Ethanol production from clean chips and whole-tree chips poplar feedstocks: effects of preprocessing on ethanol yield and process economics

Danielle Uchimura Pascoli

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Committee:

Renata Bura

Richard Gustafson

Heidi Gough

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Abstract

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Danielle Uchimura Pascoli

Chair of the Supervisory Committee:
Renata Bura
School of Environmental and Forest Sciences

Biomass feedstock represents up to 40% of the operating costs of ethanol production. The overarching goal of this work is to develop an economically viable process to convert poplar biomass to ethanol. Two different types of poplar feedstocks were compared: poplar clean pulp chips (CPC) with low non-structural components (NSCs) content and thus more expensive; and poplar whole-tree chips (WTC) with high NSCs content and consequently cheaper. NSCs can be very detrimental to the overall ethanol production yields, so a biomass preprocessing step can be used to partially remove these components from the biomass prior to the pretreatment. The objective of the present work was to evaluate and compare the technical and economic feasibility of three biorefinery scenarios: 1) CPC feedstock via pretreatment, enzymatic hydrolysis, overliming, and sugars to ethanol fermentation, 2) WTC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation, and 3) CPC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation. Three different preprocessing conditions were tested (acidic, alkaline, and neutral washes at 80°C for 3 hours). All untreated and preprocessed samples were subjected to steam pretreatment, enzymatic hydrolysis, and fermentation. An additional detoxification step (i.e., overliming) was investigated for the CPC samples. All preprocessing conditions tested were effective in removing NSCs from both types of biomass at different extents. Acidic preprocessing was the most effective, removing 81% and 66% of ash from CPC and WTC, respectively; and removing 42% and 57% of extractives from CPC and WTC, respectively. At the same time, acidic preprocessing achieved the highest improvement in monomeric sugar yield after steam pretreatment and enzymatic hydrolysis (85 kg and 38 kg increase per tonne of WTC and CPC, respectively, when compared to the untreated). This is due to the removal of ash during preprocessing, which lowered the buffering capacity of the biomass and resulted in better solubilization of the monomeric sugars during pretreatment. Fermentation of the liquid fraction after steam pretreatment showed that similar improvements on ethanol yield can be achieved via preprocessing (49-56% ethanol yield) and overliming (45% ethanol yield). However, sugar losses of 20-30% were observed during the overliming step, which reduced the final ethanol yield. Finally, a preliminary economic assessment showed that using WTC via preprocessing (scenario 2) resulted in a return on investment of 920% when compared to scenario 1. This is due to the low price of WTC, and to the high monomeric sugar yield from acidic preprocessed WTC, which in turn increased the ethanol production. Thus, this work will have important implications for increasing the ethanol yield and lowering the lignocellulosic ethanol production costs.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

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1. INTRODUCTION

1.1 ETHANOL FROM LIGNOCELLULOSIC BIOMASS

For the last decades, fossil fuels have been the most used energy source in the world, representing up to 80% of total global energy consumption in 2014 [1]. However, the rapid consumption of these fuels intensified greenhouse gas emissions, contributing to global warming. Not to mention, fossil fuels are non-renewable energy sources, which predicts a supply shortage in the near future. As a result, this scenario promoted a search for renewable, sustainable and non-toxic alternatives. Second-generation ethanol is an attractive renewable fuel that it can be produced from lignocellulosic biomass, an abundant feedstock that does not compete with the food industry. However, the cost of lignocellulosic ethanol production makes this biofuel unable to compete economically with fossil fuels and first-generation biofuels (i.e., corn ethanol). Hence, this work investigated alternatives to lower the costs of ethanol production from lignocellulosic biomass.

According to the National Renewable Energy Laboratory (NREL) [2], the process of converting lignocellulosic biomass into ethanol consists of the following major steps: 1) feedstock handling and preprocessing, 2) biomass pretreatment to release cellulose and hemicellulose carbohydrates, 3) detoxification of the liquid stream to remove fermentation inhibitors, 4) enzymatic hydrolysis of solid stream to break down cellulose into glucose monomers, 5) fermentation of monomeric sugars into ethanol by microorganisms, and 6) product recovery. Techno-economic studies have reported that the biomass feedstock can represent up to 40% of the total operating cost of ethanol production in a biorefinery [3], [4]. There are many different types of lignocellulosic biomass feedstocks, such as agricultural residues (stover, straw, bagasse), forest biomass (softwood and hardwood), and municipal solid waste[5]. Thus, it is critical to evaluate the cost, availability, and chemical composition of a potential feedstock for bioconversion purposes.

1.2 POPLAR FEEDSTOCKS: CLEAN CHIPS VERSUS WHOLE-TREE CHIPS

In the Pacific Northwest (PNW), a potential feedstock candidate for biofuels production is poplar. Poplar trees have high sugar content, are available all year round, have a fast growth rate and can grow on marginal land with minimum water and nutrient requirements. The wood logs from fully-grown poplar trees go through a cleaning and debarking process, resulting in clean pulp chips (CPC) (Figure 1.1). The productivity of these trees is quite low, varying from 6 to 16 dry tonne per hectare per year, and they are harvested every 8 to 20 years. Also, the decade-long harvest cycles, very energy-intensive harvesting, and extra cost for cleaning and debarking the wood logs make this homogeneous woody biomass quite expensive [6]. According to R. Stonex from GreenWood Resources Portland, OR (personal communication, March, 2019), the price of poplar CPC is in average $116 per dry tonne.

Short-rotation coppice is another type of poplar plantation system that has very high biomass yields (up to 25 dry tonnes per hectare per year), and a whole-tree harvesting system that does not require cleaning or debarking [6]. Because short rotation coppice poplar is usually harvested every 2 to 5 years, this biomass has a higher juvenile wood content than CPC [7]. This combination offers a lower-cost poplar feedstock of $77 per dry tonne, according to R. Stonex from GreenWood Resources (personal communication, March, 2019). Because of its whole-tree harvesting system, short rotation coppice is comprised of a heterogeneous mixture of different parts of the tree (wood chips, bark, branches, and leaves). Dou et al. [6] studied the bioconversion of poplar short rotation coppice biomass, and they found that leaves are problematic because of their low sugar content and high ash and extractives content. They stated that the removal of leaves from short-rotation coppice is essential to achieve good bioconversion yields [6]. With this in mind, the present work will use poplar whole-tree chips (WTC), comprised of wood chips, bark, and branches only (Figure 1.1).


Poplar clean pulp chips (CPC)	Poplar whole-tree chips (WTC)

Figure 1.1. Types of poplar biomass.

1.3 LIGNOCELLULOSIC BIOMASS CHEMICAL COMPOSITION

The chemical composition of lignocellulosic biomass varies according to the type of biomass, but overall it comprises mainly of cellulose, hemicellulose, and lignin; and these components are strongly associated within the plant cell wall. Poplar has a cellulose content ranging from 42-49%, hemicellulose from 16-23%, and lignin from 21-29% of the biomass dry weight [8].

Cellulose is a linear homopolymer comprised of D-glucose (hexose) units bound through β (1→4) glycosidic bonds (Figure 1.2). Cellulose chains can form different intra and intermolecular bonds, resulting in a very rigid, crystalline structure. The recalcitrance of cellulose makes it difficult to be depolymerized. The degree of polymerization (DP) of cellulose in various materials range from 305 (rayon fibers) to 15,300 (unopened cotton), while poplar's cellulose DP is approximately 4,500 [7], [9].

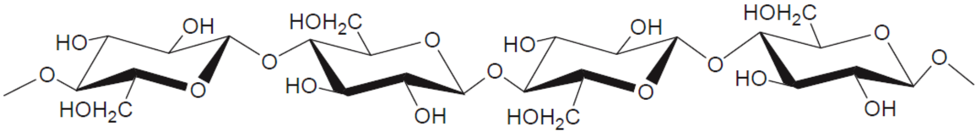


Figure 1.2. Structure of cellulose (reprinted from [8]).

Hemicellulose is comprised of a branched heteropolymer of low molecular weight, containing different 5C and 6C carbohydrate units, such as xylan, mannan, glucan, and galactan. In hardwoods, including poplar, the predominant type of hemicellulose is O-acetyl-4-O-methylglucuroxylan (or glucuronoxylan). This hemicellulose consists of a backbone of xylose units liked by β (1→4) glycosidic bonds, with small branches of 4-O-methylglucuronic acid. Hydroxyl groups at C2 and C3 of the xylose units are substituted by O-acetyl groups in an approximate ratio of about 7 O-acetyl groups per 10 xylose units (Figure 1.3) [7], [10].

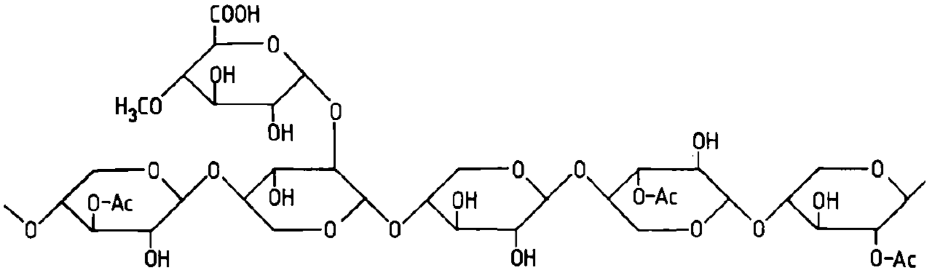


Figure 1.3. Structure of glucuronoxylan from hardwood (reprinted from [7]).

Lignin is a highly branched aromatic polymer comprising of three phenolic compound precursors: *p*-coumaryl, coniferyl, and sinapyl alcohols (Figure 1.4). Lignin provides the mechanical strength to plantsto grow upwards. Hardwoods lignin is the "guaiacyl-syringyl" type, which is composed of coniferyl and sinapyil alcohols with mostly β-O-4 linkages. The majority of the functional groups of hardwood lignin are methoxyl groups [10].

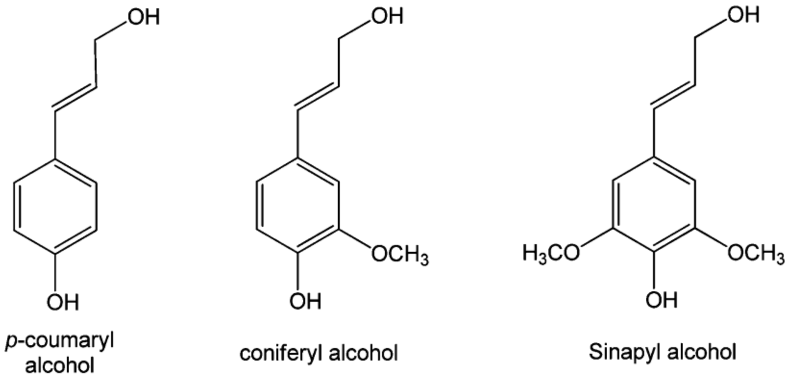


Figure 1.4. Lignin precursors (reprinted from [7]).

