`Detoxification of C5 Rich Prehydrolysate from FPInnovations Modified TMP-Bio Process for Lactic Acid Fermentation

by

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ABSTRACT

This work studied a combination of methods for detoxifying prehydrolysate produced by FPInnovations' modified TMP-Bio process from hard wood chips for fermentation based bio-refining. The optimal conditions for pH adjustment based removal of fermentation inhibiting compounds was investigated as well as the results of further refining overlimed pressate by membrane filtration in order to remove additional inhibitors. Initial pH adjustment to 11 with continual stirring in a 25°C water bath removed between 14-19% of the total phenols detected in the pressate (in vanillin equivalents), while reducing the concentration of xylose by 25-28%. Samples were subsequently passed through membrane filtration at 200Da, removing up to 28% of the acetic acid detected after overliming. Dilute pressate samples treated with this combination of detoxification steps was fermentable by *Bacillus coagulans*, consuming 97% or greater of the available xylose within 24 hours.

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Table of Contents

List of Tables

List of Figures

Nomenclature

IFBR	Integrated Forest Bio-refinery
H-Lignin	Hydrolysis Lignin
AS-Lignin	Acid Soluble Lignin
HMF	Hydroxymethylfurfural
PHL	Pre-Hydrolysis Liquor
PHP	Pre-Hydrolysis Pressate (Modified TMP-Bio)
TPHL	Treated Pre-Hydrolysis Liquor
NF	Nano-filtration
TMP	Thermomechanical Pulp
IC	Ion Chromatography

Chapter 1

Introduction

1.1 Integrated Forest Biorefinery

Possessed of abundant natural woodland, Canada is well established globally as a major producer of wood products such as pulp and paper. Wood chip production has reached record highs in recent years, totaling greater than 3 million tonnes annually in 2012 alone. [1] Improvements in forestry techniques and harvesting methods has resulted in significant increases in production of materials for this industry over the last decade, but this excess of product is in stark contrast to a decline in demand for paper products. [2] Newsprint quality paper is one such product that has experienced a significant shift in demand in recent years due to a sea-change in media toward a digital presentation format. The economic constraints created by this trend have reduced saleable material prices and raised concerns regarding growing fuel costs and environmental protection movements.

The excess of raw materials available to mills due to this shift in demand has caused a push for many mills to investigate alternative manufacturing schemes by which they might diversify their product portfolio, as economic co-products have been shown to be an effective means of optimizing production efficiency. [3] Biorefining presents one such means of diversification, offering a plethora of options for mills to convert lignocellulosic material into other valuable products capable of meeting the growing market demand for sustainably sourced materials. New advancements in biomass preparation and process

refinements provide sustainable pathways for energy and material consumables that see increased demand in evolving modern markets.

The ideal production center for such products is an Integrated Forest Biorefinery (IFBR) which incorporates multiple biomass converting technologies into a cohesive framework in order to effectively make use of lignocellulosic material as a feedstock for the combined production of pulp and paper as well as specialty chemicals and energy. The addition of such technologies into a pulp mill is especially promising, as these facilities already possess much of the requisite infrastructure as well as established biomass supply, and processing experience. A complete IFBR is in many ways analogous to the petroleum refinery, converting a single base material into a spectrum of value-added products; however, in contrast to oil-based industrial processes biorefineries perform this task in a sustainable manner, producing materials with the potential to be non-toxic, biodegradeable, and reusable. [4]

Biomasses have complex structures whose components are strongly connected, necessitating treatments to fractionate and disrupt the original cell structure in order to permit separation and refinement of each. Ideally these pretreatment steps would employ simple equipment and low-cost chemicals, as well as being applicable for use with a large range of biomass types; however, current technologies will require significant refinement to achieve these goals pursuant to the development of a true bio-based economy. As the primary source of lignocellulosic biomass for prospective IFBR facilities, it is important to look at the composition of wood in order to determine how such a facility might make use

of each available resource. Table 1 on the following page shows an overview of the primary components of coniferous (soft) and deciduous (hard) wood. [5] The distinction between hard and softwood trees is based solely upon their reproductive methods in regard to seed structure, with angiosperm trees producing a fruit enclosed seed, and gymnosperms shedding seeds uncoated from structures such as cones. The exact composition of wood from any particular species of tree may vary somewhat in available extractives as many have defensive adaptations specific to their preferred habitat, but generalizations regarding their overall composition have been found to be accurate in many cases. [6]

Table 1: Composition of Wood

Wood Components	Concentration (%)	
	Hardwood	**Softwood**
Cellulose	41-48	46-55
Hemicellulose	26-35	23-25
Lignin	19-28	24-33
Extractives	3-10	3-10

From this we can see that there are four different resources available for extraction from wood chips, which then leads to the possibility of a system such as that shown in Figure 1 on the following page which accounts for each in turn. By staging the processes of the facility such that each portion of the biomass is extracted and converted into final products in turn, they can each be optimized for use. The original cellulose based pulping portion of the mill is thus able to continue production while the remaining fractions are employed separately.

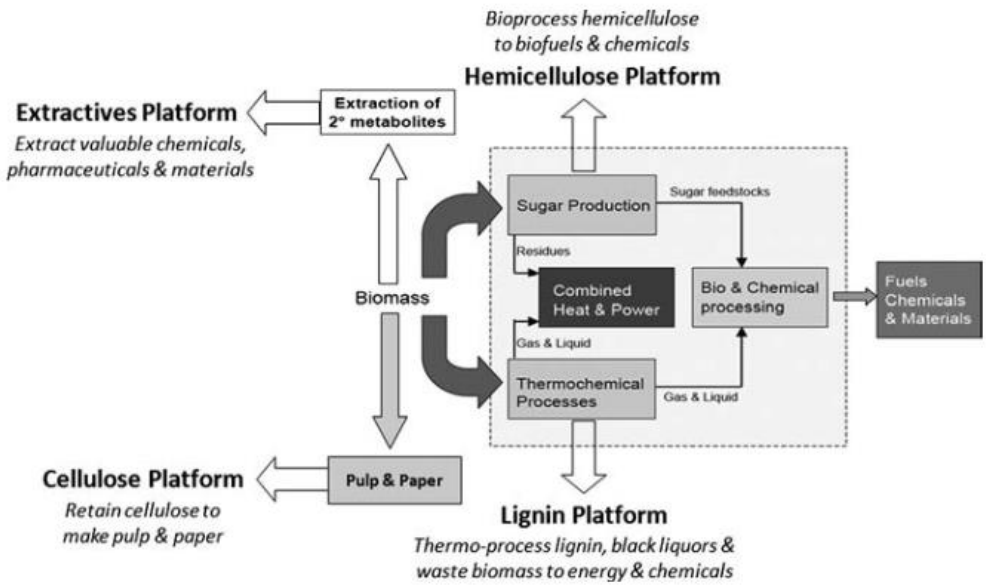

Figure 1: The Integrated Forest Biorefinery Concept

1.1.1 TMP-Bio

One promising method for such biorefining based production is TMP-Bio. Developed at FPInnovations, the process uses chemical, enzymatic and mechanical pretreatment as well as hydrolysis to convert the hemicellulose and lignin in wood chips into a stream of mixed sugars and hydrolysis lignin (h-lignin) for use in bio-processing. The sugars extracted through this process can then be fermented to form useable products such as biofuels, sweeteners, and many other saleable goods. TMP-Bio is a stand-alone process that makes use of the existing supply infrastructure of pulp-mills to produce products in addition to their pulp, making it a unique alternative for established businesses that will not adversely affect their other operations and may in fact reduce effluent loads in certain sectors. [7] The process flow diagram below in Figure 2 shows an overview of the TMP-Bio process, beginning with untreated wood chips as biomass, followed by patented treatments produce

digestible biomass ready for hydrolysis into a mixed steam of C6 and C5 sugars as well as hydrolysis lignin (H-Lignin).

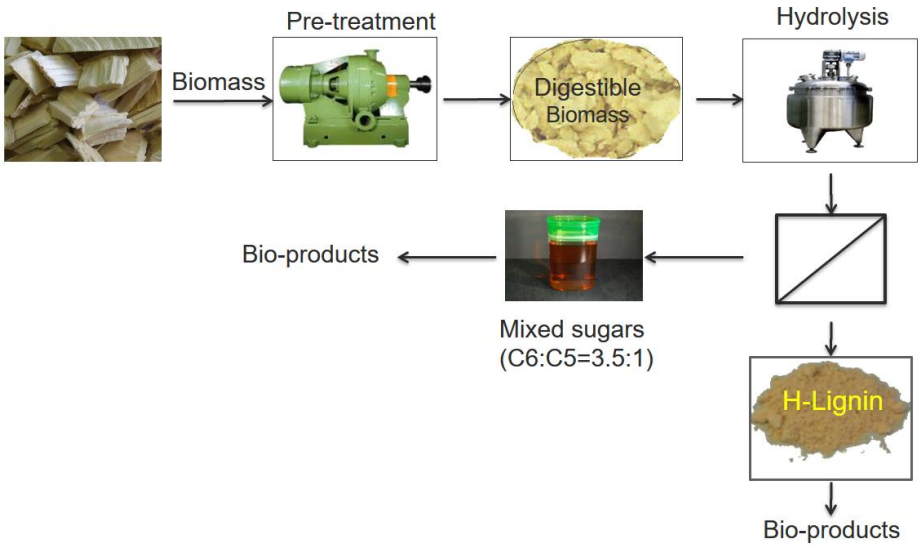

Figure 2: TMP-Bio Process

Under high solids loaded conditions TMP-Bio has the potential to reach high sugar yields, demonstrating results in excess of 90% in batch tests, with the potential to be adapted for use in large scale operations. [8] The mixed sugar steam produced by TMP-Bio may be fermented or purified to produce saleable products, but the composite of differing carbohydrates makes the design of targeted processes less efficient. Refinements to the original TMP-Bio technology in order to specialize it for different applications have resulted in a separate technique referred to as the modified TMP-Bio process, which permits the separation of a pre-hydrolysis pressate stream composed primarily of C5 sugars with a low lignin content. Figure 3 on the following page shows the modified pretreatment process of the Modified TMP-Bio process.

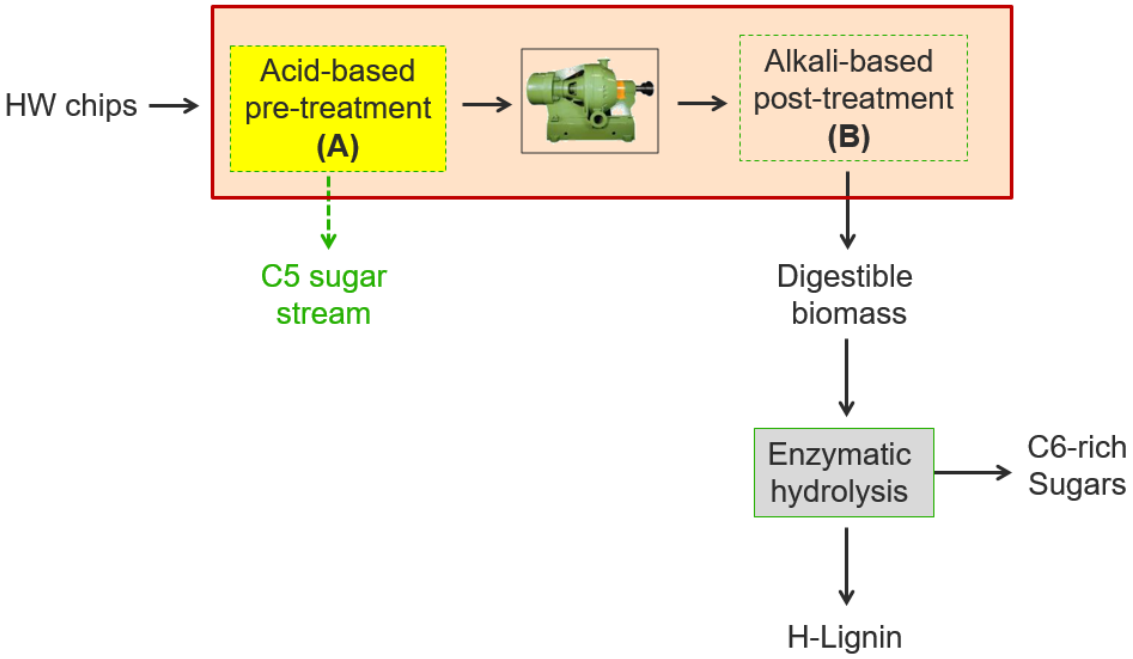

Figure 3: Modified TMP-Bio Process

The high concentration of xylose in the pressate resulting from the modified TMP-Bio process gives it the potential to act as a feedstock for the production of a wide range of commercial products including naturally sourced lactic acid, useable in detergents, as well as xylitol, a sweetener that does not cause dental caries making it a popular addition to chewing gum. [9] [10] [11] It is important to note that treatment of lignocellulosic biomass with mineral acids (such as sulphuric acid) leads to the formation of other chemical byproducts through dehydration of monomeric sugers such as xylose into byproducts. This may result in the production of a number of compounds including furfural, and hydroxymethylfurfural, amongst others. [12] [13] The former is of particular import in many applications, as shown in the diagram below it holds the potential to act as a feedstock for a number of valuable products; however, it is known to act as a fermentation inhibitor for many yeasts. [14]

