Production of Bioethanol and Furan Derivatives from Softwood Hemicellulose

by

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Abstract

Woody biomass provides a great potential for the biofuels production in the United States. Hemicellulose accounts for 20-30% of dried woody biomass, which can be readily broken down to simple sugars (primarily xylose and mannose). It is important for us to develop value added chemicals from hemicelluloses. In this study, we have extracted the hemicelluloses sugars from the pine wood. The prehydrolysate was from the acid pretreatment, which contains sugars and fermentation inhibitors; it was directly examined for the productivity of furan production under hydrothermal condition. The second hydrolysis was then applied and the fermentation was also tested for the inhibition level of the hemicellulose sugars. After that, two groups of detoxification methods were applied to the prehydrolysate. The sugars and toxic compounds were compared before and after fermentation, the detoxified prehydrolysate was tested in 48 hours fermentation, the level of toxic compounds and sugar content were monitored and compared. The results showed that the original control group from the prehydrolysate can hardly be fermented. The chemical methods showed advantages for the higher preservation of sugars, the ion resins and activated charcoal methods showed benefits for removing of more inhibitors and higher fermentation rate.

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Introduction

To decrease our heavy dependence on fossil fuels, lignocellulosic biomass is being considered as a potential resource for the production of bioethanol, a substitute for current hydrocarbon fuels. Lignocellulosic biomass is mainly composed by hemicellulose, lignin and cellulose. Hemicellulose has a random, amorphous structure with little strength, which could be easily hydrolyzed into monosaccharides. Cellulose is composed by entanglements of long chains of β-1, 4 linked glucose monosaccharides. Hemicellulose is composed of many different monosaccharides, including xylose, mannose, galactose, rhamnose, and arabinose. Sugars of hemicellulose and cellulose could be used in fermentation by yeast. After pretreatment of woody biomass, hydrolysis of sugars, fermentation and separation, bioethanol could be produced as the final product. While lignin is also an important raw material of adhesives production in chemical industry, the value of the whole bioconversion process will be increased if lignin is efficiently separated from lignocellulosic biomass.

Problem Statement

Due to the high cost of the biorefinery schemes for the bioethanol, it is critical to generate the high quality lignin and other potentially valuable co-products (e.g., furfural and acetic acid) from woody feedstocks. However, after hydrolysis, especially to organosolv pretreatment and acid hydrolysis, the chemical reactions will take place and produce inhibitors which can be toxic to the microorganism fermentation. Although different methods of detoxification are examined by many researchers, they are not so effective to increase the fermentability of the hydrolysate generated by the organosolv pretreatment. Part of the

reasons is that most of the inhibitors are organic compounds (e.g. furfural and HMF), which could hardly be removed from the hydrolysate. The short term goal of this research is to develop a detoxification method which could make hydrolysates after acid pretreatment fermentable and then the long term goal is to separate the value added co-products from woody biomass associated with the biofuels production. The specific objective for this study is to find a furfural/HMF oriented hydrothermal conversion process based on the sugars from woody biomass for value added co-products and biofuels production.

Scope of Research

In the US, plenty of underutilized forest biomass with low cost is available for the potential biofuels and value added co-products production. While we do have plenty of natural forest resource in Alabama, the large scale of bioconversion of underutilized and low cost forest biomass into bioethanol is in its infancy but is potentially viable in Alabama.

One target of this research is to generate value added co-products from the hemicellulose stream which could generate additional revenue for cellulosic ethanol from lignocellulosic biomass. These value added co-products will significantly improve the cost-effectiveness of biofuels production from underutilized forest biomass. The results from this research will potentially help to solve the issues of excess woody biomass material in Alabama, and providing economical opportunities for local communities. It is expected that the transformative technologies developed from this study will help revive the forest industry in Alabama, transforming conventional solid wood, and pulp and paper manufacture to forest biorefinery in the near future.

Contributions

The overall objective is to develop a furfural/HMF oriented hydrothermal conversion process for biofuels and test the fermentability of the detoxified hydrolysates from lignocellulosic material. The specific objectives of this study are as follows:

(1) Develop an efficient method to produce co-products from the hemicelluloses stream.

(2) Develop an effective way to remove the inhibitors in the hydrolysate after the acid pretreatment.

The detoxification methods and the potential generation approaches of value added co-products from this study will certainly contribute to the diversification of chemicals from woody biomass. The results will be published and presented as scientific papers.

Dissertation Organization

This dissertation is organized as follows. In Chapter 2, related work in the literature is briefly reviewed. In Chapter 3, the new conditions to extract out the hemicellulose sugars from the loblolly pine have been examined and the value added co-products have been produced under a series experiments. In Chapter 4, detoxification methods have been applied and selected to the prehydrolysate generated by the acid pretreatment of loblolly pine. Fermentation results were presented and compared to select suitable detoxification methods for the prehydrolysate from the acid pretreatment of loblolly pine. In Chapter 5, we summarize the main contributions of this dissertation and discuss future directions for this research.

Literature Review

Introduction

This world is heavily dependent on the petroleum-based fuels and chemicals. However, there will be a shortage of petroleum supplies and the energy problem will exceed a critical threshold eventually. To response these issues, the government has provided incentives to establish greater energy independence by promoting research on environmentally friendly and sustainable biofuels like bioethanol and biodiesel (Fukuda et al. 2009). Recently, considerable research has been carried out for the production of bioethanol from various biomass resources. Ethanol from lignocellulosic feedstocks is being proved by many scientists for its potential role of the final answer to the sustainable energy development of the whole world (Spatari et al. 2010). Lignocellulosic biomass, such as wood, is composed of a complex mixture of cellulose, hemicellulose, and lignin (Han et al. 2007). Cellulose is a long chain which is composed by glucose with the β-1, 4 linkages. Compare with cellulose, hemicellulose structure is more complex with different forms (branch) and compositions (arabinose, mannose, etc.). The structure and compositions of hemicellulose is described briefly below.

Hemicellulose Structure and Compositions

In lignocellulosic biomass, branched hemicellulose structure with a DP (degree of polymerization) 200 occupies 25-35% (weight percentage), which are mainly composed by pentoses (D-xylose, D-arabinose), hexoses (D-mannose, D-glucose, -galactose) and sugar acids. Hardwoods hemicelluloses are rich in xylan polymers with small amount of mannan,

while softwood hemicelluloses are rich in mannan polymers (Kumar et al. 2008). These structures are represented in Figure 2.1 below.

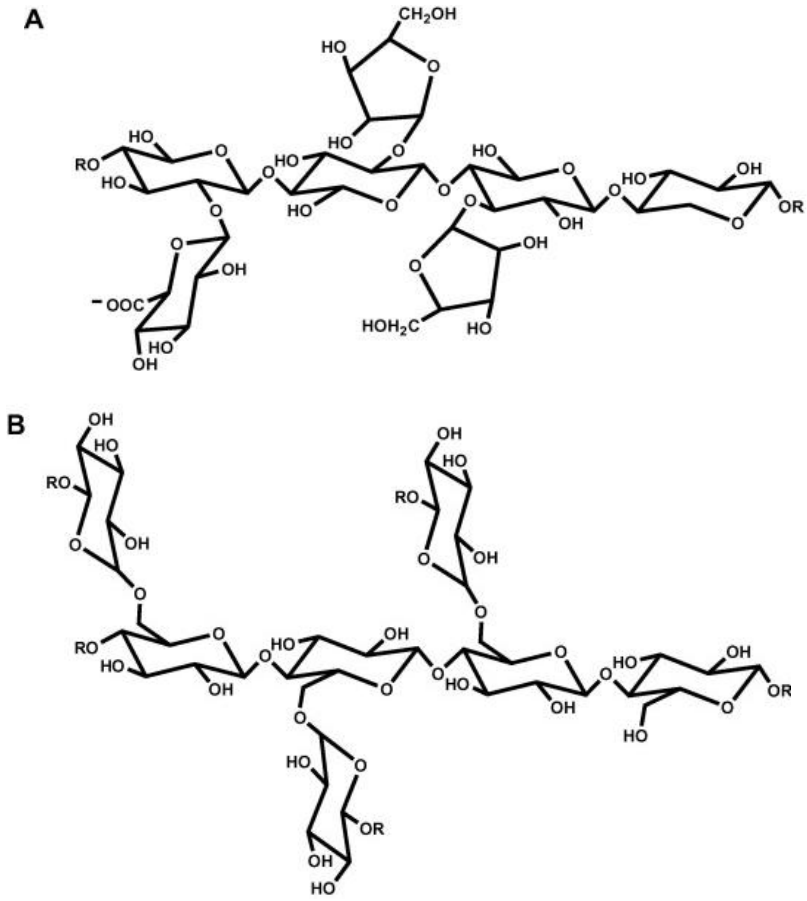


Figure 2.1. The basic structure of xylan and xyloglucan of hemicellulose.