Lignocellulosic biomass also contains a variety of non-chemically bound components besides cellulose, hemicellulose, and lignin. These components are inorganic minerals and organic extractives, which are often called non-structural components (NSCs) [4]. In general, fully-grown poplar are comprised of 0.7- 2.2% inorganic minerals and 1.4 - 3.6% extractives [8]. However, the amount of NSCs in woody biomass varies among the different parts of the tree (stem < branches < bark) and the age of the tree (juvenile wood has higher extractives content than mature) [4], [7], [10], [11]. Thus, poplar WTC has a higher NSCs content than CPC because it contains more juvenile wood, bark, and branches in its composition.

The inorganic NSC (i.e., ash) is comprised of inorganic minerals that can be either physiological (i.e., part of the plant’s structure), or originated from deposited metal salts from the soil during harvesting. The most abundant metal component in hardwoods is calcium, followed by potassium and magnesium [7], [10]. He et al. [12] investigated the effects of ash on bioconversion of corn stover into ethanol, and they found that ash negatively affected the dilute-acid pretreatment efficiency because it increased the buffering capacity of the biomass. They also reported that cations in ash caused cellulase inhibition during simultaneous saccharification and fermentation (SSF), decreasing the enzymatic hydrolysis efficiency and consequently the ethanol yield [12].

Thus, biomass ash content is an important consideration for the overall process due to these negative effects.

The organic NSC (i.e., extractives) is comprised of an extremely heterogeneous group of phytochemicals including alkaloids, terpenoids, flavonoids, fatty acids, and a large variety of phenolic substances, among others. More than 160 different compounds are found in poplar species [13]. The main extractives in poplar are phenolics (including phenolic glycosides, phenolic acids, phenolic aldehydes, and others), alkaloids, and fatty acids. Many of these chemicals have antimicrobial, antioxidant, and anti-inflammatory properties that offer protection against microbial and insect attacks [13].

1.4 FERMENTATION INHIBITORS

Fermentation inhibition can occur due to the presence of various compounds in the liquid fraction (also called hydrolysate) after pretreatment. These compounds may be degradation products formed during the pretreatment step, or may be originally present in the original biomass and extracted during pretreatment. Ranatunga et al. [14] reported that organic extractives from hardwoods are highly toxic to xylose fermentation by *Zymomonas mobilis* due to their antimicrobial properties. In addition, the pretreatment severity has a direct effect on the toxicity of the hydrolysate. For example, when the biomass pretreatment is carried out under acidic conditions, high temperature and pressure, sugars and lignin can degrade into compounds (furans and phenols) that can be toxic during fermentation [15]. Overall, the main types of compounds responsible for fermentation inhibition are furan aldehydes, aliphatic acids, and phenols. Furan aldehydes (furfural and 5-hydroxymethylfurfural (HMF), are formed from the degradation of 5C and 6C sugars, respectively, due to the severe conditions of pretreatment (combination of temperature, time and pH). So, the higher the severity, the higher the concentration of furans.

Aliphatic acids are comprised primarily of acetic acid, formic acid, and levulinic acid. Acetic acid is formed from hydrolysis of the acetyl groups present in the hemicellulose structure of hardwoods; so, it is an inevitable by-product. Formic acid and levulinic acid are generated from the further degradation of furfural and HMF when the pretreatment severity is too high. Finally, phenols can be originated either from phenolic extractives present in woody biomass or from lignin degradation [15]. Because these various compounds can inhibit the fermentation and reduce the ethanol yield, a detoxification step is usually applied in order to achieve good fermentability [15].

1.5 DETOXIFICATION

Detoxification of the hydrolysate is typically performed to remove fermentation inhibitors. There are many different chemical, physical, and biological detoxification methods, such as ion exchange, activated charcoal, addition of alkali, vacuum evaporation, microbial or enzymatic treatment, among others [16], [17]. For example, the addition of calcium hydroxide, a process named overliming, has been suggested to be one of the most effective methods to remove furan aldehydes inhibitors [18]. This method was included in the NREL 2002 model of ethanol production from corn stover [2]. Although the specific mechanisms of overliming are still being investigated, some potential mechanisms have been proposed [15], [19], [20]. For example, the detoxifying effect of overliming can be due to the precipitation and vulnerability of some inhibitors at high pH [20]. While a commonly known process, overliming removes only some classes of inhibitors. Thus, it is usually combined with a complementary detoxification step, normally activated charcoal, in order to thoroughly remove inhibitors.

It should be noted that overliming can react in somewhat different ways depending on the feedstock and pretreatment of choice, considering that different feedstocks have different chemical compositions and that different pretreatment conditions (especially pH) can generate different types/amounts of inhibitors. Also, previous studies [15], [16], [21] have reported that overliming causes sugar degradation of about 20% of the initial sugar content in the hydrolysate , thus reducing the final ethanol yield. Since pentose sugars are less stable than hexoses, xylose suffers greater degradation than glucose, for example [18]. Another drawback of overliming is the formation of a solid waste (gypsum) during the reaction, which requires proper separation and disposal, thus increasing operating costs. Under those circumstances, although overliming is necessary in order to achieve good fermentability, it is detrimental for the overall process.

1.6 BIOMASS PREPROCESSING

Overall, two critical challenges for ethanol production from lignocellulosic biomass were investigated in this study: 1) selecting a relatively inexpensive feedstock with a chemical composition favorable for ethanol production, and 2) removing inhibitors from hydrolysate without compromising ethanol yield. In the Pacific Northwest, poplar whole-tree chips (WTC) is recognized as a potential low-cost feedstock for ethanol production. Dou et al. [6] assessed the possibility of using poplar WTC instead of CPC as feedstock for bioconversion, and they reported that leafless WTC achieved an overall sugar recovery as good as that from CPC. However, complications remain, including WTC's high NSCs content. Hence, to turn WTC biomass into a viable feedstock for bioconversion, it is necessary to diminish the effects of its high NSCs content.

Some work toward this goal has been conducted focused on biomass preprocessing, i.e., washing the biomass before pretreatment. Hörhammer et al. [22] investigated the removal of NSCs by preprocessing poplar whole-tree chips with neutral and dilute acid washes, and they reported that ash removal reduced the buffering capacity of the biomass, resulting in higher monomeric sugar yield in the liquid stream. Also, the removal of inorganics resulted in a better digestibility of solid stream during enzymatic hydrolysis. They also reported that dilute-acid preprocessing increased the ethanol fermentation yield from 5% to 55% without any detoxification step due to the removal of organic extractives. In a like manner, Castro et al. [23] discussed the effects of alkaline deacetylation of rice straw, and they reported that the removal of acetyl groups from the biomass before pretreatment improved the ethanol production by about 4-fold without any detoxification.

As can be seen, biomass preprocessing can remove NSCs and significantly improve bioconversion yields, therefore making possible to 1) use cheaper feedstocks, such as heterogeneous WTC or other low-rate materials, and 2) achieve good hydrolysate fermentability without a detoxification step.

1.7 OBJECTIVES

The present work investigated the technical and economic feasibility of three different biorefinery scenarios (Figure 1.5):

1) CPC feedstock via pretreatment, enzymatic hydrolysis, overliming, and sugars to ethanol fermentation,

2) WTC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation,

3) CPC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation.

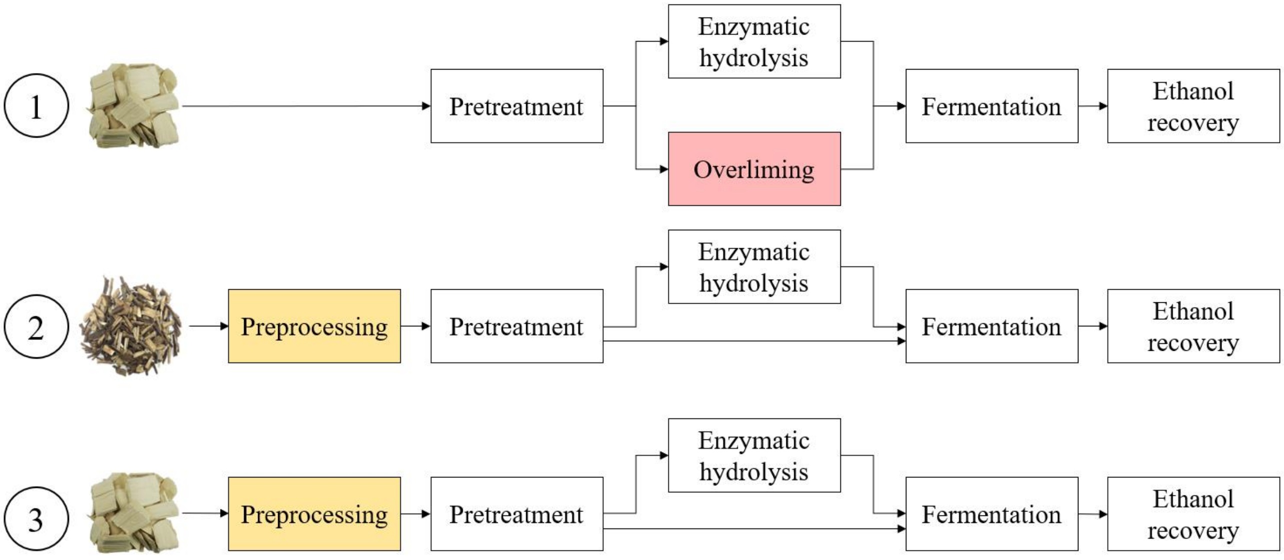


Figure 1.5. Biorefinery scenarios.

The objectives of the study were:

- Compare three preprocessing conditions (acidic, alkaline, and neutral) using both poplar WTC and CPC feedstocks, and determine their overall ethanol yield.
- Assess the possibility of removing overliming before hydrolysate fermentation when preprocessing is done.
- Evaluate preliminary economic assessment of the biorefinery scenarios.

2. MATERIALS AND METHODS

Two poplar feedstocks were used in this work: poplar clean pulp chips (CPC) and poplar whole-tree chips (WTC). Both feedstocks were preprocessed under acidic, alkaline, or neutral conditions at 80ºC for 3 hours. Next, untreated and preprocessed samples were steam exploded at 195ºC for 5 min with 3% (w/w) SO_2 impregnation. The resulting slurry was vacuum filtered using paper filter. Enzymatic hydrolysis of the solid fraction was performed at 50ºC, pH 4.8 for 96 hours. A complete mass balance was conducted to determine the monomeric sugar yield and recovery after steam pretreatment and enzymatic hydrolysis. Both the liquid fraction after pretreatment (i.e., hydrolysate) and the liquid obtained after enzymatic hydrolysis were fermented at 30ºC, pH 6. Overliming before fermentation was assessed for CPC hydrolysates. Finally, a preliminary economic assessment was performed to compare the technical and economic feasibility of three biorefinery scenarios.

2.1 RAW MATERIALS

The two poplar feedstocks used (CPC and WTC) are both hybrids of *Populus trichocarpa* and *Populus deltoids* obtained from a plantation near Jefferson, OR managed by GreenWood Resources (Portland, OR). The feedstocks were harvested and chipped in fall 2016, but under different harvesting systems. The clean pulp chips comprised of 12-year-old poplar trees that were harvested, cleaned, and debarked, resulting in a homogeneous feedstock comprised exclusively of white wood chips. The poplar whole-tree chips comprised of 3-year-old trees harvested without leaves and chipped, resulting in a heterogeneous mixture of white wood (stem), bark, and branches. Both raw materials were stored at -20°C until used.

