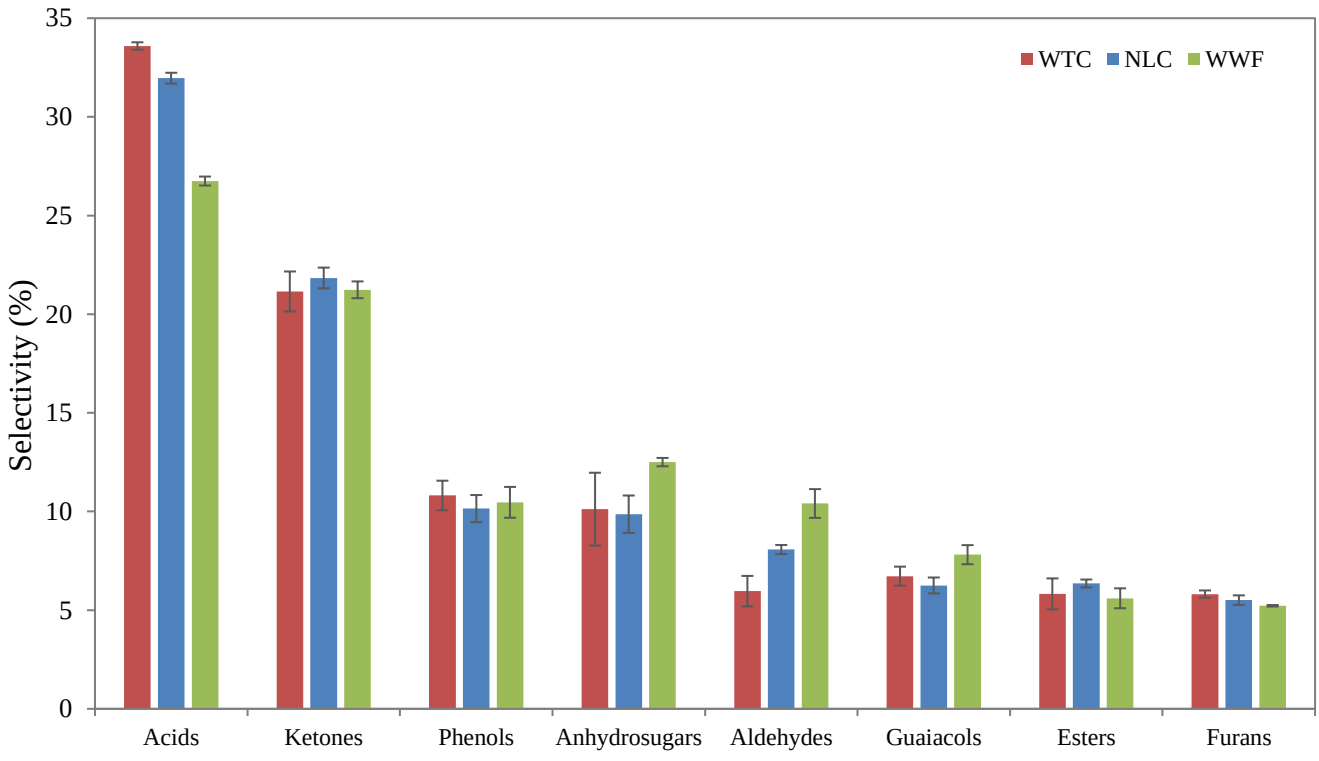

Figure 3.4 Organic compound groups in bio-oil of WTC, NLC, and WWF from the fluidized bed reactor

The bio-oil from WWF showed different organic compound selectivity compared to SRC samples (Figure 3.4). In particular, it exhibited higher selectivity to anhydrosugars (12.5%), and aldehydes (10.4%), but lower selectivity to acids (26.6%). Overall, the selectivity of carbohydrate derived compounds – anhydrosugars, aldehydes, and furans were found in positive correlation with the carbohydrate content of raw biomass ($R^2 = 0.98$, Figure S2). In addition to the different carbohydrate content, ash may also contribute to the differences in bio-oil selectivity. Since WWF comprises the least amount of ash content and alkali/alkaline metals, secondary reactions promoted by inorganics are suppressed for this feedstock, resulting in lower yields of degradation compounds, such as acids.

Char yield and characterization

The general understanding about the mechanism for char formation is that it occurs during depolymerization of the bulk aromatic structure [102]. As a macromolecular assembly of aromatic rings, lignin is reported to facilitate higher char yield than polysaccharides do during fast pyrolysis [103–105]. Likewise, the char yields were found to be associated to the total lignin/phenolic constituents in raw biomass and varied across three poplar samples. As shown in Figure 3.3, WTC had the highest char yield of 19.7%, which is significantly higher than the char yield from NLC (13.6%). Not surprisingly, the smallest amount of char was collected from WWF (8.5%). Different than WWF, where phenolic compounds are lignified in the mature wood, the foliage phenolic compounds are more likely to be present in the form of polyphenols, such as tannin and some flavonoids [23,154]. As polyaromatics are generally known as precursors to char formation, the higher content of polyphenolic compounds may induce more char formation from WTC. In addition, a previous study suggested that bark and juvenile wood comprise higher amounts of polyphenolic compounds over mature wood [10], which may further increase the char yield from SRC samples. Besides phenolic content and lignification level, the high ash content of SRC samples might be another factor positively affecting char agglomeration (Table 3.1). In particular, alkali and alkaline minerals act as catalysts for the secondary reactions and favor subsequent char formation [103,104]. Overall, leaf removal significantly decreased ash and phenolic content, leading to a markedly reduction of char yield by 6.1%.

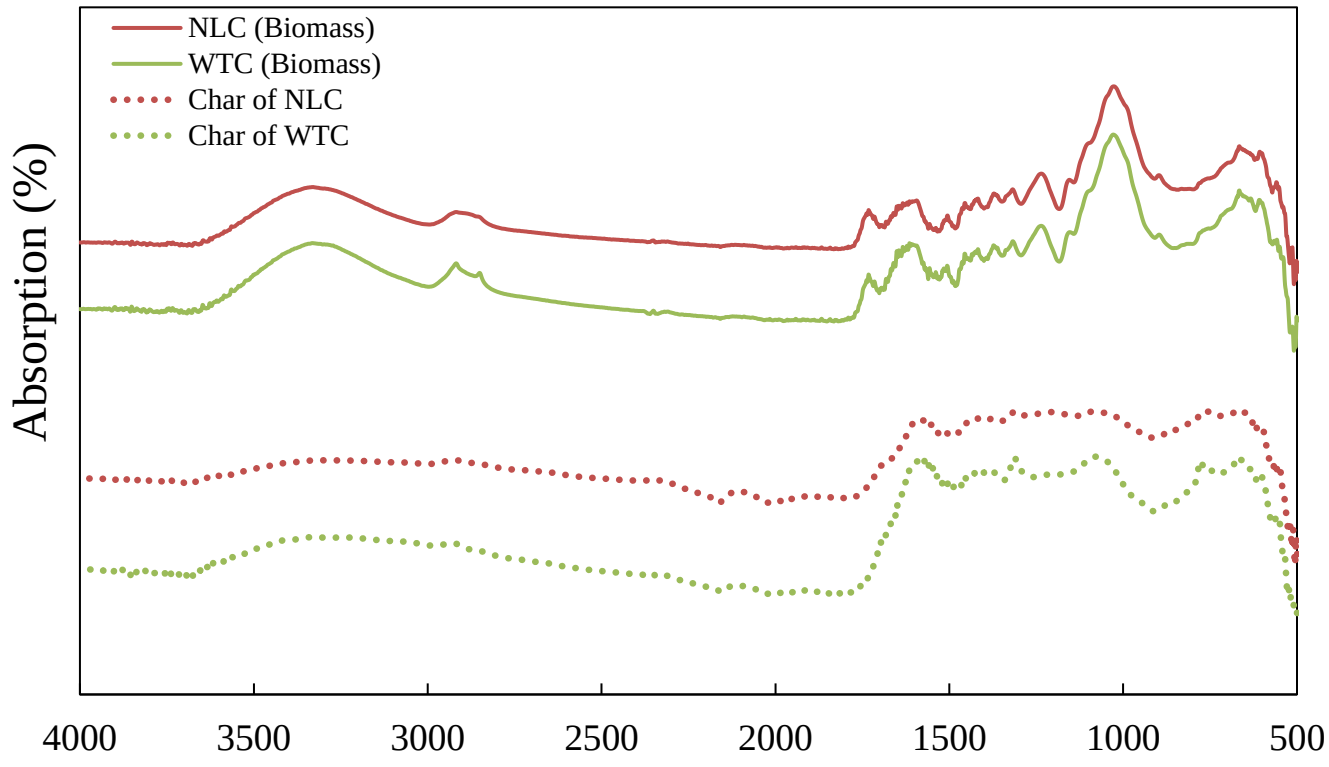

Figure 3.5 FTIR spectra of raw biomass and char samples of WTC and NLC

FTIR spectra of both raw biomass and char samples of WTC and NLC are shown in Figure 3.5. For raw biomass, WTC and NLC show spectra with strong lignocellulosic fingerprint region in the frequency range of 600-1800 cm^{-1} [155]. The strong absorption of C-O stretching and vibration at the band of 900-1100 cm^{-1} indicates the glycosidic linkage of cellulose and hemicellulose [155]. Aromatic ring vibration and C=C/C=O stretching (1500-1750 cm^{-1}), C-H stretching (2800-3000 cm^{-1}), along with O-H stretching (3300-3600 cm^{-1}) represent the alcohol and aromatic groups of phenolic compounds [155,156]. Evidently, the WTC has stronger adsorption of phenolic compounds than NLC. The spectrum of char shows flattened peaks for both WTC and NLC in virtue of lignocellulose decomposition. Stronger absorption for the char at the band of 1500-1750 cm^{-1} implies more C=C and C=O stretching of the carbonyl/carboxyl groups within aromatic rings [156], which is consistent with the fact that char is formed by

phenolic polymerization. The char spectra of WTC and NLC share a similar trend, but stronger bonds in the frequency range of 1500-1750 cm^{-1} of WTC char suggest higher content of phenolic compounds. Recall Table 3.1 and Figure 3.4, where NLC shows a significantly lower phenolic content than WTC, but similar phenol selectivity in the bio-oils. It appears that the difference of phenolic content in the feedstocks affected the composition of the char rather than the composition of the bio-oil. In other words, the results suggest that the phenolics from the leaves end up in the char after pyrolysis.

Gas yield and composition

Besides bio-oil and char, large amounts of non-condensable gases were generated during fast pyrolysis. From Table 3.2, it is apparent that the major gas products are carbon monoxide and carbon dioxide, with compositions ranging from 37.1-50.8% and 41.6-57.0%, respectively. Leaf removal significantly altered the gas product selectivity, generating 2.9% more carbon monoxide and 2.4% less carbon dioxide. This is mainly attributed to the chemical composition change due to leaf removal. Leaf removal increased the cellulose content such that more thermal cracking of cellulose carbonyl induced a higher CO yield [156]. Leaf removal also decreased the ash content, whereby carbon dioxide derived from secondary reactions (e.g. decarboxylation) of polysaccharides was reduced [104,157]. Interestingly, although WWF displays no significant difference in gas yield when compared to SRC samples (Figure 3.3), it had higher selectivity of carbon monoxide (50.8%) and lower selectivity of carbon dioxide (41.6%). In general, the presence of ash, especially the alkali/alkaline minerals, act as catalysts of secondary reactions such as thermal cracking and generates more CO_2. Other low-molecular-weight gaseous species detected were low in content and not statistically different between WTC and NLC.

Table 3.2 Chemical composition in non-condensable gas of WTC, NLC, and WWF from fluidized bed reactor (as mass percentage of gas)

	Formula	WTC (%)	NLC (%)	WWF (%)
Carbon monoxide	CO	37.1 ± 0.8	40.0 ± 1.8	50.8 ± 1.0
Carbon dioxide	CO_2	57.0 ± 1.0	54.6 ± 0.7	41.6 ± 1.1
Methane	CH_4	4.0 ± 0.4	4.8 ± 0.4	5.2 ± 0.2
Ethylene	C_2H_4	1.3 ± 0.2	0.9 ± 0.3	1.6 ± 0.1
Ethane	C_2H_6	0.8 ± 0.2	1.3 ± 0.3	0.7 ± 0.1

Interestingly, taking the standard deviation into consideration, the combined yields of char and gas exhibit no significant difference between WTC (34.0%) and NLC (31.3%). It appears that removal of leaves shifts the pyrolysis process from one that favors the production of char to one that favors the production of gas, though the overall byproducts (char and gas) remained unchanged. We hypothesize that this takes place because of the difference in the extent of competitive secondary reactions during fast pyrolysis of WTC and NLC.

During primary pyrolysis reactions, biomass is decomposed into volatiles, char, and gas. During secondary reactions, some of the reactive volatiles will further be consumed by two competing types of reactions: those that produce char, and those that produce non-condensable gas [154,158]. Because of the presence of foliage phenolics, WTC is likely to favor the char formation. That, in turn, outcompetes the secondary cracking reactions, consuming more carbon that otherwise could end up as gas. Additionally, char is reported to catalyze the decomposition of volatiles into coke [154]. The coke, after re-polymerization and condensation, further forms into (secondary) char. The combination of these effects led WTC to produce more char and less gas than NLC in fast pyrolysis. To the best of our knowledge, the current work is the first to report the effect of leaf removal on product distribution in fast pyrolysis.

3.3.4 Energy recovery comparison between thermochemical conversion (fast pyrolysis) and biochemical conversion

Table 3.3 shows the elemental composition of crude bio-oil from the fluidized bed reactor. Based on that, the higher heating value (HHV) of the bio-oil was calculated using an empirical equation (Equation 1). Compared to bio-oil from NLC, bio-oil from WTC has higher H (9.1% vs. 7.5%) and O (64.8% vs. 56.7%), but lower C (25.3% vs. 35.4%). The elemental composition of bio-oil was mainly affected by its water content; namely, higher water content led to a higher oxygen content and a lower carbon content. The calculated HHV of bio-oil from WTC is 2.1 MJ/kg lower than that of NLC. The leaf removal process, despite not affecting the bio-oil yield, increased the HHV of the bio-oil by 16%. Additionally, the HHV of the bio-oil from WWF is 2.2-4.2 MJ/kg higher than bio-oil from SRC samples. The difference in HHV correlates inversely with oxygen content due to the water content in bio-oil.

As previously reported, the biochemical conversion yields were markedly different across WTC, NLC, and WWF [139]. The corresponding ethanol yields were 122.5 liter/tonne, 203.5 liter/tonne, and 285.3 liter/tonne for WTC, NLC, and WWF, respectively (Table S2). In the present research, it is apparent that the difference in bio-oil yields for fast pyrolysis was smaller compared to the difference in ethanol yields for biochemical conversion. Since the fuels for the two processes are different and are expressed in different bases, it is necessary to define a common platform which could allow one to establish a meaningful comparison between the products from both processes.