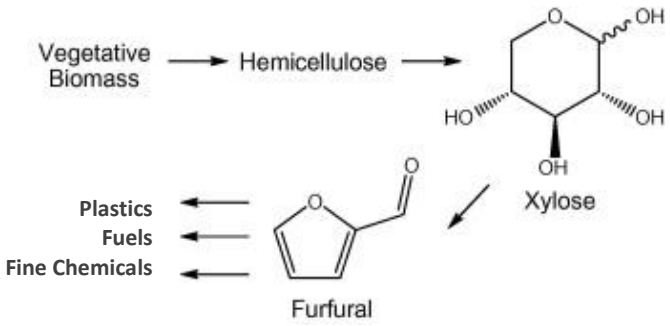

Figure 4: Conversion of Biomass to Furfural

The dehydration of pentoses (such as xylose) to furfural takes place most readily under acidic conditions and high temperatures. [15] This reaction is hindered when working with feedstock containing mixed sugars rather than pure pentoses, which is attributed to the degradation of furfural in the presence of complex saccharide solutions, as well as the effects of other degradation products formed from hexoses. [16] This is promising in some respects for the production of fermentable feedstocks with the modified TMP-Bio process, as the ideal conditions for formation of furfural are not reached and thus its concentration within the initial pre-hydrolysis pressate is low. The table below shows the concentrations of various wood extractives and processing byproducts detected in pre-hydrolysis pressate produced by a hardwood run of the modified TMP-Bio process.

Table 1: Wood Extractives in PHP

Components	Concentration
Furfural (ppm)	247
HMF (ppm)	29.2
Acetic Acid (g/L)	12.37
AS-Lignin (g/L)	5.60
Total Phenols (g/L)	4.50

In a similar fashion to furfural, HMF is a useful industrial chemical with a wide variety of applications in bioenergy production, but both are present in PHP in negligible amounts. [17] Industrial applications for TMP-Bio are likely to focus upon preparation of the PHP for fermentation and pursuant to this the higher concentration of fermentation inhibiting compounds such as acetic acid and phenols is of greater concern.

1.1.2 Fermentation of Prehydrolysates

Prehydrolysate such as that produced by the modified TMP-Bio process cannot directly be used as a fermentation feedstock; however, as they contain many wood extractives and by-products that act as inhibitors to the process. Previous works have shown promising results from the use of activated charcoal to remove fermentation inhibitors from hydrolysates, but investigation of other techniques is an important step in optimizing the process for commercial use. [18] Other methods of detoxification include reactive extraction, membrane filtration, and overliming. [19] [20] [21] Of these, overliming is heavily dependent upon the pH the material is raised to, the temperature of the sample, and the length of time it is kept at the adjusted pH. Identifying the optimal conditions for overliming of pressate produced via the modified TMP-Bio process developed by FPInnovations is therefore an important milestone in determining the most commercially viable method of detoxifying its prehydrolysate for fermentation.

1.2 Significance of Study

With existing pulp mills searching for ways to diversify their production in order to reduce their susceptibility to fluctuations in market demand for wood pulp-based products, bio-refining based technologies like TMP-Bio have an important role to play. Development of methods by which the products of the modified TMP-Bio process may be converted into saleable products on-site is an important part of establishing it as a stand-alone system. The demand for naturally and renewably sourced products such as those produced from biomass hydrolysates is growing as many companies have begun to prioritize "green" solutions in order to better conform to environmental regulations and improve the sustainability of their processes, making this technology even more appealing. [22]

Any process designed to ferment the sugars produced by TMP-Bio must; however, account for the presence of several fermentation inhibiting compounds present in the pressate. Raw hardwood prehydrolysate is known to contain sufficiently high concentrations of acetic acid, amongst other compounds, to make fermentation impossible in many cases. [23] Identification of viable methods for removal of these inhibitors as well as refinement of the technology is therefore an important step in opening new avenues of sustainable production for Canada's forestry industry.

1.3 Objectives

The major objectives of this study are as follows:

- Study the effects of overliming on PHL produced by FPInnovations modified TMP-Bio process from hardwood chips.
- Determine the optimal conditions for pH adjustment based detoxification of such pressate.
- Study the effects of combining membrane filtration and overliming to remove fermentation inhibitors from said PHL.
- Demonstrate the fermentability of prehydrolysate treated with these detoxification methods.

Chapter 2

Literature Review

2.1 Biorefining Hydrolysate Components

The modern pulp industry has been introduced to the concept of integrated forest biorefinery, which approaches the creation of value-added elements from the by-products of existing manufacturing processes. Attention toward this methodology has increased significantly of late in part due to the growing interest in green processes, and technologies that has become prevalent in modern society. Utilization of sustainable biomass, such as excess wood chips, to produce ethanol or similar biofuels is only one-such process that has received immense attention in recent years thanks to its potential to replace fossil fuels in some applications. Biorefinery based techniques have also been investigated in such endeavours as the production of chemicals ranging from furfural, and acetic acid to hemicellulose in the prehydrolysis Kraft method of pulp production, and holds potential in use with prehydrolysis liquor or prehydrolysate.

As previously shown, wood based lignocellulosic biomass is composed primarily of three components: cellulose, hemicellulose, and lignin. [24] Hydrolysis of this material can be catalyzed by acids and enzymes to produce a mixture of sugars, h-lignin, and various byproducts. Dilute acid hydrolysis is a quick and relatively simple method of performing this separation, but may result in degradation of monosaccharides and the formation of high concentrations of fermentation inhibitors.

2.1.1 Cellulose

As the most abundant macromolecule in nature, being found in plant-life worldwide, cellulose is an important component to consider when determining the optimal use of forest based bioresources. Composed primarily of glucose channeled from the carbohydrate metabolism of plant cells, microfibrils of cellulose become intermingled with hemicellulose, proteins and lignin to form the biocomposite that is generally referred to as wood. [25] The structures formed from this macromolecular composite provide the exceptional load bearing qualities plant life is known for. Commercially cellulose is well studied, with wood based forms making up the basis of the pulp and paper industry, while cotton and hemp based celluloses are similarly fundamental for textile production. [26] The daily processes of modern pulp mills are aimed at improving the process of refining and purifying this material for use and thus the development of IFBR systems must focus on applications revolving around the remaining components of lignocellulosic biomass.

2.1.2 Lignin

Found intermingled with cellulose microfibrils within plants, lignin is the second most abundantly present natural polymer and it provides much of the rigidity required for the formation of cell walls. Lignocellulosic composites also possess significantly greater resistance to natural decomposition due to hydrolytic enzyme resistance imparted by the presence of lignin. [27] Composed primarily of phenolic monolignol units, lignin has a complex structure that is varied in its forms, hampering the development of an accurate, complete model. This modeling is further complicated by the fact that its makeup can vary

strongly depending upon whether source wood is from a soft or hardwood tree as well as which species it is sourced from. The figure below shows fragmental structures found within the Sakakibara model of softwood lignin. [28]

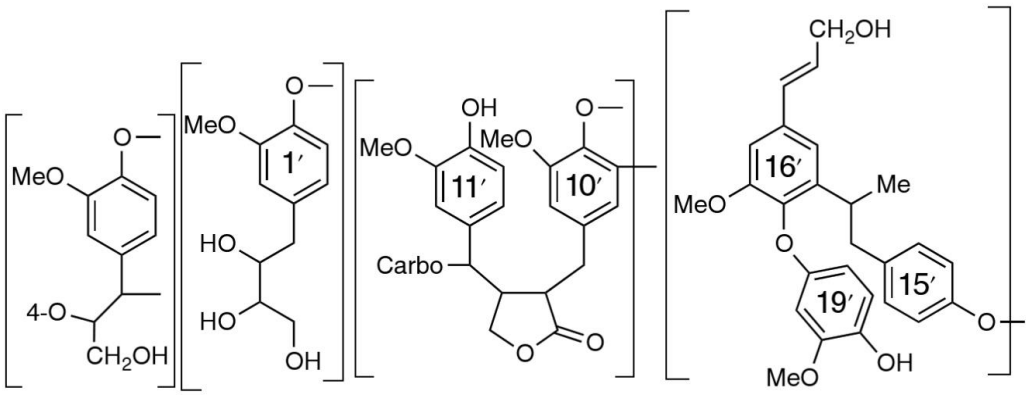

Figure 5: Fragmental Structures within Lignin

The molecular weight of lignin in natural wood samples varies greatly, ranging from as low as 4000 atomic mass units (Daltons) rising to 33900 Da in some tested cases. [29] In many processes lignin can act as a hindrance, impeding separation operations due to the way it reinforces the structure of the feed biomass, preventing extraction of targeted components.

Dissolved lignin in hydrolyzed wood extracts can be precipitated out of the solution in some part by acidification, though this separated only a small fraction of the detected material. More effective methods such as adsorption onto activated carbon and lime mud have shown much higher rates of removal, reaching up to 86% after an hour-long treatment. [30] Adsorption onto charcoal still faces significant challenges; however, as the material costs for industrial application as well as those required for regeneration of the absorption

surface imposes a practical limitation. The development of a more robust list of industrial applications for lignin will provide additional impetus for research into new extraction methods that may address these concerns.

2.1.3 Hemicellulose

Unlike cellulose, hemicellulose is a non-homogenous biopolymer composed primarily of xylose with arabinose, mannose, and galactose branches with a low degree of polymerization. As previously shown the fraction of hemicellulose making up lignocellulosic biomass varies between 23% and 35% by weight. While structural components of cellular biomass are bound within the matrix, some carbohydrates can be removed through extraction and washing steps. [31] Fractionation and removal may also be performed through techniques such as acid hydrolysis or alkali treatment with enzymatic extraction. The latter of these allows for process tuning to focus upon individual components of the targeted biomass by selection of various enzymes and adjustment of dosage. [32]

Acid hydrolysis of hardwood extracts such as those produced by FPInnovations' TMP-Bio process contain high concentrations of hemicellulose oligomers such as glucuronoxylan (or simply xylan as it is commonly called) which has a backbone structure composed of xylose molecules. [33] After hydrolysis xylan and xylose make up a clear majority of the sugar profile of the pressate, with other oligomers and their polymers averaging less than 6% each as shown in the table on the following page.