2.2 PREPROCESSING

Preprocessing was carried out using three different solutions (acidic, alkaline, or neutral), which were conducted using 0.05 M sulfuric acid solution, 0.1 M sodium hydroxide solution, or DI water, respectively. A liquid-to-biomass ratio (volume:mass) of 10:1 was used for all conditions, and the washes were performed at 80ºC for 3 hours in water bath. Acidic and alkaline preprocessed biomass were rinsed and soaked (50:1 water-to-biomass) with DI water at room temperature. Water was changed daily until the pH reached between 5 and 7. All preprocessed biomass were drained and centrifuged for 10 minutes, and the solids were stored at -20ºC until used.

A portion of each WTC sample (including untreated) was manually separated into white wood (stem) and bark. Branches were peeled and separated.

All samples, including the fractions, were analyzed regarding sugars, lignin, total ash content, elemental composition, and total extractives.

2.3 STEAM PRETREATMENT

Steam pretreatment was performed in duplicate for all samples. Briefly, 300 g of oven-dried (OD) biomass was impregnated with anhydrous 3% (w/w) SO_2 overnight, and then steam exploded at 195ºC for 5 minutes in a 2.7 L batch reactor (Aurora Technical, Savona, BC, Canada). After steam explosion, the pretreated biomass slurry was vacuum filtered using filter paper to separate the solid and liquid fractions. The solid fraction was washed using DI water, and the wash-water was analyzed by High Pressure Liquid Chromatography (HPLC), as described in section 2.4.1. The solids were washed until no residual soluble sugars and inhibitors originated from the liquid stream were detected.

2.4 COMPOSITIONAL ANALYSIS

2.4.1 High-Pressure Liquid Chromatography (HPLC) analysis

Monomeric sugar concentration was determined using a Dionex HPLC ICS-3000 system (Sunnyvale, CA) equipped with an electrochemical detector and anion exchange column (Dionex, CarboPac PA1), using DI water at 1 mL/min flow rate as eluent and post-column addition of 0.2 M NaOH at 0.5 mL/min.

Acetic acid, furfural, 5-hydroxymethylfurfural (HMF), and ethanol concentrations were determined using refractive index detection (RID) on a Shimadzu Prominence LC equipped with an anion exchange column (Rezex RHM Monosaccharide H+ (8%) Phenomenex, Inc., Torrance, CA), using mobile phase 5 mM H_2SO_4 at 0.6 mL/min flow rate.

2.4.2 Ash and extractives

The total ash content of all solid samples, including untreated and preprocessed biomass, as well as the solid fraction after steam pretreatment, was measured gravimetrically according to National Renewable Energy Laboratory (NREL) procedure, by heating 0.5 g of oven-dried 40 mesh-ground sample in a muffle furnace at 575ºC for 12 hours [24]. Total extractives content of untreated and preprocessed biomass was determined according to NREL procedure [25], with reflux time of 12 hours for both water and ethanol Soxhlet extraction.

2.4.3 Elemental analysis

The specific mineral content of all solid samples was determined by elemental analysis. Briefly, oven-dried 40 mesh-ground samples were digested in series with nitric acid, hydrogen peroxide, and hydrochloric acid at 155°C for 5 hours. The sample digest was filtered, and the filtrate was analyzed for mineral composition using inductively couple plasma optical emission spectrometry (ICP-OES, Thermo-Scientific, iCAP 6300) [26].

2.4.4 Carbohydrates, acetate groups, and lignin content

The chemical composition of all solid samples was determined according to methods derived from TAPPI Standard Method T 222 [27] and NREL procedure [28]. Briefly, 0.2 g of 40 mesh-ground OD sample was treated with 3 mL of 72% H_2SO_4 for 2 hours at room temperature, followed by dilution with 112 mL of DI water and then autoclaved at 121ºC for 60 minutes. Acid-insoluble lignin content was determined gravimetrically by filtration through tared sintered glass crucibles. Following filtration, the filtrate was analyzed by HPLC, as described in section 2.4.1 (for sugars and acetyl content determination) and by UV/Vis spectrophotometer (Shimadzu UV-1800, Tokyo, Japan) at 205 nm (for acid-soluble lignin content determination).

2.4.5 Liquid fraction carbohydrates and degradation products

Monomeric and oligomeric soluble carbohydrates present in the liquid fraction after steam pretreatment (i.e., hydrolysate) were quantified based on the NREL procedure [29]. Briefly, 0.7 mL of 72% H_2SO_4 and 4.3 mL of DI water were added to 15 mL of liquid sample. The mixture was autoclaved at 121ºC for 60 minutes and analyzed by HPLC, as described in section 2.4.1. Oligomeric sugars were calculated by subtracting monomeric sugar content from the total sugar content.

Concentration of furfural, 5-hydroxymethylfurfural (HMF), and acetic acid was determined by HPLC, as described in section 2.4.1. Total phenolic concentration was determined by the Folin-Ciocalteu method [30] using UV/Vis spectrophotometer at 765 nm (Shimadzu UV-1800, Tokyo, Japan). Gallic acid was used as a calibration standard for total phenolics.

2.5 BUFFERING CAPACITY TEST

The buffering capacity of untreated and preprocessed biomass was determined by titration as described by Hörhammer et al. (2018) [22]. Briefly, 50 g OD weight of biomass was soaked in 1 L of DI water at 80ºC for 30 minutes in a water bath. Next, biomass was separated by vacuum filtration, and 800 mL of filtrate was titrated with 50 mL of 0.004 M H_2SO_4. DI water was titrated as blank.

2.6 ENZYMATIC HYDROLYSIS

Enzymatic hydrolysis of the solid fraction after steam pretreatment was carried out at 5% (m/v) solids consistency in a total volume of 50 mL in an orbital shaker at 50ºC and 175 rpm. 50mM sodium citrate buffer was used to maintain the pH at 4.8. Cellulase (Celluclast 1.5L, Sigma) was added at 67 mg protein/g cellulose to the pretreated biomass, supplemented with β-glucosidase (Novozyme 188, Sigma) at 9 mg protein/g cellulose. 1 mL samples were collected at multiple time points to quantify the cellulose to glucose conversion. Clean pulp chips achieved maximum cellulose to glucose conversion at 48 hours, while whole-tree chips achieved maximum conversion at 96 hours. Regardless of the maximum conversion point, all glucose-rich liquids were filtered after 96 hours of reaction and boiled at for 10 minutes. Finally, the liquid samples were stored at -20ºC until used for fermentation.

2.7 MONOMERIC SUGAR YIELD AND RECOVERY CALCULATION

The monomeric sugar yield and recovery were calculated based on the input feedstock mass and its original sugar composition, respectively. The output total monomeric sugar was obtained by combining the monomeric sugars present in the liquid fraction after steam pretreatment with the amount of monomeric glucose and xylose released after enzymatic hydrolysis of the solid fraction.

Monomeric sugar yield is the total mass of monomeric sugars found in both solid (after enzymatic hydrolysis) and liquid fractions divided by the initial oven-dried mass of raw biomass (in kg monomeric sugars per tonne of oven-dried raw biomass). Monomeric sugar recovery, on the other hand, was defined as the total mass of monomeric sugars found in both solid and liquid fractions divided by the initial monomeric sugar mass in the raw biomass (as a percentage out of the initial mass of sugars).

2.8 OVERLIMING

Hydrolysates were conditioned by overliming following the procedure described by NREL [21], with modifications. Briefly, the hydrolysate pH was increased to 10 with $Ca(OH)_2$ (calcium hydroxide) and incubated at 50ºC for 1 hour with rotation at 150 rpm. Next, the hydrolysate was filtered through a 0.2 µm filter (Fisherbrand disposable PES filter), and the filtrate pH was readjusted to 6 by adding 10N H_2SO_4 solution. The filtered hydrolysates were incubated at 50ºC for 1 hour with rotation at 150 rpm, followed by a final sterile filtration (0.2µm Fisherbrand disposable PES filter).

2.9 FERMENTATION

2.9.1 Microorganism and media

Scheffersomyces stipitis ATCC 58376, also known as *Pichia stipitis* Y-7124, was obtained from ATCC, Manassas, VA. Single colonies were transferred from agar plates to a sterile medium containing 10 g/L glucose, 20 g/L xylose, 3 g/L yeast extract, 5 g/L peptone, 2.3 g/L urea, and 1 g/L $MgSO_4 \cdot 7H_2O$. The inoculum was grown at 30ºC with constant orbital mixing (175 rpm) for 48h. Cells were harvested by centrifugation at 3,000 rpm for 5 minutes at room temperature. The pellets were washed with sterile DI water, and re-suspended in sterile DI water to obtain a concentrated yeast culture. Cell concentration was measured using a UV/Vis spectrophotometer (Shimadzu UV-1800) based on standard curve relating the dry cell weight (DCW) per liter with its corresponding absorbance at 600 nm.

2.9.2 Fermentation

Fermentation was performed on (a) WTC and CPC liquids obtained after enzymatic hydrolysis, (b) WTC and CPC hydrolysates, and (c) CPC overlimed hydrolysates. For the hydrolysate fermentation (with and without overliming), one sample of each type (acidic, alkaline, and neutral) was randomly selected. All samples were supplemented with 3 g/L yeast extract, 5 g/L peptone, 2.3 g/L urea, and 1 g/L $MgSO_4 \cdot 7H_2O$, and the pH was brought to 6 using a 50% NaOH solution, and filter-sterilized (0.2 µm Fisherbrand disposable PES filter) prior to inoculation. A control was prepared using reagent-grade sugars at similar concentrations to those in the experimental samples. The fermentation was performed in duplicate using 125 mL foam-plugged Erlenmeyer flasks (semi-aerobic) at 30ºC and 175 rpm, with 50 mL total volume. Concentrated yeast culture was added to achieve 5 g of DCW per liter. Samples were aseptically collected at the time of inoculation and at multiple time points. 1 mL samples were immediately centrifuged at 10,000 rpm for 5 minutes at room temperature, and the cell-free supernatant was analyzed by HPLC for sugar and ethanol quantification, as described in section 2.4.1.

2.9.3 Ethanol yield and percent theoretical yield calculations

The ethanol yield was calculated based on the ratio between the maximum ethanol concentration achieved during fermentation and the total initial sugar concentration. A theoretical maximum ethanol yield of 0.51 per unit of sugar (g/g) was used to calculate the percent theoretical yield ($Y_{\%T}$) [22] (Equation 2.1).

Equation 2.1. Percent theoretical ethanol yield.

$$\mathrm{Y}_{\%\mathrm{T}} = \frac{\text{maximum ethanol concentration}}{\text{initial sugars concentration } \times 0.51} \times 100$$

2.10 STATISTICAL ANALYSIS

Biomass chemical composition and total monomeric sugars yield were subjected to one-way analysis of variance (ANOVA) followed by a Tukey's test based on 5% alpha level (95% confidence interval). Data were analyzed using Minitab 18 software. Each experiment was analyzed in triplicate unless otherwise stated. Any data analysis mentioned as "significant" represents statistically significant ($p < 0.05$).