Table 3.3 Elemental composition and HHV of bio-oil from WTC, NLC, and WWF and energy recovery rate of thermochemical conversion (fast pyrolysis) and biochemical conversion

	Crude bio-oil						Energy recovery rate	
	Elements				Water content	HHV	Thermochemical conversion[2]	Biochemical conversion[3]
	C	H	N	O[1]				
	(%)	(%)	(%)	(%)	(%)	(MJ/kg)	(%)	(%)
WTC	25.3	9.1	0.9	64.8	55.2	12.8	29.4	15.6
NLC	35.4	7.5	0.5	56.7	41.4	14.9	34.1	25.5
WWF	38.5	7.8	0.3	53.4	29.4	17.0	42.9	35.7

[1]Calculated by difference

[2]upgraded bio-oil with a conceptual energy recovery of 78% by hydrotreating and refining [150]

[3]ethanol yields in biochemical conversion based on previous research [139] (Table S2)

Energy recovery rate, also known as energy efficiency, is expressed as the fraction of feedstock energy inputs into the process that ends up as chemical energy of the liquid fuel [149,159]. It allows comparison between different conversion processes [160]. Given the HHV and yield of ethanol and the energy content of the input biomass, the energy recovery rate for the biochemical conversion was calculated by Equation 2. Similarly, the energy recovery rate for fast pyrolysis was determined based on the HHV (Table 3.3) and yield of crude bio-oil (Figure 3.3). However, the crude bio-oil is highly oxygenated and does not meet the criteria for direct use as transportation fuel. To properly establish a comparison between two processes based on liquid fuel products, the crude bio-oil requires further upgrading. Herein, hydrotreating and refining were assumed to be carried out to upgrade the bio-oil with a conceptual energy recovery of 78% [150], as described in the methods. The data presented in Table 3.3 consider the upgrading of bio-oil. In summary, we are making the following assumptions: 1) Equation 1 is valid for the HHV estimation for feedstock and bio-oil as previous fast pyrolysis papers applied, 2) The energy recovery rate of upgrading the crude bio-oil to refined fuel product is 78%, as reported by Bridgewater [150], 3) the fermentation yield of ethanol is same as 2011 NREL

biochemical conversion report, where glucose (95%), arabinose (85%), and xylose (85%) are fermented into ethanol (Supporting Information Table S2).

The comparison of energy recovery rate between two conversion processes is shown in Table 3.3. For fast pyrolysis, WTC had 29.4% energy recovery rate, which is 4.7% lower than that of NLC. Not surprisingly, the highest energy recovery rate was obtained in the fast pyrolysis of WWF (42.9%). In contrast, the energy recovery rates of biochemical conversion ranged from 15.6% to 35.7% and were relatively lower than those of fast pyrolysis. Consistent with previous studies, our findings confirmed that thermochemical conversion recovers more energy than biochemical conversion in its liquid fuel products [159,160]. In particular, the energy recovery rate of WTC in fast pyrolysis was 13.8% higher than that in biochemical conversion. It is noteworthy that leaf removal made a substantial increase in energy recovery rate in biochemical conversion (9.9%). To the contrary, no such large improvement was observed in thermochemical conversion by leaf removal (4.7%). Moreover, in contrast to the biochemical conversion, where WWF achieved 10.2-20.1% higher energy recovery rate over SRC samples, WWF showed less pronounced advantage (8.8-12.5%) in thermochemical conversion. Our findings from the perspective of energy efficiency underscore the advantage of thermochemical conversion in converting SRC poplar (especially WTC) into biofuel, yet no assessment was made with respect to economic or environmental aspects. Future work on the techno-economic analysis and life cycle assessment may help to further evaluate the coppice-to-fuel process and make more comprehensive comparisons between thermochemical and biochemical conversions.

The results demonstrate that leaf materials will not cause substantial losses of yield and energy recovery when being included in the fast pyrolysis of SRC poplar biomass. Leaf separation may not be a necessary step for conversion of SRC poplar to bio-oil, which might lead to large

savings from the aspect of feedstock supply chain. On the other hand, if the savings associated with the use of NLC outweigh the cost of the leaf removal process, one shall consider performing leaf separation prior to fast pyrolysis of SRC poplar. A recent study by Thompson et al. [28] has shown that an air classification method can separate the heterogeneous biomass mixture into anatomical unique fractions at a cost-effective level. Once economics of leaf separation is proven, this could be a promising way to lower the cost of leaf removal and allow a better use of NLC in fast pyrolysis.

In general, WTC exhibits much higher energy recovery rate in thermochemical conversion than in biochemical conversion. From this, we conclude that it will be more reasonable to convert WTC to biofuel via thermochemical conversion.

3.4 Conclusions

This study explored fast pyrolysis of SRC poplar as a realistic feedstock for the biorefinery. As the first rotation coppice, the 2-year-old SRC poplar contains leaves and is very heterogeneous. Based on the temperature for maximum yield of volatiles determined in a micropyrolyzer, fast pyrolysis of WTC and NLC was performed in a fluidized bed reactor. One of the key findings is that although leaf removal significantly changed the chemical composition of raw biomass, no substantial difference was observed in bio-oil yield and composition during fast pyrolysis. Leaf removal affected the char and gas yields as well as their chemical composition. The HHV of bio-oil from NLC was 2.1 MJ/kg higher than that of WTC, which was mainly affected by the water content. Leaf removal increased the energy recovery rate from 29.4% to 34.1%, both of which were significantly higher than the energy recovery rates of biochemical conversion. Though the presence of leaves decreased the energy content of bio-oil, our work indicates that fast pyrolysis

is an attractive method to convert SRC poplar into fuels because of its higher energy recovery rate. Overall, leaf removal may not be essential for fast pyrolysis of SRC poplar.

Abbreviations

SRC, short rotation coppice; SFR, short rotation forestry; WTC, whole tree coppice; NLC, noleaf coppice; WWF, whitewood forestry; HHV, higher heating value; SLPM, standard liters per minute; HPLC, high-pressure liquid chromatography; GC, gas chromatography; MS, mass spectrometry, FID, flame ionization detector; TCD, thermal conductivity detectors; ANOVA, analysis of variance.

Supporting Information

Process flow diagram of feedstock harvest, collection, and preparation (Figure S1); ; Relationship between selectivity of carbohydrate derived bio-oil compounds (including anhydrosugars, aldehydes, and furans) and carbohydrate content of raw biomass (Figure S2); Yield of volatile constituents for WTC, NLC, and WWF at 575°C in micropyrolyzer (Figure S3); Organic compounds selectivity in bio-oil of WTC, NLC, and WWF from fluidized bed reactor (Table S1); and Sugar yield and ethanol yield of WTC, NLC, and WWF in biochemical conversion (Table S2).

Supplementary materials and methods

Fast pyrolysis in micropyrolyzer (Py-GC/MS-FID)

Micropyrolysis experiments were carried out in a commercial micropyrolyzer (Pyroprobe model 5200, CDS Analytical Inc.) connected to an online Gas Chromatography/Mass Spectrometry equipped with a Flame Ionization Detector (GC/MS-FID). Inside the pyroprobe, a platinum coil holds and heats a quartz tube which contains the biomass. When preparing the samples in the quartz tube, biomass was loaded in the middle of the tube with loose quartz wool packed at both ends. 0.5 mg of poplar samples were weighed in an analytical balance (AD 6000 Ultra Microbalance, Perkin Elmer, Waltham, MA) with an accuracy of 0.1 µg, as described previously [161].

The pyrolysis was carried out at the filament temperatures ranging from 475°C to 675°C at a heating ramp of 1000°C/s. Upon reaching the set point temperature, the probe temperature was kept constant for 60 s. Vapors produced were swept into a trap at 50°C to allow collection of condensable products. After the time elapsed, the trap was then heated up to 300°C to desorb the condensed vapors. Vapors produced from micropyrolysis were then carried by helium gas

(99.9999%) via a transfer line into the GC column for analysis. The temperature of the pyroprobe interface and the transfer line between micropyrolyzer and GC were maintained at 300°C to minimize the condensation of the vapor products.

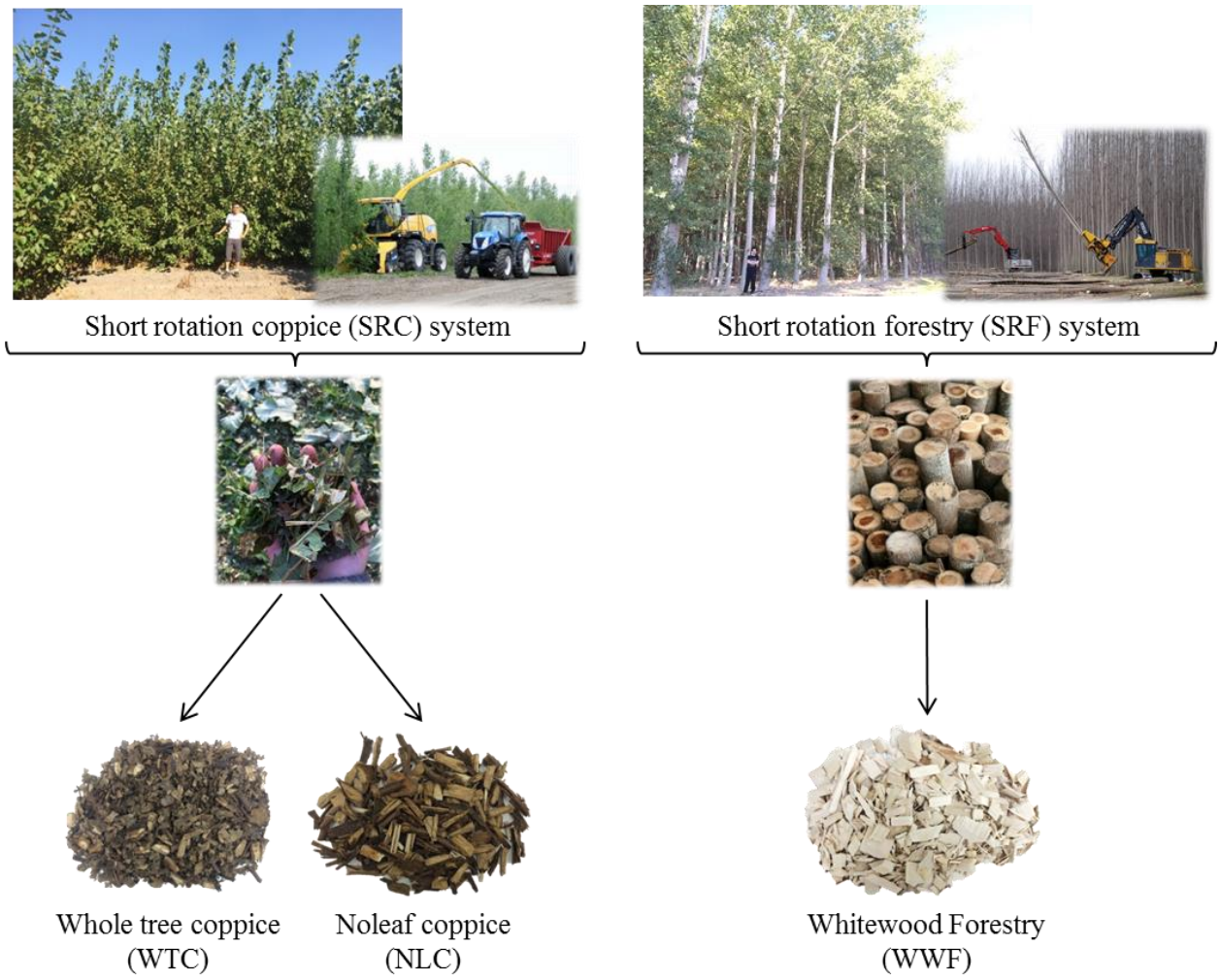

Figure S1. Process flow diagram of feedstock harvest, collection, and preparation.

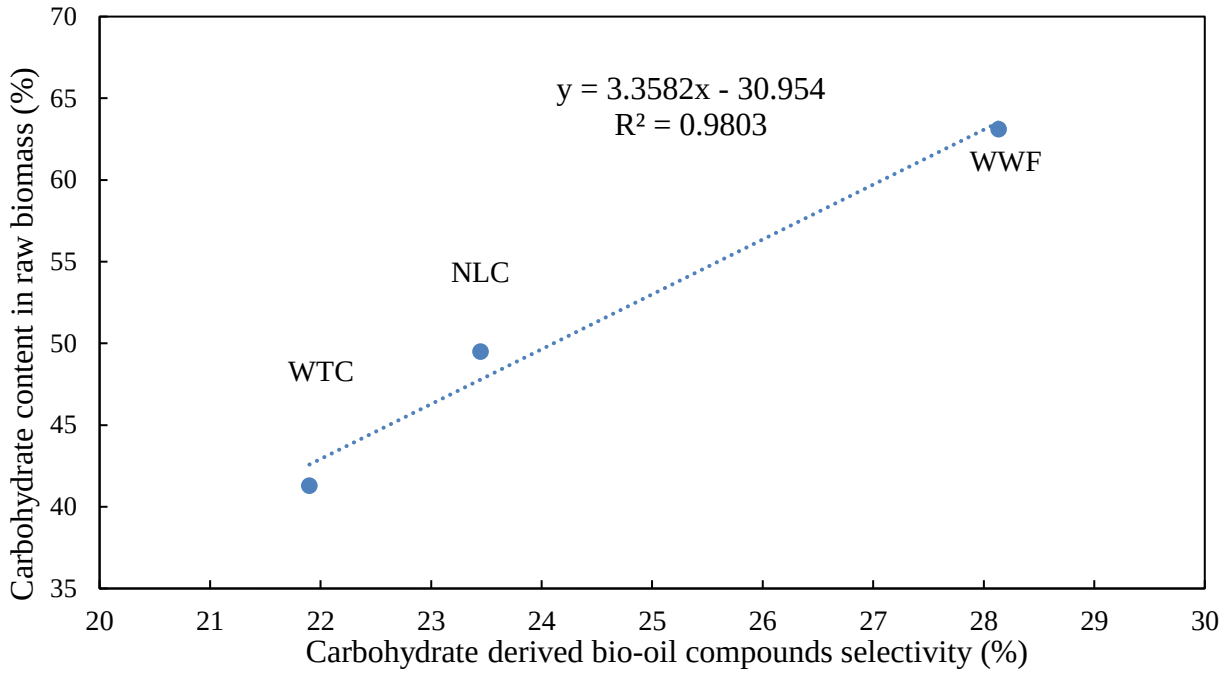

Figure S2. Relationship between selectivity of carbohydrate derived bio-oil compounds (including anhydrosugars, aldehydes, and furans) and carbohydrate content of raw biomass.

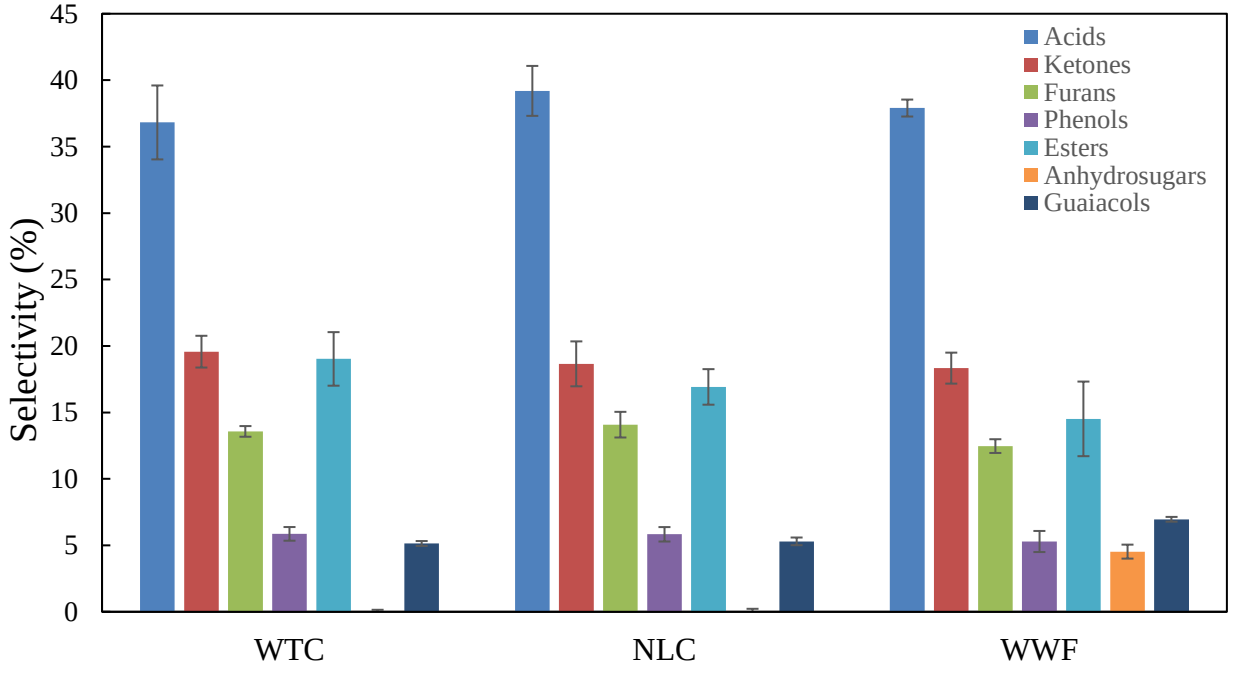

Figure S3. Yield of volatile constituents for WTC, NLC, and WWF at 575°C in micropyrolyzer