Table 2: Sugar Profile of Hydrolysed TMP-Bio Pressate

Components	Fraction
Arabinose	2.2%
Arabinan	0.22%
Galactose	2.4%
Galactan	0.22%
Glucose	3.5%
Glucan	1.5%
Xylose	73%
Xylan	11%
Mannose	4.0%
Mannan	1.8%

Alongside the sugars released, hydrolysis causes acetyl groups to be liberated from xylan in the hemicellulose, which forms the source of much of the toxicity of the resulting hydrolysate. The diagram below shows the structure of xylan and the oxygen bonds between components that are broken during hydrolysis. [34]

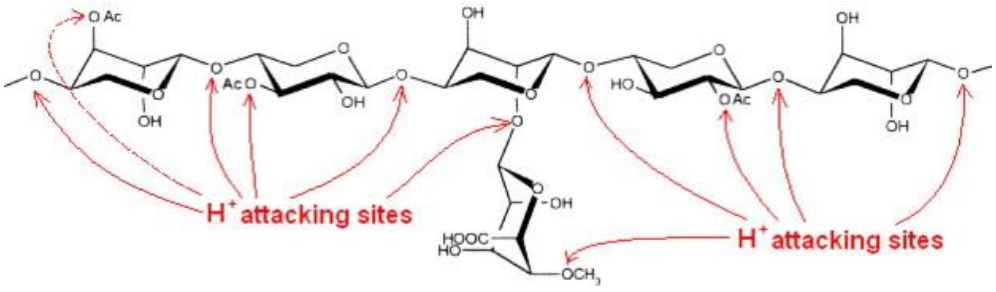

Figure 6: Hydrolysis of Xylan

The acetic acid resulting from this degradation can pass through the cell membrane of the micro-organisms used in fermentation based biorefining, causing changes in internal pH which can lead to cell death. [33] The presence of this inhibitor can also block pores within

the cell membrane preventing nutrient absorption and stunting growth. [35] The concentration of acetic acid resulting from this can vary widely between different biomass sources and the specifics of the extraction process. Sources have listed results with concentrations of acetic acid as low as 1.3 g/L from corn stover hydrolysate, with TMP-Bio pressate showing higher results between 11.2 and 12.4 g/L in different batches. [36]

2.1.3.1 Acetic Acid

While dissolved acetic acid can have detrimental effects on fermentation-based production processes, it has significant value in and of itself as an industrial feedstock completely separate from it's culinary applications in the form of household vinegar. The global acetic acid market is expected to grow to 10.31 billion USD by the end of 2018 from the 5.93 billion USD observed in 2011 when the global demand reached 8,720,313 tonnes annually. [37]

The growth of global demand for acetic acid is expected to continue as saturated demand from end-use industries is sustained by increased consumption of adhesives and packaging materials throughout the global market. Economic analyses of a Kraft pulp mill have shown that with optimized recovery processes as much as 31.6 tonnes of acetic acid could be produced per 1000 tonnes of pulp without adversely affecting normal production, presenting a significant source of added revenue with the potential for upward scaling. [38]

2.1.3.2 Applications of Acetic Acid

Acetic acid is commonly employed as a food preservative, acidity regulator and condiment, making up 4-18% of household vinegar, which is typically produced by fermentation of ethanol. [39] Many different varieties of household vinegar are available, each being sourced from different alcohol containing base materials. Dilute vinegar such as this has a plethora of uses as a household cleaner and has attained some degree of acclaim as a 'green' alternative to many commercial products.

In 2016 the majority of the global supply of acetic acid was employed in the manufacture of vinyl acetate monomer (VAM) and terephthalic acid (TPA) whose production combined to consume 56% of the world's supply. [40] VAM is widely used in polymer manufacturing and the production of adhesives, paint, films, and coatings, and has shown increased market demand in recent years. [41] TPA is employed in many of the same fields, seeing wide use in the production of commonly used plastics such as polyethylene terephthalate (PET), solidified packaging resins, treated natural fibers, and films. [42] The ever growing demand for such products creates an area of significant potential for biorefinery as a renewable source of feedstock.

The recovery of acetic acid from biorefinery based processes has been an area of significant interest of late. Various groups have tested a number of methods to find the most efficient for the extraction of useable material from PHLs. Attempts to employ reactive extraction with tri-octyl amine dissolved in octanol was shown to remove up to 63.53% of the detected acetic acid in treated material, though its efficacy suffers due to hindrance by other

chemicals and sugars dissolved in the medium. [19] Ion exchange resin based extraction from treated PHLs has also shown significant promise. Adsorption of treated PHLs onto microporous polystrenic weak base anion resins via formation of hydrogen bonds removed up to 70% of dissolved acetic acid. [20] Subsequent desorption and recovery of the resins is then possible via washing with a NaOH solution. Such adsorption techniques do require some refinement before they can be employed commercially however, as they demand significant overhead due to the high cost of resins and solvents.

2.2 Fermentation Applications for PHLs

While wood extractives and hydrolysis byproducts can be valuable products in and of themselves, the carbohydrates released during the breakdown of hemicullosic biomass are viable for use in a wide spectrum of applications through fermentation. Such processes are already widely used industrially for the creation of everything from beverages and foodstuffs, to fuels such as bioethanol. The lattermost of these has been an area of great interest in recent years, as the growing price of oil combined with a demand that is projected only to increase in the coming years. Current estimates indicate that the demand for petrol in Pakistan alone will exceed 8 million tonnes by the end of 2020. [43] The development of a sustainable fuel industry through bio-based fuel production is one of the few means by which a carbon-neutral future might be attained without a complete shift in transportation infrastructure. Biofuels also present an opportunity for areas without existing supply systems for petroleum and oil through fermenter based biogas production. [44] Biofuel production is hindered currently by the high cost of production, as purification

of fermentation products often demands high consumption of steam and electricity. [45] While production of bioethanol as a potential fossil fuel alternative has garnered the most media attention, assessment of the possible end-products from an IFBR approach to diversification of dissolving pulp mill liquor have shown that xylitol production is the most economically viable target. Creation of commercial products through fermentation such as this is, in some ways, a more approachable start-point for industrial diversification, as it permits expansion into existing markets.

The prehydrolysis liquor produced from hardwood dissolving pulp processes such as FPInnovation's modified TMP-Bio system, contain a number of potentially valuable commodities, including the aforementioned acetic acid, as well as furfural, lignin, and various sugars. The lattermost of these was the focus of this work, as such prehydrolysates contain a high concentration of pentose which when purified is a valuable feedstock for bioconversion into xylitol and lactic acid, amongst other products. Fermentation of pentose can be optimized for a desired end-product, depending upon the microorganism employed. Optimization of these methods may focus on several factors such as: Feed conditions, bacterial strain selection, nutrient additives, and feed detoxification amongst others.

The microorganisms used in fermentation processes may employ a number of different biological methods to convert sugars into the end products an IFBR system selects for. Micro-organisms such as *Bacillus coagulans* will ideally carry out lactic acid production through the pentose phosphate pathway (PPP). This metabolic conversion sequence produces lactate alone rather than the phosphoketalase pathway (PKP) which results in the

heterolactic production of acetic acid. The diagram below shows the expected sequence for each of these systems with xylose as the initial feedstock. [46]

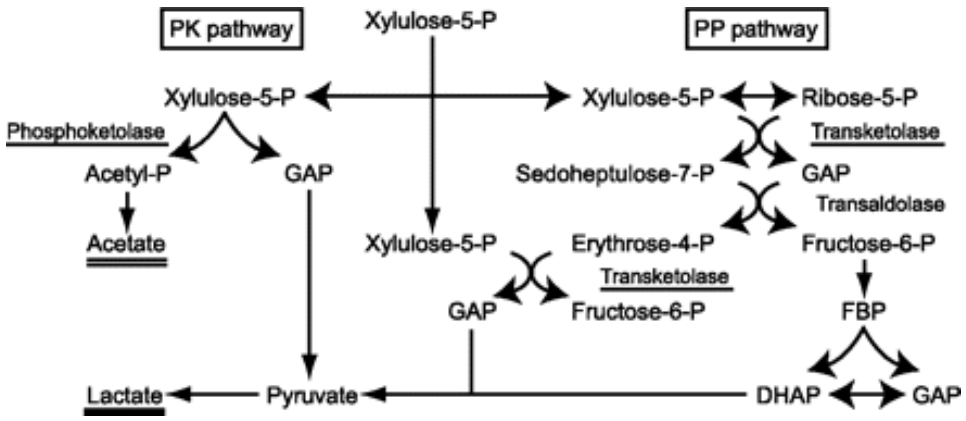

Figure 7: Pathways for Lactate Production

Employing xylose as the primary food source for the fermenting microorganism functions similarly, as direct conversion into D-xylulose-5-phosphate is possible for *B. coagulans* via xylose isomerase and xylulokinase. [47] The selection between PPP and PPK is especially important with hardwood PHLs that are expected to have high initial concentrations of acetic acid that can inhibit cellular function. Studies have shown that the selectivity of *B. coagulans* for PPP can further be enhanced by fermentation at 50-55°C and 5.5 pH. [47] Optimally selected strains of *B. coagulans* have shown yields as high as 99% on a gram per gram basis in fermentations of pure xylose into lactic acid. [48]

Before an industrial fermentation can begin however, it is necessary to remove several inhibiting compounds from raw prehydrolysate, as works by FPInnovations and many others have shown it to be unfermentable in its untreated state. Lactic acid fermentation

trials of untreated PHL against a control sample of pure xylose can be seen in Figures 8 & 9 below.

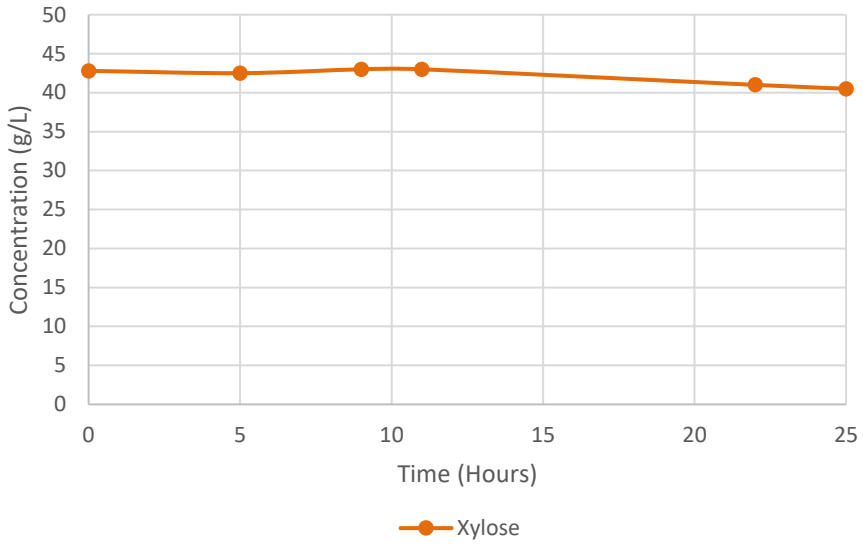

Figure 8: Lactic Acid Fermentation of Untreated Prehydrolysate

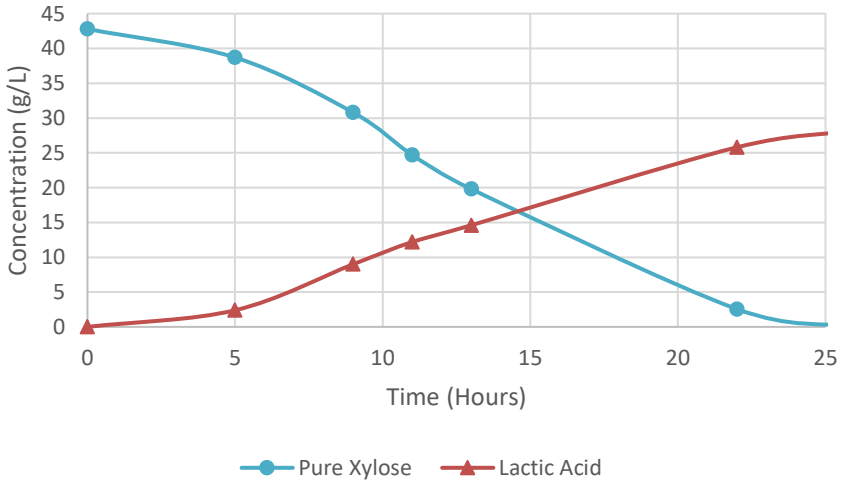

Figure 9: Lactic Acid Fermentation of Pure Xylose

These fermentation trials demonstrate that untreated TMP-Bio PHL with an initial concentration of 42.8 g/L of xylose did not show any sugar consumption by *B. coagulans* during the observation period. A control sample with the same initial concentration of xylose and without any inhibitory compounds was completely consumed over the same span of time with commensurate production of lactic acid. [49]

While low concentrations (below 100 mM) of aliphatic acids have been found to support the growth of alcohol producing bacteria, they exhibit toxic effects at higher concentrations. [50] The impact of these inhibitors is believed to be exacerbated by compounding effects that occur when they are present together, and thus any proposed purification system would need to be effective against a wide range of contaminants. [51]