3. RESULTS AND DISCUSSION

This study investigated the impacts of preprocessing on the overall bioconversion yields using two poplar feedstocks, and assessed if preprocessing could replace overliming in the ethanol bioconversion process. The preprocessing conditions were chosen based on biomass washing techniques established by Hörhammer et al. (2018) [22] and on deacetylation studies from Chen et al. (2012) [31], which have obtained promising results on the removal of NSCs from the biomass with low sugar loss. A comparison between the monomeric sugar yield and recovery after steam pretreatment, and ethanol fermentation yields of untreated and preprocessed samples was performed. Finally, a preliminary economic assessment was performed of three different biorefinery scenarios: 1) CPC feedstock via pretreatment, enzymatic hydrolysis, overliming, and sugars to ethanol fermentation, 2) WTC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation, and 3) CPC feedstock via preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation.

All percentage increase/decrease showed in this discussion were calculated based on the untreated biomass as the original value. Any data analysis mentioned as “significant” represents statistically significant ($p < 0.05$).

3.1 CHEMICAL COMPOSITION OF UNTREATED AND PREPROCESSED BIOMASS

Table 3.1 shows the chemical composition of untreated and preprocessed poplar whole-tree chips (WTC) and clean pulp chips (CPC).

Table 3.1. Chemical composition of untreated and preprocessed poplar biomasses (as a percentage of the OD weight).

		Ash (%)	**Extractives (%)**	**Glucan (%)**	**Xylan (%)**	**Total sugars* (%)**	**Total lignin (%)**	**Acetic acid (%)**
Whole-tree chips (WTC)	**Untreated**	1.6 ± 0.1^{a}	10.7 ± 0.1^{a}	42.1 ± 0.9^{a}	14.3 ± 0.3^{a}	60.6 ± 1.2^{ab}	28.6 ± 0.8^{a}	4.8 ± 0.2^{a}
	Acidic	0.5 ± 0.1^{b}	4.6 ± 0.3^{b}	41.4 ± 0.9^{a}	15.3 ± 0.2^{b}	59.5 ± 1.2^{bc}	28.1 ± 0.2^{ab}	5.7 ± 0.7^{a}
	Alkaline	1.6 ± 0.1^{a}	4.3 ± 0.4^{b}	42.5 ± 0.5^{a}	15.6 ± 0.1^{b}	61.8 ± 0.7^{a}	27.6 ± 0.2^{ab}	2.7 ± 0.1^{b}
	Neutral	1.4 ± 0.1^{c}	6.8 ± 0.2^{c}	39.6 ± 0.3^{b}	14.6 ± 0.1^{a}	57.9 ± 0.5^{c}	27.4 ± 0.2^{b}	5.2 ± 0.1^{a}
Clean pulp chips (CPC)	**Untreated**	0.6 ± 0.0^{a}	3.5 ± 0.3^{a}	47.9 ± 0.4^{a}	14.4 ± 0.1^{a}	64.7 ± 0.7^{a}	26.1 ± 0.1^{a}	5.0 ± 0.6^{a}
	Acidic	0.1 ± 0.1^{b}	2.0 ± 0.1^{b}	48.4 ± 1.3^{a}	14.4 ± 0.4^{a}	64.9 ± 1.7^{a}	26.1 ± 0.2^{a}	4.4 ± 0.2^{a}
	Alkaline	0.8 ± 0.1^{c}	3.0 ± 0.0^{c}	52.4 ± 0.9^{b}	15.7 ± 0.3^{b}	70.7 ± 1.3^{b}	25.8 ± 0.1^{a}	1.4 ± 0.1^{b}
	Neutral	0.4 ± 0.0^{d}	2.6 ± 0.1^{d}	49.4 ± 0.7^{a}	14.9 ± 0.3^{a}	66.8 ± 1.0^{a}	26.3 ± 0.5^{a}	4.8 ± 0.1^{a}

Data represented as the mean values of triplicate analysis with standard deviation, extractives as duplicates.
Different superscript letters indicate statistically significant differences ($p<0.05$) within each column by Tukey's test (WTC and CPC treatments were compared separately).
*Total sugars include glucan, xylan, arabinan, galactan, and mannan.

3.1.1 Non-structural components (ash and extractives)

Untreated WTC had a greater NSCs content (12% ash plus extractives), than CPC (4%) (Table 3.1). This difference in NSCs content was due to the chemical composition of different parts of the tree. Untreated white wood fraction of WTC contains approximately 7% of NSCs (including ash and extractives), while the bark fraction contains 35% of NSCs (Appendix, Table A.1). Therefore, the untreated WTC biomass showed a higher NSCs content than the CPC primarily because of the presence of bark in the sample. This finding of higher NSCs in bark agrees with earlier reports [32]. Dou et al. [6] compared the chemical characteristics of different fractions of 2-year-old poplar, and they reported that extractives content in bark was about two times higher than that in white wood, while ash content in bark was about five times higher than that in white wood.

However, the higher concentration of NSCs in bark is somewhat mitigated by their lesser mass contribution on the total tree's composition. According to Devappa et al. [13] the white wood (stem) accounts for 52% of the poplar tree mass, while the bark portion accounts for 9%. WTC biomass has a higher juvenile wood content than CPC because it was harvested at a younger age, and trees produce juvenile wood during its first years of growth [7]. Previous studies reported that, overall, juvenile wood has higher extractives content than mature wood [11]. In good agreement with those studies, here the white wood fraction of WTC (mostly comprised of juvenile wood) had 6.3% of total extractives content (Appendix, Table A.1), while the CPC had 3.5%.

The acidic preprocessing was the most effective in removing ash of both WTC and CPC biomass (66% and 81% removal, respectively), followed by neutral preprocessing (11% and 32% removal, respectively) (Table 3.1). These findings are consistent with the literature, where Hörhammer et al. [22] was able to remove 59% of ash from 2-year-old whole-tree poplar chips using an acidic-neutral wash, and He et al. [12] removed 20% ash after neutral washing of corn stover using water at 1:10 water-to-biomass ratio. Alkaline preprocessed CPC biomass showed an approximate 40% increase in total ash content when compared to untreated biomass. This finding might be explained by an accumulation of sodium cations originated from the sodium hydroxide (NaOH) used during the preprocessing step, which is further discussed with the biomass specific mineral content below (Table 3.2). It should be noted that different from CPC, the alkaline preprocessing did not significantly change ($p<0.05$) the total ash content of WTC poplar (only 3% increase when compared to untreated). Although Kundu et al. [33] and Cho et al. [34] also studied deacetylation of homogeneous yellow poplar biomass using diluted sodium hydroxide solution, they did not report its effects on the NSCs content.

Extractives were removed by all preprocessing conditions. Acidic preprocessing removed 57% and 42% of extractives from WTC and CPC biomasses, respectively (Table 3.1). Neutral preprocessing removed almost 20% less than acidic (only 37% and 25% from WTC and CPC, respectively), almost 20% less than acidic. Similar results were reported by Hörhammer et al. [22], where acidic-neutral and neutral washes removed 43% and 51% of extractives from 2-year-old whole-tree poplar chips, respectively.

3.1.2 Sugars, lignin, and acetic acid

The total sugars and lignin content of both WTC and CPC showed minimal variations after preprocessing (Table 3.1). The fact that preprocessing did not compromise the sugar content was favorable for its application in ethanol production.

Acetic acid was removed by alkaline preprocessing (48% and 73% from WTC and CPC, respectively), and no significant ($p<0.05$) removal was obtained with acidic and neutral conditions. These findings are consistent with those from Chen et al. [35], where deacetylation was able to remove 80% of acetyl groups from corn stover.

3.1.3 Elemental composition

An elemental analysis was performed to characterize the mineral composition of untreated and preprocessed biomasses (Table 3.2). Calcium and potassium were predominant in both untreated WTC (3140 µg/g and 1893 µg/g, respectively) and CPC (831 µg/g and 793 µg/g, respectively), followed by magnesium, phosphorus, and sulfur. According to Fengel [7], the main inorganic components found in woody biomass are calcium, potassium, and magnesium. Acidic preprocessing was more effective in removing minerals from both WTC and CPC: potassium and magnesium were completely removed, while calcium was partially removed (43% and 83% removal from WTC and CPC, respectively). Calcium is present in the biomass in different forms, such as acid-soluble salts, non-leachable salts, and as organically bound metal ions which are also very difficult to be removed [36]. Alkaline preprocessing did not remove calcium from WTC, but removed 89% of potassium. CPC completely removed potassium. Not surprisingly, alkaline preprocessing added sodium to both WTC and CPC (692 µg/g and 1594 µg/g, respectively), which can be explained by the occurrence of diffusion process between the wood fibers and the sodium hydroxide solution used during preprocessing. This diffusion occurs due to a concentration gradient between the wood fibers and the external solution, where cations migrate from the most concentrated medium (sodium hydroxide solution) to the least concentrated one (wood fibers). More specifically, the literature describes this process as an ion exchange reaction where the wood cellulose fibers act as cation exchangers when exposed to a solution, being able to exchange its original hydrogen ions for other metal ions present in the surrounding solution [37], [38].

Table 3.2. Elemental composition of untreated and preprocessed poplar biomasses.

		Ca (µg/g)	**K (µg/g)**	**Mg (µg/g)**	**Na (µg/g)**	**P (µg/g)**	**S (µg/g)**
Whole-tree chips (WTC)	**Untreated**	3140 ± 142	1893 ± 25	429 ± 10	0.0 ± 0.0	560 ± 35	228 ± 10
	Acidic	1776 ± 56	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	183 ± 8	152 ± 5
	Alkaline	3248 ± 90	211 ± 19	358 ± 4	692 ± 0.0	250 ± 25	123 ± 7
	Neutral	3256 ± 77	1015 ± 27	391 ± 23	0.0 ± 0.0	495 ± 11	196 ± 4
Clean pulp chips (CPC)	**Untreated**	831 ± 28	793 ± 12	237 ± 19	0.0 ± 0.0	170 ± 5	77 ± 2
	Acidic	140 ± 6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	66 ± 2
	Alkaline	778 ± 52	0.0 ± 0.0	225 ± 20	1594 ± 24	57 ± 3	50 ± 6
	Neutral	744 ± 20	0.0 ± 0.0	157 ±10	0.0 ± 0.0	108 ± 14	70 ± 4

Data represented as the mean values of duplicate analysis with standard deviation.
Other elements were analyzed (including barium, iron, manganese, and silica), but they were either not detected or they were present at trace amounts (lower than 50 µg/g).

3.2 BUFFERING CAPACITY

Buffering capacity of untreated and preprocessed biomass was measured to determine how the biomass pH changes with the addition of a dilute-acid solution. The steam pretreatment step is carried out under acidic conditions, and it has been suggested that ash can buffer the pH reduction during pretreatment, thus decreasing the pretreatment efficacy [39]. Figure 3.1 shows the titration curves for water extracts of untreated and preprocessed biomasses. DI water was used as a reference. Different preprocessing conditions had different initial pH for both WTC and CPC biomasses, possibly due to the presence of residual chemicals from preprocessing step.