Table S1. Organic compounds selectivity in bio-oil of WTC, NLC, and WWF from fluidized bed reactor

		WTC	NLC	WWF
Group	Compounds	Selectivity (%)		
Acids		**33.59**	**32.96**	**26.75**
	Acetic Acid	33.59	32.96	26.75
Ketones		**21.15**	**22.84**	**21.24**
	Acetone	2.51	2.57	3.28
	2,3-Butanedione	1.12	1.09	0.97
	Acetol	10.91	9.79	8.53
	1-Hydroxy-2-butanone	1.52	2.82	2.57
	2-Cyclopenten-1-one, 2-hydroxy	2.11	3.05	3.61
	1,2-Cyclophexanedione	0.99	0.74	0.80
	1,2-Cyclopentanedione, 3-methyl-	1.99	1.77	1.48
Phenols		**10.81**	**10.15**	**10.47**
	Phenol	2.70	2.91	4.56
	o-Cresol	1.33	1.26	0.82
	m-Cresol	0.73	0.59	0.25
	Catechol	1.89	1.44	1.07
	3-methoxycatechol	2.47	2.57	2.52
	Hydroquinone	0.23	0.18	0.18
	4-Methylcatechol	1.16	0.94	0.41
	4-ethylcatechol	0.30	0.26	0.65
Anhydrosugars		**10.12**	**9.86**	**12.50**
	Levoglucosan	10.12	9.86	12.50
Guaiacols		**6.72**	**6.25**	**7.81**
	O-Guaiacol	0.55	0.54	0.47
	Creosol	1.19	0.55	1.22
	2-Methoxy-4-vinylphenol	2.57	2.69	3.10
	Syringol	1.05	1.07	1.10
	Isoeugenol	0.14	0.18	0.61
	Methoxyeugenol	0.52	0.54	0.69
	Coniferyl alcohol	0.70	0.69	0.63
Aldehydes		**5.96**	**8.07**	**10.41**
	Glycolaldehyde	5.59	7.48	9.32
	Succindialdehyde	0.38	0.59	1.09
Esters		**5.82**	**6.35**	**5.60**
	n-Propyl Acetate	2.90	3.28	2.36
	Propanoic acid, 2-oxo, methyl ester	2.92	3.07	3.24

Furans	**5.82**	**5.51**	**5.22**
Furfural	2.92	3.44	3.16
2(5H)-Furanone	2.24	1.52	1.62
2,3-dihydrobenzofuran	0.65	0.55	0.44

Table S2. Sugar yield and ethanol yield of WTC, NLC, and WWF in biochemical conversion

	Sugar yield		**Fermentation yield of ethanol (*Z. mobilis*)[3]**			
	C5[1]	C6[2]	C5[1]	C6[2]	Sum	
	(kg/tonne)	(kg/tonne)	(kg/tonne)	(kg/tonne)	(kg/tonne)	(L/tonne)
WTC	50.2	166.1	21.0	75.7	96.7	122.5
NLC	95.2	268.3	39.8	120.8	160.6	203.5
WWF	111.5	387.8	46.6	178.4	225.1	285.3

* Data obtained and calculated based on biochemical conversion [139]

[1] C5 stands for 5 carbon sugars, including arabinose and xylose

[2] C6 stands for 6 carbon sugars, including galactose, glucose, and mannose

[3] Fermentation conversion based on 2011 NREL Biochemical Conversion Report [43], where only glucose (95%), arabinose (85%), and xylose (85%) are fermented into ethanol

Chapter 4. **Bridging the gap between feedstock growers and users: a study of poplar coppice based biorefinery 3**

Abstract

In the advanced biofuel industry, land productivity is important to feedstock growers and conversion process product yield is important to the biorefinery. The crop productivity, however, may not positively correlate with bioconversion yield. Therefore, it is important to relate sugar yield to biomass productivity. In this study, 2-year old poplar trees harvested in the 1st coppice cycle, including one low-productive hybrid and one high-productive hybrid, were collected from two poplar tree farms. Through steam pretreatment and enzymatic hydrolysis, the bioconversion yields of low and high productive poplar hybrids were compared for both sites. Although they have the similar chemical composition, the low-productive hybrids had 9% to 19% higher sugar yields than the high-productive hybrids. Economic calculations show the impact on the grower and biorefinery of using the two feedstocks. Growing a high-productive hybrid means the land owner would use 11% to 26% less land (which could be used for other crops) or collect $2.53 to $3.46 MM/yr extra revenue from the surplus feedstock. In contrast, the biorefinery would receive 7% to 12% additional revenue by using the low-productive hybrid. We propose a business model based on the integration of the plantation and the biorefinery. In this model, different feedstocks are assessed using a metric of product tonnage per unit land per year. Use of this new economic

[3] Prepared for submission at Green Chemistry, C. Dou, R. Gustafson, and R. Bura.

metric bridges the gap between feedstock growers and users to maximize the overall production efficiency.

4.1 Introduction

Lignocellulose provides many opportunities to produce fuels and chemicals that are sustainable alternatives to production from current fossil resources. The success of lignocellulose-based products, however, is strongly dependent on having a cost competitive feedstock and high conversion yields.

Feedstock quantity plays a key role in the biofuels industry. Due to the relatively low energy density of lignocellulosic biomass, a commercial-scale biorefinery requires a large feedstock supply [7,31]. For feedstock growers, the goal is to increase the land productivity, maximize the biomass yield, and ultimately generate more profits. To address these issues, many studies have been done to develop fast growing energy crops using breeding and/or genetic tools [33,162]. Higher productivity results in less land use and the feedstock growing, harvesting, and handling can be conducted in a smaller area to reduce the production cost.

Feedstock quality is critical to the conversion process. High feedstock quality is generally related to high sugar content, low lignin content, and ease of conversion, which may lead to high fermentable sugar and final product yields [163]. Overall, the characterization of high quality feedstock is dependent on the conversion yield per unit biomass. Upon receipt of harvested biomass, the feedstock users – the biorefineries – are most interested in the efficiency of biomass conversion. Sourcing a single type of feedstock with uniform compositional properties year-round would be ideal to ensure a stable conversion process. In reality, feedstock quality varies

depending on the crop variety, where it is grown, and how it is processed before reaching the biorefinery. Inconsistent feedstock quality will lead to fluctuations of the conversion yield [24,35,42]. Over the decades, substantial efforts have been made to improve the biomass conversion yield by optimizing process conditions for specific feedstocks [37,38,73]. Even for the same type of feedstock, however, heterogeneity of the biomass quality is prevalent and the result is an uncertain final product yield [41].

With the current expansion of lignocellulosic bioeconomy, there is a demand to treat lignocellulosic biomass as a fungible commodity [164,165]. This would require the establishment of an efficient, large-capacity, and reliable supply system for producing and trading the lignocellulosic feedstocks [166]. Commodity goods are generally defined by their volume and standardized quality. However, unlike other commodities (e.g. corn grain and sugar), biofuel feedstocks do not share a uniform format.

To reconcile the need for feedstock quantity in the supply chain and the need for feedstock quality in the conversion process, there must be a better understanding of feedstock from both sides. The overarching goal of the present work is to bridge the gap between the growers and users to facilitate future commoditization of lignocellulosic feedstock. This work investigates the sugar yield in bioconversion of two poplar clones, one high-productive hybrid and one low-productive hybrid, from two poplar plantation sites. With the plantation productivity data and bioconversion experimental results, we can assess the economics from the perspectives of feedstock growers and feedstock users. In addition, a new business model integrating plantations and biorefineries is introduced to account for the performance of both enterprises' needs and to enhance the overall efficiency from feedstock production through biofuel conversion.

4.2 Methods

The materials used in this research were two-year-old short rotation coppice poplars harvested after the first rotation. Two hybrids – one low-productive and one high-productive – were collected from two planation sites (Jefferson, OR and Clarksburg, CA, USA). Previous research in our group showed that leaf material impedes bioconversion and lowers the overall sugar yield of the poplar [139]. Therefore, all feedstocks in this study were leaf-free samples. After analyzing the chemical composition, four coppice poplar samples were processed through steam pretreatment at 195°C for 5 minutes with SO_2 (3% w/w) impregnation. The water insoluble fractions (WIF) and water soluble fractions (WSF) were separated and analyzed. WIF were then enzymatically hydrolyzed at 5% (w/v) consistency and 5 FPU/g cellulose enzymes loading. A complete mass balance was conducted to assess difference in sugar productions of the four poplar samples. Economic analyses were made from both feedstock grower and user perspectives by using the conversion results of experiments and land productivity data of the tree farm.

4.2.1 **Raw material**

Two poplar hybrids were studied in this research; one low-productive hybrid of *Populus trichocarpa* and *Populus deltoides* and one high-productive hybrid of *Populus deltoides* and *Populus maximowiczii*. Both hybrids were cultivated in two plantation sites, one located in Jefferson, OR and one located in Clarksburg, CA. Poplars were established using a short rotation coppice management regime and harvested using a fully mechanized whole tree harvester. The samples used here were 1st rotation poplar trees harvested after their first two growing seasons.

Leaf materials negatively affect the bioconversion and were removed from the feedstock as described previously [139]. All samples were kept frozen at -20 °C until use.

4.2.2 **Pretreatment and processing conditions**

Four poplar feedstocks were processed in the same condition. 600 g oven-dried (OD) weight of each feedstock was pre-impregnated overnight with anhydrous SO_2 in plastic bags at atmospheric pressure. The amount of SO_2 added to the bag corresponded to 3% (w/w) loading, and was determined by weighing the bag before and after the addition of gas.

Steam explosion pretreatment was performed in a 2.7-liter batch reactor (Aurora Technical, Savona, BC, Canada). Briefly, samples were loaded and heated at temperature 195 °C for 5 min. Following the reaction time, the pneumatic valve was opened to explode and discharge the biomass into a collection container. After steam explosion, the pretreated biomass slurry was separated into WSF and WIF using vacuum filtration. The WIF was then washed with a volume of deionized water equivalent to 20 times the dry weight of the sample to remove the free sugars.

4.2.3 **Enzymatic hydrolysis**

Enzymatic hydrolysis was carried out using cellulase (Celluclast 1.5 L, Sigma) at 5 Filter Paper Units (FPU)/g cellulose and β-glucosidase (Novozyme 188, Sigma) at 10 cellobiase units (CBU)/g cellulose. The WIF was hydrolyzed at 5% (w/v) consistency in a total volume of 50 ml in 125 ml Erlenmeyer flasks. The flasks were incubated at 50°C and 175 rpm in a New Brunswick shaker. Additionally, 50 mM citrate buffer was added to maintain the pH at 4.8, and tetracycline (40 µg/ml) and cycloheximide (30 µg/ml) were used to inhibit microbial

contamination. 1 ml samples were taken periodically, boiled for 10 min to denature enzymes, filtered through a 0.22 μm syringe filter, and stored at -20 °C until analysis.

4.2.4 High Pressure Liquid Chromatography (HPLC) analysis

The concentration of monomeric sugars from chemical composition analysis and enzymatic hydrolysis was 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 0.2 M NaOH. Standards were prepared to encompass the same range of concentrations as the samples. Fucose was added to all samples and standards as an internal standard.

Acetic acid, 5-hydroxymethylfurfurals (HMF) and furfural were measured using refractive index detection on a Shimadzu Prominence LC. Separation of these compounds was achieved by an anion exchange column (Rezex RHM Monosaccharide H^+ (8%), Phenomenex, Inc., Torrance, CA) with an isocratic mobile phase that consisted of 5 mM H_2SO_4 at a flow rate of 0.6 ml/min. The column oven temperature was maintained at a constant temperature of 63 °C. Standards were prepared and used to quantify the unknown samples.

4.2.5 Compositional analysis

Ash and extractives

Ash content of raw biomass samples was measured gravimetrically by heating 20-mesh-milled dry biomass to 575 ± 25°C for 18 ± 6 hours [122]. Water and ethanol extractives of raw biomass were determined according to National Renewable Energy Laboratory (NREL) methods [123].

Soluble fraction carbohydrates and degradation products

Monomeric/oligomeric soluble carbohydrates and degradation products were determined using NREL LAP TP-510-42623 [124]. Briefly, 0.7 ml of 72% 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.

Insoluble fraction carbohydrates, acetate groups, and lignin

The chemical composition of raw biomass and WIF were determined according to a modified method derived from TAPPI Standard Method [126] and NREL protocols [127]. Briefly, 0.2 g of finely ground oven dried sample is treated with 3 ml 72% H_2SO_4 for 120 min at room temperature, then diluted into 120 ml total volume and autoclaved at 121 °C for 60 min. Klason lignin contents were determined by gravimetric methods. After filtration through tared sintered-glass crucibles, the carbohydrate and acetyl composition of the filtrate is analyzed by HPLC and the acid soluble lignin in the filtrate is analyzed by UV at 205 nm.

4.2.6 **Sugar yield calculation**

A complete mass balance was calculated using the composition and total mass of each WSF and WIF leaving pretreatment and enzymatic hydrolysis as described previously [62]. Glucose and xylose are the major sugars presented in the biomass and recovered during the process. Arabinose, galactose, and mannose were calculated as minor sugars. The sugar yield was defined

as the total mass of monomeric sugars in the hydrolyzed solid and liquid phases normalized by the initial oven dry mass of biomass (kg monomeric sugars/tonne biomass).

4.2.7 **Economic modelling from feedstock growers and feedstock users**

The economic potentials for growing and using different poplar hybrids were assessed from both the perspectives of feedstock growers and feedstock users. The annual feedstock processing capacity in the simulated cellulosic ethanol plant was set as 700,000 tonne. For the economic model of feedstock users, the annual ethanol production was calculated by implementing the sugar yield results in the current research and the fermentation conversion of NREL 2011 report [43]. The annual ethanol revenue was determined from the annual ethanol production at ethanol selling price of $1.59/gallon ($6.00/liter) [129]. From that, the revenue difference was calculated to illustrate the influence of feedstock quality in using different poplar hybrids.

For the economic model of feedstock growers, the average annual revenue was calculated based on the hybrid's land productivity and feedstock price. The feedstock price ($53/tonne) was determined from the heating value of the poplar with the assumption that the only other realistic market for the 1st rotation two-year-old poplar would be hog fuel [139]. A higher heating value (HHV) at 19.8 MJ/kg and a price of $2.8/MMBtu ($0.00265/MJ) were used for the calculation [129,167]. In addition, given the feedstock processing capacity of the commercial-scale cellulosic ethanol plant and the land productivity of each poplar sample, the overall land requirement was calculated to indicate the impact of feedstock productivity on land usage.

The land sugar productivity is proposed in this paper to illustrate the plantation productivity with respect to the sugar production. Expressed as annual sugar yield per unit of land (kg

sugars/acre/year), that metric is determined by using the sugar conversion yield from the data and land productivity from the tree farm.

4.2.8 **Statistical analysis**

The results were subjected to one-way analysis of variance (ANOVA) analysis followed by a Tukey's test. All data are represented as the mean of triplicates with standard deviation. Chemical composition, sugar conversion of enzymatic hydrolysis, sugar yield following steam pretreatment, and enzymatic hydrolysis were analyzed based on 5% alpha level (95% confidence interval). Statistical differences in chemical composition and sugar yield were determined from p-values ($p < 0.05$). Data were analyzed using R (version 3.0.1) software. In this manuscript, any data analysis mentioned as "significant" represents statistically significant (p value <0.05).