Glucuronoarabinoxylan (A) is β-(1, 4)-d-xylan substituted by glucuronic acid at the O-2 and by arabinose at the O-2 and O-3. Xyloglucan (B) is β-(1,4)-d-glucan, the most common pattern of XG backbone substitution is a regular pattern of 3-substituted glucose residues followed by a single free glucose (Caffall et al. 2009).

The woody cell wall consists of lignin impregnated in a network of cellulose and hemicelluloses resulting in composite structure with the cell wall (Ye et al. 2003). Within the tree, this composite network helps to provide mechanical strength to the wood cell. But

during processing, the presence of lignin can be counterproductive and act as an inhibitor. As such, the process of converting biomass into ethanol requires the right pretreatment, hydrolysis and downstream fermentation processing.

Pretreatment and Fractionation Process

Most pretreatment methods today are focused on acid pretreatment, uncatalyzed steam explosion, liquid hot water pretreatments, flow-through acid pretreatment, lime pretreatment and ammonia pretreatment (Mosier et al. 2005). An efficient and effective method of pretreatment is undoubtedly a promise for the following hydrolysis and saccharification. The goal for all of these methods of pretreatment is to lower the cost of chemical manufacture, lower the energy necessary during processing, and increase yields during fermentation (Yang et al. 2008). Among all these methods, acid pretreatment and ethanol organosolv pretreatment will be discussed specifically in this review, for they could generate the most inhibitors.

Organosolv pretreatment process

Historically, the organosolv process has been explored widely from the industry of paper production (Diaz et al. 2004, Gilarranz et al. 1998, Jimenez et al. 2004, Ni et al. 1995). Presently, studies have obtained the analysis of the lignin fraction extracted during the organosolv pulping process (Hepditch et al. 1997, Ibarra et al. 2005, Bonini et al. 2005), including its promising future of production of various industrial products such as adhesives or biodegradable polymers (Kubo et al. 2004). Due to the high cost of the biorefinery schemes for the bioethanol, it is critical to partition the high quality lignin and other potentially valuable co-products (e.g., furfural and acetic acid) from woody feedstock (Pan et al. 2005). Organosolv is perhaps one of the most promising methods for separation of

co-products while still generating a high level of bioethanol level. The ethanol organosolv pretreatment process is shown in Figure 2.2.

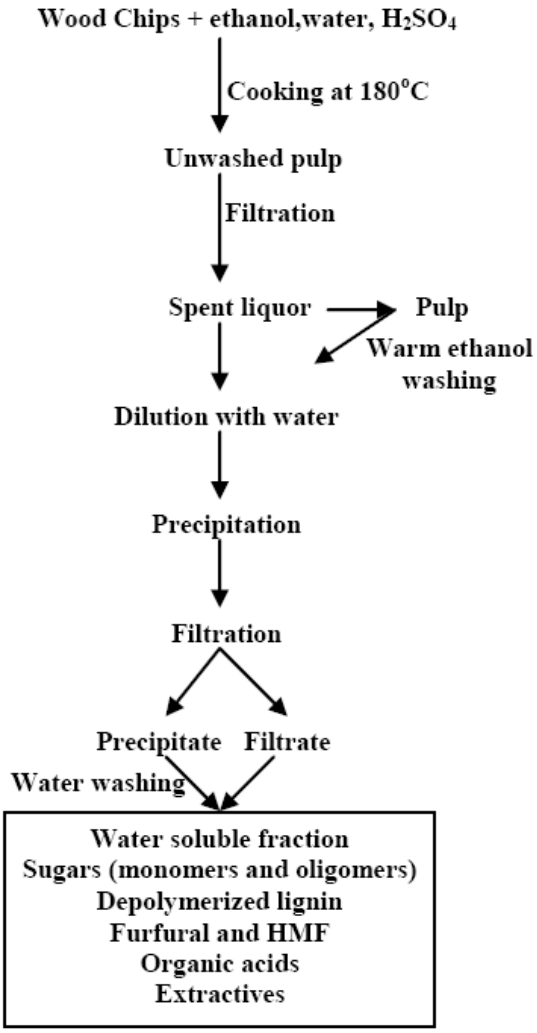


Figure 2.2. The flowchart of the ethanol organosolv process for bioethanol production.

As shown in the Figure 2.2, the final product contains sugars and other extractives. There is thus a potential for a suite of potential co-products that could be generated by future biorefineries. However, extractives and some organic chemicals can be problematic by inhibiting the fermentation downstream. Since the fermentation process consumes only

monomers sugars; the oligomers need further cellulase hydrolysis or acid hydrolysis to be broken down into monomers.

Acid pretreatment process

Considerable attention has been paid to acid pretreatment for a long history (Tsao et al. 1982, Bienkowski et al. 1984, McMillan et al. 1994, Jacobsen et al. 1999), dilute sulfuric acid have been used for hydrolyzing hemicellulose to xylose and other sugars (Zeitsch et al. 2000). For cellulose, the addition of sulfuric acid will also break down the glucose chain and the digestibility of the cellulose will be enhanced in the following enzymatic hydrolysis since the hemicellulose is removed at the beginning of the acid pretreatment (Knappert et al. 1981, Brownell et al. 1984, Converse et al. 1985, Grous et al. 1985).

The acid pretreatment process is illustrated in Figure 2.3. Acid and biomass can be mixed together and then heated indirectly through the vessel walls or by the direct steam explosion.

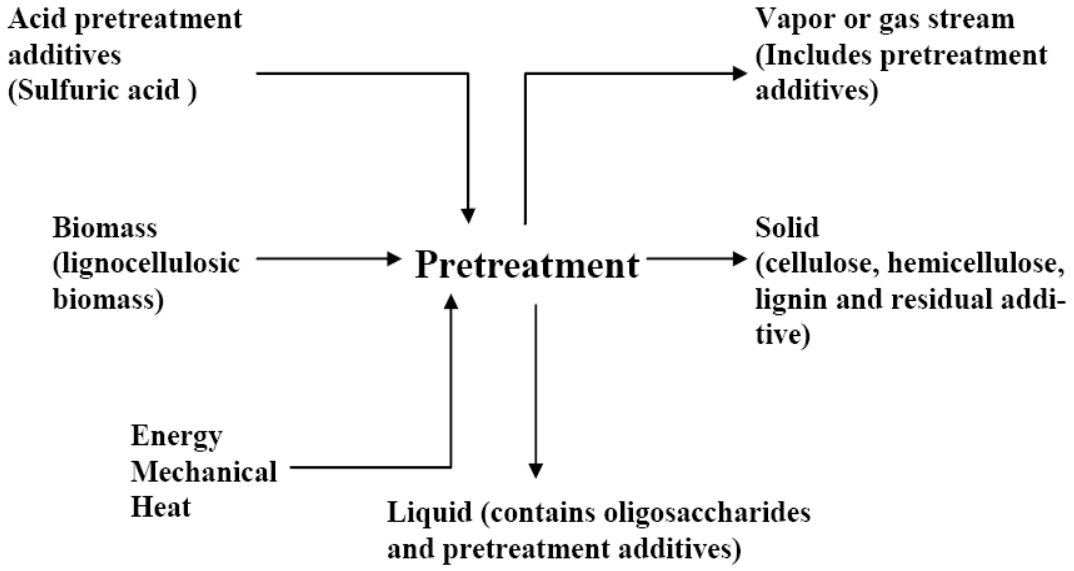


Figure 2.3. The flow chat of the acid pretreatment process (Mosier et al. 2005).

Fermentation and Detoxification

After pretreatment, the prehydrolysate contains the fermentation sugars most of which are monomers. However, before downstream fermentation, another step is required for successful fermentation. Detoxification is needed due to the formation of toxic compounds, such as furfural, hydroxylmethylfurfural (HMF), acetic acid, formic acid, levulinic acid etc. in the pretreatment process (Kumar et al. 2009).

Fermentation inhibitors

The use of different combination of biomass feedstock and pretreatment methods resulted in different inhibitory compounds and different concentration of inhibitors (Palmqvist et al. 2000, Chandel et al. 2007). For instance, more than 35 potential inhibitors were identified in *Saccharomyces cerevisiae* fermentation in dilute nitric acid hydrolyzates of hybrid poplar (Luo et al. 2002). Regardless of the mechanism of inhibition, inhibitors should be removed before fermentation. Generally, there are three groups of inhibitors.

1st: aliphatic acids (acetic, formic and levulinic acid), 2nd: furan derivatives furfural and 5-hydroxymethylfurfural (HMF) and 3rd: phenolic compounds (phenol, vanillin, p-hydroxybenzoic acid).