The pH of untreated WTC extract remained quite stable with the addition of a dilute-acid solution (pH dropped from 5.4 to 4.8), reflecting the high buffering capacity of the biomass due to higher ash content (Figure 3.1a). Accordingly, alkaline preprocessed WTC had an initial pH of 7.2 due to the formation of sodium hydroxide during water extraction, which in turn buffered the dilute-acid solution during the titration. It is noted that this sample required a high volume of dilute-acid solution to reach a stable pH (approximately 40 mL), demonstrating its higher buffering capacity. In contrast, acidic preprocessed WTC biomass showed a similar behavior as the water blank, indicating a low buffering capacity as a result of its low mineral ash content. Hörhammer et al. had similar results where acidic and neutral preprocessing decreased the buffering capacity of 2-year-old poplar whole-tree chips when compared to untreated biomass due to a lower ash content [22]. The CPC samples presented similar trends as the WTC, as it is shown in Figure 3.1b. It should be noted that alkaline preprocessed CPC started at pH 9 as a result of its higher sodium content (1594 μg/g) when compared to the alkaline WTC sample (692 μg/g).

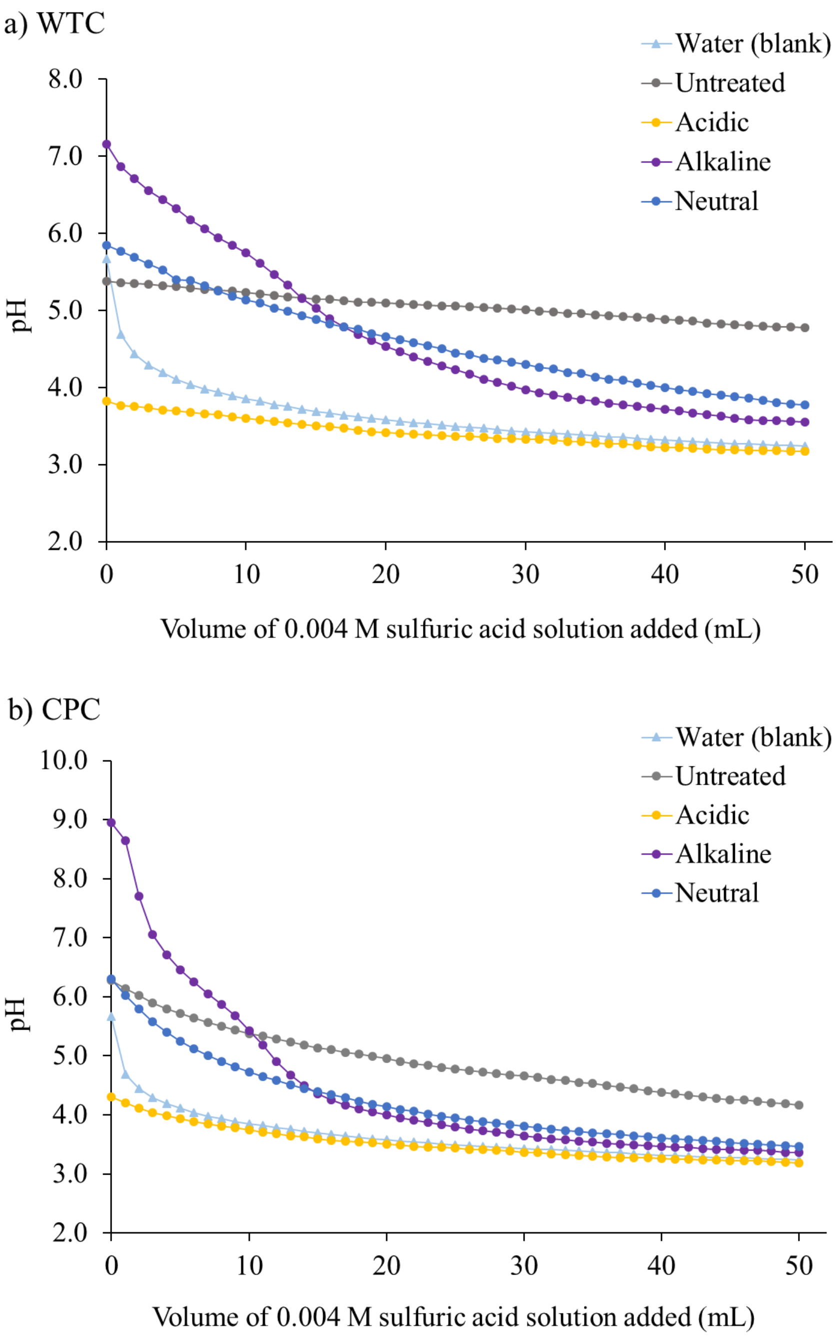


Figure 3.1. Titration curves of untreated and preprocessed poplar biomasses of both (a) WTC samples and (b) CPC samples, along with DI water (blank) as a reference.

3.3 CHEMICAL COMPOSITION OF LIQUID AND SOLID FRACTIONS AFTER STEAM PRETREATMENT AND ENZYMATIC HYDROLYSIS

3.3.1 Chemical composition of liquid fraction (hydrolysates)

Table 3.3 shows the total sugar yield in the hydrolysate, as well as the monomeric sugar percentage. For WTC, acidic and neutral preprocessing had the highest total sugar yield, 258 and 257 kg/tonne respectively, thus recovering approximately 8% more kilograms of sugars per tonne of biomass than untreated WTC. The majority of sugars recovered in all hydrolysates was xylose, representing 50-70% of the total sugars (varying with the preprocessing condition), followed by glucose, which corresponds to 19-40% of the total sugars (Appendix, Table A.3). Minor sugars, such as arabinose, galactose, and mannose were also present in the hydrolysates at lower amounts, which was expected since these sugars were present at trace amounts in the original biomass [6]. Acidic preprocessing released 91% of monomeric sugars from the biomass, approximately 12% greater than all other samples, including untreated biomass. Previous studies reported that the removal of NSCs from biomass can enhance the hydrolysability of hemicellulose during the pretreatment step as a result of a lower buffering capacity effect [12], [22]. In good agreement with those studies, acidic preprocessing showed the highest removal of NSCs (66% removal of ash) and lower buffering capacity, which improved the hemicellulose hydrolysis into monomers.

For CPC, acidic preprocessing had the highest total sugar yield of 213 kg/tonne of biomass, approximately 27% higher than untreated biomass (168 kg/tonne). Unlike WTC, all CPC samples had a monomeric sugar percentage above 93% as a result of the original low NSCs of this biomass, with the highest percentage of 101% for acidic preprocessing. When comparing WTC and CPC total sugar yield in the liquid fraction, WTC recovered a greater mass of sugars per tonne of biomass than CPC as a result of higher hemicellulose solubilization during pretreatment. This outcome is possibly related to differences in anatomical properties between the WTC and CPC wood. According to Bao et al., the fibers in juvenile wood are about 24% shorter than those in mature wood, thus being more susceptible to the steam pretreatment reactions and resulting in a better hemicellulose extraction and more sugars in the liquid fraction [11].

Table 3.3. Chemical composition of liquid and solid fractions after steam pretreatment, and enzymatic hydrolysis conversion.

		Liquid fraction			**Solid fraction**					
					Solid recovery	**Chemical composition**				**Enzymatic hydrolysis**
		pH	**Total sugars** [1]			**Glucan**	**Xylan**	**Lignin**	**Ash**	**Glucose conversion** [2]
			kg/tonne	mon % [3]	%	%	%	%	%	%
Whole-tree chips (WTC)	**Untreated**	1.8	239 ± 7	79.0%	63.4	60.7 ± 0.5	2.4 ± 0.0	36.6 ± 0.3	1.0 ± 0.3	67.7 ± 1.4
	Acidic	1.4	258 ± 2	91.1%	63.0	62.7 ± 0.1	1.3 ± 0.0	38.4 ± 0.9	0.2 ± 0.0	75.7 ± 1.2
	Alkaline	1.6	245 ± 5	78.7%	67.7	63.1 ± 0.7	2.6 ± 0.0	35.2 ± 0.1	0.5 ± 0.2	72.7 ± 1.2
	Neutral	1.6	257 ± 9	78.9%	62.7	61.9 ± 0.5	1.6 ± 0.1	37.6 ± 0.3	1.0 ± 0.1	72.8 ± 0.6
Clean pulp chips (CPC)	**Untreated**	1.4	168 ± 5	96.2%	63.9	68.0 ± 1.1	1.2 ± 0.1	34.4 ± 0.4	<0.1%	78.2 ± 2.3
	Acidic	1.4	213 ± 4	101.2%	63.6	66.8 ± 1.1	0.0 ± 0.0	33.6 ± 0.2	<0.1%	76.7 ± 1.2
	Alkaline	1.6	176 ± 1	93.4%	72.3	72.9 ± 0.6	2.4 ± 0.0	28.3 ± 0.2	<0.1%	69.8 ± 1.4
	Neutral	1.5	201 ± 5	93.5%	68.6	70.6 ± 0.3	1.4 ± 0.1	31.5 ± 0.5	<0.1%	73.2 ± 0.1

Data represented as the mean values of triplicate analysis, which in turn comes from two steam pretreatments replicates.

[1] Total sugars include glucan, xylan, arabinan, galactan, and mannan.

[2] Maximum glucose conversion: WTC samples after 96h of hydrolysis, and CPC samples after 48h.

[3] Monomeric sugar percentage of the total sugars

3.3.2 Chemical composition of solid fraction and enzymatic hydrolysis conversion

Table 3.3 shows the maximum cellulose to glucose conversion after enzymatic hydrolysis (EH) of WTC (after 96h of reaction) and CPC solids (after 48h of reaction). Conversion is calculated based on the final mass of glucose obtained after EH, divided by the initial mass of unconverted glucose in each solid fraction. For WTC, all preprocessed samples had higher conversions than untreated. Untreated WTC had the lowest conversion of 68% as a result of the combination of higher xylan and ash contents (2.4% and 1.0%, respectively) when compared to the preprocessed samples. Several studies [40]–[42] have reported that xylan (hemicellulose) has a negative effect on cellulose digestion because it behaves as physical barrier blocking the access of enzymes to the cellulose fibers. Additionally, He et al. [12] and Bin [12], [43] reported that certain cations, including calcium, can negatively affect the hydrolysis by inhibiting the activity of endoglucanases and exoglucanases. Accordingly, acidic preprocessed WTC resulted in the highest conversion of 76% (8% improvement when compared to untreated WTC) due to its low xylan and ash contents (1.3% and 0.2%, respectively). The lower xylan content is associated with greater solubilization of the hemicellulose during pretreatment, resulting in less residual xylan in the solid fraction [44].