A variety of methods of removing or accounting for inhibitors to bacteria growth have been investigated in recent years. Some focus on the organism itself, attempting to cultivate resistant strains that can survive in environments with elevated levels of acetic acid or phenols, while others attempt to use chemical or physical techniques to reduce the concentration of these substances in the feedstock. [52] [53] Various additives including activated charcoal, char, and lime have all been tested for removal efficiency and optimal process conditions with a variety of hydrolysate sources. [54]

2.2.1 Overliming

Of the many available purification techniques, overliming, the addition of alkali such as calcium hydroxide to adsorb or precipitate inhibitors from the hydrolysate, is one of the most common, due in part to its cost efficiency. Optimization of this process requires careful consideration of temperature, timing and final pH of the mixture, as it is known to degrade a portion of the available sugars during the process. The mechanisms of overliming are thought to arise from the formation of complex ions between the Ca^{2+} cation and components of the hydrolysate. The specific reactions have yet to be identified, but the models described below are thought to represent the essential mechanisms according to works by Purwadi *et al*. [21]

$$Z + A \underset{k2}{\overset{k1}{\Leftrightarrow}} \{ZA\}$$

$$\{ZA\} \overset{k3}{\rightarrow} P + Z$$

In these equations Z is the Ca^{2+} ions, and A is the reactants (sugars, phenols, furfural, etc.) being affected. ZA is therefore the complex ion formed from their interactions, which can then degrade, resulting in the formation of the end-product P while the cation is released back into solution. Purwadi's work was unable to effectively determine the order of the reactions that occur during overliming; however, their results did indicate that both reaction time and temperature were important to the process. Other works have shown that phenolic compounds can be removed by the overliming process. [55] [56] [11] Testing at multiple pHs of 10, 11 and 12 showed various levels of removal efficiency. Trials at a pH of 11 and

60 °C showed the highest removal from spruce chip based hydrolysates at 25%; however, trials at a higher pH of 12 resulted in an increase in detected total phenols. [55] Similar overliming attempts with dilute hydrolysates of olive stones resulted in a maximum of 29% removal of total phenolic compounds after treatment at 1 hour, a pH of 12 and 60 °C. [57] Works attempting to use overliming in order to detoxify phenol rich pyrolytic sugar syrup have indicated that it can be an effective method to prepare them for fermentation, removing approximately 20% of the detected inhibitors. [58]

Alkaline treatment of carbohydrates has been shown to result in peeling reactions and endwise degradation of polysaccharides which can lead to the formation of additional inhibitors as found at the more extreme treatment conditions. [59] Aromatic carboxylic acids formed during this process are often classed as phenylic compounds, as they act to inhibit cell development in the same ways. Due to these considerations it is necessary to test a variety of overliming conditions for a particular hydrolysate to ensure optimal detoxification results.

When focusing on the detoxification of hydrolysates, various combinations of inhibitor removal technology have been tested. Many studies focus on activated charcoal as the detoxification additive with the highest removal rate. This method however, can incur high costs in an industrial setting and requires filtration to recover the additives after adsorption. Overliming followed by treatment with activated charcoal has typically proven to have the highest removal efficiency going up to 78.44% reduction in total furfurals and phenols in certain cases. [24] Alternatives for activated charcoal such as modified pyrochar have also

been investigated, but require additional infrastructure to produce the modified treatment material on-site. [54] Ion exchange resins have also shown considerable promise for the removal of phenols, furfural, and hydroxymethyl furfural, however, they did not appreciably affect acetic acid concentrations in the hydrolysate. [60] Membrane filtration has however, been shown to remove acetic acid from wood hydrolysates effectively, while also concentrating the available sugars. [20] A combination of techniques would therefore appear to be the most promising solution.

2.2.2 Membrane Filtration

Employed almost-ubiquitously throughout industry for water processing, nano-membrane filtration employs a specialized membrane composed of materials that are permeable to targeted compounds while retaining others via size exclusion. By varying the membrane specifications as well as the processing conditions it is possible to tune such systems to retain larger or smaller molecules. Industrial applications of membrane filtration must also consider the specifics of the filtration devices themselves which may employ differing configurations of membrane cartridges or flow direction systems to reduce fouling and wear on the device. The use of nanomembrane filtration to separate and concentrate valuable components from waste streams has garnered significant interest of late in a number of industrial applications. Studies have shown that ceramic membrane nanofiltration was able to effectively remove sugars, amino acids, and small peptides from pre-filtered herring marinade, resulting in an overall reduction in waste water of 37.5%. [61] Other works by IFBR focused research have shown considerable success in separating

hemicelluloses and acetic acid from PHLs drawn from a kraft pulp mill using a combination of nanofiltration and reverse osmosis to create a sequential process for the recovery of useable bio-products. Nanofiltration succeeded in attaining a rejection of lignin and oligomeric sugars greater than 90%, concentrating the filtered PHL to a useable level for conversion into furfural. [12] In the filtration of products from TMP-Bio PHLs, two streams of material are produced; a concentrated sugar stream and another composed primarily of acetic acid and removed water as shown in the diagram below.

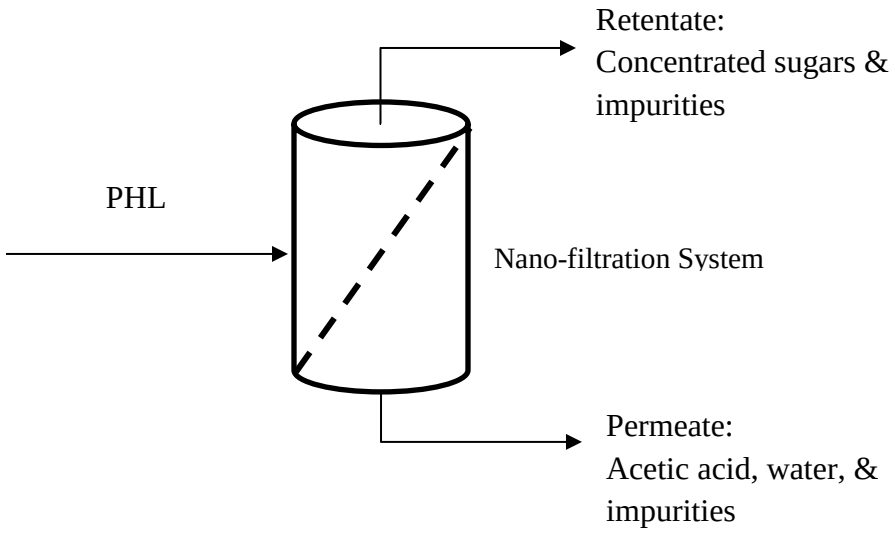

Figure 10: Process flow for Membrane Filtration

Other impurities and compounds present in the PHL such as phenols and other wood extractives will remain in the retentate for the most part, with only small fractions being removed in the permeate, as the selection of the process membrane is tuned to favor removal of acetic acid. During the separation a small portion of the sugars present in the feed may also be lost in the permeate if the membrane is not sufficiently stringent, as well

as material becoming entrapped in the filter itself. In an industrial setting membrane fouling may be reduced by backflushing of permeate through the system or using water jets to loosen and disperse accumulated material, though this technique is not suitable for all filtration systems. Chemical cleaning with alkaline or acid washes and detergents is also common, however; frequent cleaning does reduce the lifespan of the membrane. [62]

Chapter 3

Materials and Methods

3.1 Hardwood Prehydrolysate

Mixed hardwood chips were provided by FPInnovations for use in production of the prehydrolysis liquor for testing. Such chips are similar to those used in pulp mills throughout Canada. As per the modified TMP-Bio process, the raw biomass was treated with sulphuric acid to bring a slurry of chips and water to a pH of 2, after which the mixture was cooked in a boiler for 30 minutes at a temperature of 140 °C. The softened chips were then put through a pneumatic press to extract the xylose rich prehydrolysate. The complete batch of prepared prehydrolysate was divided and frozen immediately after production for storage.

3.2 Detoxification of Prehydrolysate

Samples were thawed overnight in a refrigeration unit before pH adjustment and subsequent membrane filtration. Overliming was carried out using solid $Ca(OH)_2$ (Anachemia ACS Reagent Grade) to increase the pH and H_2SO_4 (Anachemia ACS Reagent Grade) was used to lower it after processing. Four different sets of conditions were investigated during the overliming portion of the project:

I) pH increase to 10 at 25°C

II) pH increase to 11 at 25°C

III) pH increase to 10 at 60°C

IV) pH increase to 11 at 60°C

Each sample was maintained at the target temperature in a water bath for one hour after pH adjustment with continual stirring. The prehydrolysate was adjusted to pH 6 after overliming and then vacuum filtered through glass microfiber filters (Whatman 934-AH) to remove precipitates before membrane filtration. A desktop column from Koch Membrane Systems shown in the image below was prepared, for testing with 500 mL of the treated PHLs.

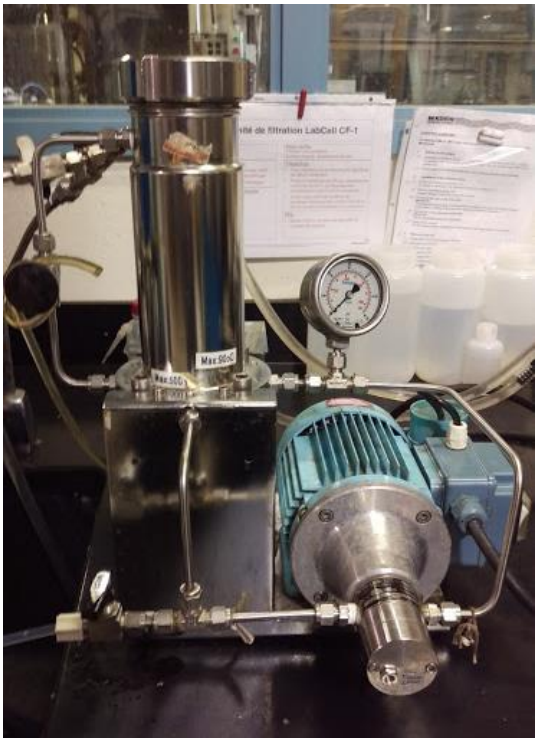

Figure 11: Membrane Filtration Column

Pressure within the column was raised to 440 Pa using compressed nitrogen, while a pump and water heater were used to keep the column at 40 °C throughout the filtration process. Overlimed prehydrolysate was passed through a disc of 200 Da membrane (SelRO® MPF-34, Koch Membrane Systems) and allowed to process until they reached a final volume of

approximately 200 mL. Samples were made up with distilled water after filtration in preparation for fermentation and testing.

3.3 Media and Fermentation Conditions

For each fermentation trial 500 mL of detoxified hardwood pressate from each set of overliming conditions was filtered down to 0.22 μm under sterile conditions after membrane filtration and stored at 10 °C for 24 hours prior to fermentation while the inoculum was being prepared. The sugar-rich filtrates were fermented to lactic acid as described by Bischoff et al. [10] with slight modifications. The prepared reactor setup can be seen in image below.

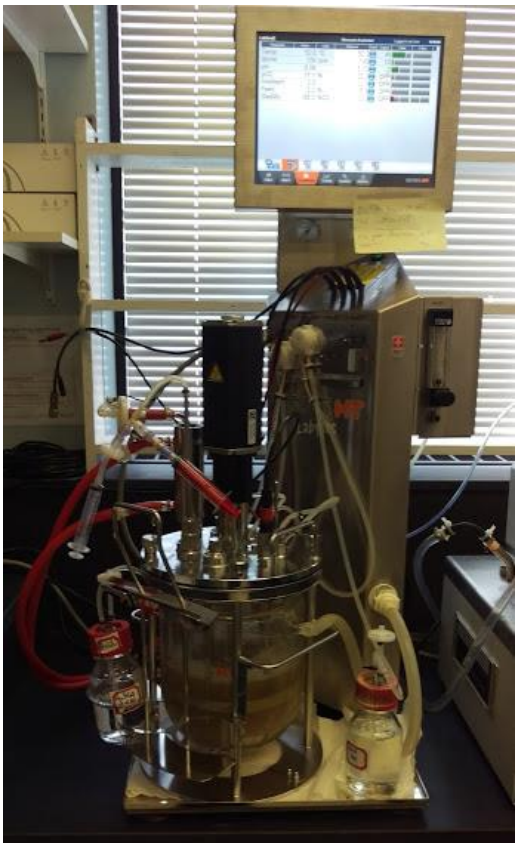

Figure 12: Fermentation Reactor

The 1200 mL INFORS anaerobic bioreactor was prepared with an inoculum of *Bacillus coagulans* GBI 30, 6086 was produced by recovering cultures from a BSX agar plate (10 g tryptone, 5 gL^{-1} yeast extract, 2 g K_2HPO_4, and 15 gL^{-1} agar, after being grown from the stock culture at 50 °C, static overnight) by inoculating 50 mL BSX media (10 gL^{-1} tryptone, 5 gL^{-1} yeast extract, 2 gL^{-1} K_2HPO_4, and 10 gL^{-1} xylose) with a colony and incubating at 50 °C, static overnight.