Detoxification Methods

Biological detoxification methods

Biological detoxification methods utilizes enzyme to degrade the inhibitors and is effective to complete removal of phenolic monomers and phenolic acids (Palmqvist et al. 2000). The first method of biological detoxification encompasses treating the prehydrolysate with perxidase and laccase which are obtained from the ligninolytic fungus *Trametes*

versicolor (Jonsson et al. 1998). This detoxifying mechanism of the enzyme treatment was suggested to be oxidative polymerization of low molecular weight phenolic compounds (Jonsson et al. 1998). Another method of treatment can be the use of the filamentous fungus *Trichoderma reesei* which could degrade inhibitors of acetic acid, furfural and benzoic acid derivatives, resulting in a 3 fold increase in maximum ethanol productivity and four times increased ethanol yield (Palmqvist et al. 1997).

Physical detoxification methods

Evaporation approaches

Basically, the mechanism of the evaporation methods is utilizing the different vapor points of inhibitors and water. However, for evaporation, the non-volatile fraction was found to be considerably more toxic in the referred study. After obtaining 10% (v/v) of the original volume by roto-evaporation, only a little ethanol was produced after the fermentation and suggests evaporation is not the most effective method to remove the inhibitors (Palmqvist et al. 1996)

Extraction approaches

Extracting the inhibitors from hydrolysates is one approach to separate part of the inhibitors from the hydrolysates by the mechanism of different solubility inside the water and extracted solvents. Ethyl acetate and diluted $NaHCO_3$ are two potential extraction solvents for woody biomass (Wilson et al. 1989). For ethyl acetate extraction, the major inhibitors were relatively soluble both in the aqueous and in the organic phase (Clark et al. 1984).

Adsorption approaches

Activated charcoal and ion exchange resins are regular methods based on their ability of adsorption (Chandel et al. 2007, Villarreal et al. 2006). The activated charcoal could absorb inhibitory compounds in the hydrolysate and after its adsorption saturation, it could be reactivated or regenerated by heating (Converti et al. 2000) However, during adsorption saturation, there would be a loss of glucose adsorbed as well as the inhibitory compounds.

Ion exchange resin is a famous detoxification method, which could be used for different polymeric adsorbents (resin) and different size of columns (Joseph et al. 2002). It is effective in absorbing the inhibitors inside the hydrolysates and can be utilized to remove the Hibbert's ketones, the related inhibitory compounds of which could be the most toxic compounds inside the softwood hydrolysates (Tenborg et al. 1998).

Chemical detoxification methods

Chemical detoxification methods are used by treating the hydrolysate with the chemical reagents, alkali and reducing agents are the most frequently used (Palmvist et al. 2000). Commonly, the adjustment of pH with $Ca(OH)_2$ is preferred to the NaOH due to better fermentability and precipitation of 'toxic compounds' that occurs with NaOH (van Zyl et al. 1988). A large precipitate will form after the overliming treatment (pH 10), which will result in increased ethanol productivity. The mechanism of overliming treatment has been demonstrated by the fact of precipitation of toxic components and the instability of some inhibitors at high pH (Jonsson et al. 1998). Some researchers treated the dilute-acid hydrolysates with sodium sulphite, which had been proved to decrease the concentrations of furfural and HMF (Larsson et al. 1999).

Biochemical and Hydrothermal Conversion of Softwood Hemicellulose to Ethanol and Furan Derivatives

Abstract:

The purpose of this study was to explore the biochemical and hydrothermal conversion of hemicellulose into biofuels and value-added co-products. It was found that extraction of loblolly pine with 0.4% H_2SO_4 (w/w) at 150 ℃ for 2 h can dissolve most of the hemicellulose. The addition of surfactant at 0.4% (w/w) showed significant improvement on hemicellulose extraction, hemicellulose yield increased from 8.14±0.16% (w/w) to 10.15±0.02% (w/w) based on raw biomass. Pre-extracted hemicellulose was fermented to ethanol by *Saccharomyces cerevisiae*. In the presence of 0.4% surfactant, the glucose consumption rate in the hydrolysate fermentation increased from 0.28 g g^{-1} h^{-1} to 0.67 g g^{-1} h^{-1}, and mannose consumption rate was kept around 0.42 g g^{-1} h^{-1} without considerable changes. The final ethanol concentration increased from 7.45±0.18 g/L to 10.68±0.26 g/L. It was also observed that the hemicellulose extract could be effectively converted to levulinic acid at low pH (~2) by a hydrothermal process. Under the condition of 200 ℃ for 45 min, 38.61±1.06% of hexoses from the hydrolysate extracted by 0.4% of sulfuric acid converted to HMF, and 64.48±1.20% of pentoses to furfural. The addition of surfactant (0.4%w/w) significantly increased the selectivity of furan by 41% for HMF and 30% for furfural.

Introduction

Lignocellulosic biomass, a renewable and sustainable resource of feedstocks, provides a great potential for developing and producing biofuels and chemicals (Galbe et al. 2002,

Hoyer et al. 2009, Wingren et al. 2003, Huber et al. 2006, Ogbonna et al. 2010, Kovacs et al. 2009, Suryawati et al. 2009, Kim et al. 2008). Hemicellulose, which accounts for 20-30% of dried woody biomass, can be readily broken down to simple sugars (primarily xylose and mannose) and other degradation compounds during the biomass fractionation process. However, the utilization of the hemicellulose stream is extensively limited by its toxicity and low conversion yield during ethanol or butanol fermentation process (Ezeji et al. 2007, Qureshi et al. 2008, Martinez et al. 2001). It has been observed that pre-extraction of hemicellulose under mild conditions before lignocellulosic biomass pretreatment and fractionation will enable an effective fermentation of hemicellulose sugars to ethanol or butanol (Tunc et al. 2008, Um et al. 2009). Typically, two major types of galactoglucomannan and (galacto)glucomannan were found in softwood. Glactoglucomannan (5-8%) was a galactose-rich fraction and (galacto)glucomannan (10-15%) was a galactose-low fraction. Their corresponding ratios of galactose: glucose: mannose is 1:1:3 and 0.1:1:3, respectively (Sjöström et al. 1993).

Hot water autohydrolysis, alkaline and dilute acid prehydrolysis have been previously explored for hemicellulose extraction from lignocellulosic biomass (Um et al. 2009, Yoon et al. 2008, Kim et al. 2001, Sattler et al. 2008, Al-Dajani et al. 2008, Um et al 2010). Specifically, Springer and Harris (Springer et al. 1982) compared the hemicellulose extraction from aspen wood with hot water and 0.4% sulfuric acid at 170 ℃. They found that more monomeric xylose was present in the dilute acid prehydrolysate than in the water prehydrolysate, and that acid prehydrolysis resulted in higher yields of xylan removal (Springer et al. 1982). Conner et al. (Conner et al. 1984, Conner et al. 1985, Conner et al.

1986) made a series of investigation on hemicellulose removal from hardwood (aspen, birch, maple and red oak) by water, dilute hydrochloric acid and dilute acetic acid. Kinetic modeling of hardwood prehydrolysis was established and compared between hot water and dilute acid extraction processes (Conner et al. 1984, Conner et al. 1985, Conner et al. 1986). It was discovered that xylan removal basically followed two parallel first-order reactions, and dilute acids could reduce the reaction temperature and time as compared to hot water pre-hydrolysis. Typically during the hot water extraction of hardwood, the acetyl groups were cleaved from xylan hemicellulose and released as acetic acid catalyst for the further hemicellulose extraction. The addition of dilute mineral acids could further increase the hemicellulose solubilization rate and yields (Springer et al. 1982). Although hot water prehydrolysis is appealing for hardwood hemicellulose removal due to the reduced cost of mineral acid and its neutralization, dilute acid is required for hemicellulose extraction from softwood. Nguyen et al. used dilute acid to pretreat softwood for biofuels production (Nguyen et al. 2000). Lundqvist et al. explored the microwave heat extraction of hemicellulose from spruce (Lundqvist et al. 2002, Lundqvis, et al. 2003). Pre-extraction of hemicellulose has also been investigated with Kraft pulping (Al-Dajani et al. 2008). Um and van Walsum explored the pre-pulping extraction of hemicellulose by alkaline from mixed hardwoods (Um, et al. 2009, Um, et al. 2010). Alkaline extraction of hemicellulose with NaOH (1-2 M) was performed at low temperature (50-90 ℃) from aspen chips (Al-Dajani et al. 2008). However, the recovery and recycle of alkaline chemicals such as NaOH could be a challenge for this process. Consequently, a cost-effective approach to hemicellulose

extraction is needed. In this connection, the addition of surfactant purposely to reduce the usage level of acids in the hemicellulose extraction process was investigated.