Overall, the enzymatic hydrolysis of all CPC samples was faster than that of WTC, which may be explained by an overall lower ash and lignin content of CPC solids, resulting in better hydrolysis. Surprisingly, alkaline preprocessed CPC solids had the lowest enzymatic hydrolysis conversion of the CPC samples (70% after 48 h of hydrolysis), including untreated. Despite the fact that all CPC solid fractions had ash content lower than 0.1% (because of the initial low ash content of this biomass, shown in Table 3.1), the low cellulose to glucose conversion of alkaline preprocessed biomass is probably associated with the higher xylan content of 2.4%, which prevented enzyme accessibility to the cellulose fiber, as discussed previously. The higher residual xylan content in the alkaline preprocessed CPC solid fraction is a result of a lower hemicellulose solubilization during pretreatment, which was caused by the higher buffering capacity of this biomass. Additionally, alkaline preprocessed CPC resulted in the highest solid recovery after steam pretreatment (72%), which is another indicator of the lower solubilization of sugars during pretreatment.

3.3.3 Elemental composition of solid fraction

The elemental composition of solid fraction is shown in Table 3.4. Potassium, magnesium, sodium, and phosphorus were utterly removed in all WTC and CPC samples during steam pretreatment. Calcium, conversely, was removed to a lesser extent due to its lower solubility [36], as mentioned earlier in section 3.1.3. In comparison to the original calcium content of WTC samples before steam pretreatment (Table 3.2), alkaline preprocessing showed the greatest calcium removal during pretreatment (29% removal from the initial calcium content in alkaline preprocessed biomass), followed by acidic (24% removal), and neutral (15%). Untreated WTC had a minor calcium removal of only 4% when compared to the original calcium content in untreated biomass.

In contrast, calcium was removed to a greater extent from all CPC solid samples. Neutral preprocessing had the highest calcium removal of 92% (Table 3.4). Additionally, untreated CPC solids showed a calcium removal of 85% when compared to the original calcium content in CPC biomass (Table 3.2), which is 20 times greater than the removal in untreated WTC solids (4%). As discussed previously in this manuscript, the difference in calcium removal between WTC and CPC biomasses can be attributed to the presence of bark in WTC.

Finally, an increase in sulfur content after stem pretreatment was observed in all samples, which originates from the SO_2 used during biomass impregnation. When compared to the sulfur content of the biomass prior to pretreatment (Table 3.2), WTC samples had an increase of sulfur content in the order of 4 to 9-fold, while CPC samples increased by 6 to 11-fold.

Table 3.4. Elemental composition of solid fractions after steam pretreatment.

		Ca (μg/g)	**S (μg/g)**
Whole-tree chips (WTC)	**Untreated**	3000 ± 171	1062 ± 13
	Acidic	1349 ± 55	811 ± 22
	Alkaline	2315 ± 10	1084 ± 16
	Neutral	2766 ± 81	1144 ± 19
Clean pulp chips (CPC)	**Untreated**	126 ± 16	486 ± 20
	Acidic	97 ± 19	408 ± 12
	Alkaline	99 ± 10	562 ± 21
	Neutral	57 ± 6	431 ± 11

Data represented as the mean values of duplicate analysis with standard deviation.
Magnesium, potassium, phosphorus, and sodium were completely removed (0 μg/g) during steam pretreatment from both WTC and CPC.
Other elements were analyzed (including barium, iron, manganese, and silica), but they were either not detected or they were present at trace amounts (lower than 50 μg/g).

3.4 MONOMERIC SUGAR YIELD AND RECOVERY AFTER PREPROCESSING, STEAM PRETREATMENT, AND ENZYMATIC HYDROLYSIS

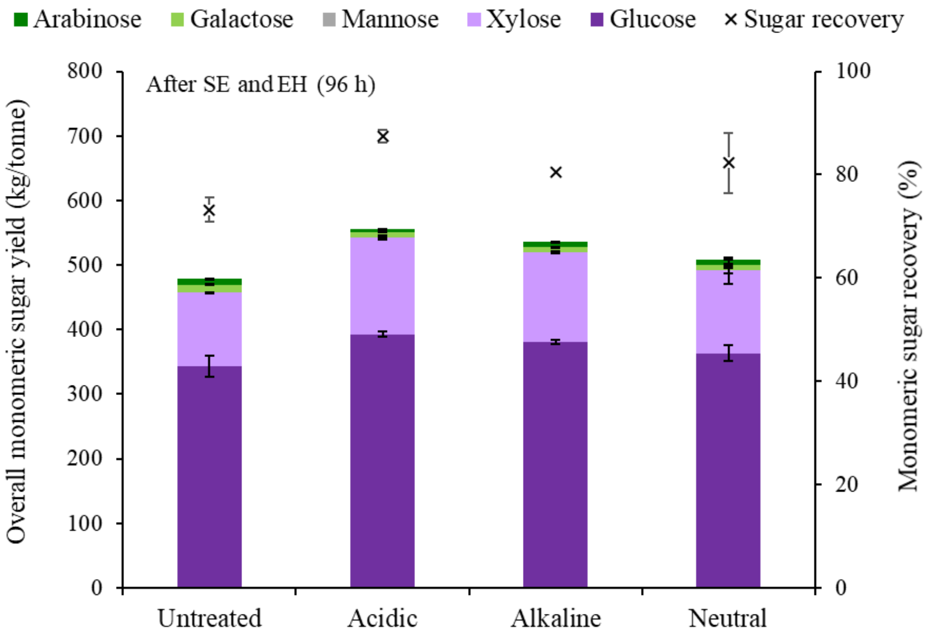
Figure 3.2 shows the total monomeric sugar yield and recovery after steam explosion (SE) pretreatment and enzymatic hydrolysis (EH) for both WTC (Figure 3.2a) and CPC (Figure 3.2b). The complete data, including statistical analysis, can be found in the Appendix, Table A.4. Untreated WTC had a monomeric sugar yield of 493 kg/tonne. WTC preprocessed under acidic condition resulted in a significantly ($p<0.05$) higher yield of 578 kg/tonne, followed by alkaline

(553 kg/tonne) and neutral (529 kg/tonne). Acidic preprocessing significantly increased ($p<0.05$) the monomeric sugar yield by 85 kg per tonne of biomass when compared to untreated possibly due to 1) lower buffering capacity of acidic preprocessed biomass (Figure 3.1), 2) higher enzymatic hydrolysis conversion (Table 3.3), and 3) better hemicellulose hydrolysis during steam pretreatment resulting in more monomeric sugars in the liquid fraction (Table 3.3). Therefore, these results suggest that acidic preprocessing improved the monomeric sugar yield of WTC biomass. Hörhammer et al. [22] reported similar results using 2-year-old poplar WTC, where acidic wash resulted in 90 kg increase in monomeric sugar yield when compared to untreated.

For the CPC samples (Figure 3.2b), acidic (577 kg/tonne), alkaline (573 kg/tonne), and neutral (580 kg/tonne) preprocessing showed significant ($p<0.05$) improvement in the monomeric sugar yield when compared to untreated CPC (539 kg/tonne). There was no statistically difference ($p<0.05$) in monomeric sugar yield between the three preprocessing conditions.

The monomeric sugar recovery of WTC improved with preprocessing (Figure 3.2a). Acidic preprocessing achieved remarkable 87.5% monomeric sugar recovery – 14% more than untreated WTC (recovery of 73%). Neutral and alkaline preprocessed WTC had monomeric sugar recovery of 82% and 81%, respectively. CPC samples only showed minor differences in monomeric sugar recovery (Figure 3.2b). The highest recovery was achieved by acidic preprocessing (80%), only 5% higher than untreated CPC (75%). Overall, WTC samples recovered more monomeric sugars after steam pretreatment and enzymatic hydrolysis than CPC samples. This can be explained by the different morphology of these biomasses that led to higher hemicellulose solubilization, as discussed previously.

a) WTC



b) CPC

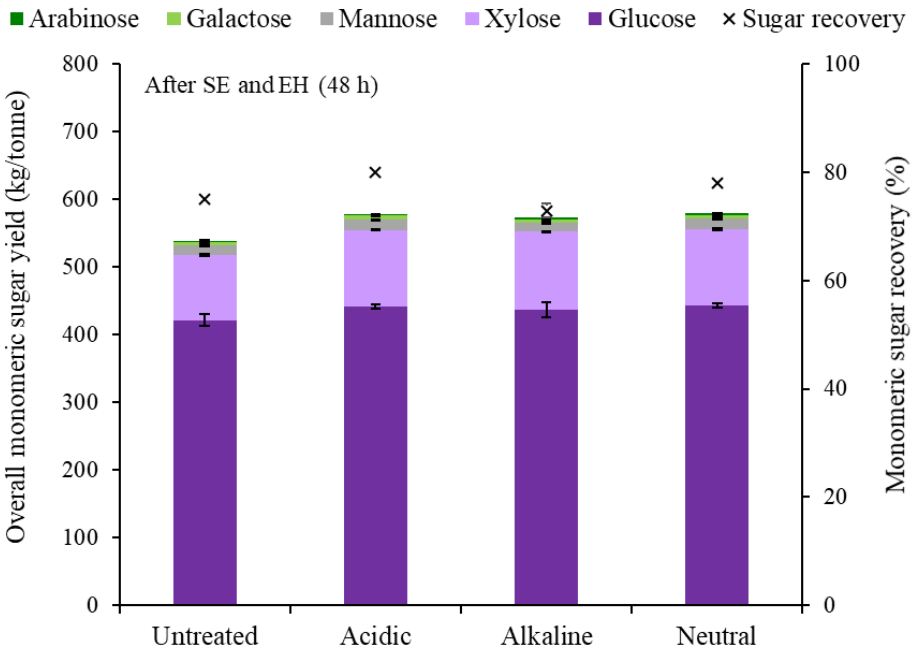


Figure 3.2. Total monomeric sugar yield (kilograms of monomeric sugars obtained per tonne of oven-dried raw biomass) and monomeric sugar recovery (percentage of monomeric sugars recovered from original sugars) of (a) WTC samples and (b) CPC samples.

3.5 FERMENTATION AND OVERLIMING

Table 3.5 shows the ethanol fermentation yields of hydrolysates (without overliming) and liquid resulted from enzymatic hydrolysis. General trends were observed in the fermentation of both WTC and CPC hydrolysates. First, untreated samples showed negligible ethanol yields (1.9% and 1.1% of theoretical ethanol yield for untreated WTC and CPC, respectively). In contrast, all preprocessed WTC and CPC samples showed significant improvement in ethanol conversion, with yields ranging from 49% to 56%. Thus, preprocessing improved the fermentability of both WTC and CPC hydrolysates without requiring extra overliming detoxification step. This might be explained by the removal of specific types of extractives with antimicrobial characteristics during preprocessing (Table 3.1), which were inhibiting the fermentation of untreated samples [13], [14]. Similar trends were reported by Hörhammer et al. [22], who observed a 50% increase in ethanol yield when acidic preprocessing was performed with 2-year-old whole-tree chips poplar biomass.

Table 3.5 also shows the fermentation yields of enzymatic hydrolysis liquid. Since this liquid contained only monomeric sugars and no inhibitors, all samples reached a maximum ethanol conversion after 8h of reaction, as well as very similar yields ranging from 82 to 85% (fermentation control showed an ethanol yield of 84.6% ± 3.8%).

Table 3.6 shows the fermentation results of overlimed CPC hydrolysates, as well as the sugar loss resulted from overliming. Overliming increased the ethanol fermentation yield of untreated CPC from 1.1% (Table 3.5) to 45%. Similar results were observed by Cantarella et al. [45], when comparing different detoxification methods for steam-exploded poplar and reported that overliming increased ethanol production rate by 30%, while untreated samples were reported as unfermentable.