4.3 Results and Discussion

In this study, poplar hybrids were planted at two sites, Jefferson, OR and Clarksburg, CA. At each site, two hybrids were grown for two years, harvested, and prepared by removing the leaf materials. Based on the productivity, two poplars were labelled as low-productive hybrid and high-productive hybrid. The prepared poplar samples were steam pretreated followed by enzymatic hydrolysis to assess the overall sugar yields (in kg monomeric sugars/tonne biomass). From that, we estimated the corresponding ethanol production from each poplar feedstock. Further evaluation was made to determine the impact of feedstock quality on the economics of commercial-scale cellulosic ethanol production. Given the land productivity (in tonne/acre/year) of each poplar feedstock, we compare the economics of feedstock growers by using different hybrids on different plantation sites. Further, the annual sugar yield per unit of land is calculated,

leading to a new performance metric for the biofuels industry which integrates the plantations and biorefineries and provides a basis for commodity trading of lignocellulosic feedstocks.

4.3.1 **Bioconversion of coppice poplar for sugar production**

Chemical composition of poplar feedstocks

Table 4.1 Chemical composition of low productive and high productive hybrids of two plantation sites (shown as weight percentage) [a]

Site	Hybrid	Raw Biomass composition (%)							
		Glucan	Xylan	Minor sugars	Total sugars	Lignin	Acetic acid	Ash	Extractives
Jefferson	Low-productive	33.2	11.9	4.4	49.5	26.8	6.1	3.4	16.7
		0.5	0.3	0.1	0.2	1.0	0.0	0.0	0.0
	High-productive	34.3	12.9	4.0	51.2	26.4	6.8	4.9	10.0
		0.2	0.1	0.1	0.3	1.8	0.1	0.5	0.7
Clarksburg	Low-productive	36.6	12.3	4.1	53.0	27.0	6.2	3.2	16.4
		0.3	0.1	0.1	0.4	0.6	0.2	0.0	0.9
	High-productive	37.5	12.5	3.6	53.6	27.5	6.2	2.7	14.5
		0.5	0.2	0.1	0.7	0.5	0.2	0.1	0.5

[a] Standard deviations (SDs) are shown under each mean value

Before bioconversion, all four feedstocks were characterized to determine the chemical composition. The compositional information is listed in Table 4.1. For the Jefferson site, the low-productive hybrid had 1.7% lower total sugar content, 1.5% lower ash content, and 6.7% higher extractives content than the high-productive hybrid. For the Clarksburg site, the chemical composition between two coppice samples were similar. The only significant differences were found in ash and extractives, where the low-productive hybrid is 0.5% and 1.9% higher in ash and extractives than the high-productive hybrid, respectively. Interestingly, both hybrids showed 2.4% - 3.5% higher sugar content in the Jefferson site than the Clarksburg site. Other

constituents, however, did not show these differences between sites. Overall, the difference in the chemical composition, especially the sugar content and the lignin content, was minimal between two hybrids in both sites.

Chemical composition of water insoluble fraction (WIF) and water soluble fraction (WSF) after pretreatment

Following pretreatment and liquid-solid separation, the compositions of the water insoluble fraction (WIF) and the water soluble fraction (WSF) were analyzed. Expressed as percent of dry matter, Table 4.2 shows the WIF chemical composition. The sugar and lignin content ranged from 55.2% to 58.7% and from 36.8% to 37.4%, respectively within the four coppice poplar samples. The trends of WIF sugar content were generally consistent with the raw biomass composition. Comparing between different hybrids of each site, the low-productive hybrid comprised 1.3% less sugar and 1.5% more lignin than the high-productive hybrid in Jefferson, while the low-productive hybrid had 1.0% more sugar and 0.8% less lignin than the high-productive hybrid in Clarksburg. The WIFs of Jefferson site had higher sugar content than those of Clarksburg site, which agrees with the sugar composition in raw biomass.

Table 4.2 Composition and sugar recovery resulting from steam pretreated of coppice samples including WIF yield (expressed as recovered solids in kg/tonne), chemical composition of WIF (as percentages of the solid weight), and monomeric sugar yields in WSF samples (expressed as kg/tonne raw biomass) [a]

Site	Hybrid	WIF yield (kg/tonne)	Chemical composition of WIF (%)					Sugar recovered in WSF (kg/tonne)			
			Glucan	Xylan	Minor sugars	Total sugars	Lignin	Glucose	Xylose	Minor sugars	Total sugars
Jefferson	Low-productive	468.5	52.2	2.4	0.7	55.2	37.4	49.4	78.2	28.7	156.3
		21.6	0.3	0.3	0.1	0.1	0.1	1.5	4.0	1.1	6.3
	High-productive	467.4	53.0	2.8	0.7	56.5	35.9	27.2	65.7	20.5	113.3
		5.6	1.0	0.1	0.0	0.9	0.4	0.4	2.1	1.0	2.6
Clarksburg	Low-productive	508.5	55.3	2.6	0.7	58.7	37.6	62.2	80.4	30.4	173.0
		9.3	0.6	0.1	0.0	0.5	0.5	2.6	3.9	0.4	6.1
	High-productive	529.0	54.4	2.6	0.7	57.7	36.8	53.4	72.0	27.1	152.4
		6.4	0.4	0.2	0.0	0.2	0.7	4.0	4.6	2.5	11.1

[a] Standard deviations (SDs) are shown under each mean value. kg/tonne values refer to kg of sugar recovered per tonne of raw OD biomass

Table 4.2 shows that the monomeric sugar yields in the WSF differ between poplar hybrids. As expected, the majority of minor sugars resided in the WSF since most hemicellulose was dissolved during pretreatment. Contrary to the small difference in WIF composition, a significant difference in WSF monomeric sugar yields was observed between different hybrids (Table 4.2). For samples from the Jefferson site, the yields of glucose, xylose, and minor sugar were 45%, 16%, and 29% higher in the low-productive hybrid than the

high-productive hybrid. The difference was relatively smaller for samples from Clarksburg site. For Clarksburg the yields of glucose, xylose, and minor sugar were 14%, 10%, and 11% higher in the low-productive hybrid than the high-productive hybrid, respectively.

Enzymatic hydrolysis of water insoluble fraction (WIF)

The WIF of all samples were enzymatically hydrolyzed at 5% consistency with 5 FPU/g cellulose cellulase enzyme loading. In Figure 4.1 shows the extent of cellulose and xylan conversion for the different hybrids. The cellulose and xylan conversion highlights the differences in hydrolyzability between different hybrids from two sites. The maximum conversion was obtained after 96hr enzymatic hydrolysis for all the samples and these results were used to compare the hydrolysis of the different hybrids. For coppice poplar samples from the Jefferson site, there was no significant difference between hybrids; the cellulose to glucose conversion was 78-79% and xylan to xylose conversion was 58-60% for both the hybrids. For the two hybrids from Clarksburg, the glucan conversion was similar, while the low-productive hybrid (79%) had higher xylan conversion than the high-productive hybrid (75%). Interestingly, although there was no substantial difference in WIF hydrolyzability between hybrids, the cellulose and xylan conversions differed between sites. Poplar samples from the Jefferson had higher glucan but lower xylan conversion compared to samples from Clarksburg.

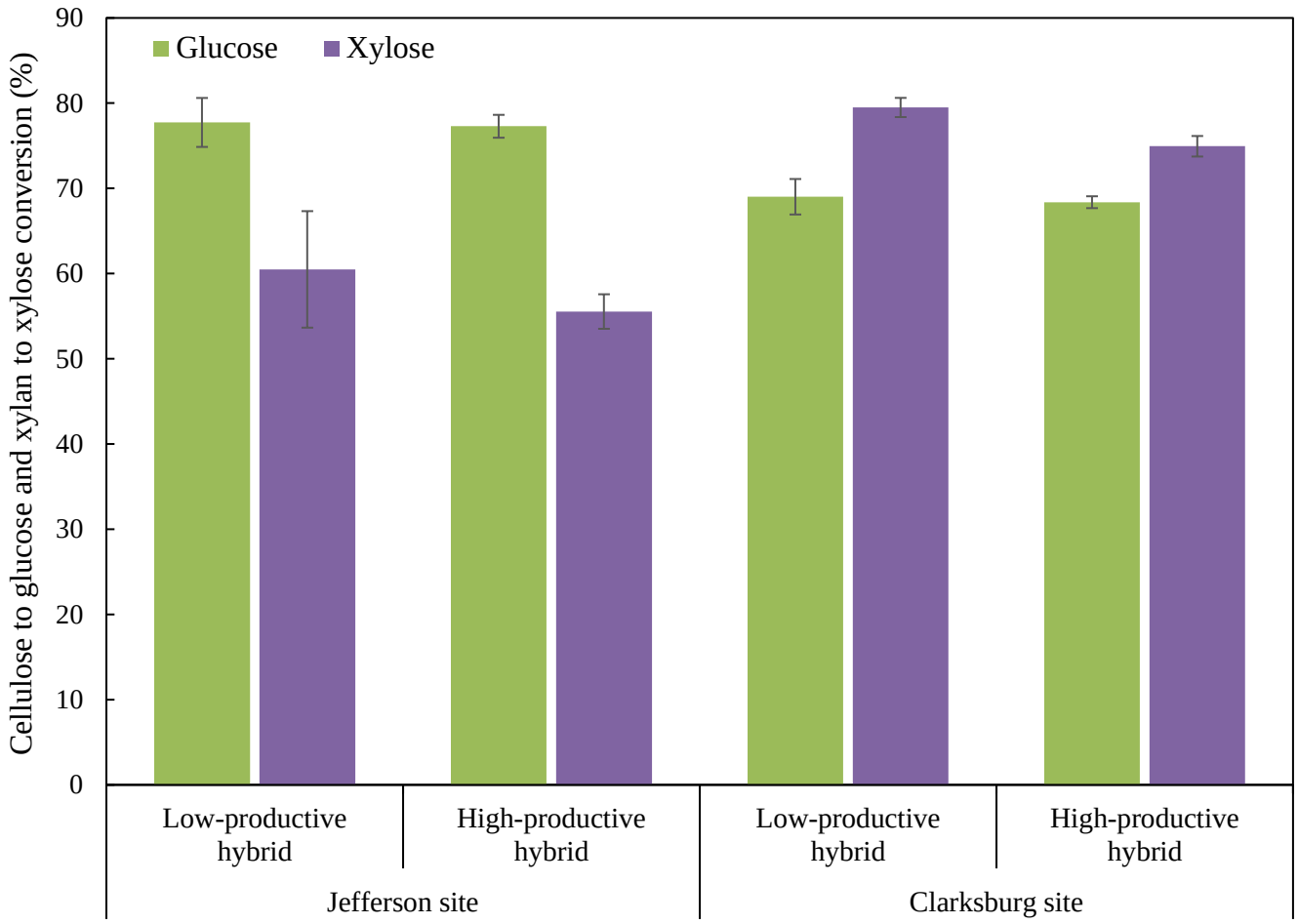

Figure 4.1 96 h cellulose to glucose and xylan to xylose conversion of water insoluble fraction (WIF) of steam pretreated coppice poplar samples. Error bars indicate standard deviation from triplicates

Overall sugar yield

The overall sugar yield was calculated based on the monomeric sugars present in WSF and monomeric sugars from hydrolyzed WIF. It indicates the total amount of fermentable sugars that can be obtained from each coppice poplar sample in the bioconversion process. Figure 4.2 presents the total sugar yield for two hybrids from two sites. It can be seen that the yields range

from 306 kg/tonne to 395 kg/tonne. For the Jefferson coppice poplar samples, 71 kg/tonne more monomeric sugars were recovered from the low-productive hybrid compared to the high-productive hybrid. For the Clarksburg coppice poplar samples, the overall monomeric sugar yield of the low-productive hybrid was 395 kg/tonne – 34 kg/tonne higher than that of the high-productive hybrid.

It is noteworthy that, contrary to the similar original sugar content in raw biomass, the overall sugar yields were substantially different between hybrids for both sites. As Table 4.1 shows, the differences in sugar composition of the raw biomass were relatively small between hybrids in each site. Despite slightly lower original sugar content, the low-productive hybrid achieved 19% higher sugar yield compared to the high-productive hybrid in Jefferson site (Figure 4.2). For the Clarksburg site, both hybrids had identical sugar content in raw biomass, but the sugar yield of the low-productive hybrid was 9% higher than that of the high-productive hybrid. The original sugar composition in raw biomass showed no correlation to the sugar yield. This highlights the difficulty in predicting the bioconversion yield when only given the compositional characteristics.

Biomass sugar content, especially the cellulose content, has been proposed as one of the prime targets to improve the feedstock quality by using classic breeding and genetic transformation [46]. However, the findings in this study indicate that a higher sugar content in feedstock may not yield more sugar in bioconversion. Predicting the conversion yield by only knowing the feedstock chemical composition appears to be difficult; previous research has shown that biomass recalcitrance is affect by multiple elements and cannot be simply judged on one factor [41,168]. As such, test trials that represent the conversion process are necessary to evaluate the

process-oriented quality of lignocellulosic feedstock and potential product yields. Even for the same type of feedstock, trials are needed to better understand the processing performance of feedstock and to eliminate uncertainty from species variabilities [41].

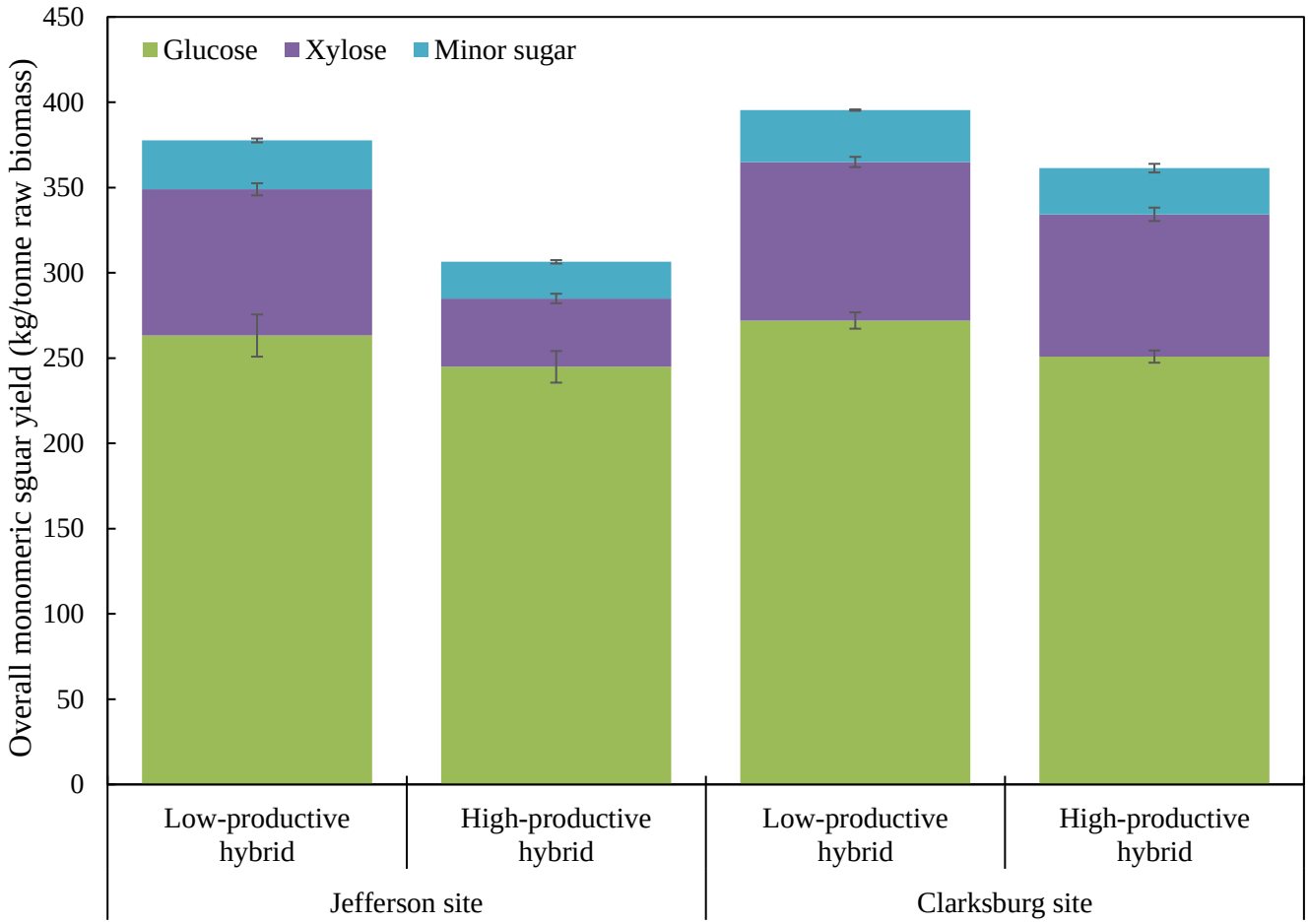

Figure 4.2 Overall sugar yield expressed as monomeric sugar per unit raw biomass (kg/tonne) of coppice poplar samples after pretreatment and enzymatic hydrolysis. Error bars indicate standard deviation from triplicates

4.3.2 **Economics from growers and users vantage**

The contrast between rapid growth and ease of conversion for the poplar hybrids discussed above motivated us to investigate the economic impacts of adopting specific hybrids for use in a biorefinery. Feedstock growers generally evaluate their economics based on the total biomass harvested from the plantation over a given year. Table 4.3 reveals the land productivity varies between hybrids and sites. The low-productive hybrid delivered 26% and 11% less feedstock than the high-productive hybrid in Jefferson site and Clarksburg site, respectively. The difference between sites was more significant. For each hybrid, the Clarksburg site produced 43% and 53% less biomass than the Jefferson site.