It should be noted also that the development of high-value co-products such as furan derivatives from hemicellulose sugars will generate extra revenue to the bioconversion process, and will become a critical component for the economic viability of a regional bioenergy production system. Furan derivatives are the principal materials that are considered very important in the manufacture of industrial chemicals (Chheda et al. 2007, Tong et al. 2010). Catalytic conversion of biomass waste stream to furans will provide an alternative route based on renewable feedstock to replace the non-sustainable petroleum approach. Furfural and HMF can be produced from the dehydration of xylose, arabinose, mannose and glucose (Chheda et al. 2007). Likewise, levulinic acid and formic acid can also be formed during further degradation of HMF and furfural during the hydrothermal conversion process. Selective production of furfural and HMF from sugars has been improved recently by using a biphasic reactor system or in the presence of ionic liquid (Chheda et al. 2007, Lima et al. 2009). However, expensive organic solvents (dimethyl sulfoxide and methyl isobutyl ketone) and special ionic salts (1-ethyl-3-methylimidazolium hydrogen sulfate) are required for these processes (Chheda et al. 2007, Lima et al. 2009). Therefore, further research work to improve selectivity with a simple technique is essential for developing value-added co-products. In connection to this, the other significance of this work is to explore an inexpensive approach to improve the selective production of furfural and HMF from hemicellulose sugars in the presence of surfactant. In this study, dilute sulfuric acid was used to extract the hemicellulose from loblolly pine with the addition of surfactant. Our main focus is to

investigate the potential to maximize hemicellulose recovery at low acid concentration for value-added products. Subsequently, the hydrolyzed hemicellulose stream was fermented to ethanol using *Saccharomyces cerevisiae*. The hemicellulose stream was also used to produce furan derivatives (furfural and HMF) via hydrothermal conversion. The effect of surfactant on hemicellulose sugars fermentation and hydrothermal conversion was also explored.

Materials and Methods

Woody biomass and substrates

Loblolly pine (*Pinus taeda*) wood chips were collected from a local forest products mill. The initial moisture content of these wood chips is 9.0 wt%. Wood chips were ground by a Brinkmann mill, and the wood mill between 40-60 mesh was collected for chemical composition analysis. The average size of wood chips was reduced to 1.0 × 0.5 ×0.3 cm (L ×W ×H) by a Waring commercial blender prior to hemicellulose extraction.

Chemical analysis

The extractives content of loblolly pine (wood powders, 40 mesh) was determined using acetone extraction according to the standard method as described by the National Renewable Energy Laboratory (Ehrman et al. 1994). The lignin and carbohydrate composition of loblolly pine were determined on the extractive-free samples according to National Renewable Energy Laboratory protocol (Ruiz et al. 1996). A Shimadzu high performance liquid chromatography (HPLC) equipped with a Bio-Rad Aminex HPX-87P column was sued to separate and quantify individual sugars. The amount of ethanol, furfural, hydroxymethylfurfural (HMF), formic acid, acetic acid and levulinic acid were quantified on Shimadzu HPLC by using a Bio-Rad Aminex HPX-87H column. H_2SO_4 (5mM) is used as the

mobile phase at an isocratic flow rate of 0.6 mL/min to separate ethanol, furfural, and HMF and the temperature of HPX-87H column was maintained at 60 ^{o}C during the elution. To separate formic acid, acetic acid and levulinic acid, the column temperature was changed to 30 ℃ and the flow rate of 5 mM H_2SO_4 was changed to 0.7 mL/min.

Microorganism and culture media

Saccharomyces cerevisiae strain (ATCC 32120, commercial baking yeast) is used for sugar fermentation in this study. *S. cerevisiae* was maintained in solid YPG medium (yeast extract, peptone, glucose) containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 15 g/L agar. Colony of *S. cerevisiae* was cultured in liquid YPG containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose. After the yeast was cultured at 30^{o}C and 150 rpm for 6 h, the liquid YPG with yeast inside was centrifuged and washed by sterile water 3 times, and then the dry weight of the yeast biomass was measured at 600 nm with a UV-Vis spectrometer (Genesys 10, Thermo Fisher).

Hemicellulose extraction

The extraction of hemicellulose sugars from loblolly pine was carried out in a 4 L Parr reactor equipped with 120 mL of Chemglass pressure vessels. The liquid to wood ratio during the extraction was at 3.5 to 1. Thirteen grams of wood chips (o.d.) and 45.8 mL of DI water containing different concentrations of H_2SO_4 and/or Tween 80 (Table 3.2, first and second columns on the left) were added to each of the 120 mL of Chemglass pressure vessel. The hemicellulose extraction was carried out in duplicates. After sample addition, the Chemglass cylindrical pressure vessels were put into a 4 L Parr steel reactor equipped with a 4842 controller. The hemicellulose extraction was carried out at 150 ^{o}C for 2 h (time at temperature)

with additional 15 min temperature ramping time. After the extraction, the reactor was cooled to 60 ^{o}C in 5 min before removing the samples from the Chemglass vessels for subsequent separation and a secondary acid hydrolysis.

Separation and secondary acid hydrolysis of extracted hemicellulose

A Whatman No. 2 filter paper was used to filter and separate the solid biomass residue from the prehydrolysate after the hemicellulose extraction. Then 20 mL of the prehydrolysate from each Chemglass pressure vessel were transferred to separate serum bottles (125 mL) to carry out a secondary acid hydrolysis to convert to monomeric sugars. A 4% sulfuric acid solution (w/w) was used for the secondary acid hydrolysis. The serum bottles were sealed after acid addition with rubber stopper and aluminium cap and transferred into an autoclave for 1 h at 121 ^{o}C. After the autoclaving, the serum bottles were removed and the pH of the liquid in each bottle was adjusted to 6.0 with NaOH for analyzing samples by HPLC with Aminex HPX-87P column. A small amount of the hydrolysate liquid (0.1 mL) was taken and mixed with 0.9 mL sterile DI water to perform chemical analysis. The rest of the hydrolysate in each serum bottle was sealed with sterile rubber stopper for further fermentation.

Fermentation of hemicellulose sugars to ethanol

Yeast was added into each serum bottle at a concentration of 2 g/L. The serum bottles were then sealed with rubber septa, vented by a needle. No additional media supplementation was added into the hydrolysate. Bottles were incubated in an orbital shaker at 30 ^{o}C and 150 rpm for 48 h. Samples of fermentation liquid were taken out through a needle at 0 h, 1 h, 4 h, 8 h, 12 h, 24 h and 48 h. A 0.2 mL of the aliquots were transferred into a 1.5 mL centrifuge tube and centrifuged at 13,400 × g for 3 min. After the centrifugation, 0.1 mL of the supernatant was

withdrawn and mixed with 0.9 mL DI water for chemical analysis. Glucose and mannose consumption rate (g g^{-1} h^{-1}) was estimated from the change of sugar concentration over the first 4 h during the fermentation, and the initial yeast biomass concentration was 2 g/L. It was assumed that the yeast biomass concentration did not change in the first 4 h fermentation. The ethanol yield was calculated as % of the theoretical yield by using the following formula:

$$\%Yield = \frac{[EtOH]}{0.51 \times [Glu\cos e + Mannose]} \times 100\%$$

Hydrothermal conversion of hemicellulose to furfural and HMF

Hydrothermal conversion of hemicellulose prehydrolysate to furfural and HMF is also carried out in the Chemglass cylindrical pressure vessels (120 mL). These vessels have an internal thread for use with a teflon bushing as a pressure seal. The Chemglass vessels containing 5 mL of prehydrolysate were placed into the 4 L Parr steel reactor with a 4842 controller. The hydrothermal treatment was carried out 200 ℃ for 45 min (time at temperature) with an additional 30 minutes temperature ramping time. After the treatment, the Parr reactor was cooled down to 60 ℃ in 15 min. The sample was then taken out of the pressure vessels for composition analysis by HPLC. A different set of experiments was carried out to determine the effect of initial pH, reaction time and secondary acid hydrolysis on the yield of furfural and HMF during the hydrothermal conversion. During the hydrothermal treatment conditions, pentoses (xylose and arabinose) could be converted into a mixture of furfural, formic acid, and polymer and hexoses (glucose, galactose and mannose) could be converted into a mixture of HMF, levulinic acid, formic and polymer. We therefore determine pentoses-to-furfural conversion selectivity based on as the mole concentration of furfural produced over each mole concentration of pentoses consumed and hexoses-to-HMF

conversion selectivity based on the mole concentration of HMF over each mole concentration of hexoses in the hydrolysate.