For the duration of the fermentation the temperature was maintained at 50 °C with a water jacket and stirring was constant at 220 rpm, while pH was kept between 6-6.5 by manual addition of 5 N NaOH. The process was anaerobic, but not strictly (i.e. the bio-reactor was not purged with nitrogen before inoculation). Instead inoculation began in an aerobic environment, and the bacteria quickly consumed all available oxygen and the process became anaerobic. Growth media (tryptone, yeast extract, K_2HPO_4) were autoclaved at 121 °C for 20 min to sterilize them prior to aseptic additions while the sugar solution was filtered through a Nalgene™ Rapid-Flow™ Sterile disposable filter equipped with a 0.45 μm polyethersulfone (PES) membrane.

For each trial 20 mL of (26.1 gL^{-1} K_2HPO_4, 11.3 g KH_2PO_4, and 25 g NH_4NO_3) and 1 mL of (1.05 M nitrilotriacetic acid, 0.59 M $MgSO_4{\cdot}7H_2O$, 0.91 M $CaCl_2{\cdot}2H_2O$, and 0.04 M $FeSO_4{\cdot}7H_2O$) were aseptically added per liter of the working volume. The inoculum represented 6-9% (v/v) of the working volume. A control sample for fermentation was prepared from distilled water and 14 g/L of xylose (VWR, Reagent grade).

3.4 Analysis of Sugars, By-products, & Lactic Acid

The sugar and acetic acid content of the prehydrolysate and detoxified samples was assessed by FPInnovations staff using the in-house apparatus. Monosaccharides were determined with a DX-60 Ion Chromatography system (Dionex, Sunnyvale, CA), equipped with an anion exchange column (Dionex CarboPac PA1) and an ED40 electrochemical detector, with 2-deoxyglucose (0.25 mg mL^{-1}) as the internal standard. The column was eluted with deionized water at a flow rate of 1 mL min^{-1}. Aliquots (20 µl) were injected after passing through a 0.45µm nylon syringe filter (Chromatographic Specialties Inc., Brockville, ON, Canada). Baseline stability and detector sensitivity were optimized by post column addition of 0.2 M NaOH at a flow rate of 0.5 mL min^{-1} using a Dionex AXP pump. The column was reconditioned using 1 M NaOH after each analysis. Monosaccharides (arabinose, galactose, glucose, xylose and mannose) were quantified with reference to standards.

The total concentration of phenols in the sample as vanillin equivalents was determined via colorimetric assay [63]. An aliquot of diluted pressate was added to 3 mL of distilled water. 250 µL of Folin-Ciocalteu reagent was added to each before being vortexed and allowed to stand for 5 minutes before the addition of 750 µL of 20% Na_2CO_3. The solution volume was then adjusted to 5 mL with distilled water and allowed to incubate for 90 minutes, vortexing every half hour to ensure adequate mixing. After incubation, the absorbance of the sample at 760 nm was read against a prepared blank. All samples were analyzed in duplicate.

Chapter 4

Detoxification of Modified TMP-Bio Prehydrolysate

4.1 Initial Prehydrolysate Composition

The prehydrolysate produced in FPInnovations Pointe-Claire Pilot Plant had a composition as outlined in Table 1. As expected of the modified TMP-Bio process the initial concentration of C5 sugars such as xylose far surpassed that of the C6 sugars.

Table 3: Composition of Untreated Prehydrolysate

Components	Concentration (g/L)
Arabinose	2.34
Arabinan	0.18
Galactose	2.15
Galactan	0.13
Glucose	3.40
Glucan	0.19
Xylose	46.5
Xylan	6.77
Mannose	2.61
Mannan	0.20
Phenols	3.84
Acetic Acid	12.37

The strong presence of acetic acid and phenols in the untreated pressate necessitated detoxification before fermentation, as both are known to have significant inhibitory effects. [51] Previous studies into detoxification of hemicellulosic hydrolysates have indicated that

overliming can effectively reduce the total concentration of phenols and subsequently reduce their inhibitory effects on fermentation. [55] According to work by Luis Fernando del Rio of FPInnovations previous batches of pressate had been fermentable using the same methodology after total detoxification attempts had reduced the total concentration of detected phenols to below 3 g/L without observation of the concentration of acetic acid. The threshold for other inhibitory compounds when fermenting with *B. Coagulans* were unknown however.

4.2 Detoxification of Prehydrolysate by Overliming and Membrane Filtration

For this study four different sets of conditions were evaluated to determine the most effective combination of pH and temperature for detoxification of modified TMP-Bio prehydrolysate. The effects of this treatment on the concentration of xylose, total phenols (in vanillin equivalents), and acetic acid was studied as percentage removal from the untreated pressate after overliming, and then from the post-overliming conditions to the concentrations after membrane filtration as shown in the tables below.

Table 4: Effects of Detoxification on Prehydrolysate Sugar Composition

	Xylose (g/L)	Removal (%)
Raw Prehydrolysate	46.5	
After Overliming		
Condition I	32.60	29.9
Condition II	33.45	28.1
Condition III	35.10	24.5
Condition IV	25.80	44.5
After Membrane Filtration		
Condition I	29.90	8.3
Condition II	34.00	-1.6
Condition III	31.50	10.3
Condition IV	25.30	1.9

Table 5: Effects of Detoxification on Inhibiter Composition

	Total Phenols (g/L)	Removal (%)	Acetic Acid (g/L)	Removal (%)
Raw Prehydrolysate	3.84		12.369	
After Overliming				
pH 10, 25 °C	3.22	16.1	14.21	-14.9
pH 11, 25 °C	3.11	19.0	14.34	-15.9
pH 10, 60 °C	3.34	13.0	15.31	-23.8
pH 11, 60 °C	3.54	7.8	14.49	-17.1
After NF				
pH 10, 25 °C	2.75	14.6	10.81	23.9
pH 11, 25 °C	2.80	10.0	11.35	20.9
pH 10, 60 °C	2.69	19.5	11.07	27.7
pH 11, 60 °C	3.19	9.9	10.69	26.2

The removal of phenols from prehydrolysate by overliming is attributed to the formation of complex ions from the Ca^{2+} ions and furans present in the untreated pressate. [21] As the availability of calcium ions is limited by the presence of OH^-, increases in pH affect the efficacy of treatment with alkali. The effects of overliming on the concentration of total phenols was comparable to literature values of approximately 20% for the hardwood pressate produced by FPInnovations' modified TMP-Bio process. [51] The increase in concentration of acetic acid after overliming can be attributed to the liberation of acetyl groups bound to dissolved hemicellulose present in the prehydrolysate. [56] Further refinement of the prehydrolysate with a more stringent membrane could remove additional acetic acid, as it has been demonstrated that multi-stage reverse osmosis can recover up to 70%. [20]

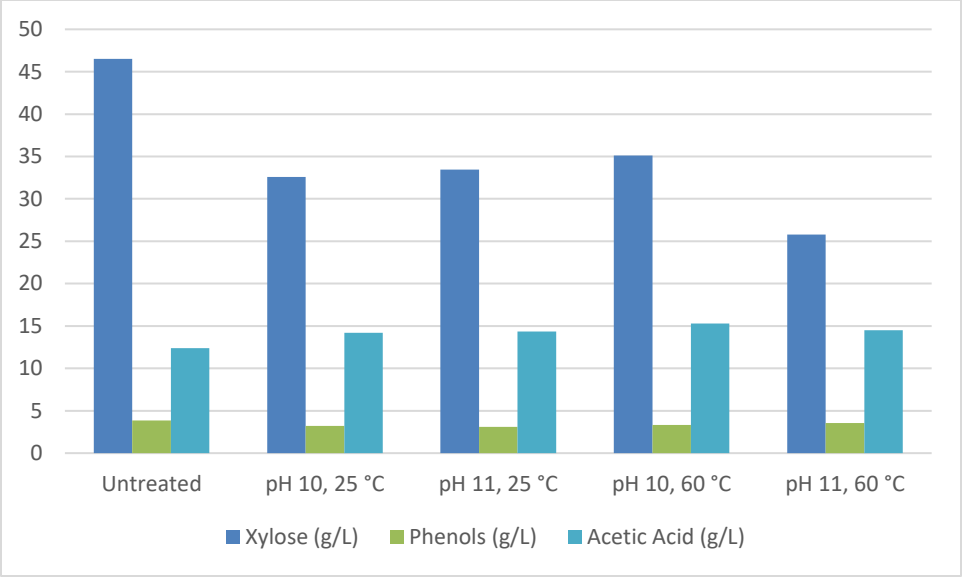

Figure 13: Effects of Overliming on Pressate Composition

Overliming at a temperature of 60 °C resulted in the highest xylose losses when raised to pH 11. On average adjustment to pH 10 resulted in a greater removal of phenols to sugar losses and would be the optimal target for detoxification of such prehydrolysates. The change in the concentration of xylose varied significantly between the various conditions used for overliming. The lowest removal of sugars occurred during the overliming trials under condition III at 24.5% of the initial concentration. The most severe overliming under condition IV removed another 20% of the detected xylose in the pressate beyond the results of the condition III trials, which resulted in a final xylose concentration of only 25.80 g/L of pressate.

Spruce and Bagasse hydrolysate overlimed with calcium hydroxide under similar circumstances to Conditions I & III resulted in a 19 and 17% reduction in total phenols respectively, comparable to that shown in the prehydrolysate produced at FPI. [51] The

harshest overliming conditions at 60 °C and a pH of 11 resulted in only a 7.8% removal of the detected phenols in the pressate, less than half of the removal shown by the samples overlimed at 25 °C. This low rate of removal paired with a high loss in xylose indicates that condition IV overliming is not suitable for detoxification of modified TMP-Bio prehydrolysate.

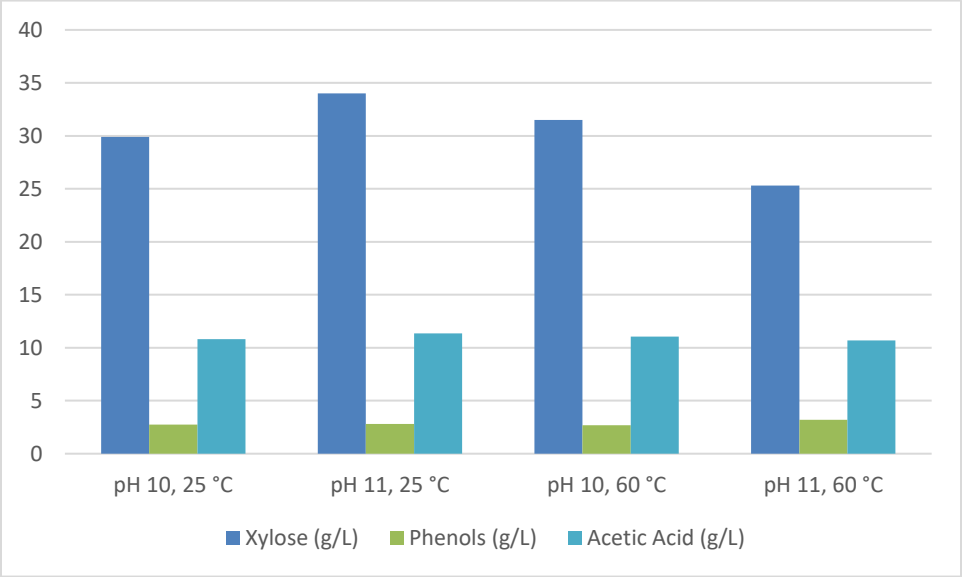

Figure 14: Composition of Membrane Filtration Treated Pressate

The effects of membrane filtration through a membrane with pores sized to prevent the passage of molecules larger than 200 Da (SelRO® MPF-34, Koch Membrane Systems) at 440 Pa and 40 °C were roughly consistent, averaging a phenol removal of 14.6% and a 24.7% removal of acetic acid from the concentrations found after overliming. These membranes were readily available at FPInnovations; however, membranes with a lower cut-off (closer to 150 Da) would remove higher concentrations of acetic acid.