The land area required to produce feedstock for the biorefinery is highly dependent on the crop productivity. By assuming one feedstock grower provides all the feedstocks for a biorefinery that processes 700,000 tonne biomass each year, we calculated the land area needed to meet the feedstock supply for each hybrid grown at each site. As shown in Table 4.3, the difference was significant. At the Jefferson site, growing the high-productive hybrid requires 0.65 MM acres less land than the low-productive hybrid, which represents an over 25% land reduction. At the Clarksburg site, selecting the high-productive hybrid could save 0.48 MM acres (11%) of the land area.

Alternatively, switching from a low productivity to a high productivity hybrid would yield more feedstock on a given land base and more revenue for the farmer. If the extra feedstock obtained by switching hybrids is sold as hog fuel at price of $53/tonne [139], replacing the low-productive hybrid by the high-productive one could result in a $2.53 to $3.46 MM/yr additional revenue (Table 4.3). Of note, even modest improvements in land productivity will dramatically reduce

the land area requirement or generate additional revenue. Taken together, the difference in productivity is substantial and will be a key factor for feedstock growers to consider in selecting which clone to plant.

Table 4.3 Plantation economics including land feedstock productivity, land needed to meet the capacity of a commercial-scale biorefinery, and additional revenue of selling surplus feedstock and biorefinery economics including conversion efficiency, annual ethanol production, and annual ethanol revenue using different poplar hybrids from two sites

Site	Hybrid	Plantation economics			Biorefinery economics		
		Land feedstock productivity	Land needed[1]	Additional revenue[3]	Ethanol conversion yield	Annual ethanol production	Annual ethanol revenue
		(tonne/acre/yr)	(MM acre/yr)[2]	($MM/yr)	(liter/tonne)	(MM liter/yr)	($MM/yr)
Jefferson	Low-productive	2.78	0.25	-	214	150	902
	High-productive	3.75	0.19	3.46	179	125	754
Clarksburg	Low-productive	1.59	0.44	-	223	156	941
	High-productive	1.78	0.39	2.53	205	143	864

[1] Land area required to meet the biorefinery with feedstock capacity of 700,000 dry tonne/year

[2] MM stands for million

[3] Feedstock grower additional revenue calculated based on selling surplus feedstock at the price of $53/tonne [139]

The economic perspective of the feedstock user, who is mostly concerned with conversion yields, is different than that of the feedstock grower. Experimental data from this study was applied to economic models investigate the impact of feedstock quality on

ethanol production in a commercial-scale cellulosic biorefinery. We compared the revenue difference in selling ethanol as the final product using different coppice poplars as biorefinery feedstocks. The annual feedstock processing capacity of the simulated biorefinery was set as 700,000 tonne. As Table 4.3 shows, the ethanol conversion yield ranged from 179 to 223 liter/tonne, and the corresponding biorefinery ethanol production of varied from 125 to 156 MM liter/yr. The annual ethanol production using the low-productive hybrid was 16% and 8% higher than the high-productive hybrid for the two sites. Given the ethanol price of $1.59/gallon ($6.00/liter, 2017) [129], the annual revenue of biorefinery using low-productive hybrid as feedstock was $148 MM and $ 78 MM higher than using high-productive hybrid for Jefferson site and Clarksburg site, respectively.

4.3.3 **An overarching performance metric**

To date, no standard has been established for the quality of lignocellulosic feedstocks. Quality standards will be required, however, for lignocellulosic biomass feedstocks to be traded as commodity products.

The current lignocellulosic feedstocks trading is based on the dry weight of biomass. Our findings suggest that if the feedstock conversion facilities do not consider quality variations inherent in the feedstocks, they may experience considerable fluctuations in bioconversion product yield. Even knowing the compositional properties of feedstock, it might still be risky to estimate the final product yield just based on the sugar content in raw biomass. From this study, we suggest biorefineries have to appreciate the quality variations of feedstocks and pay attention to the inconsistency in final product yields.

The definition of commodity-type lignocellulosic feedstock could be reconsidered: not the dry weight of raw biomass or total sugar in the raw biomass, but the total amount of sugars that can be obtained in the conversion process. A more reasonable pricing strategy should be set up based on the feedstock quality that accounts for bioconversion yield.

Given that different hybrids will have different productivity and different conversion yields it would be useful to have an overarching metric that accounts for both of these important economic drivers. A metric of product volume (or mass) per acre per year combines the effects of land productivity and conversion yield. Table 4.4 shows the annual sugar productivity and ethanol output per unit of land between different hybrids and sites. For every acre of land in Jefferson site, the high-productive hybrid could obtain 8.7% higher sugar production and 11% higher ethanol output than the low-productive hybrid. The difference between hybrids in Clarksburg site was smaller - 2.4% for sugar productivity and 3% for ethanol output. In general, the ethanol output from Jefferson site was 40% to 46% higher than Clarksburg site.

Table 4.4 Land productivity in terms of sugar yield, ethanol output, and revenue

Site	**Hybrid**	**Integrated model economics**		
		Land sugar productivity	Land ethanol output*	Land revenue
		(tonne/acre/yr)	(liter/acre/yr)	($/acre/yr)
Jefferson	Low-productive	1.05	594	3,577
	High-productive	1.15	670	4,035
Clarksburg	Low-productive	0.63	355	2,135
	High-productive	0.64	365	2,196

* Fermentation conversion calculated based on NREL 2011 biochemical conversion report [43]

Reconciling feedstock growers and users will be challenging given the current status of the lignocellulosic supply chain and biorefineries. A business model that integrates feedstock plantations and biorefineries would ultimately solve this problem and drive the industry to an overall greater productivity. This strategy is being applied today for different types of crops, including sugarcane and pulpwood in South American and oil palm in Southeast Asia. For example, Klabin – the Brazilian paper manufacturer – has its own nursery, plantation, and pulp mill and uses a metric of tonnes of pulp per hectare per year to assess their overall pulping performance [169]. This allows the company to breed and choose the most productive crop with the best pulp quality. A similar integrated approach in the biofuels industry could enable a more holistic approach to developing the industry.

4.4 Conclusions

The quality of two-year-old coppice poplar varies between hybrids and plantations, leading to different product yields in biochemical conversion. Although having the similar sugar content, the low-productive hybrid showed 9-19% higher sugar yield compared to the high-productive hybrid from two sites. Selection of hybrids can significantly impact the economics for the feedstock users. However, the economics of feedstock growers are more likely to be influenced by the land productivity. A metric that combines the plantation productivity and bioconversion yield would provide an overarching measure of performance. An integrated business model with the plantation being economically tied to the biorefinery would eliminate differences between feedstock growers and users and would improve the overall efficiency of biofuel production.

Chapter 5. **The removal of non-structure components from short rotation coppice poplar as an economic feasible preprocess in biofuel plant** [4]

Abstract

Depending on the lignocellulosic species and the growing conditions, the organic and inorganic non-structural components (NSCs) can contribute to more than 10% of the short rotation coppice poplar. However, the influence of NSCs of biomass on the production of fuels and chemicals is not well known. In this study, we assessed the impact of NSCs removal on the overall sugar recovery and fermentation yield of short rotation coppice poplar after pretreatment and enzymatic hydrolysis. In addition, we focused on evaluating the economics of preprocessing as a new unit process in the biorefinery. Coppice poplar was preprocessed by neutral or acidic washing before steam pretreatment, enzymatic hydrolysis, and fermentation. Preprocessing of poplar significantly reduced as much as 70% ash and 50% extractives contents. The overall sugar yield after pretreatment and hydrolysis was 18-22% higher when the biomass had been preprocessed, which was explained by higher sugar yield in liquid fraction and more efficient enzymatic hydrolysis of solid fraction. The ethanol yield was 36-50% higher for the preprocessed biomass during fermentation of liquid fraction. It appears that preprocessing methods changed the buffering capacity of the biomass via ash removal and thereby improves the enzymatic hydrolysis. Meanwhile, removal of extractives during preprocessing reduced the

[4] Prepared for submission at Applied Energy, C. Dou, H. Hörhammer, R. Gustafson, and R. Bura.

inhibitor formation and improved the fermentation yield. The economic modelling shows that introduction of one preprocessing unit in the biorefinery could bring in $43.1 MM additional gross ethanol revenue and greatly benefit the economics. Based on the results from this study, there is great technical and economic potential for preprocessing of coppice poplar in biochemical conversion.

Keywords: Biomass, preprocess, acidic-neutral wash, steam pretreatment, enzymatic hydrolysis, fermentation, short rotation coppice, economic assessment

5.1 Introduction

The ideal chemical characteristics of lignocellulosic biomass for conversion to fuels and chemical via sugar platform are high carbohydrate content and low lignin content. Considerable research has been done to decrease lignin and increase sugar content in biomass by using genetic engineering techniques [170]. However, there has been minimal research on the role of biomass non-structural components (NSCs) on bioconversion to fuels and chemicals.

Biomass NSCs are a variety of non-chemically bound components of lignocellulosic cell wall that can be extracted using different polar and non-polar solvents such as water, ethanol, benzene, phenols, toluene, and their mixtures [123,171]. They include inorganic minerals i.e. ash, and organic components such as phenols, protein, terpenes, fatty acids and their esters, waxes, polyhydric alcohols, alkaloids, non-structural carbohydrates, and other aromatic compounds [123,172].

The inorganic NSCs are the removable ashes presented in biomass. According to origin and location in the plant, the ash can be divided into separate groups, i.e., (i) soil and sand contamination during harvest, storage, and handling, (ii) inherent vascular ash, and (iii) structural ash [35]. Introduced ashes are the inorganic minerals that adhere to the biomass and can be removed by washing the material. Vascular and structural ashes, also known as physiological ashes, are minerals bound within the cells and cell walls. They are incorporated into the lignocellulosic structure and resistant to washing [35,173]. These types of ash are feedstock specific and are governed by the physiology of the plants, growth stage, and growing conditions. Woody feedstocks are typically lower in inorganic NSCs than herbaceous feedstocks; in the review by Tao et al. [59], the ash content was measured as 0.1–6.4% (mean 1.9%) and 1.0–

26.2% (mean 7.0%) for woody and herbaceous biomass, respectively. In addition, the distribution of ash in different parts of woody biomass differs. For example, ash accounts for only 1.3% of the stem wood for short rotation coppice poplar, but branches, bark, and leaves contain higher level of inorganics of 5.7%, 6.9%, and 10.5%, respectively [139].

The organic NSCs are important for protecting the plant, and are therefore a rich source of bioactive compounds [174]. Organic NSCs (i.e. extractives) content is generally higher for woody biomass than for herbaceous biomass [10]. However, various parts of the tree, e.g. stem, branches, roots, bark, and leaves/needles, differ markedly with respect to both their amount and composition of extractives. The amount of extractives in poplar stem wood is about 10%, whereas the extractives content in the bark fraction can be as high as 36% [22]. Although there are similarities in the occurrence of wood extractives within families, there are distinct differences in the composition even between closely related wood species [22,174]. More than 160 different types of compounds have been reported from poplar species with the majority belonging to the groups of phenolic glycosides, esters, sterols, fatty acids, flavonoids, alkaloids, lactones, lignans, and resins, etc. [22,175].

Depending on biomass species and growing conditions, the NSCs can make up more than 20% of the woody biomass [139]. They can contribute to convertible biomass content decrease since biochemical conversion only uses sugars for production fuels and chemicals. However, we still do not know how the NSCs influence the conversion process of biomass to biofuels and biochemicals, nor if they should be removed prior to conversion process.

The removal of inorganic NSCs has been practiced via deashing process prior to thermochemical conversions to mitigate the risks in catalytic poisoning, slagging, and equipment fouling

[176,177]. Physical ash separation by air classification and ash leaching using water or low concentration acid treatment were developed for thermochemical conversion processes [28,178]. For example, Chin et al. [179] found that ash removal from fast growing woody biomass (*Acacia spp.*) by water leaching (48.5% removal) and acetic acid leaching (56.1% removal). Meanwhile, it also showed that the ash removal considerably improved ash melting characteristics under high temperature and increased the higher heating value (HHV) [179]. Aston et al [178] reported that when corn stover was leached using 0.5 M sulfuric acid, the ash removal increased from 41.3% to 50.4%. In addition to thermochemical conversion, several studies investigated the inorganic NSCs removal in biochemical conversion. A study by He et al. [180] reduced the ash content of corn stover from 9.6% to 5.0% by water washing prior to dilute acid pretreatment, which, thereby, increased the hydrolysis yield from 43.3% to 71.0% and the ethanol yield from 51.7% to 73.5%.

While many studies have investigated the effects of inorganic NSCs in the production of fuels and chemicals from lignocellulosic biomass, there has been less focus on the impact of organic NSCs. For thermochemical conversion, no extensive research has been done on the impact of organic NSCs, mainly because their presence does not show negatively effect on the process. For biochemical conversion, little is known about the effectiveness in the removal of organic NSCs (i.e. extractives). In fact, we believe removal of organic NSCs may occur during deashing processes, but such change was not mentioned or discussed before. To the best of our knowledge, no research conducts a deep discussion about the economic benefits of NSCs removal for the biochemical conversion.

The current research aims to test the influence of organic and inorganic NSCs removal on the sugar yield and ethanol production from short rotation coppice (SRC) poplar after pretreatment, enzymatic hydrolysis, and fermentation. By comparing the conversion yield results between untreated and preprocessed poplar samples, we assess preprocess as an additional unit process in the conversion of SRC poplar to ethanol. The overarching objective of this research is to evaluate the technical and economic feasibility of preprocessing in an industrial scale biochemical-based biorefinery. In general, several factors of the proposed preprocessing unit were modelled and discussed here, including: (i) cost of installing and operating preprocessing unit; (ii) water usage and cost of wastewater treatment; and (iii) increment of ethanol revenue.

5.2 Methods

Poplar biomass was preprocessed with neutral or acidic washing before steam explosion, enzymatic hydrolysis, and fermentation. The neutral wash was conducted with water, whereas the acidic wash was carried out with a dilute sulfuric acid solution. Untreated and preprocessed biomass was steam exploded at 195°C for 5 minutes with SO_2 (3% w/w) impregnation. After separation, the chemical compositions of the solid fractions and liquid fractions were analyzed. The solid fractions were then enzymatically hydrolyzed at 5% (w/v) consistency with 5 FPU/g cellulose enzyme loading. The liquid fractions were fermented. A complete mass balance was conducted to assess the sugar yields for different scenarios. The economics of preprocess was further assessed by comparing the additional cost of preprocessing unit versus the ethanol sale increase.

5.2.1 **Raw material**

2-year-old 2nd cycle short rotation coppice poplar used in this research is a hybrid of *Populus trichocarpa* × *Populus deltoide* obtained from a plantation near Jefferson, OR managed by GreenWood Resources (Portland, OR). The poplar trees were harvested without leaves and chipped in October 2015. All samples were stored and kept frozen at -20 °C until use.