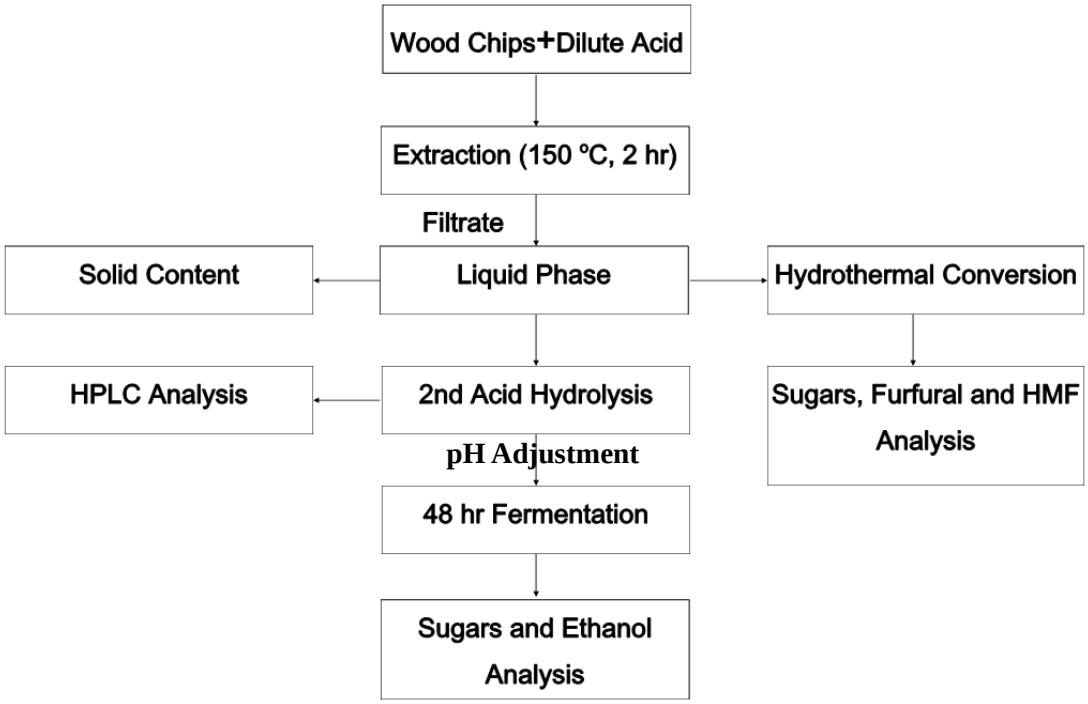


Figure 3.1. Experimental procedures for hemicellulose extraction, fermentation and hydrothermal conversion.

Statistical analysis

Data was analyzed using the single-factor or two-factor (with replicates) analysis of variance (ANOVA) with Microsoft Excel 2003. All the significance tests were performed at the 95% confidence level.

Results and Discussion

Chemical composition of loblolly pine

The chemical composition of loblolly pine was shown in Table 3.1. The carbohydrates (cellulose and hemicellulose) represents approximately 60.71wt% of loblolly pine. The total lignin content was 28.67 wt%. These results are in agreement with previous reports on the

composition of pine wood (Sievers et al. 2009, Frederick et al. 2008, Zhu et al. 2010).

Table 3.1. Chemical composition of Loblolly Pine

Chemical composition	Amount (%)	STDEV
Glucan	42.38	1.80
Xylan	6.34	0.21
Galactan	1.80	0.06
Arabinan	1.10	0.09
Mannan	9.09	0.10
Extractives	3.52	0.12
Acetyl groups	1.00	0.02
Acid insoluble lignin	27.99	0.62
Acid soluble lignin	0.68	0.02

Effect of sulfuric acid and surfactant on hemicellulose extraction from loblolly pine

The effect of sulfuric acid and surfactant on the hemicellulose extraction from loblolly pine was first examined (Table 3.2). Two hours of hot water extraction of wood chips at 150 ℃ without the presence of sulfuric acid and surfactant produce 4.03% of soluble sugars from wood chips. The addition of sulfuric acid to the hot water during wood chip extraction significantly increased the amount of soluble hemicellulose (ANOVA, $p<0.05$). At 0.1% (w/w) of sulfuric acid addition approximately 5.38 g of hemicellulose sugars were released from 100 g of wood chips (o.d.). Further increasing the concentration of sulfuric acid to 0.6% resulted in higher hemicellulose yield up to 8.70% (based on the raw material). By increasing the sulfuric acid concentration from 0 to 0.6%, a total soluble hemicellulose yield increase from 4.03% to 8.70% was obtained. When the amount of individual hemicellulose sugar is examined, mannose is the dominant sugar in the extracted hemicellulose prehydrolysate. At the highest concentration of sulfuric acid (0.6%), the amount of mannose released almost tripled compared to using hot water alone, 1.37 % vs 3.95%. It appears that the increase in glucose, xylose, galactose and arabinose was less pronounced with the addition of sulfuric

acid.

The addition of surfactant, Tween 80, in the range of 0.0%-0.4% (w/w) led to an increase in soluble sugars from 8.14 g to 10.15 g based on 100 g wood chips (ANOVA, $p<0.05$) under the presence of 0.4% of H_2SO_4 (Table 3.2). This result suggests that addition of surfactant can improve hemicellulose extraction without an increase in acid concentration. As mentioned earlier, hot water autohydrolysis, alkaline and dilute acid prehydrolysis have been evaluated for hemicellulose extraction from woody biomass (Yoon et al. 2008, Kim et al. 2001, Sattler et al. 2008, Al-Dajani et al. 2008). Hot water extraction was shown effective for hardwood hemicellulose removal under mild condition (150 ℃), this probably was due to the release of acetic acid from the acetylated carbohydrates in the hardwood (Tunc et al. 2008). For softwood materials, effective pre-extraction of hemicellulose requires a higher temperature (180 ℃ and 190 ℃) (Yoon et al. 2008). Alternatively, inorganic acid can be used to catalyze hemicellulose extraction at relatively mild conditions (,Kim et al. 2001). The dominant hemicellulose component in loblolly pine was mannan (9.09%). During the extraction, the maximum yield of mannan was 3.95% (based on raw material) with 0.6% of sulfuric acid. Without sulfuric acid, the mannan yield was only 1.37%. From this study, it is apparent that dilute sulfuric acid could significantly improve the mannan yield and total hemicellulose yield at 150 ℃. It is recognized that the use of inorganic acid will bring potential corrosion issue and require subsequent neutralization. In order to minimize the use of sulfuric acid, we investigated the effect of surfactant addition on hemicellulose extraction.

Surfactant-based pulping previously has provided potential benefits including reduction of acid or alkali consumption, reduced rejects and reduced lignin content in pulp (Chen et al.

1994, Duggirala et al. 1999, Hamzeh et al. 2009, Wei et al. 2005). In conventional pulping, the addition of a surfactant-based additive enhances the penetration and diffusion of cooking liquor into the wood chips. The cooking liquor penetrates the biomass faster, resulting in a better delignification reaction. We assume that addition of surfactant could increase the acid penetration and improve the hemicellulose extraction. Interestingly, only the yield of xylan was increased from 1.61% to 2.29% with the addition of 0.4% of Tween 80 under 0.4% of sulfuric acid. However, the yield of mannan was increased from 3.54% to 3.74% under the same condition. The yields change of glucan, galactan and arabinan seemingly followed the same trend as mannan with the addition of surfactant. The significant increase of total hemicellulose yield in the presence of surfactant indicated that acid penetration into wood chips probably was a potential issue for hemicellulose extraction (ANOVA, $p<0.005$).

Table 3.2. Monomeric sugars in hemicellulose extract after secondary acid hydrolysis

Extract conditions		Monomeric sugars in hemicellulose extract (g/100g)[a]					
H_2SO_4	Tween	Glucose	Xylose	Galactose	Arabinose	Mannose	Total
(w/w)	(w/w)	47.09	7.20	2.00	1.35	10.10	(initial)
0.00%	0.00%	0.58±0.01	0.92±0.06	0.05±0.02	0.66±0.05	1.37±0.08	4.03±0.22
0.10%	0.00%	0.83±0.03	1.24±0.02	1.12±0.04	1.37±0.04	3.45±0.07	5.38±0.16
0.20%	0.00%	1.08±0.02	1.50±0.05	0.92±0.03	0.85±0.05	2.68±0.07	7.04±0.13
0.40%	0.00%	1.45±0.03	1.61±0.04	1.09±0.03	0.46±0.02	3.54±0.10	8.14±0.16
0.60%	0.00%	1.30±0.01	1.74±0.04	1.05±0.12	0.67±0.02	3.95±0.02	8.70±0.08
0.40%	0.05%	1.41±0.13	1.88±0.10	1.09±0.13	0.94±0.03	3.35±0.18	8.67±0.51
0.40%	0.10%	1.32±0.04	1.83±0.07	1.14±0.00	0.91±0.00	3.15±0.09	8.35±0.20
0.40%	0.20%	1.60±0.10	2.15±0.07	1.21±0.00	1.00±0.05	3.72±0.12	9.68±0.24
0.40%	0.40%	1.64±0.01	2.29±0.03	1.45±0.02	1.03±0.07	3.74±0.05	10.15±0.02

[a] The extracted monomeric sugars were estimated based on the monomeric sugars concentration after secondary acid hydrolysis and their collected volumes (25-30 mL) after extraction.