Regarding the preprocessed CPC samples with overliming, the overall results were quite similar (53-54% ethanol yield). As an exception, alkaline preprocessed CPC that reached an ethanol yield of 65% (Table 3.6). Thus, it can be seen that overliming did not add improvements to the already preprocessed CPC samples, which ethanol yields were ranging from 52 to 55% (Table 3.5). Also, when comparing the increase in CPC fermentation yield exclusively via overliming (from 1.1% to 45%) with that via preprocessing only (from 1.1% to 52-55%), it can be seen that similar improvements were achieved by both methods.

Also, it can be noted in Table 3.6 that overliming resulted in a sugar loss of 30% in untreated CPC, and about 20% in all preprocessed CPC samples. Even though the fermentation yields (ratio between maximum ethanol concentration and initial sugar concentration) increased, the fact that the initial sugar concentration decreased by 20-30% automatically resulted in a lower concentration of ethanol. This represents a problem for the biorefinery, because it would have a lower ethanol production per tonne of biomass. Martinez et al. [18] also reported sugar losses during overliming of sugarcane bagasse hydrolysates, especially on pentose sugars because they are less stable, which caused a reduction on the final ethanol concentration. Under those circumstances, preprocessing seems to be a better option because it improves the ethanol fermentation yield without compromising the sugars.

It should be highlighted that the goal of the fermentation experiments in the current study was to show the main effects of biomass preprocessing and overliming on the ethanol yield. It was not in the scope of this study to identify which preprocessing condition would lead to the highest yield. In addition, this fermentation experiment was carried out using a non-genetically engineered microorganism. NREL commonly uses a recombinant co-fermenting bacteria in order to maximize ethanol production, thus achieving 85 to 95% conversion of sugars to ethanol [46]. Hence, in this particular case where the fermentation is already optimized, the concentration of monomeric sugars available for fermentation is the determining factor for the final ethanol yield.

To summarize, biomass preprocessing showed great potential in increasing the monomeric sugar yield after steam explosion and enzymatic hydrolysis using both poplar CPC and WTC (Figure 3.2), thus improving the ethanol production. Additionally, it was shown that overliming caused big sugar loss in the CPC hydrolysates, which decreased the final ethanol yield. Therefore, a preliminary economic evaluation was performed to determine the economic feasibility of different biorefinery scenarios using either preprocessing or overliming.

Table 3.5. Fermentation results of hydrolysates (without overliming) and liquid after enzymatic hydrolysis of the solid fraction.

		Ethanol fermentation yield [1] ($Y_{\%T}$)	
		Hydrolysate	**Solid fraction after enzymatic hydrolysis**
	Control [2]	79.1 ± 1.6	84.6 ± 3.8
Whole-tree chips (WTC)	**Untreated**	1.9 ± 0.2	82.2 ± 2.7
	Acidic	53.2 ± 1.3	82.8 ± 0.6
	Alkaline	55.0 ± 0.2	82.3 ± 0.9
	Neutral	49.0 ± 0.4	83.4 ± 0.6
Clean pulp chips (CPC)	**Untreated**	1.1 ± 0.0	83.7 ± 0.8
	Acidic	52.0 ± 3.2	82.5 ± 1.4
	Alkaline	55.1 ± 1.5	81.8 ± 1.7
	Neutral	55.6 ± 1.9	85.5 ± 2.4

Data represented as the mean values of duplicate analysis with standard deviation

[1] Ethanol fermentation yield is expressed as a percent of theoretical yield ($Y_{\%T}$) using a theoretical maximum yield of 0.51 g/g

[2] The fermentation control contained reagent-grade sugars at similar concentrations to those in experimental samples

Table 3.6. Fermentation results of overlimed CPC hydrolysates and total sugar loss percentage.

		Ethanol fermentation yield [1] ($Y_{\%T}$)	**Total sugar loss after overliming [2] (%)**
Clean pulp chips (CPC)	**Untreated**	45.3 ± 0.5	29.5
	Acidic	53.3 ± 1.0	19.4
	Alkaline	64.6 ± 0.6	20.4
	Neutral	54.6 ± 1.3	19.2

Data represented as the mean values of duplicate analysis with standard deviation

[1] Ethanol fermentation yield is expressed as a percent of theoretical yield ($Y_{\%T}$) using a theoretical maximum yield of 0.51 g/g

[2] Accounting for glucose and xylose only, which are the main sugars present in the liquid fraction

3.6 PRELIMINARY ECONOMIC ASSESSMENT

Three biorefinery scenarios were used for this economic assessment: 1) CPC feedstock via pretreatment, enzymatic hydrolysis, overliming, and sugars to ethanol fermentation; 2) WTC feedstock via acidic preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation; and 3) CPC feedstock via acidic preprocessing, pretreatment, enzymatic hydrolysis, and sugars to ethanol fermentation.

In this preliminary economic assessment, the focus was on the influence of acidic preprocessing and overliming on the biorefinery revenue using either poplar CPC or WTC. First, the capital cost of preprocessing and overliming were assessed: the overliming unit operation was assumed to be the same as the one in the 2002 NREL report [2], while the preprocessing unit was assumed to be the same as the configuration used in the deacetylation process in the 2015 NREL report [47]. Chemical Engineering Plant Cost Index (CEPCI) was used to properly adjust the price of the equipment to the year 2018, and a scaling exponent of 0.6 was used to adjust the capital cost of each equipment to our biorefinery size. The biorefinery size ratio was calculated based on the feedstock consumption rate (dry tonne/day). Second, the direct unit operating costs were calculated: operating cost of overliming includes chemicals (calcium hydroxide and sulfuric acid), and gypsum disposal to a landfill; while the operating cost of preprocessing includes water, sulfuric acid, and the cost to send wastewater to a municipal treatment facility. All operating costs were calculated based on the latest pricing quotes and properly scaled to the biorefinery flow rates. The adjusted capital and direct operating costs of both processes are presented in Table 3.7.

Table 3.7. Capital and operating costs of overliming and preprocessing units.

	Capital cost (MM $)	**Direct operating cost (MM $/year)**
Overliming	$ 1.73	$ 0.96
Preprocessing	$ 3.35	$ 7.52

It can be seen in Table 3.7 that the capital cost of preprocessing is about twice higher than that for overliming. Also, the operating cost of preprocessing is approximately 7 times higher than that of overliming, which is primarily due to the high cost of clean water (at a liquid-to-wood ratio of 4) and wastewater treatment required for preprocessing. The complete individual equipment and chemical prices used in this calculation can be found in the Appendix, Tables A.5 and A.6.

It should be noted that Table 3.7 provides an incomplete picture of the overall process economics. The use of WTC via preprocessing results in higher biorefinery income due to higher ethanol yields and perhaps lower overall operating costs due to the use of a cheaper feedstock. To assess the economic benefits of switching from a process that uses CPC and overliming to one that can use cheaper WTC but requires preprocessing, the incremental return on investment (as shown in Equation 3.1) associated with making these process changes was calculated. Further, it was of interest to compare the economics of two biorefineries where both use CPC, but one uses preprocessing and the other uses overliming to obtain a fermentable hydrolysate.

Equation 3.1. Incremental Return on Investment (ROI) equation.

$$\mathrm{ROI}\ (\%) = \frac{\text{additional revenue}}{\text{additional capital cost}} \times 100$$

$$= \frac{(\text{revenue} - \text{operating costs})_{\mathrm{A}} - (\text{revenue} - \text{operating costs})_{\mathrm{B}}}{(\text{capital cost})_{\mathrm{A}} - (\text{capital cost})_{\mathrm{B}}} \times 100$$

The total operating costs for each scenario was calculated from the direct operating costs shown in Table 3.7 plus the cost associated with the feedstock used in the biorefinery. Assuming a feedstock usage of 275,000 dry tonnes per year for both cases, the feedstock cost of poplar CPC is equal to $ 31.88 MM per year, while the feedstock cost of poplar WTC is equal to $ 21.16 MM per year. Hence, it can be seen that using WTC could substantially reduce the operating cost of the biorefinery.

To calculate the annual revenue of each scenario, their final ethanol production (liters per year) was multiplied by the cellulosic ethanol selling price of $0.94 per liter. This price includes the selling price of the fuel plus the cellulosic waiver credit (CWC) and D5 RIN, which would be attached to produced ethanol as regulated by the Renewable Fuel Standard program [48]. The CWC and D5 RIN prices were determined using the latest U.S. EPA 2019 calculations [49]. The ethanol production was calculated based on the total monomeric sugars yield (kg/tonne of biomass) obtained for each case in the experimental part of this work (Appendix, Table A.4), a feedstock rate of 275,000 tonnes/year, and assuming a 95% glucose conversion to ethanol, and an 85% xylose conversion to ethanol during the fermentation by using a recombinant co-fermenting bacteria [46]. Table 3.8 shows a summary of capital and total operating costs (including feedstock cost), as well as the annual revenue from ethanol selling of the three scenarios mentioned above.

Table 3.8. Capital cost, total operating cost, and revenue of three scenarios proposed.

	Capital cost (MM $)	**Total operating cost* (MM $/year)**	**Annual revenue (MM $/year)**	**Description**
Scenario 1	$ 1.73	$ 32.84	$ 76.88	CPC feedstock, with overliming
Scenario 2	$ 3.35	$ 28.68	$ 87.66	WTC feedstock, with acidic preprocessing
Scenario 3	$ 3.35	$ 39.40	$89.51	CPC feedstock, with acidic preprocessing

* Total operating cost including the feedstock cost.

It can be seen in Table 3.8 that the feedstock cost plays a big role in the total operating costs. When accounting for preprocessing only, the operating costs were higher than those of overliming. However, if using the WTC feedstock (scenario 2), it turns out being cheaper than using the more expensive CPC feedstock with overliming (scenario 1). Also, preprocessing considerably increased the final annual revenue in scenarios 2 and 3 because of its higher monomeric sugar yield (shown in Appendix, Table A.4), thus resulting in more fermentable sugars that were converted to ethanol.

Finally, the Return on Investment (ROI) was assessed (Equation 1), pairwise between scenarios 1 and 2, and between scenarios 1 and 3. The ROI between scenarios 1 and 2 was determined to be 920%. Thus, investing in WTC feedstock via acidic preprocessing instead of CPC feedstock via typical layout with overliming will yield a return of 920% of the initial investment. This staggering return on investment is due to two main reasons: a) WTC feedstock is substantially cheaper than CPC, and it is known that the feedstock is the biggest contributor to the operating cost; and b) acidic preprocessing significantly improved the monomeric sugar yield, which in turn results in much higher ethanol production. The ROI between scenarios 1 and 3 was 374%, showing that using CPC via preprocessing still generates higher revenue than using CPC via typical layout with overliming, even though the capital and operating costs for preprocessing were higher. This excellent ROI is mainly attributable to the 30% sugar loss resulting from overliming that reduces the final ethanol production and consequently the revenue.