5.2.2 **Preprocess**

The preprocessing conditions were decided based on preliminary experiments for the maximal ash removal and minimal sugar loss. Briefly, the dilute acidic wash was conducted using a 0.1 N sulfuric acid solution at a liquid to biomass ratio of 10 to 1 in a water bath at 80 °C for 3 hours. Following acidic wash, sufficient amount of deionized (DI) water was applied to wash half of the acid processed biomass, in order to remove the residual acids and adjust the pH to neutral condition. At 50:1 water to biomass ratio, dilute acidic washed biomass was soaked in DI water at 25 °C for 4 days with daily water changes. In parallel, neutral wash was carried out by soaking biomass in DI water for 4 days at the same condition. Overall, three different preprocessing scenarios were conducted prior to pretreatment: 1) neutral wash (neutral), 2) dilute acidic wash (acidic), and 3) dilute acidic wash with subsequent neutral wash (acidic-neutral).

After preprocessing the biomass was drained and then centrifuged for 2 minutes. Both the untreated and the preprocessed biomass samples were analyzed for the buffering capacity, total ash content, elemental composition (i.e. specific minerals), extractives, sugars, and lignin. Based on these analyses the influences of different preprocessing methods were compared and discussed.

5.2.3 **Steam pretreatment**

For all the preprocessed biomass and untreated biomass, 300 g oven-dried (OD) biomass was impregnated with 3% (w/w) SO_2 overnight, and then steam pretreated at 195 °C for 5 minutes in a 2.7-liter batch reactor (Aurora Technical, Savona, BC, Canada) [181]. After steam explosion, the pretreated biomass slurry was separated into solid and liquid fractions using vacuum filtration. Anlyses were conducted for the chemical composition of both solid and liquid fractions as mentioned below.

5.2.4 **Compositional analysis**

Ash and extractives

Ash content of raw biomass samples was measured gravimetrically by heating 20-mesh-milled dry biomass to 575 °C for 12 hours [122]. Water and ethanol extractives of raw biomass were determined by weighing the biomass before and after Soxhlet extraction for 20 hours [123].

Elemental analysis

Elemental analysis was conducted to determine the inorganic constituents of biomass samples using ICP methods, as described before [182]. Oven dry samples ground to 40 mesh were digested in accordance with EPA Method 3050. In brief, each sample was digested with nitric acid, hydrogen peroxide, and hydrochloric acid in series at $115^0C \pm 5^0C$ for 5 hours. The sample digest filtrate was then analyzed with inductively coupled plasma mass spectrometry ICP 61E (Thermo Fisher Scientific).

Liquid fraction carbohydrates and degradation products

Monomeric and oligomeric soluble carbohydrates and degradation products were determined using NREL LAP [124]. Briefly, 0.7 mL of 72% H_2SO_4 was added to 15 mL of the liquid samples, and made up the volume to 20 mL with water. Samples were autoclaved at 121˚C for 60 minutes and analyzed by HPLC. Oligomeric sugar was calculated by subtracting monomeric sugar content from total sugar content determined after acid hydrolysis.

Degradation products, such as acetic acid, furfural and 5-HMF were determined using HPLC by analyzing the original liquid samples. Phenolic concentration in the liquid fraction was tested by the Folin-Ciocalteu method [125] using a UV spectrophotometer (Shimadzu, Tokyo, Japan) at 765 nm. Gallic acid was used as calibration standard.

Solid fraction carbohydrates, acetate groups and acid soluble lignin

The chemical composition of raw biomass and solid fraction was determined according to a modified method derived from TAPPI Standard Method [126] and NREL protocols [127]. Briefly, 0.2 g of finely ground oven dried sample was treated with 3 mL 72% H_2SO_4 for 2 hours at room temperature, then diluted into 120 mL total volume and autoclaved at 121 °C for 60 minutes. By filtration through tared sintered glass crucibles, Klason lignin contents were determined gravimetrically. After filtration, the carbohydrate and acetyl composition of the filtrate was analyzed by HPLC and the acid soluble lignin in the filtrate is analyzed by UV spectrophotometer (Shimadzu, Tokyo, Japan) at 205 nm.

High Pressure Liquid Chromatography (HPLC) analysis

The concentration of monomeric sugars from chemical composition analyses and enzymatic hydrolysis was determined with 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) as described before [62]. Deionized water at 1 mL/min was used as an eluent, and postcolumn addition of 0.2 M NaOH at a flow rate of 0.5 mL/min ensured optimization of baseline stability and detector sensitivity. Acetic acid, furfural, 5-hydroxymethylfurfurals (HMF), and ethanol were measured using refractive index detection on a Shimadzu Prominence LC [139]. Separation of these compounds was achieved by an anion exchange column (Rezex RHM Monosaccharide H^+ (8%), Phenomenex, Inc., Torrance, CA) with an isocratic mobile phase that consisted of 5 mM H_2SO_4 at a flow rate of 0.6 mL/min.

5.2.5 **Enzymatic hydrolysis**

Enzymatic hydrolysis was carried out using cellulase (Celluclast 1.5 L, Sigma) at 5 Filter Paper Units (FPU)/g cellulose and β-glucosidase (Novozyme 188, Sigma) at 10 cellobiase units (CBU)/g cellulose [181]. The solid fraction was hydrolyzed at 5% (w/v) consistency in a total volume of 50 mL at 50°C and 175 rpm in a shaker. 50 mM citrate buffer was added to maintain the pH at 4.8, and tetracycline (40 µg/mL) and cycloheximide (30 µg/mL) were used to inhibit microbial contamination [62]. 1 mL sample were taken periodically, and analyzed with HPLC.

5.2.6 **Sugar yield and recovery calculation**

A complete mass balance was calculated using the composition and total mass of each solid and liquid fraction leaving pretreatment and enzymatic hydrolysis [73,139]. Yields and recoveries were calculated based on the input feedstock mass and original sugars available in the raw feed, respectively. The yield was defined as the total mass of sugars in the solid and liquid fractions normalized by the initial oven dry mass of biomass (kg sugars/tonne biomass). Recovery was defined as the total mass of sugars in the solid and liquid fractions normalized by the initial mass

of sugars in the biomass (kg sugars/kg original sugars × 100%). Similarly, monomeric sugar yield was defined as total mass of monomeric sugars in the hydrolyzed solid and liquid fractions normalized by the initial oven dry mass of biomass (kg monomeric sugars/tonne biomass) and monomeric sugar recovery was defined as the total mass of monomeric sugars in the hydrolyzed solid and liquid fractions normalized by the initial mass of sugars in the biomass (kg monomeric sugars/kg original sugar × 100%).

5.2.7 **Fermentation**

The yeast, *Pichia stipitis* ATCC 58376, was used in the fermentation. The strains were taken from -80 °C and maintained on the agar plate (10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose, and 18 g/L agar). Prior to fermentation, cells were grown in seed cultural medium containing glucose (10 g/L), xylose (10 g/L), yeast extract (3 g/L), peptone (5 g/L), urea (2.3 g/L), and $MgSO_4 \cdot 7H_2O$ (1 g/L) at 30 °C and 175 rpm for 48 h in an orbital shaker. After seed growth, cells were harvested, washed, and inoculated at 5 g/L into the fermentation medium. The fermentation medium was prepared based on the liquid fraction after steam pretreatment. The sugar concentration (glucose and xylose) of liquid fraction was brought up to 17 g/L and 30 g/L, respectively using reagent-grade sugars. Besides sugars, other ingredients added into the fermentation medium were same as the seed cultural medium. The fermentations were performed in triplicate using foam-plugged 125 mL Erlenmeyer flasks (semi-aerobic) at 30 °C and 175 rpm. 1 ml sample were taken at the time of inoculation and periodically thereafter for analysis. Ethanol yields, percent theoretical yields and ethanol production rates were calculated based on initial glucose and xylose concentrations using the equations formulated by Keating et al. [183].

Ethanol yields were assessed based on the glucose and xylose consumption and expressed as percent of theoretical ($Y_{\%T}$):

$$Y_{\%T} = \frac{[EtOH]_{max}}{[Sugar]_{ini} \times 0.51} \times 100$$

where $[EtOH]_{max}$ is the maximum ethanol concentration achieved during fermentation (g/L), $[Sugar]_{ini}$ is the total initial sugar concentration during fermentation (g/L), and 0.51 is the theoretical maximum ethanol yield per unit of sugar (g/g) [183].

5.2.8 **Buffering capacity test**

The buffering capacity of poplar biomass was investigated by titration [128,139]. Briefly, 25 g OD weight of raw biomass was soaked in 0.5 L deionized water at a temperature of 80 °C for 30 minutes. Biomass was then removed by filtration and 400 mL of liquid was titrated by 0.004 M H_2SO_4. Dionized water was used as blank for reference.

5.2.9 **Economic assessment methods**

Conceptual Process Design

The overarching goal of the economic assessment is to study the potential economic benefits of introducing preprocess as a new unit process in biochemical conversion. The economic assessment in this study applied the NREL 2011 biochemical design report as the basis [43]. We choose a plant size with a feedstock capacity of 700,000 tonne/year (2,000 tonne/day with 8,400 annual operating hours). The main modifications of the NREL 2011 design include the addition of preprocessing unit and the scale-up of the wastewater treatment unit. Accordingly, we made a conservative estimation of the capital and operating costs of the preprocessing unit and the

increment of capital and operating cost of wastewater treatment unit. Hereinafter, the feasibility of preprocess was assessed by comparing the additional costs and ethanol sale increase.

Preprocessing unit

So far, there is a lack of data for the similar preprocessing unit we propose. Herein, we introduced three sets of acidic holding tanks by using the quotes of deacetylation reaction vessels reported by Davis et al [184]. A belt conveyor is used for spray washing the preprocessed biomass while transferring.

Wastewater treatment unit

Preprocess uses a large volume of water, which eventually increases the load of wastewater treatment unit. The increment of capital and operating cost resulted from the scale-up is calculated following the 2011 NREL design case [43].

Fermentation yield, production, and gross revenue

The ethanol production for the liquid fraction was determined using the experimental results in the current study. The ethanol production for the hydrolyzed sugars from the solid fraction was calculated based upon NREL 2011 report, assuming 95% conversion of monomeric glucose and 85% conversion of monomeric xylose to ethanol, with a theoretical yield of 0.51 g ethanol / g sugar [43]. Based on the recent market, the ethanol selling price was set at $1.50/gal ($5.68/liter) in our calculation [129].

5.2.10 Statistical analysis

The results were subjected to one-way analysis of variance (ANOVA) analysis followed by a Tukey's test. All analyses were carried out in triplicate, unless otherwise stated. The results were

presented as the mean with standard deviation. Chemical composition, enzymatic hydrolysis conversion, and fermentation yield were analyzed based on 5% alpha level (95% confidence interval). Data were analyzed using R (version 3.0.1) software. In this manuscript, any data analysis mentioned as "significant" represents statistically significant ($p<0.05$).

5.3 Results and discussion

2-year-old short rotation coppice poplars were preprocessed in three different ways before steam explosion, enzymatic hydrolysis, and fermentation. In order to clarify the effectiveness of the preprocess, we compared the bioconversion yields between preprocessed biomass with the untreated biomass. The overall monomeric sugar yields (kg monomeric sugars/tonne biomass) and recoveries (kg monomeric sugars/kg original sugars × 100%) and ethanol yields were used to evaluate the impact of preprocess on the steam pretreatment and subsequent enzymatic hydrolysis and fermentation. The economic assessment predicts the feasibility of preprocess as an additional unit process in the biochemical conversion scheme.

5.3.1 Chemical characteristics of untreated and preprocessed poplar biomass

Table 5.1 lists the chemical composition of the untreated and the preprocessed poplar biomass. The total amount of NSCs in the untreated biomass was over 12%, including 2% ash and 10% extractives. Preprocessing methods extensively changed the NSC composition by reducing both ash and extractives contents. The ash contents decreased to 1.5% and 0.6% with neutral wash and acidic wash, which correspond to 26% and 80% of ash removal, respectively. The acid-neutral wash lowered the ash content to 0.8%, resulting in an ash removal of 59%. These

findings were in accordance with the literature, where ash reduction from corn stover increased more than twice when acid was introduced in water leaching [178]. Preprocessing of the poplar biomass also reduced the amount of extractives. The extractives content decreased from 10.6% in the untreated biomass to 5.2-6.1% in the preprocessed biomass – a removal of 45-50% extractives. Das et al., [185] reported similar findings that up to 50% of the extractives were removed during water and acid treatment of sugarcane bagasse. Across all the samples, there was no significant difference in total sugar content ($p = 0.81$). In addition, the compositions of total lignin ($p = 0.07$), and acetic acid ($p = 0.11$) were similar for all the samples. It is critical that the preprocess did not influence the main chemical composition, especially the sugar content, of the raw poplar biomass, but reduced the ash and extractives contents.

Table 5.1 Chemical composition of untreated and preprocessed poplar biomass

	Arabinan	**Galactan**	**Glucan**	**Xylan**	**Mannan**	**Total sugars**	**Total lignin**	**Total ash**	**Extractives**
	%	%	%	%	%	%	%	%	%
Untreated	1.0	1.0	40.8	14.0	1.5	58.3	25.8	2.0	10.6
SD	0.1	0.1	1.5	1.2	0.4	2.8	1.1	0.1	1.3
Neutral	1.0	1.0	40.5	15.1	1.8	59.4	26.2	1.5	5.2
SD	0.0	0.0	0.8	0.3	0.1	1.1	0.2	0.1	0.6
Acidic-neutral	0.4	1.0	40.8	15.8	1.9	59.9	26.6	0.8	6.1
SD	0.0	0.1	1.3	0.7	0.0	2.1	1.5	0.0	2.3
Acidic	0.5	0.9	42.8	13.1	1.5	58.8	28.6	0.6	n.a.
SD	0.1	0.1	2.1	0.6	0.2	2.9	1.4	0.2	

Data represented as the mean of triplicate measurements with standard deviation, extractives as duplicates.

To better understand the effects of ash removal, we analyzed the minerals content for untreated and preprocessed samples. As shown in Table 5.2, the untreated samples contained high amount of calcium (3250 µg/g) and potassium (2225 µg/g) as well as moderate amount of magnesium (449 µg/g), phosphorus (434 µg/g), and sulfur (200 µg/g). Some minerals which are likely to

present in the form of water-soluble components were partially removed through neutral wash [186]; the contents of potassium, phosphorus, and magnesium were reduced by 88%, 76%, and 27%, respectively after neutral wash. The acidic wash removed, in the descending order of percentage, 100% of zinc, 71% of magnesium, 70% of manganese, 68% of the potassium, 49% of calcium, and 44% of phosphorus. Apparently, the acidic preprocessing removed some minerals more effectively than the neutral wash. Chin et al. [179] reported the similar trends in ash removal between water and acidic conditions. The presence of acid plays a big role in ash removal because some ash forming elements tend to have higher solubility at lower pH [179]. Different processing methods reduced different minerals at certain levels. Table 5.2 reveals that neutral wash was more capable in removing minerals like potassium and phosphorus, while acidic wash removed some minerals, such as calcium, iron, and magnesium, more extensively. A subsequent neutral wash following acidic wash was found to further remove most minerals from the biomass, including all potassium and manganese and 94% of magnesium. The combination of the neutral and acidic wash appeared to be more effective in lowering the mineral content. It is likely due to the effect of acidic wash. The acidic condition may induce the mineral solubility and thus allowed water to remove them from the external and internal surfaces of the preprocessed biomass. Different from other minerals, there was limited silica removal in all preprocessing methods. Unlike other minerals, silica plays a role as structural component in biomass as it forms a rigid microstructure that supports the plant tissue structure [186]. Removing silica is difficult without breaking the fiber microstructure and usually requires very basic solutions [179]. For all the biomass treated with acid, the sulfur content increased because of the use of the sulfuric acid in the preprocessing step.