Effect of sulfuric acid and surfactant on the generation of Furfural and HMF

Furfural and HMF can be produced during the hemicellulose extraction and acid hydrolysis due to the acid dehydration reaction at higher temperature or pressure (Larsson et al. 1999, Nguyen et al. 1998). In this study, the concentration of furfural and HMF were determined before the fermentation process (Table 3.3). As shown in Table 3.3, the production of furfural and HMF increased with an increase in acid concentration. Without the addition of sulfuric acid and surfactant, the furfural concentration was 0.08 g/L and the HMF concentration was 0.36 g/L in the hemicellulose extract after the secondary acid hydrolysis,. The furfural concentration increased to 0.22% with the addition of 0.6% of sulfuric acid while the HMF concentration increased to 0.83 g/L. With the same concentration of sulfuric acid, the addition of surfactant (0.1-0.4%) resulted in the same concentration of furfural and HMF. This indicated that the surfactant Tween 80 had no impact on the generation of furfural and HMF during hemicellulose extraction process (ANOVA, $p>0.05$). Previously, we reported that furfural and HMF could be metabolized by *S. cerevisiae* T1 in the separate hydrolysis fermentation and simultaneous saccharification fermentation (Tu et al. 2009, Tian et al. 2010). Although different conditions were used in this study, similar results were found that after 48 h of fermentation, the furfural and HMF were quickly consumed by yeast in the fermentation.

Table 3.3. Sugars consumption rate and ethanol yield during fermentation (after secondary acid hydrolysis)

Extract conditions		Initial furfural	Initial HMF	Glucose consum.	Mannose consum.	Final ethanol	Ethanol yield
H_2SO_4 (w/w)	Tween (w/w)	(g/L)[a]	(g/L)	rate ($g\ g^{-1}\ h^{-1}$)[b]	rate ($g\ g^{-1}\ h^{-1}$)	(g/L)	(%)[c]
0.00%	0.00%	0.08±0.00	0.36±0.14	0.36	0.30	3.01±0.4	59.79±6.24
0.10%	0.00%	0.13±0.01	0.48±0.01	0.46	0.35	5.79±0.22	81.60±3.34
0.20%	0.00%	0.12±0.02	0.52±0.03	0.46	0.35	6.88±0.12	77.86±0.67
0.40%	0.00%	0.18±0.02	0.69±0.00	0.28	0.42	7.45±0.18	65.73±0.20
0.60%	0.00%	0.22±0.01	0.83±0.06	0.26	0.35	8.96±0.37	71.37±3.26
0.40%	0.05%	0.18±0.01	0.67±0.05	0.43	0.38	9.51±0.07	83.12±2.78
0.40%	0.10%	0.17±0.02	0.63±0.06	0.60	0.46	9.44±0.43	88.59±5.09
0.40%	0.20%	0.18±0.02	0.66±0.04	0.56	0.35	10.01±0.15	84.09±0.49
0.40%	0.40%	0.19±0.01	0.71±0.03	0.67	0.42	10.68±0.26	85.33±0.63

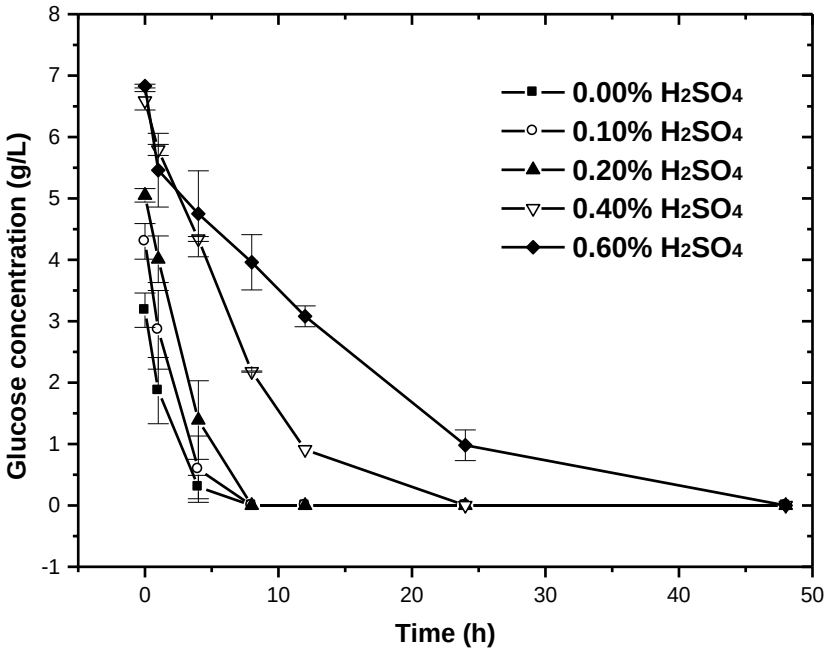
[a] Furfural and HMF concentration: initial furfural and HMF concentration after secondary acid hydrolysis of hemicellulose extract.

[b] Glucose and mannose consumption rate was estimated from the change of sugar concentration over the first 4 h during the fermentation, and the initial yeast biomass concentration was 2 g/L.

[c] Ethanol yield=percentage of theoretical yield of glucose and mannose.

Effect of initial surfactant on sugars consumption in the fermentation

The consumption of sugars (mannose and glucose) from 0 to 48 h is shown in Figure 3.2 and Figure 3.3, respectively. Since xylose, galactose and arabinose cannot be effectively utilized by *S. cerevisiae* during the 48 h fermentation, consumption of these three sugars was not reported. Only the uptake of glucose and mannose were calculated in ethanol yield. Without the presence of Tween, the released glucose under 0.0%, 0.10% and 0.20% of sulfuric acid was consumed within 8 h. The higher sulfuric acid concentration (0.4% and 0.6%) in the hemicellulose extraction resulted in longer consumption time (>24 h) for the glucose. The specific consumption rate of glucose in the hemicellulose extract with 0.4%-0.6% of H_2SO_4 was 0.26-0.28 g g^{-1} h^{-1}, considerably lower than that (0.36-0.46 g g^{-1} h^{-1}) from hemicellulose extract with 0.0%-0.2% of H_2SO_4 (Table 3.3). This indicated that a higher concentration of sulfuric acid could result in slow consumption of glucose, which probably was due to more inhibitors produced during hemicellulose extraction. The inhibition for glucose consumption during the fermentation is most likely coming from phenolic compounds, not from furfural or HMF, because the initial concentration of furfural and HMF was less than 0.22 g/L and 0.83 g/L, respectively (Table 3.3). Low concentration of furfural and HMF will not affect the fermentation of lignocellulosic hydrolysate (Tu et al. 2009, Taherzadeh et al. 1997). *S. cerevisiae* can convert furfural and HMF to their corresponding alcohols via NADH-dependent alcohol dehydrogenase (Liu et al. 2004, Palmqvist et al. 1999). Phenolic compounds have been shown to be the major inhibitors in the dilute acid hydrolysis of softwood (Larsson et al. 1999).



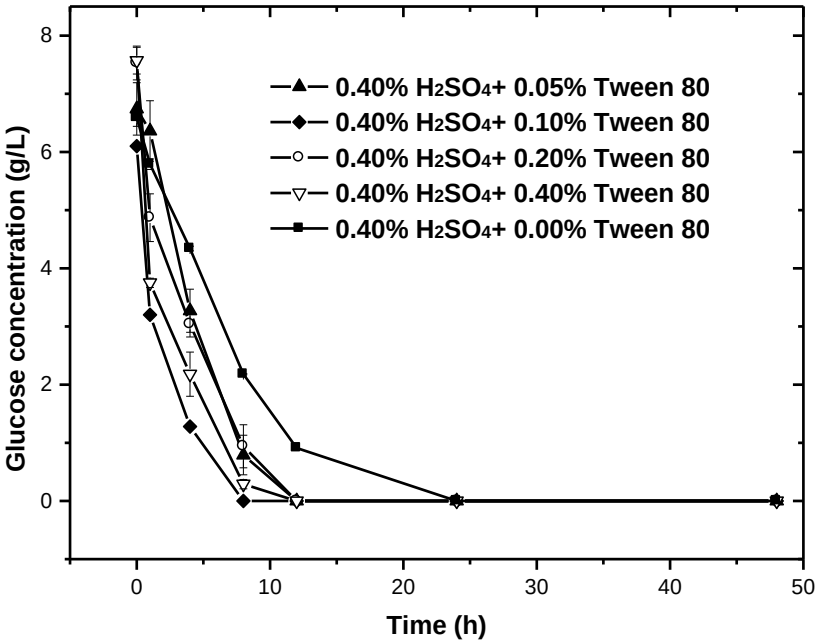


Figure 3.2. Glucose consumption during 48 h fermentation of hemicellulose sugars. Fermentation conditions: 30 ℃ and 150 rpm for 48 h with 2 g/L of initial yeast.