In sum, it appears that preprocessing is a superior approach to conditioning the hydrolysate via overliming for subsequent fermentation because it eliminates the large sugar loss associated with overliming and enables the use of low quality, but much cheaper, biomass feedstock. It should be noted that some sort of biomass preprocessing is probably necessary in a biorefinery to remove the dirt and grift from the feedstock that would erode the downstream process equipment. In the present work, we propose that the preprocessing can be engineered such that it cleans the feedstock and eliminates the need for an expensive overliming process.

4. CONCLUSIONS

By testing three preprocessing conditions (acidic, alkaline, and neutral), we found that all of them were able to remove NSCs from both types of biomass (poplar clean pulp chips and whole-tree chips). Acidic preprocessing had the highest NSCs removal from both biomasses: ash was removed by 81% and 66% from CPC and WTC, respectively; while extractives were removed by 42% and 57% from CPC and WTC, respectively. Alkaline preprocessing showed the highest acetic acid removal from both biomasses (73% and 48% removal from CPC and WTC, respectively). At the same time, we noticed that sodium cations originated from the sodium hydroxide solution were absorbed by the biomass during the preprocessing step.

Acidic preprocessing also resulted in the highest monomeric sugar yield and recovery after steam pretreatment and enzymatic hydrolysis. Acidic preprocessed WTC had a yield of 578 kg/tonne of biomass (85 kg higher than untreated WTC), while CPC had a yield 577 kg/tonne (38 kg higher than untreated CPC). At the same time, acidic preprocessed WTC achieved a remarkable 87.5% monomeric sugar recovery (14% higher than untreated WTC), while acidic preprocessed CPC had 80% recovery (5% higher than untreated CPC). This was explained by the removal of ash during acidic preprocessing, which decreased the buffering capacity of the biomass and resulted in better solubilization of sugars during pretreatment

The ethanol fermentation yield of both CPC and WTC untreated hydrolysates were negligible (about 1-2%). However, all preprocessing conditions showed significant improvement in the ethanol fermentation yield of both biomasses, increasing from 1-2% (untreated) to 49-56%. We believe that the removal of certain organic extractives with antimicrobial characteristics enabled this improvement without requiring overliming. Overliming was effective in improving the fermentability of CPC hydrolysate samples: untreated CPC fermentation yield increased from 1% to 45% when treated with overliming. However, a sugar loss of 30% was also observed during overliming, which compromises the final ethanol yield. Overall, it was noted that preprocessing and overliming improved the fermentability of untreated hydrolysates at similar extents, but it must be remembered that overliming also causes major sugar losses in the hydrolysate.

The preliminary economic assessment showed that using the cheaper WTC via preprocessing resulted in a staggering return on investment of 920%, as a result of the low feedstock price, and the big improvements on monomeric sugar yields via preprocessing, which in turn increased the ethanol production. Hence, it was shown that preprocessing enabled the use of the cheaper and lower-quality WTC biomass without requiring the overliming step.

5. FUTURE WORK

The work shown in this thesis explored the overall outcomes from various scenarios: poplar clean pulp chips versus whole-tree chips, preprocessing versus overliming, and preliminary economic assessment. The following future work is recommended:

- Include a water-recycling system in the preprocessing models to reduce the clean water requirements. Preprocessing requires large volumes of clean water. Investigation on possible ways to recycle process water has potential to lower the preprocessing operating costs.
- Perform a full techno-economic analysis using Aspen Plus of the biorefinery scenarios discussed in this work. By doing this, more evidence can be obtained to determine which model is more techno-economically feasible for ethanol production.
- Characterize the organic extractives removed during preprocessing. This can be achieved by analyzing ethanol extracts of untreated and preprocessed samples using Gas Chromatography Mass Spectrometry (GC/MS). By comparing the organic compounds found in each sample, the specific compounds removed during preprocessing can be identified. This information will help to better explain the improvement in fermentability of the preprocessed samples.

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APPENDIX

Table A.1. Chemical composition of white wood and bark fractions of WTC poplar samples.

		Glucan (%)	Xylan (%)	Total sugars* (%)	Total lignin (%)	Acetic acid (%)	Total ash (%)	Total extractives (%)
Untreated	**White wood**	45.0 ± 0.8	18.0 ± 0.1	65.8 ± 0.9	28.5 ± 1.6	5.5 ± 0.1	0.5 ± 0.0	6.3 ± 0.1
	Bark	33.2 ± 0.6	6.8 ± 0.1	46.0 ± 0.8	33.8 ± 1.2	2.0 ± 0.0	6.5 ± 0.0	28.2 ± 0.7
Acidic	**White wood**	47.8± 0.4	19.1 ± 0.1	69.4 ± 0.5	29.6 ± 0.8	5.6 ± 0.2	0.2 ± 0.1	4.2 ± 0.0
	Bark	32.5 ± 0.6	8.9 ± 0.4	45.5 ± 1.2	34.4 ± 1.6	2.6 ± 0.2	6.1 ± 0.2	13.2 ± 0.1
Alkaline	**White wood**	49.1 ± 0.5	19.6 ± 0.2	71.6 ± 0.7	28.0 ± 0.8	4.3 ± 0.0	0.5 ± 0.1	3.6 ± 0.1
	Bark	34.5 ± 0.6	9.4 ± 0.2	51.6 ± 0.9	30.6 ± 0.5	1.1 ± 0.0	7.8 ± 0.2	7.4 ± 0.5
Neutral	**White wood**	46.3 ± 1.7	18.1 ± 0.4	67.1 ± 2.2	26.9 ± 0.6	5.8 ± 0.4	0.5 ± 0.0	5.2 ± 0.3
	Bark	31.0 ± 1.9	7.6 ± 0.6	46.1 ± 3.0	27.9 ± 1.2	2.5 ± 0.0	7.6 ± 0.0	13.6 ± 0.3

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

*Total sugars include glucan, xylan, arabinan, galactan, and mannan.

Table A.2. Elemental composition of white wood and bark fractions of WTC samples.

		Ba (μg/g)	**Ca (μg/g)**	**K (μg/g)**	**Mg (μg/g)**	**Na (μg/g)**	**P (μg/g)**	**S (μg/g)**	**Si (μg/g)**
Untreated	**White wood**	8 ± 0.1	1234 ± 30	1143 ± 18	292 ± 38	0.0 ± 0.0	426 ± 38	143 ± 1	43 ± 1
	Bark	87 ± 4	13145 ± 171	6723 ± 674	1187 ± 106	0.0 ± 0.0	1137 ± 15	622 ± 29	48 ± 2
Acidic	**White wood**	4 ± 0.2	239 ± 13	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	118 ± 0.7	78 ± 2	0.0 ± 0.0
	Bark	87 ± 1	16455 ± 279	0.0 ± 0.0	212 ± 4	0.0 ± 0.0	993 ± 35	687 ± 12	32 ± 1
Alkaline	**White wood**	8 ± 0.0	917 ± 22	0.0 ± 0.0	220 ± 16	532 ± 0.0	178 ± 11	75 ± 5	32 ± 2
	Bark	127 ± 2	21180 ± 430	298 ± 4	1103 ± 84	1430 ± 25	772 ± 61	505 ± 20	24 ± 1
Neutral	**White wood**	7 ± 0.1	872 ± 19	461 ± 15	229 ± 10	0.0 ± 0.0	334 ± 9	112 ± 1	28 ± 1
	Bark	98 ± 3	17580 ± 425	2568 ± 111	1203 ± 58	0.0 ± 0.0	1008 ± 3	585 ± 17	47 ± 1

Data represented as the mean values of duplicate analysis with standard deviation.

Table A.3. Chemical composition of liquid fraction after steam pretreatment and combined severity.

		Combined severity	**Glucose**		**Xylose**	
			kg/tonne	mon %	kg/tonne	mon %
Whole-tree chips	**Untreated**	1.74	70.0 ± 2.4	76.2%	127.1 ± 2.1	78.9%
	Acidic	2.08	69.7 ± 2.3	86.0%	152.5 ± 0.1	92.1%
	Alkaline	1.88	50.1 ± 0.2	72.6%	156.2 ± 3.3	79.0%
	Neutral	1.94	65.8 ± 2.5	73.9%	148.3 ± 5.6	79.5%
Clean pulp chips	**Untreated**	2.09	50.5 ± 1.6	87.4%	97.0 ± 3.0	99.0%
	Acidic	2.14	84.2 ± 1.0	93.7%	107.2 ± 2.4	105.8%
	Alkaline	1.95	33.6 ± 1.0	84.7%	122.8 ± 1.6	94.6%
	Neutral	1.98	56.1 ± 5.3	86.8%	120.8 ± 0.4	94.2%

Table A.4. Monomeric sugar yield and recovery after steam pretreatment and enzymatic hydrolysis.

	Total monomeric sugar recovery (%)		**Total monomeric sugar yield (kg/tonne)**	
	Whole-tree chips	**Clean pulp chips**	**Whole-tree chips**	**Clean pulp chips**
Untreated	73.2 ± 2.4^{a}	75.0 ± 0.9^{ac}	493.0 ± 16.0^{a}	539.1 ± 6.1^{a}
Acidic	87.5 ± 1.2^{b}	80.0 ± 0.6^{b}	578.3 ± 8.2^{b}	577.5 ± 4.4^{b}
Alkaline	80.6 ± 0.1^{ab}	72.9 ± 1.4^{c}	553.0 ± 0.7^{ab}	573.1 ± 10.7^{b}
Neutral	82.3 ± 5.8^{ab}	78.1 ± 0.2^{ab}	528.7 ± 37.6^{ab}	579.7 ± 1.7^{b}

Different superscript letters indicate statistically significant differences ($p<0.05$) within each column by Tukey’s test (WTC and CPC treatments were compared separately).

Table A.5. Individual equipment prices (based on NREL reports and adjusted to in-house size).

Equipment Name	**Installed cost in 2018$ (in-house size)**
Overliming Tank Agitator	$ 36,658.50
Reacidification Tank Agitator	$ 111,597.94
Lime Solids Feeder	$ 5,510.73
Overlimed Hydrolyzate Pump	$ 98,986.00
Filtered Hydrolyzate Pump	$ 100,574.58
Lime Unloading Blower	$ 151,751.76
Reacidified Liquor Pump	$ 100,147.00
Hydroclone & Rotary Drum Filter	$ 285,243.22
LimeDust Vent Baghouse	$ 233,596.55
Overliming Tank	$ 157,390.36
Lime Storage Bin	$ 194,301.79
Reacidification Tank	$ 252,978.15
OVERLIMING TOTAL	**$ 1,728,736.58**
Preprocessing reactor	$ 2,938,248.79
Preprocessing reactor conveyors	$ 414,578.25
PREPROCESSING TOTAL	**$ 3,352,827.03**

Table A.6. Individual price quotations used for calculating operating costs.

Description	**Cost 2019 ($/tonne)**	**Quote source**
CPC chips (dry)	$ 116	Greenwood Resources
WTC chips (dry)	$ 77	Greenwood Resources
Calcium hydroxide	$ 165	Kemcore
Sulfuric acid	$ 41	Echemi.com
Gypsum disposal	$ 55	Waste Business Journal
Water	$ 5	EPA 2017 Report
Wastewater treatment	$ 6	EPA 2017 Report