Table 5.2 Mineral content of untreated and preprocessed poplar biomass

	Ba	Ca	Fe	K	Mg	Mn	Na	P	S	Zn	Si
	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g	μg/g
Untreated	23.3	3250	23.8	2225	449	5.9	36.5	434	200	19.9	41.6
SD	0.7	133	1.4	62	22	0.1	1.6	12	10	0.3	8.5
Neutral	24.9	2989	35.4	278	327	5.0	36.5	102	223	20.0	39.6
SD	0.6	113	3.0	75	12	0.1	2.7	3	8	1.0	1.0
Acidic-neutral	23.3	2136	30.3	0.0	0.0	0.0	36.8	219	281	0.0	40.1
SD	0.2	140	3.1	0.0	0.0	0.0	2.5	4	48	0.0	9.0
Acidic	16.2	1667	22.8	703	131	1.8	32.9	245	3459	0.0	44.4
SD	0.6	182	2.1	10	25	0.1	0.7	8	48	0.0	3.0

Data represented as the mean of triplicate measurements with standard deviation (SD).

For all the samples, buffering capacity was measured to evaluate the impacts of different processing methods (Figure 5.1). As an indicator of acid resistance, the buffering capacity describes how the biomass reacts to acidity and is critical for pretreatment [128]. High buffering capacity mitigates the acidic condition and makes the pretreatment less severe towards the biomass [139]. Figure 5.1 presents the titration curves for the water extracts of untreated and preprocessed poplar biomass, where deionized water was used as reference. It appears that for any level of acid addition, the pH of extractant from the untreated biomass is higher than the preprocessed biomass, demonstrating larger buffering capacity. Although started from different pH levels, the water extracts from biomass with neutral wash and acidic-neutral wash reached at the same pH after consumption of 30 mL titrant, indicating the similar buffering capacity. This can be explained by the removal of inorganic NSCs of the biomass. By lowering the contents of inorganic minerals, preprocessing methods reduced the buffering capacities of the biomass [139]. It should be noted that the pH for water extracts from the biomass with acidic wash was already low such that the pH did not change much during titration.

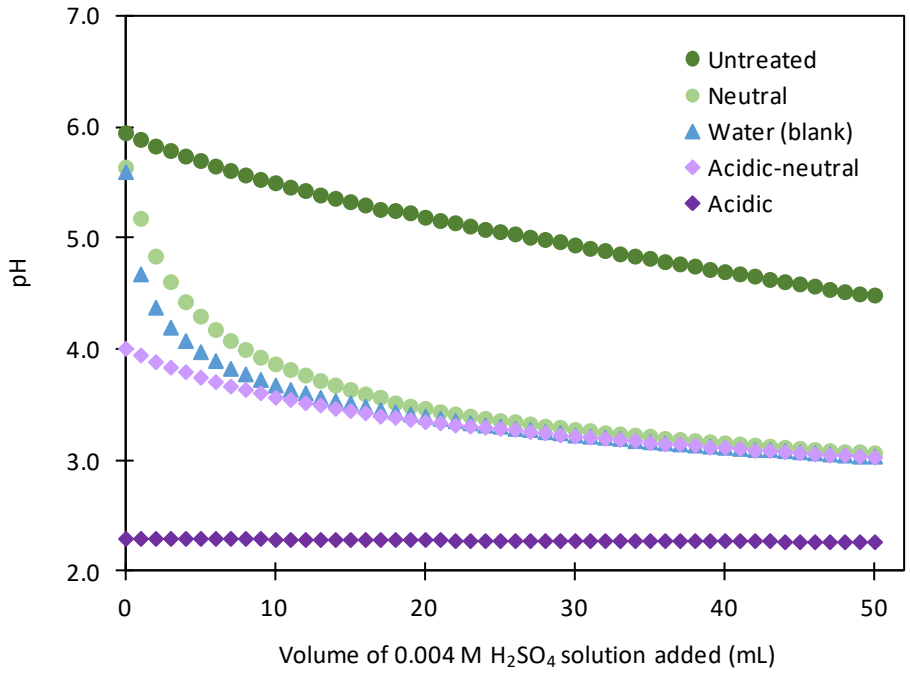

Figure 5.1 Titration curves with 0.004 M H2SO4 for water extractant of untreated and preprocessed poplar biomass, and the deionized water (blank)

5.3.2 Composition of liquid and solid fractions after steam pretreatment

Following pretreatment and liquid–solid separation of all samples, the compositions of the liquid and solid fractions were analyzed. As Table 5.3 shows, preprocess increased both the sugar yield and the percentage of monomeric sugars in the liquid fraction. In particular, the neutral wash released the highest amount of total sugars in the liquid fraction, i.e. 47 kg/tonne, which was 20% higher than that from the untreated biomass. The percentages of monomeric sugars were significantly higher for all the preprocessed samples, representing 8.6% to 11.4% increase compared to the untreated biomass. The difference of total sugar yield and monomeric sugar percentage in liquid fraction can be explained by the pretreatment severity, which is correlated to the biomass buffering capacity. By virtue of reduced buffering capacity, the processed biomass

was subjected to lower pH at higher pretreatment severity (Table 5.3), and thereby released more sugars in the monomeric form in the liquid fraction [34]. The increment of monomeric sugar percentage is critical because it provides more fermentable sugars in the liquid fraction. Previous studies on corn stover have indicated that removing inorganic minerals and organic extractives facilitate the hemicellulose solubilization during pretreatment [180]. In good alignment with those studies, our findings are the first that reveal the preprocess enhanced both the total sugars yield and monomeric sugar percentage in the liquid fraction after pretreatment of woody biomass.

Table 5.3 presents the compositions of the pretreated solids after pretreatment. Apparently, ash content in solid fraction of preprocessed biomass (0.3-1.7%) was significantly lower compared to the untreated biomass (1.4%). It is noted that, regardless of the following natural wash, the acid washed biomass had the lowest amount of residual ash in the solid fraction (0.3-0.4%). The glucan was at the same content ($p = 0.81$) for all the solid fractions. Less xylan (0.6%) was found in the solid fraction from biomass preprocessed with acidic wash, reflecting a positive relationship between the pretreatment severity and xylan removal. Interestingly, the higher lignin content (38.9%) was found in biomass with acidic wash than the others (35.0-36.9%). This is may due to the increased formation of pseudo-lignin at low pH condition (Table 5.3). Previous work by Hu et al., [187] suggested that carbohydrates may convert into pseudo-lignin during acidic pretreatment, especially under high-severity conditions.

Table 5.3 Chemical composition of liquid and solid fraction after steam explosion and cellulose to glucose conversion during enzymatic of untreated and preprocessed poplar biomass

	Pretreatment condition		Liquid fraction						Solid fraction					
			Sugar recovery						Solid yield	Chemical composition				Enzymatic hydrolysis
	Combined Severity	pH	Glucose		Xylose		Total sugars*			Glucan	Xylan	Lignin	Ash	Glucose conversion
			kg/ tonne	mon %	kg/ tonne	mon %	kg/ tonne	mon %	%	%	%	%	%	%
Untreated	1.72	1.78	79.1	75.4	114.8	70.8	232.4	72.9	54.8	59.3	1.7	36.9	1.4	62.4
SD			0.1		1.5		1.8			0.8	0.0	0.6	0.1	0.8
Neutral	1.84	1.66	85.6	83.0	148.5	80.3	279.4	81.5	54.4	58.7	1.9	35.0	0.7	68.3
SD			0.0		0.7		1.3			1.1	0.0	2.5	0.0	0.7
Acidic-neutral	1.88	1.62	66.1	86.0	135.3	81.1	235.1	82.8	58.8	59.8	2.3	36.4	0.3	74.1
SD			0.2		0.5		0.3			1.1	0.0	0.3	0.1	1.0
Acidic	2.08	1.42	88.1	85.8	118.6	83.1	241.6	84.3	53.7	58.1	0.6	38.9	0.4	76.0
SD			0.1		0.8		0.9			0.2	0.0	0.6	0.1	5.2

* Total sugars represent the combination of glucose, xylose, arabinose, galactose, and mannose.
Data represented as the mean of duplicates (liquid fraction) or triplicates (solids fraction) measurements with standard deviation.

The elemental composition of the solid fractions after steam explosion was analyzed and presented in Table 5.4. Some minerals, including potassium, magnesium, manganese, and phosphorus, were totally removed from the pretreated biomass, mainly due to the high solubility [179]. In contrast, some minerals like calcium and silica, remained in the solid fractions at a high content level, as they are likely to be more resistant to the low pH of steam pretreatment condition.

Table 5.4 Elemental composition of solid fraction after steam explosion of untreated and preprocessed poplar biomass

	Ba µg/g	**Ca** µg/g	**Fe** µg/g	**K** µg/g	**Mg** µg/g	**Mn** µg/g	**Na** µg/g	**P** µg/g	**S** µg/g	**Zn** µg/g	**Si** µg/g
Untreated	17.4	2809	24.0	0.0	0.0	0.0	32.2	0.0	1865	0.0	46.5
SD	0.3	263	1.2	0.0	0.0	0.0	2.6	0.0	135	0.0	7.3
Neutral	11.1	1765	19.6	0.0	0.0	0.0	26.3	0.0	1326	0.0	33.7
SD	0.1	64	0.8	0.0	0.0	0.0	0.3	0.0	122	0.0	1.4
Acidic-neutral	5.9	1154	24.6	0.0	0.0	0.0	31.8	0.0	712	0.0	33.6
SD	0.2	23	1.2	0.0	0.0	0.0	0.5	0.0	33	0.0	2.2
Acidic	7.1	1244	23.4	0.0	0.0	0.0	30.4	0.0	772	0.0	46.3
SD	0.1	81	1.9	0.0	0.0	0.0	1.4	0.0	32	0.0	1.0

Data represented as the mean of triplicate measurements with standard deviation.

5.3.3 **Enzymatic hydrolysis of solid fraction**

After steam explosion, enzymes were applied to the solid fractions for both untreated and preprocessed biomass to hydrolyze monomeric sugars which are fermentable. As shown in Table 5.3, preprocess demonstrated an improvement in the digestibility of steam pretreated biomass. After 72 h hydrolysis, higher cellulose to glucose conversions were achieved of solid fractions from the preprocessed samples than the untreated biomass. Particularly, the solid fraction from preprocessed biomass from the acidic wash had the highest conversion of 76%, while the untreated one had a much lower conversion of 62%. The change of digestibility of solid fraction can be explained by the difference in pretreatment severity. As discussed above, because of the removal of ash, the preprocessed biomass attributed to lower buffering capacity. Those samples, pretreated in more acidic and severe conditions, were extensively deconstructed and thereby had higher digestibility in enzymatic hydrolysis [11]. In addition to the impact of pretreatment severity, the different ash contents and mineral compositions of solid fractions can be the other

reason for the difference of enzymatic conversion. Inorganic minerals, especially the metal ions, has been reported to negatively affect cellulase activity and inhibit the hydrolysis yield [27,180]. As Tables 3 and 4 show, the solid fractions from preprocessed biomass contained less ash and lower mineral content. He et al., [180] described a 27.7% increase in enzymatic hydrolysis yield when half of the ash was removed from corn stover prior to the pretreatment. In another study on steam pretreated rice straw, Bin et al., [27] reported the inhibitive effects of cations (e.g. Ca^{2+}) on the cellulase activities once they exceeded certain content thresholds.

5.3.4 **Sugar yield and recovery**

Figure 5.2 summarize the total sugar yield and the sugar recovery from the untreated and preprocessed poplar biomass. The sugar yield and recovery were calculated both after steam pretreatment (Figure 5.2a) and after enzymatic hydrolysis (Figure 5.2b).

After steam pretreatment, the total sugar yield (expressed as kg monomeric sugars per tonne raw biomass) was calculated by combining the sugars in solid fractions and liquid fractions. As Figure 5.2a shows, the total sugar yield for the untreated biomass was 614 kg/tonne after steam pretreatment. Compared to the untreated biomass, 34-37 kg/tonne more sugars were obtained from the preprocessed biomass. Interestingly, the biomass preprocessed with acidic wash gave 601 kg/tonne sugar yield – significantly lower than other preprocessed samples. Similar trend was observed for the sugar recovery, which describes the percentage of theoretical sugar yield from the raw biomass. The sugar recoveries for the biomass with neutral wash and acidic-neutral wash were approximate to 100%, whereas the recovery was only 87% for the biomass preprocessed with acidic wash. This is mainly attribute to the pretreatment severity. As the acidic wash lowered the pH of preprocessed biomass and increased the pretreatment severity [62], it

resulted in more sugar degradation and therefore lower sugar yield and recovery as observed. In contrast, by removing the residual acid from the acidic washed biomass, neutral wash increased pH of preprocessed biomass and suppressed the increment of pretreatment severity, thereby maintained the sugar yield and recovery at the same level as neutral wash.

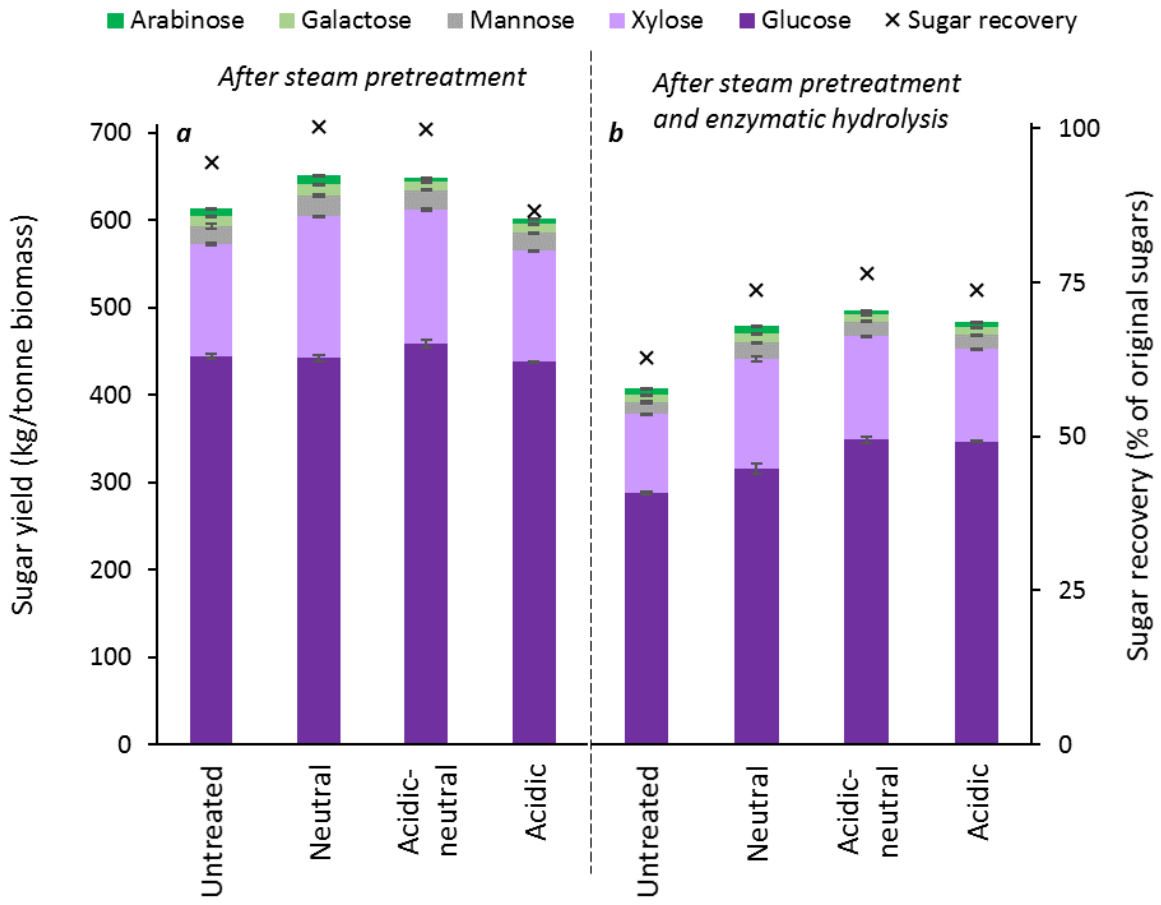

Figure 5.2 Overall sugar yield (kg of sugar per tonne of raw biomass) and sugar recovery (% recovered sugars of original sugars) after steam pretreatment (a) and after steam pretreatment and enzymatic hydrolysis (b) of untreated and preprocessed poplar biomass. Error bars indicate standard deviation from triplicate measurements.