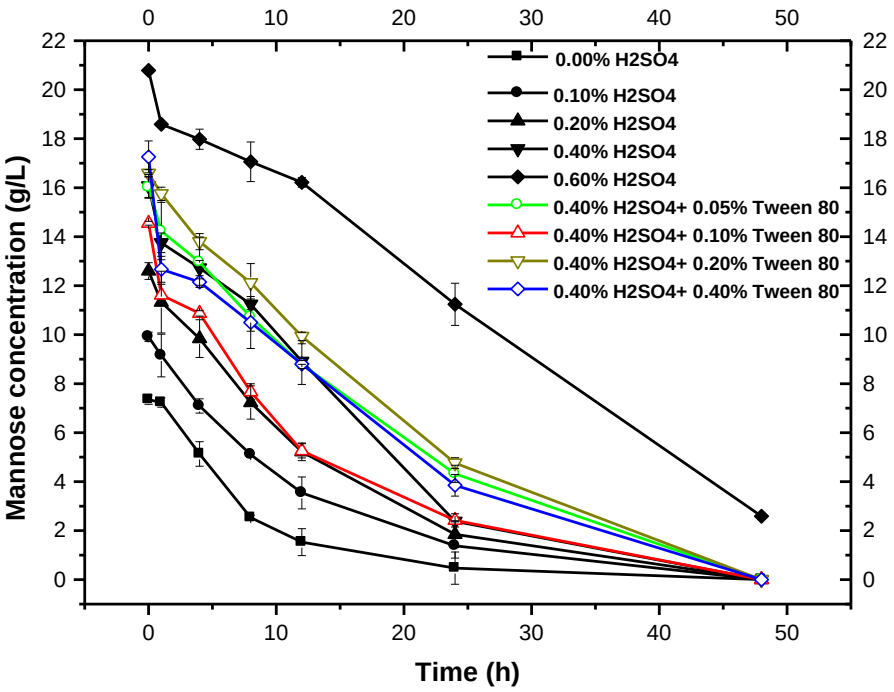


Figure 3.3. Mannose consumption during 48 h fermentation of hemicellulose sugars. Fermentation conditions: 30 ℃ and 150 rpm for 48 h with 2 g/L of initial yeast.

As is shown in Table 3.3, the specific consumption rate of glucose was similar to that of mannose during the fermentation of hemicellulose sugars without the presence of Tween 80. Interestingly, we found that the presence of surfactant could enhance sugars consumption rate, especially for glucose (Table 3.3). The glucose consumption rate increased from 0.26-0.46 g g^{-1} h^{-1} (the first five different extraction conditions) to 0.43-0.67 g g^{-1} h^{-1} (the last four different extraction conditions), and mannose consumption rate improved from 0.30-0.35 g g^{-1} h^{-1} (the first five different extraction conditions) to 0.35-0.48 g g^{-1} h^{-1} (the last four different extraction conditions). Most glucose could be consumed within 12 h, while consumption of mannose required 48 h. Compared to the first 12 h fermentation of glucose and mannose, there is a trend that mannose would be used slowly by *S. cerevisiae* in the

hemicellulose extraction liquid, similar results have been reported (Tian et al. 2010, Smith et al. 1997).

Effect of residual surfactant on ethanol yield in the fermentation

Fermentation was carried out at 30^{o}C while being shaken on a gyrotatory shaker at 150 rpm. Ethanol concentration of fermenting liquid was measured and reported with the unit g/L. The final ethanol concentration after 48 h fermentation varied from 3.01 g/L to 10.68 g/L with extracted hemicellulose sugars from different extraction conditions (Table 3.3 and Figure 3.4). The higher ethanol concentration was coming from the higher concentration of extracted hemicellulose sugars, especially glucose and mannose. However, the percentage of theoretical ethanol yield did not follow the same trend. Without the addition of surfactant in the initial extraction, the percentage of theoretical ethanol yields for most groups were lower than 80%, the average percentage of theoretical ethanol yield of first five extractions (Table 3.3) was 71%. With the presence of surfactant, the percentage of theoretical ethanol yields were from 83% to 88%, the average percentage of theoretical ethanol yield of last four extractions reached 85%. This indicated the surfactant enhanced the percentage of theoretical ethanol yield in the batch fermentation of hemicellulose sugars. Compared to the control group, the groups with H_2SO_4 showed higher ethanol concentration and higher theoretical ethanol yield. Compared to the groups with Tween 80 and the group with 0.4% sulfuric acid only, both the final ethanol concentration and the percentage of theoretical ethanol yield increased significantly in the groups with Tween 80. This indicates the surfactant could enhance the percentage of theoretical ethanol yield. Similar improvement has been reported on the fermentation of glucose and lignocellulosic hydrolysate with the addition of surfactant

(Lee et al. 1996). Galindo and Salcedo (1996) also shown the surfactant could improve xanthan yield during the fermentation via affecting the fermentation parameters such as oxygen transfer rate (Galindo et al, 1996).

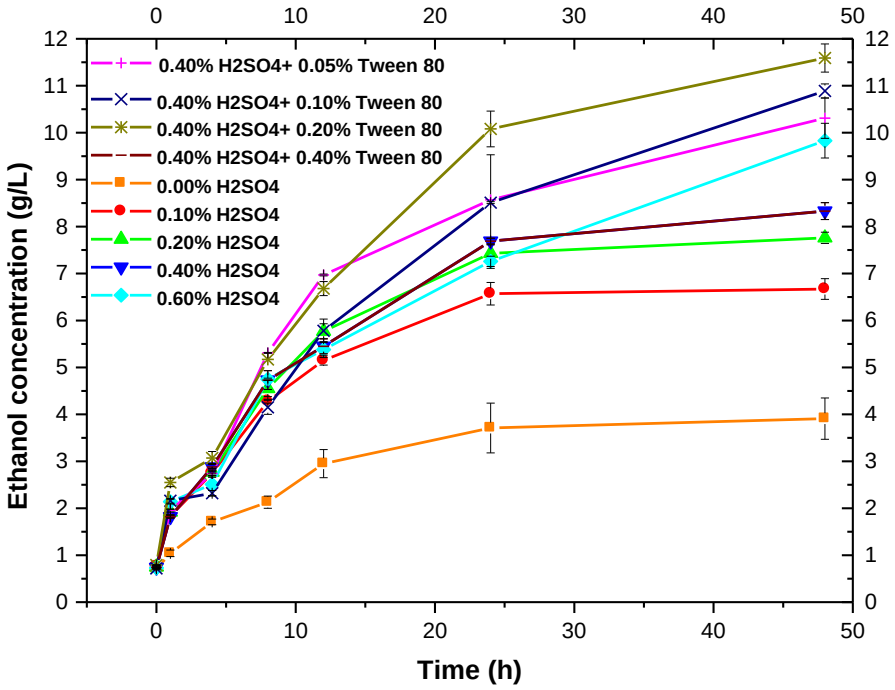


Figure 3.4. Ethanol yield during 48h fermentation of hemicellulose sugars. Fermentation conditions: 30 ℃ and 150 rpm for 48 h with 2 g/L of initial yeast.

Effect of residual surfactant on hydrothermal conversion of hemicellulose to HMF and furfural

Hemicellulose extract was also examined for producing furfural and HMF via hydrothermal conversion (Table 3.4 and Figure 3.5). Nearly 61 % of xylan and arabinan (C5 sugars) were converted into furfural at 200 ℃ for 45 min, which was much higher than that (37 %) from glucan, galactan and mannan (C6 sugars) (Table 3.4). Interestingly, we found

that the presence of surfactant significantly increased the furan selectivity in the hydrothermal conversion process. Furfural and HMF selectivity was enhanced from 53.37-67.56% (the first five extraction conditions) to 75.12-83.02% (the last four extraction conditions), and from 33.03-39.24% (the first five extraction conditions) to 47.25-56.30% (the last four extraction conditions) respectively with the presence of Tween 80. As a result, surfactant significantly increased the furan selectivity by 41% for HMF and 30% for furfural under 200 ℃ for 45 min.