The highest loss in xylose of 10.3% was found in the condition III overlimed samples. The samples overlimed under condition III also had the highest initial sugars concentration at 35.1 g/L of xylose before membrane filtration, which lead to a commensurate increase in losses due to permeability. The following mass balances show the concentration of dissolved contaminants for each membrane filtration, with the permeate stream containing any materials deposited on the filter disc.

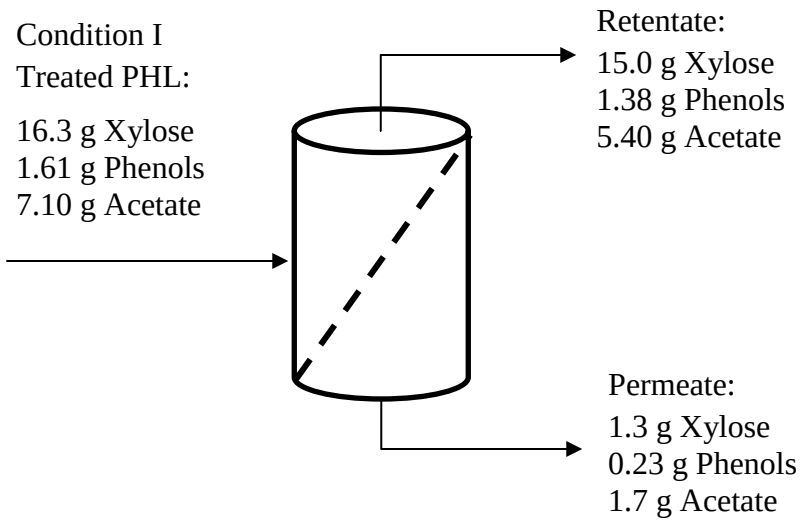

Figure 15: Condition I NF Results

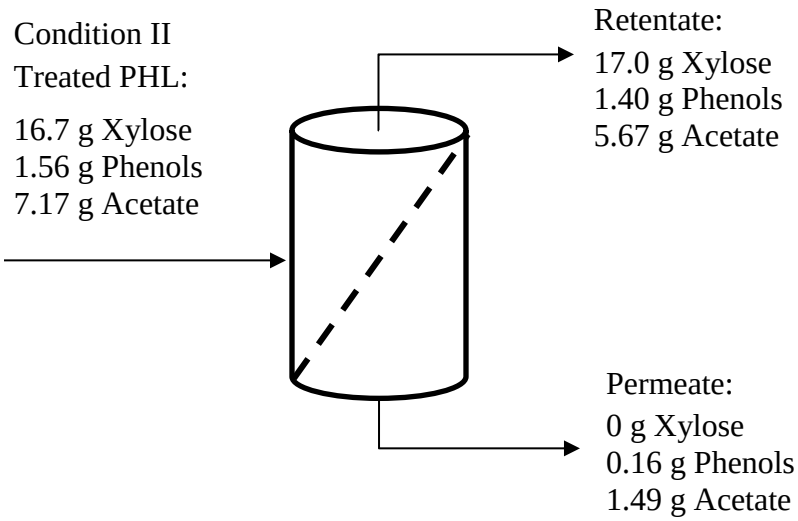

Figure 16: Condition II NF Results

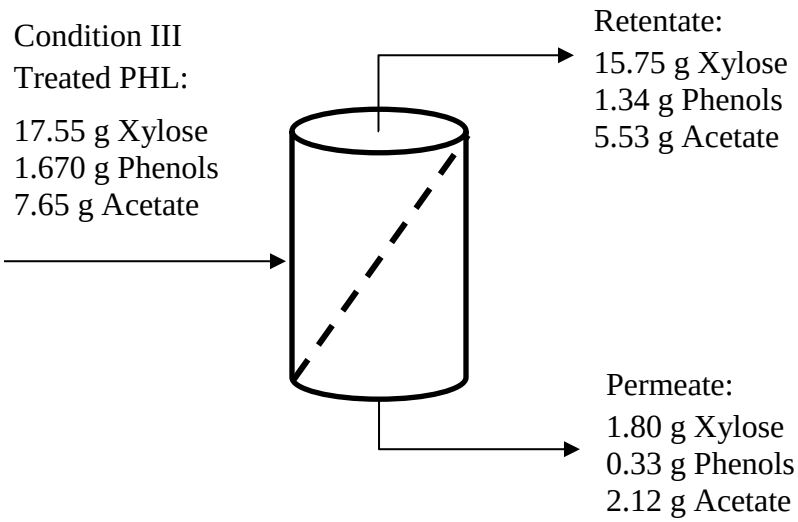

Figure 17: Condition III NF Results

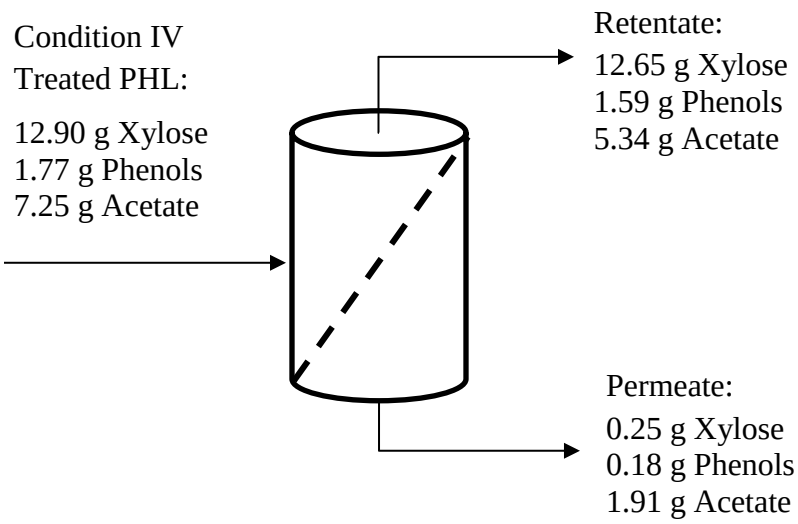

Figure 18: Condition IV NF Results

The treated PHLs prepared for nanofiltration were analyzed on a dry basis of 500 mL of dilute pressate. Pressate overlimed under condition I before membrane filtration produced prehydrolysate with a final concentration of acetic acid of 10.81 g/L, a xylose concentration of 29.90 g/L, and a total phenols concentration of 2.75 g/L. At these levels the phenols and acetic acid concentrations are low enough to allow for effective fermentation while the total losses in sugars are acceptable. Overall the retention of xylose by membrane filtration matched literature results very well, exceeding 90% in all cases, which strongly supports this method as a means of separating a useable sugar stream from treated TMP-Bio PHL. [64] Less stringent membranes with molecular weight cut offs between 150 and 300 Da have still shown considerable promise in this application, retaining up to 74% of mixed monomeric sugars from Kraft black liquor. [20] The removal rate of phenols for the membrane filtration portion of the work was found to be quite low, varying between 10-20% of the detected total across the treated PHLs, which matches with the expected high

rejection rate of these membrane filtration conditions. The separation of acetic acid from treated TMP-Bio PHL by membrane filtration averaged nearly a 75% rejection rate, which is comparable to the 70% found by Ahsan *et al*. in their investigation into NF recovery of acetic acid from PHLs. [20]

Chapter 5

Lactic Acid Fermentation of Detoxified Prehydrolysate

5.1 Fermentation of Dilute Treated Pressate

Sufficient volume of detoxified prehydrolysate was produced for fermentation trials with conditions I, III, and IV. Shortages in untreated pressate prevented the preparation of sufficient volume of condition II treated PHL for fermentation. All prehydrolysate samples were supplemented with required nutrients after filtration as described in full in Chapter 3. Figures 4, 5, and 6 show the results of fermentation of the dilute detoxified prehydrolysate against a control sample of pure xylose. As shown in previous fermentations by FPInnovations staff, untreated pressate was unfermentable by *Bacillus coagulans*, showing no change in the concentration of xylose or lactic acid over the course of the trial.

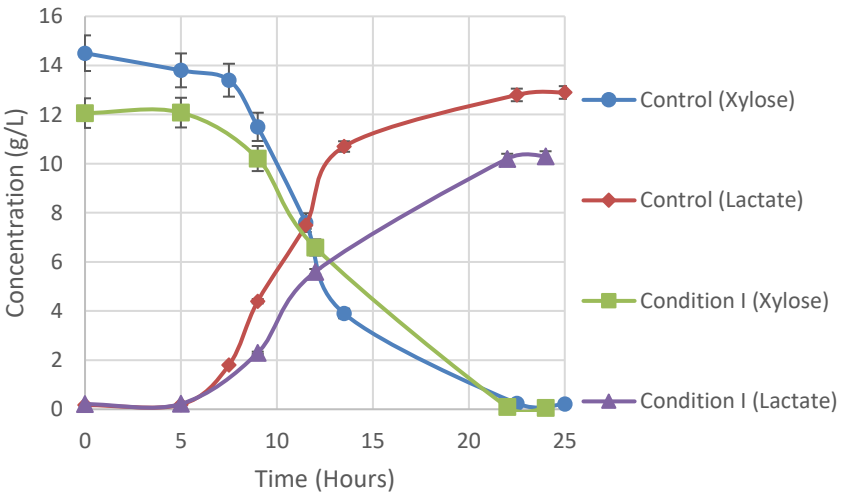

Figure 19: Lactic Acid Fermentation of Condition I Treated Prehydolysate

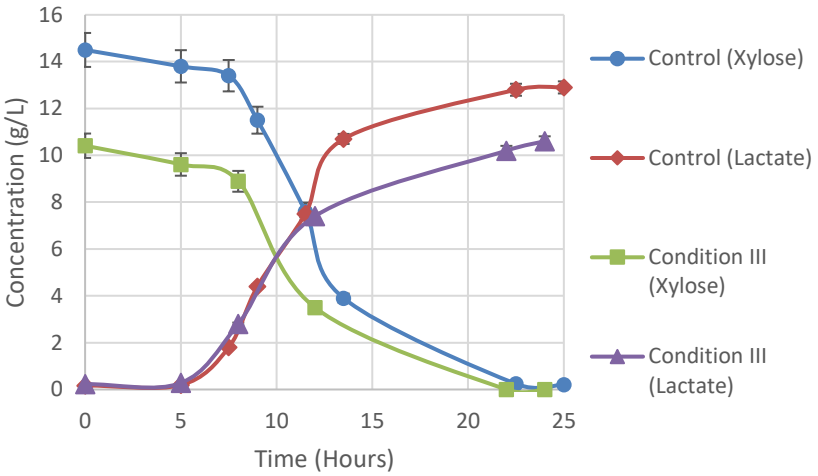

Figure 20: Lactic Acid Fermentation of Condition III Treated Prehydrolysate

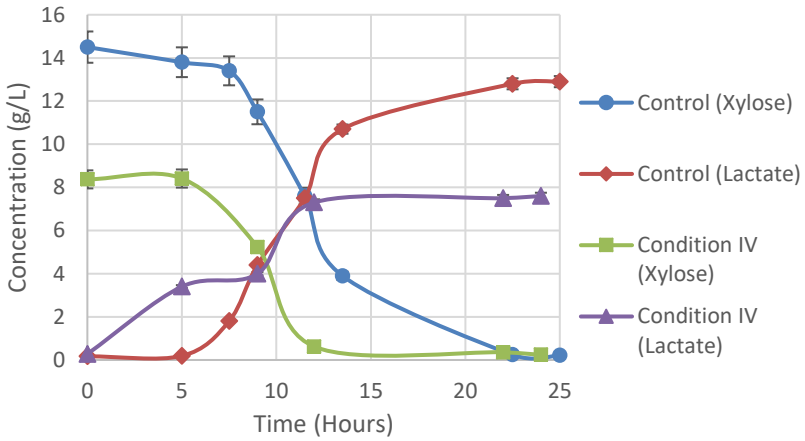

Figure 21: Lactic Acid Fermentation of Condition IV Treated Prehydrolysate

As expected, the concentration of lactate detected in samples taken from the fermentation vessel throughout the course of the process increased with a commensurate reduction in xylose. Due to the presence of additional unmonitored sugars, (glucose, mannose, etc...) within the dilute prehydrolysis liquor used, as well as the additional sugars added with the inoculum, condition I treated pressate resulted in a higher final concentration of lactate than the initial feed of xylose, but did not otherwise deviate markedly. The standard error

for IC based sugar analysis of 5% is displayed on each graph, as well as the 2% error for YSI lactic acid membrane testing. Of the tested samples, pressate detoxified using condition III overliming followed by membrane filtration had the smallest lag phase, with 8.7% of the initial xylose concentration being consumed 5 hours into the fermentation. All other samples had less than 1% of total detected xylose consumed at that point in their respective fermentation trials.