Shown in Figure 5.2b, the overall monomeric sugar yield was calculated by adding monomeric sugars in the liquid fraction and the hydrolyzed solid fraction. It determines the total amount of fermentable sugars available after the bioconversion [139]. As Figure 5.2b depicts, it is apparent that preprocess significantly increased the monomeric sugar yield. The neutral wash increased the sugar yield by 72 kg/tonne from the untreated biomass (405 kg/tonne). Coppice poplar processed with acidic wash achieved a 482 kg/tonne monomeric sugar yield, which was mainly due to its high enzymatic hydrolysis conversion. Interestingly, neutral wash following acidic wash demonstrated an additional 13 kg/tonne increase in sugar yield, resulting in the highest sugar yield of 495 kg/tonne. The sugar recovery follows the same trend as the sugar yield, where the sugar recovery of untreated biomass (63%) was much lower than the preprocessed biomass (74-76%). Among them, the biomass preprocessed with acidic-neutral wash recovered the highest proportion of sugar in the biomass. The results suggest that, by removing the NSCs, preprocess significantly improves the bioconversion efficiency.

5.3.5 **Fermentation of liquid fraction**

Table 5 shows the fermentation yields of liquid fractions obtained by steam pretreatment of untreated and preprocessed poplar using *P. stipitis*. Following fermentation, 5.0% of theoretical ethanol yield was obtained for the untreated biomass, which was distinctly lower than the ethanol yield from the control 88.0% of theoretical ethanol yield. Preprocessing methods significantly improved the fermentation yield. Neutral wash and acidic wash helped to increase the ethanol yield to 41.0% and 42.0% of theoretical ethanol yield, respectively. Among all the preprocessed biomass, the combination of neutral and acidic wash, achieved the highest fermentation yield

(55.0% of theoretical ethanol yield). It appears that the preprocessing methods favored the ethanol production during fermentation of liquid fraction.

Table 5.5 The phenolic concentration (g/L), ethanol yields expressed as maximum ethanol concentration achieved during fermentation (g/L) and as percent of theoretical yield (Y%T) in liquid fractions obtained by steam pretreatment of untreated and preprocessed biomass. The control represents the fermentation of reagent-grade sugars at the same initial sugar concentration.

	Phenolics	**Max ethanol concentration**	**Ethanol**
	g/L	g/L	Y%/T
Untreated	2.02	1.21	5.0
SD	0.03	0.02	0.1
Neutral	1.89	9.43	41.0
SD	0.08	0.02	0.1
Acidic-neutral	1.67	12.89	55.0
SD	0.06	0.06	0.3
Acidic	1.80	9.99	42.0
SD	0.03	0.03	0.1
Control*	-	19.82	83.0
SD	-	0.07	0.3

* The control represents the fermentation of reagent-grade sugars at the same initial sugar concentration.

The phenolic concentrations of liquid fractions provide clues to explain the fermentation results (Table 5.5). Phenolics formed during pretreatment have been identified as the key inhibitors in fermentation. It is notable that preprocessing method lowered the phenolic concentrations in the liquid fraction from 3.6 g/L for the untreated biomass to 3.0 g/L, 2.9 g/L, and 2.5 g/L for the acidic, neutral, and acidic-neutral washed biomass, respectively. Phenolics can be released and generated from different sources [188]. They are formed from lignin degradation during acid-catalyzed steam pretreatment. The acidic environment also contribute to the formation of phenolics from the degradation of sugars [187]. Previous research has shown that lower pH

results in higher severity and generates more phenolics during pretreatment [181]. Surprisingly, the pretreatment pH and severity shown in Table 5.3 reveals the opposite trends of phenolic concentration (Table 5.5). This is because besides lignin and sugars, extractives could act as a big source of phenolics as well [10]. The woody biomass used in this research – short rotation coppice poplar – is a mixture of bark, branch, and juvenile wood and comprises very high extractives content (more than 10%, Table 5.1). It is possible that the high amount of extractives content in the raw biomass determined the phenolic concentration in the liquid fraction.

In addition to the phenolic extractives, other non-phenolic NSCs may also contribute to suppressing the fermentation. Those include aromatic compounds (e.g. benzoic acid and terpenoids) which function as the antimicrobial chemicals and protect the poplar from microbial attack [22,175]. The antimicrobial compounds and their derivatives, are likely to dissolve into the liquid fraction during pretreatment [189]. It is believed that preprocessing methods reduced the total amount of NSCs prior to pretreatment and mitigated the inhibition on microbial performance of yeast. Overall, the removal of NSCs resulted in better ethanol conversion and higher ethanol yield for the preprocessed biomass compared to untreated biomass. The fermentation data in this work clearly indicate that liquid fraction from acidic-neutral wash could achieve the highest fermentation yield (12.89 g/L and 55.0 Y%/T) across all the preprocessed biomass.

5.3.6 **Economic assessment**

The economic assessment gives insights into the potential for introducing preprocess as an additional unit process in the biochemical conversion. The economic benefits of preprocessing are to increase the conversion yield, and, specifically, increase the ethanol production from the

poplar coppice. Nevertheless, such benefits are difficult to quantify without actual operating data. To assess the economic benefits, it is possible to calculate the total project investment increase and compare with the increase in ethanol revenue. The goal of the economic assessment is to make a conservative evaluation to tell if the ethanol revenue increase can justify the preprocess as an additional bioconversion unit.

Among all the preprocessing methods, acidic-neutral wash improved the sugar and ethanol yield most and hence was evaluated in this study. The proposed preprocess design includes three holding tanks and a belt conveyor. The feedstock materials are preprocessed in the holding tanks with a sulfuric acid loading of 4.9 mg/g dry biomass. After draining the tank, the preprocess liquid is neutralized with sodium hydroxide before being pumped to wastewater treatment unit. The preprocessed biomass is discharged from the tank and then spray washed with water on the belt conveyor. Great amount of water usage can be a concern of the preprocess. The design adjusted some assumptions for a modest reduction in water usage, including 1) the liquid to wood ratio is 1:4 in the preprocess, 2) the preprocess lasts 30 mins in the holding tank, 3) the washing water was collected and reused as preprocess liquid after adding more acids. Additionally, we assumed that the preprocessing temperature (80°C) could be achieved by using the waste heat out of the boiler and generator unit.

Table 5.6 Capital and operating cost of preprocessing and wastewater treatment units

	Capital cost ($MM)	Operating cost ($MM/year)	Description
Preprocessing unit	$8.01	$3.00	Preprocessing unit include three batch tanks consisting of discharge conveyors[1] and a belt conveyor where the preprocessed biomass is washed by spraying water.

Wastewater treatment unit	$21.68	$2.30	Numbers stand for additional capital and operating cost account for 81% increase of the wastewater treatment capacity.

[1] Tanks are 316 stainless steel vertical pressure vessels [184]

As shown in Table 5.6, for a biorefinery with 2,000 tonne/day feedstock capacity, the total capital cost of the preprocessing unit is $8.01 MM with $3.00 MM annual operating cost. Meanwhile, the preprocess increases the total flow of the wastewater, which requires $21.68 MM additional capital cost and $2.30 MM increase in the annual operating cost of the wastewater treatment unit. Assuming a payback period of 5 years and an annual equipment maintenance cost of 10% of the installed equipment cost, the annual savings needed to justify purchasing and operating the preprocessing unit can be calculated. By summarizing the yearly payback, operating cost, and maintenance cost, the required annual savings must be no less than $14.36 MM.

As described in the methods, the ethanol yield is calculated based on separated fermentation of liquid fraction and hydrolyzed solid fraction. From that, we assessed the ethanol yield from one tonne of raw biomass and annual ethanol production of the biorefinery. Both ethanol yield and annual ethanol production reveals substantial increments because of preprocess. The results illustrated in Table 5.7 show that the acidic-neutral preprocess increases the ethanol yield from 55.4 gal/tonne to 96.4 gal/tonne and the corresponding annual ethanol production rises from 38.8 MM gal/yr to 67.6 MM gal/yr – a 74% increase. Given the annual ethanol production and ethanol price ($1.50/gal), the gross annual revenue of ethanol from the preprocessed biomass is $101.4 MM/yr, which stands for $43.1 MM/yr more than that from the untreated biomass.

Comparing the investment verses the gross revenue increase, it is not difficult to envision that the preprocess could be an economic feasible unit process in biochemical conversion. The use of preprocessing unit results in a big improvement of the net benefits, though requires a big investment, and can be considered in the design of future biofuel plants.

Table 5.7 Annual ethanol yield and gross revenue for untreated biomass and preprocessed (acidic-neutral) biomass

	Ethanol yield gal/tonne (liter/tonne)	Annual ethanol production MM gal/year (MM liter/year)	Annual gross revenue[1] $MM/year
Untreated	55.4 (209.9)	38.8 (146.9)	58.2
Preprocessed (acidic-neutral)	96.4 (364.9)	67.6 (255.5)	101.4

[1] Ethanol selling price at $1.50/gal ($5.68/liter) [129]

5.4 Conclusions

Preprocess demonstrated a twofold effect on the bioconversion of coppice poplar into ethanol. First, during preprocessing the removal of inorganic NSCs decreased neutralization capacity of poplar biomass, thus increased the severity of the pretreatment which positively affected the release of monomeric hemicellulose in the liquid fraction and the efficiency of enzymatic hydrolysis. All of the factors contributed to increasing overall monomeric sugar yield after pretreatment and enzymatic hydrolysis by 72-88 kg/tonne after the removal of inorganic NSCs from poplar biomass. Second, preprocess removed over 42% of organic NSCs that induced significantly higher sugars to ethanol fermentation yield of the liquid fraction. For all the preprocessing methods, acidic-neutral wash showed the highest overall monomeric sugar yield

(495 kg/tonne) as well as best fermentation yield of 55.0% of theoretical ethanol yield. In the economic assessment, the proposed preprocessing unit demonstrated its economic potential within the current biochemical conversion scheme. These findings illustrate that the application of preprocess will benefit the economics of future biorefineries. To the best of our knowledge, the current work is the first to report the effect of preprocess on sugar yield, fermentation yield, and economics in bioconversion of lignocellulosic biomass.

Chapter 6. **Conclusions and future work**

6.1 Summary and conclusions

Short rotation coppice (SRC) describes a plantation system where woody crops like poplar are intensively cultured to maximize the land productivity. It is seen as an option to produce woody biomass efficiently in a short time, providing a competitive planation model to support the feedstock supply for commercial-scale biofuel production. Growing poplar using a SRC system requires trees to be harvested after two-year growth such that the root system is well established. The current study investigates the conversion of that two-year-old SRC poplar for sugar and fuel production in potential biochemical and thermochemical biorefineries.

The harvested SRC biomass – whole tree coppice (WTC) consists of (whitewood) chip, bark, branch, and leaf. Among them, leaf material makes up more than 1/3 of WTC but has a low sugar content, and high phenolic, ash, and extractives contents. Leaf removed SRC biomass – noleaf coppice (NLC) showed a significant change in chemical composition. Both SRC biomass (WTC and NLC) had lower sugar content compared to the whitewood from forestry plantation (WWF).

For biochemical conversion, leaf removal improved monomeric sugar yield by 147 kg/tonne after steam pretreatment and enzymatic hydrolysis. With the presence of bark and branch, the NLC achieved 67% monomeric sugar recovery, showing no significant difference compared to that of WWF (71%). The overall sugar yield of NLC was 135 kg/tonne less than that of WWF, however, due the low inherent sugar content of the NLC. These findings demonstrate that it is essential to remove the leaves prior to pretreatment to ensure a better overall sugar yield. A

minimum ethanol selling price to cover operating expenses of $1.52/gallon was established from the techno/economic analysis, assuming the NLC feedstock is available for its fuel value.

For thermochemical conversion, fast pyrolysis of WTC and NLC was performed in a fluidized bed reactor and compared with WWF. Leaf removal changed the distribution of pyrolysis products including bio-oil, char, and non-condensable gas. No substantial difference was observed in bio-oil yield and composition during fast pyrolysis of WTC and NLC. Leaf removal changed the char and gas yields as well as the non-condensable gases chemical composition. Compared to the SRC samples, WWF had higher bio-oil yield and lower char yield. The HHV of bio-oil from NLC was 1.3 MJ/kg higher than that of WTC, which was mainly affected by the water content. Leaf removal increased the energy recovery rate from 42.1% to 43.3%. Although the presence of leaves slightly decreases the energy content of bio-oil and the energy recovery rate of fast pyrolysis, our work indicates that leaf removal may not be essential for fast pyrolysis of SRC poplar, given the fact that leaf removal means a big waste of collected feedstock.

Feedstock quality can affect the biochemical conversion yield. Through steam pretreatment and enzymatic hydrolysis, the overall sugar yields differentiate between two leafless poplar hybrids from two short rotation coppice planation sites. For both sites, the low productive hybrids had a 9% to 19% higher sugar yields than high productive hybrids. Given that, the current work investigates the economics of plantation and biorefinery. In the simulated model, we assumed one feedstock grower provides all the feedstocks for a biorefinery that processes 700,000 tonne biomass each year. The economic model of feedstock user shows that the biorefinery will achieve a significantly higher revenue by using low productive hybrid – a 7% to 12% increase than using high productive hybrid. In contrast, the feedstock productivity dramatically influences

the economics of feedstock grower. Growing the high productive hybrid means either 11% to 25% cut in land use or considerable extra revenue from the surplus feedstock. To bridge the gap between feedstock grower and user, we proposed a business model with the integration of plantation and biorefinery to maximize the overall efficiency.

Non-structural components (NSCs) are a variety of components of lignocellulosic cell wall that can be extracted using different polar and non-polar solvents. They include inorganic minerals i.e. ash, and organic components such as phenols, protein, terpenes, fatty acids, and other aromatic compounds. By removing the NSCs using processing methods, we studied the role of biomass NSCs on bioconversion to fuels and chemicals. Preprocess demonstrated a twofold effect on the bioconversion of coppice poplar into ethanol. First, during preprocessing the removal of inorganic NSCs decreased neutralization capacity of poplar biomass, thus increased the severity of the pretreatment which positively affected the release of monomeric hemicellulose in the liquid fraction and the efficiency of enzymatic hydrolysis. It contributed to increased overall monomeric sugar yield after pretreatment and enzymatic hydrolysis by 72-88 kg/tonne after the removal of inorganic NSCs from poplar biomass. Second, preprocess removed over 42% of organic NSCs that induced significantly higher sugars to ethanol fermentation yield of the liquid fraction. For all the preprocessing methods, acidic-neutral wash showed the highest overall monomeric sugar yield (495 kg/tonne) as well as the best fermentation yield of 55.0% of theoretical ethanol yield. In the economic assessment, the proposed preprocessing unit demonstrated its economic potential within the current biochemical conversion scheme. These findings illustrate that the application of preprocess will benefit the economics of future biorefineries.

6.2 Future work

The following items are recommended for expanding future research of this project:

- The growth and maturity of the wood is limited in the first rotation coppice. We would anticipate that the following rotations will have more wood chip fraction, less bark/branch (the second rotation coppice is showing 60% whitewood content in recent harvest), and therefore a higher overall sugar content. The potential sugar yield could be higher during biochemical conversion. But more work in converting SRC poplar of following rotations need to be done to prove hypothesis.
- Bio-oil derived from current fast pyrolysis of SRC poplar was composed of a mixture of oxygenates with a relatively low HHV. An *in-situ* catalytic reaction has been tested in this fluidized bed reactor using other types of biomass, and will be needed to upgrading bio-oil from SRC poplar into fuel additives and chemicals.
- Future work on the techno-economic analysis and life cycle assessment may help to further evaluate the coppice-to-fuel process and make better comparisons between thermochemical conversion and biochemical conversion.

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