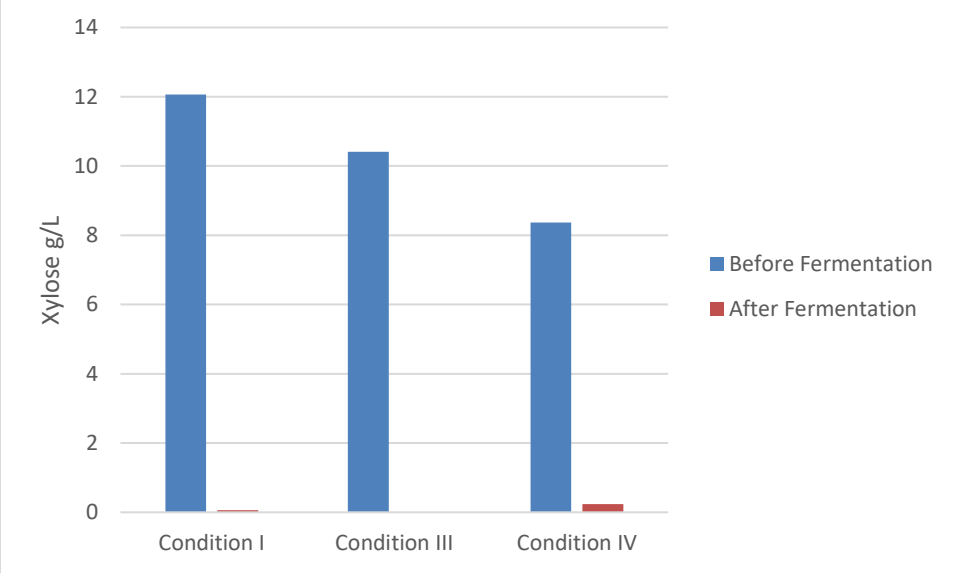

Figure 22: Concentrations of Xylose Before and After Fermentation

The treated prehydrolysate overlimed under condition IV had the lowest initial concentration of xylose due to losses from the overliming portion of the detoxification process. During fermentation over 80% of the initial concentration of xylose for that trial was consumed within 8 hours, which is reasonable given that optimized trials using a pure xylose feedstock have obtained yields as high as 99% on a gram per gram basis. [65] Condition I & III treated pressates showed results similar to those of the control sample in

respects to lag time and rate of consumption of xylose, resulting in comparable fermentation curves. The successful fermentation of all treated pressate samples indicates that this combination of detoxification processes is reducing the concentration of the primary inhibitory compounds beneath the thresholds required by *B. coagulans* for these conditions. It is important to note that while less severely limiting than some of the identified inhibitors found in prehydrolysate samples such as those used in this study, lactic acid has been identified as having inhibitory effects on the growth of *B. coagulans* if allowed to accumulate without neutralization. There is some indication that for optimal growth and yield it is preferable to employ calcium hydroxide over sodium hydroxide for this purpose, as sodium salts are reported to have higher inhibitory action. [65]

Chapter 6

Conclusions

6.1 Summary of Results and Findings

This study focused on the detoxification of hardwood prehydrolysate produced by the modified TMP-Bio process. Such pressate is known to contain fermentation inhibitors such as acetic acid and phenols, the removal of which was the primary objective. Untreated prehydrolysate was known from previous work at FPInnovations to be unsuitable for fermentation by *Bacillus coagulans* due to the presence of these inhibiting compounds and as such fermentation trials were used to test the viability of treated pressate. The control fermentation trials carried out by FPInnovations showed that a pure xylose solution with the same initial sugar concentration as an untreated PHL could be used as a feedstock to produce lactic acid while the latter did not show any change.

Overliming of the prehydrolysate at 25 °C and a Ph of 10 and 11 removed an average of 17% of the phenols detected in the untreated sample, with losses of xylose between 28.1% and 31.6% of the original concentration. Use of pH adjustment at 60 °C resulted in lower removal of total phenols and higher sugar losses of up to 56.8% of the original value in the most extreme cases. This indicates that higher temperature overliming is suboptimal for pressate produced under the present conditions, though the technique did yield satisfactory results for phenol removal. Overliming was found in practice to increase the overall concentration of acetic acid in the tested TMP-Bio PHLs, likely due to the liberation of acetyl groups on remaining hemicelluloses in the sample. These results strongly support

the use of combination detoxification for overlimed samples as pH adjustment alone is unlikely to produce a fermentable product.

Subsequent membrane filtration was employed to further reduce the concentration of all tested inhibitors in the overlimed prehydrolysate. After dilution to the original volume the retained pressate showed reductions in acetic acid concentration of up to 23.9% for the condition I treated pressate with only an 8.3% loss in xylose from the concentration remaining after pH adjustment. Some small portion of these losses likely arise from the filtration setup which employed disposable discs of sheet membrane which were discarded between trials.

Lactic acid fermentation of prehydrolysate treated under conditions I, III, and IV showed successful production of lactate over the course of the 24 hour trial. All samples reached final concentrations of xylose below 0.5 g/L. Pressate detoxified using condition III overliming and membrane filtration produced a fermentation curve with the smallest lag phase resulting in 7.7% of detected xylose being consumed within 5 hours. The low final sugar concentration achieved in these fermentations indicate that the dilute pressate has been effectively detoxified for these conditions, with a commensurate production of lactic acid. Low concentrations of phenols and aliphatic acids have been shown to increase the fermentability of pressates, which is likely a contributing factor in the high yields achieved in these trials.

Membrane filtration of prehydrolysate produced by FPInnovations modified TMP-Bio process is a promising method of detoxification to remove acetic acid with minimal losses in sugars, producing a more fermentable product. Further investigation into membrane filtration with a lower pass rate could improve acetic acid removal significantly, ultimately leading to a process that can produce a valuable feedstock for fermentation of naturally sourced chemicals.

6.2 Recommendations for Future Work

The findings of this project stand as a case study for further research into biorefining of TMP-Bio prehydrolysates. Future works may explore:

- The effects of more stringent membranes on detoxification.
- Fermentability of treated pressates as feedstock for other microbes.
- Comparisons of the investigated detoxification methods on prehydrolysates produced from softwood chips.
- Optimization of acetic acid recovery from membrane filtration.

Bibliography

[1] Wood Pellet Association of Canada, "Canadian Wood Pellet Opportunities," in *IEACCC - EPPEI Joint Workshop - Cofiring Biomass with Coal*, Beijing China, 2017.

[2] T. Kovacs, V. Naish, B. O'Connor, F. Gagne, L. Hall, V. Trudeau and P. Martel, "An ecotoxicological characterization of nanocrystalline cellulose (NCC)," *Nanotoxicology*, pp. 4, 3, 255-270, January 01, 2010.

[3] X. Pan, C. Arato, N. Gilkes, D. Gregg, W. Mabee, K. Pye, Z. Xiao and J. Saddler, " Biorefining of softwoods using ethanol organosolv pulping: Preliminary evaluation of process streams for manufacture of fuel-grade ethanol and co-products," *Biotechnology and Bioengineering,* vol. 4, no. 90, pp. 473-481, 2005.

[4] L. Christopher, Integrated forest biorefineries: Challenges and opportunities, Cambridge: Royal Society of Chemistry, 2013.

[5] R. Rowell, The Chemistry of Solid Wood, Seattle: American Chemical Society, 1984.

[6] J. G. Kisser, A. Ylinen, K. Freudenberg, F. F. P. Kollmann, W. Liese, B. Thunell, H. G. W. Winkelmann, A. Côté, J. Koch, J. E. Marian and A. J. Stamm, "History of Wood Science," *Wood Science and Technology*, vol. 1, no. 3, pp. 161-190, 1967.

[7] J. Jönsson, P. Ruohonen, G. Michel and T. Berntsson, "The potential for steam savings and implementation of different biorefinery concepts in Scandinavian

integrated TMP and paper mills," *Applied Thermal Engineering*, vol. 31, no. 13, pp. 2107-2114, 2011.

[8] C. Mao and Z. Yuan, "TMP-Bio for converting cellulosic biomass to 2nd generation sugar and near-native lignin," FPInnovations, Pointe-Claire, 2017.

[9] S. Rangaswamy, "Xylitol Production from D-Xylose by Facultative Anaerobic Bacteria," 27 January 2003. [Online]. Available: http://scholar.lib.vt.edu/theses/available/etd-03082003-172558/unrestricted/SenETD.pdf.

[10] K. M. Bischoff, S. R. Hughes, S. R. Hughes and J. O. Rich, "Fermentation of corn fiber hydrolysate to lactic acid by the moderate thermophile Bacillus coagulans," *Biotechnology Letters*, pp. 32, 6, 823-828, 2010.

[11] A. Martinez, M. E. Rodriguez, S. W. York, J. F. Preston and L. O. Ingram, "Effects of Ca(OH)2 treatments (“overliming”) on the composition and toxicity of bagasse hemicellulose hydrolysates," *Biotechnology and Bioengineering*, vol. 69, no. 5, pp. 526-536, 2000.

[12] I. Kaur, Further developments in recovering Furfural and acetic acid from an industrial pre-hydrolysis liquor (PHL), Fredericton: University of New Brunswick, 2015.

[13] Y. Liu, J. Wang and M. Wolcott, "Modeling the production of sugar and byproducts from acid bisulfite pretreatment and enzymatic hydrolysis of Douglas-fir," *Bioresource Technology*, vol. 224, pp. 389-396, 2017.

[14] E. Lam, J. H. Chong, E. Majid, Y. Liu, S. Hrapovic, A. C. W. Leung and J. H. T. Luong, "Carbocatalytic dehydration of xylose to furfural in water," *Carbon,* vol. 50, no. 3, pp. 1033-1043, 2012.

[15] C. García-Sancho, I. Agirrezabal-Telleria, M. B. Güemez and P. Maireles-Torres, "Dehydration of d-xylose to furfural using different supported niobia catalysts," *Applied Catalysis B: Environmental,* pp. 1-10, 2014.

[16] B. Danon, W. Hongsiri, L. Aa and J. W, "Kinetic study on homogeneously catalyzed xylose dehydration to furfural in the presence of arabinose and glucose," *Biomass and Bioenergy,* vol. 66, pp. 364-370, 2014.

[17] C. V. L. D. Nguyen, W.-H. Chen, H.-W. Huang, Z. A. ALOthman, Y. Yamauchi and K. C.-W. Wu, "Combined treatments for producing 5-hydroxymethylfurfural (HMF) from lignocellulosic biomass," *Catalysis Today*, vol. 278, pp. 344-349, 2016.

[18] P. Fatehi, J. Ryan and Y. Ni, "Adsorption of lignocelluloses of model pre-hydrolysis liquor on activated carbon," *Bioresource Technology*, vol. 131, no. 1, pp. 308-314, 2013.

[19] G. Yang, M. S. Jahan, L. Ahsan, L. Zheng and Y. Ni, "Recovery of acetic acid from pre-hydrolysis liquor of hardwood kraft-based dissolving pulp production process by reactive extraction with triisooctylamine," *Bioresource Technology*, vol. 138, no. 6, pp. 253-258, 2013.

[20] L. Ahsan, M. S. Jahan and Y. Ni, "Recovering/concentrating of hemicellulosic sugars and acetic acid by nanofiltration and reverse osmosis from prehydrolysis

liquor of kraft based hardwood dissolving pulp process," *Bioresource Technology,* vol. 5, no. 111, p. 155, 2014.

[21] R. Purwadi, C. Niklasson and M. Taherzadeh, "Kinetic study of detoxification of dilute-acid hydrolyzates by Ca(OH)2," *Journal of Biotechnology,* vol. 114, no. 1-2, pp. 187-198, 2004.

[22] D. V. Heiningen, "Converting a Kraft pulp mill into an integrated forest biorefinery," *Pulp and Paper Canada Ontario,* vol. 6, no. 107, pp. 38-43, 2006.

[23] J. N. Nigam, "Development of xylose-fermenting yeast Pichia stipitis for ethanol production through adaptation on hardwood hemicellulose acid prehydrolysate," *Journal of Applied Microbiology,* vol. 2, no. 90, pp. 208-215, 2001.

[24] P. K. Keshav, N. Shaik, S. Koti and V. R. Linga, " Bioconversion of alkali delignified cotton stalk using two-stage dilute acid hydrolysis and fermentation of detoxified hydrolysate into ethanol," *Industrial Crops & Products,* no. 91, pp. 323-331, 2016.

[25] R. M. Brown and I. M. Saxena, Cellulose: Molecular and structural biology : selected articles on the synthesis, structure, and applications of cellulose, Dordrecht: Springer, 2007.

[26] International Conference on Textile Engineering and Materials, Advances in Textile Engineering and Materials IV: Selected, Peer Reviewed Papers from the 4th International Conference on Textile Engineering and Materials, Shenzhen, China: Trans Tech Publishers, 2014.

[27] A. Leonowicz, A. Matuszewska, J. Luterek, D. Ziegenhagen, M. Wojtaś-Wasilewska, N. S. Cho, M. Hofrichter and J. Rogalski, "Biodegradation of lignin by white rot fungi," *Fungal Genetics and Biology*, vol. 27, 1999.

[28] F. G. Calvo-Flores, J. A. Dobado, J. I. Garcia and F. J. Martin-Martinez, Lignin and Lignans as Renewable Raw Materials: Chemistry, Technology and Applications, Hoboken: Wiley, 2015.

[29] N. Gralén, "The molecular weight of lignin," *Journal of Colloid Science*, vol. 1, no. 5, pp. 453-463, 1946.

[30] S. V. Mohan and J. Karthikeyan, "Removal of lignin and tannin colour from aqueous solution by adsorption onto activated charcoal," *Environmental Pollution*, vol. 1, no. 97, pp. 183-187, 1997.

[31] A. Sluiter, Determination of structural carbohydrates and lignin in biomass: Laboratory analytical procedure (LAP), Golden: National Renewable Energy Laboratory (US), 2008.

[32] C. Xiros, P. Katapodis and P. Christakopoulos, "Factors affecting cellulose and hemicellulose hydrolysis of alkali treated brewers spent grain by Fusarium oxysporum enzyme extract," *Bioresource Technology*, vol. 2, no. 102, pp. 1688-96, 2011.

[33] D. H. Cho and S.-J. B. Y. P. C. &. K. Y. H. Shin, "Enhanced ethanol production from deacetylated yellow poplar acid hydrolysate by Pichia stipitis," *Bioresource Technology*, vol. 13, no. 101, pp. 4947-4951, 2010.

[34] R. Hu, L. Lin, T. Liu and S. Liu, " Dilute sulfuric acid hydrolysis of sugar maple wood extract at atmospheric pressure," *Bioresource Technology,* vol. 10, no. 101, pp. 3586-3594, 2010.

[35] F. Agbogbo and K. Wenger, "Effect of pretreatment chemicals on xylose fermentation by Pichia stipitis," *Biotechnology Letters,* vol. 24, no. 28, pp. 2065-2069, 2006.

[36] J. J. Fenske, D. A. Griffin and M. H. Penner, "Comparison of aromatic monomers in lignocellulosic biomass prehydrolysates," *Journal of Industrial Microbiology & Biotechnology,* vol. 6, no. 20, pp. 364-368, 1998.

[37] Popular Plastics & Packaging, "Global acetic acid market to grow at a CAGR of 9.3% from 2012 to 2018," *Popular Plastics & Packaging,* no. 58, p. 12, 2013.

[38] H. Mao, J. M. Genco, A. Heiningen and H. Pendse, "Technical Economic Evaluation of a Hardwood Biorefinery Using the "Near-Neutral" Hemicellulose Pre-Extraction Process," *Journal of Biobased Materials and Bioenergy,* vol. 2, no. 2, pp. 177-185, 2008.

[39] A. Basile, Acetic acids: Chemical properties, production and applications, Hauppauge: Nova Science Publishers Inc., 2013.

[40] R. Heinen, V. Ledoux-Pedailles and M. Yoneyama, "CEH Marketing Research Report: Acetic Acid," *Chemical Economics Handbook,* 2016.

[41] R. Renneke, S. R. McIntosh, V. Arunajatesan, M. Cruz, B. Chen, T. Tacke, H. Lansink-Rotgerink and M. Stoyanova, "Development of high performance catalysts

for the production of vinyl acetate monomer," *Topics in Catalysis,* vol. 38, no. 4, pp. 279-287, 2006.

[42] W. E. Luttrell and R. L. Hester, "Terephthalic acid," *Journal of Chemical Health & Safety,* vol. 23, no. 6, 2016.

[43] S. Fazl-E-Haider, "Rising fuel prices - a worrisome problem," *Pakistan & Gulf Economist,* vol. 14, no. 37, pp. 13-15, 2018.

[44] B. Yimer, Alternative energy sources to combat climate change: Biogas production using cost effective material, Hamburg: Anchor, 2014.

[45] M. O. S. Dias, T. L. Junqueira, C. D. F. Jesus, C. E. V. Rossell, F. R. Maciel and A. Bonomi, "Improving bioethanol production - Comparison between extractive and low temperature fermentation," *Applied Energy,* no. 98, pp. 548-555, 2012.

[46] K. Okano, S. Yoshida, T. Tanaka, C. Ogino, H. Fukuda and A. Kondo, "Homo-D-lactic acid fermentation from arabinose by redirection of the phosphoketolase pathway to the pentose phosphate pathway in L-lactate dehydrogenase gene-deficient Lactobacillus plantarum," *Applied and Environmental Microbiology,* vol. 15, no. 75, pp. 5175-5178, 2009.

[47] Z. Zheng, X. Lin, T. Jiang, W. Ye and J. Ouyang, "Genomic analysis of a xylose operon and characterization of novel xylose isomerase and xylulokinase from Bacillus coagulans NL01," *Biotechnology Letters,* vol. 8, no. 38, pp. 1331-1339, 2016.

[48] L. Ye, X. Zhou, M. S. B. Hudari, Z. Li and J. C. Wu, "Highly efficient production of l-lactic acid from xylose by newly isolated Bacillus coagulans C106," *Bioresource Technology*, no. 132, pp. 38-44, 2013.

[49] L. Fernando del Rio, "Sugar quality and fermentability (C6) [FPInnovations TMP-Bio PHL]," FPInnovations, Montreal, 2016.

[50] S. Larsson, E. Palmqvist, B. Hahn-Hägerdal, C. Tengborg, K. Stenberg, G. Zacchi and N. Nilvebrant, "The generation of fermentation inhibitors during dilute acid hydrolysis of softwood," *Enzyme and Microbial Technology*, vol. 24, no. 3, pp. 151-159, 1999.

[51] L. J. Jönsson, B. Alriksson and N.-O. Nilvebrant, "Bioconversion of lignocellulose: inhibitors and detoxification," *Biotechnology for Biofuels*, vol. 6, no. 1, p. 16, 2013.

[52] C. Huang, T. Lin, G. Guo and W. Hwang, "Enhanced ethanol production by fermentation of rice straw hydrolysate without detoxification using a newly adapted strain of Pichia stipitis," *Bioresource Technology*, no. 100, pp. 3914-3920, 2009.

[53] S. I. Mussatto, J. C. Santos and I. C. Roberto, "Effect of pH and activated charcoal adsorption on hemicellulosic hydrolysate detoxification for xylitol production," *Journal of Chemical Technology & Biotechnology*, vol. 79, no. 6, pp. 590-596, 2004.

[54] F. Monlau, C. Sambusiti, N. Antoniou, A. Zabaniotou, A. Solhy and A. Barakat, "Pyrochars from bioenergy residue as novel bio-adsorbents for lignocellulosic hydrolysate detoxification," *Bioresource Technology*, vol. 187, no. 5, pp. 379-386, 2015.

[55] R. Millati, C. Niklasson and M. J. Taherzadeh, "Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by Saccharomyces cerevisiae," *Process Biochemistry,* pp. 38, 4, 515-522, 2002.

[56] S. Jing, F. Pedram, S. Pendar and N. Yonghao, "Lime Treatment of Prehydrolysis Liquor from the Kraft-Based Dissolving Pulp Production Process," *Industrial & Engineering Chemistry Research,* vol. 51, no. 2, 2012.

[57] J. Andary, J. Maalouly, R. Ouaini, H. Chebib, M. Beyrouthy, D. N. Rutledge and N. Ouaini, "Phenolic Compounds from Diluted Acid Hydrolysates of Olive Stones: Effect of Overliming," vol. 1, no. 103, 2013.

[58] Z. Chi, M. Rover, E. Jun, M. Deaton, P. Johnston, R. C. Brown, Z. Wen and L. R. Jarboe, "Overliming detoxification of pyrolytic sugar syrup for direct fermentation of levoglucosan to ethanol," *Bioresource Technology,* no. 150, pp. 220-227, 2013.

[59] L. J. Jönsson and C. Martín, " Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects," *Bioresource Technology,* no. 199, pp. 103-112, 2016.

[60] W. Carvalho, L. Canilha, S. I. Mussatto, G. Dragone, M. L. V. Morales and A. I. N. Solenzal, "Detoxification of sugarcane bagasse hemicellulosic hydrolysate with ion-exchange resins for xylitol production by calcium alginate-entrapped cells," *Journal of Chemical Technology & Biotechnology*, vol. 79, no. 8, pp. 863-868, 2004.

[61] L. Fjerbæk Søtoft, J. Lizarazu, B. Razi Parjikolaei, H. Karring and K. Christensen, "Membrane fractionation of herring marinade for separation and recovery of fats,

proteins, amino acids, salt, acetic acid and water," *Journal of Food Engineering,* vol. 158, pp. 39-47, 2015.

[62] R. Baker, Membrane Technology and Applications, Chichester, West Sussex: John Wiley & Sons., 2012.

[63] E. Ragazzi and G. Veronese, "Quantitative analysis of phenolic compounds after thin-layer chromatographic separation," *Journal of Chromatography,* pp. 77, 2, 369-375, 1973.

[64] M. Malmali, J. Stickel and S. Wickramasinghe, "Sugar concentration and detoxification of clarified biomass hydrolysate by nanofiltration," *Separation and Purification Technology,* vol. 1, no. 32, pp. 655-665, 2014.

[65] L. Ye, X. Zhou, M. Hudari, Z. Li and J. Wu, "Highly efficient production of l-lactic acid from xylose by newly isolated bacillus coagulans c106," *Bioresource Technology,* vol. 132, pp. 38-44, 2013.

[66] L. Gustafsson, "Characterization and fermentation of dilute-acid hydrolyzates from wood," *Industrial and Engineering Chemistry Research,* vol. 36, no. 11, pp. 4659-4665, 1997.

[67] C. Rueda, M. Marinova, J. Paris, G. Ruiz and A. Coz, "Technoeconomic assessment of different biorefinery approaches for a spent sulfite liquor," *Journal of Chemical Technology & Biotechnology,* vol. 10, no. 91, pp. 2646-2653, 2016.

[68] S. I. Mussatto and I. C. Roberto, "Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review," *Bioresource Technology*, vol. 1, no. 93, pp. 1-10, 2004.

[69] Environmental Protection Agency of the United States, Office of Water, Membrane Filtration Guidance Manual, Washington D.C.: Environmental Protection Agency of the United States, 2003.

[70] I. Kuar and Y. Ni, "A Process to Produce Furfural and Acetic Acid from Pre-Hydrolysis Liquor of Kraft Based Dissolving Pulp Process," *Separation and Purification Technology*, vol. 146, pp. 121-126, 2015.

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