The hydrothermal conversion of hemicellulose extract was initially explored for the optimal condition. Without a secondary acid hydrolysis of hemicellulose to monomeric sugars and pH adjustment, the hemicellulose extract (pH 2) contains monomeric sugars and oligosaccharides. Most of the hemicellulose could be directly converted to furfural and HMF (Figure 3.5a). However, considerable amount of sugars (peak 2) were left in the solution. Subsequently, we first converted the hemicellulose extract to monomeric sugars with a secondary acid hydrolysis (4% sulfuric acid, 121 ℃, 1 h), then carried out the 45 min hydrothermal conversion. No HMF was determined and only small amount of furfural was generated (Figure 3.5b), but significant amount of levulinic acid (peak 5), formic acid (peak 3) and acetic acid (peak 4) were produced. This probably was due to the higher concentration of sulfuric acid (pH<1), which further degraded furfural and HMF to levulinic and formic acids. The levulinic acid is also an important intermediate chemical for industrial application. Furthermore, we readjusted the pH of hemicellulose extract to 2 after a secondary acid hydrolysis (Figure 3.5c), then performed an additional 45 min hydrothermal conversion. The production of levulinic acid was significantly prohibited and the yield of HMF was close to

that in Figure 3.5a. This indicated the lower pH (<1) will favour the production of levulinic and formic acids, and higher pH (>2) will assist the production of furfural and HMF. However, the final products in the hydrothermal reaction will also depend on the reaction time. Therefore, we extended the hydrothermal reaction time to 2 h instead of 45 min (Figure 3.5d), the residue sugars (peak 2) were considerably reduced, and the yield of furfural and HMF seemly did not increase correspondingly. This probably was caused by the polymerization of furfural and HMF at high temperature and concentration. Chuntanapum and Matsumura (2010) reported similar hydrothermal reaction for HMF to form char at high pressure and temperature (Chuntanapum et al, 2009, Chuntanapum et al, 2010). This indicated that hydrothermal conversion has to be well adjusted to maximize the furan derivatives via tailoring the temperature, pH and reaction time.

Table 3.4. Hydrothermal conversion of hemicellulose extract to furfural and HMF at 200 ℃ for 45 min.

Hemicellulose		Hydrothermal conversion of hemicellulose extract to furfural and HMF extraction					
H_2SO_4 (w/w)	Tween (w/w)	Furfural (g/L)	C5 sugars (g/L)	Selectivity (mol %)	HMF (g/L)	C6 sugars (g/L)	Selectivity (mol %)
0.00%	0.00%	2.62±0.68	7.63±0.25	53.37±12.15	3.49±0.77	12.66±2.40	39.21±1.31
0.10%	0.00%	3.67±0.07	9.59±0.04	59.78±1.32	4.99±0.25	18.15±0.03	39.24±1.94
0.20%	0.00%	4.37±0.04	11.40±0.38	59.97±1.52	5.21±0.00	21.66±0.23	34.37±0.37
0.40%	0.00%	4.89±0.11	12.13±0.03	64.48±1.20	6.68±0.01	24.71±0.69	38.61±1.06
0.60%	0.00%	6.34±0.59	14.64±0.42	67.56±4.33	7.20±0.05	31.13±0.01	33.03±0.22
0.40%	0.05%	7.25±0.03	14.17±0.71	80.05±4.37	9.01±0.99	27.25±0.65	47.25±0.65
0.40%	0.10%	6.58±0.02	13.68±0.01	75.12±0.12	9.17±0.10	24.85±0.01	52.73±0.58
0.40%	0.20%	7.95±0.14	14.97±0.01	83.02±1.40	10.58±0.81	28.18±0.07	53.65±4.23
0.40%	0.40%	8.47±0.15	17.28±0.38	76.56±0.37	11.66±0.80	29.57±0.68	56.30±2.56

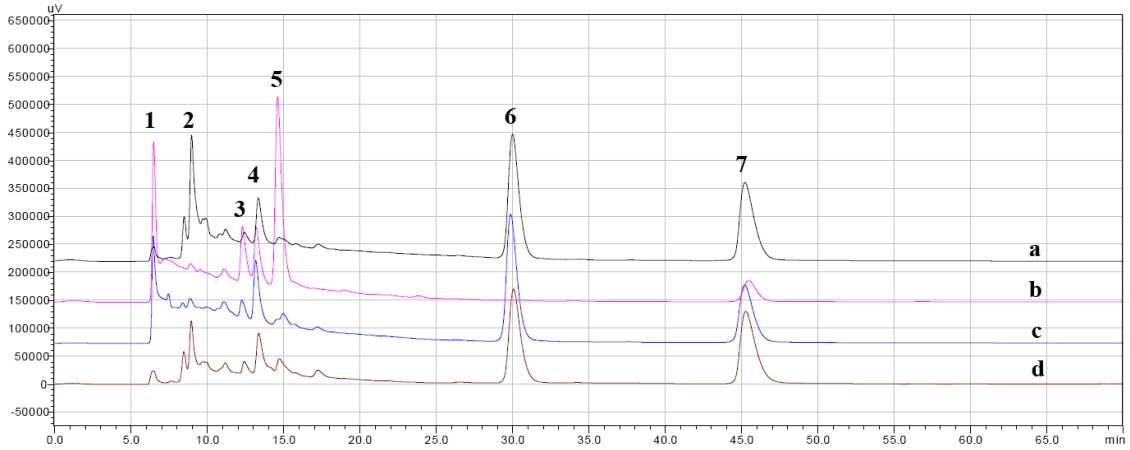


Figure 3.5. Effect of reaction time and pH on hydrothermal conversion of hemicellulose extract to furfural and HMF (HPLC chromatograpy results). Hydrothermal conversion of hemicellulose prehydrolysate was conducted at 200 ℃ for 45 min (a); at at 200 ℃ for 45 min after a secondary acid hydrolysis (4% of H_2SO_4) (b); at 200 ℃ for 45 min after a secondary acid hydrolysis and pH re-adjusted back to 2 (c); and at 200 ℃ for 120 min (d). The major peaks in the chromatography are : 1 sulfuric acid; 2 mixture of hemicellulose sugars ; 3 formic acid; 4 acetic acid; 5 levulinic acid; 6 HMF and 7 furfural.

Conclusions

In conclusion, the results showed that dilute acid and surfactant significantly improved the hemicellulose extraction from softwood at the mild condition (150 ℃). The surfactant could enhance the glucose and mannose consumption rates considerably during the biochemical conversion of hemicellulose to ethanol. The hydrolysate of hemicellulose extract could be fermented with an 85% of theoretical yield after adjusting the pH with the addition of Tween 80. The results showed that the Tween 80 is an efficient additive to the hemicellulose extract which when diluted by acid has the potential for its bioconversion to ethanol. The finding of furan selectivity increase from surfactant during the hydrothermal reaction will help us design suitable strategies for the effective conversion of hemicellulose to value-added co-products.

Detoxification of the Prehydrolysate from the Acid Pretreatment of Loblolly Pine

Abstract:

In this work, the liquid stream was from the prehydrolysate of the acid pretreatment, two groups of detoxification methods were applied to the prehydrolysate. The sugars and toxic compounds were compared before and after fermentation, the detoxified prehydrolysate was tested in 48 hours fermentation, the level of toxic compounds and sugar content were monitored and compared. The results showed that the original control group from the prehydrolysate can hardly be fermented. However, after detoxification, the indicators of inhibitors level decreased, and the sugars could be utilized in 48 hours fermentation, both the ethanol production and sugar consumption were increased. The chemical methods showed advantages for the higher preservation of sugars, the ion resins and activated charcoal methods showed benefits for removing of more inhibitors and higher fermentation rate.

Introduction:

The research of sustainable bioethanol is needed to provide an alternative to petroleum based fuels (DOE 2009). Bioethanol produced from the lignocellulosic biomass is being explored by many scientists in this world (Fukuda et al. 2009). Lignocellulosic biomass, such as wood, is majorly composed of a complex mixture of cellulose, hemicellulose, and lignin (Spatari et al. 2010). For softwood, while cellulose played an important role to provide the cellulose for hydrolysis, the hemicellulose, with a branched structure, could occupy 25-35% weight by weight of dry biomass. The chemical compositions of hemicellulose are pentoses

(xylose, arabinose), hexoses (mannose, glucose, galactose) and sugar acids (Han et al. 2007). Those sugars could be decomposed into the liquid at the process of pretreatment, but they can be hardly fermented due to the degradation of the sugars will form the inhibitors in the pretreatment. In the prehydrolysate liquid, sugars and inhibitors are composed as the major parts, sugars could be converted into ethanol or other important chemical compounds.

The maximum fermentation of hemicellulose sugars can be achieved after the detoxification. Generally, detoxification methods included three major areas: biological detoxification methods, physical detoxification methods, chemical detoxification methods. The target of these detoxification methods is to remove the inhibitors as much as possible. Many methods were explored and determined to the prehydrolysate of different pretreatment methods. Though the modern detectors such as HPLC, GC-MS could provide much more advanced platform for the research of the inhibitors, there are still some small compounds which are difficult to detect through conventional sensor. Yet, those compounds could be the key inhibitors. Common inhibitors included: furan derivatives, aliphatic acids and phenolic compounds (Palmqvist et al. 2000, Chandel et al. 2007).

In this study, chemical detoxification methods and physical detoxification methods were applied to the prehydrolysate of acid pretreatment liquid. Chemical detoxification methods are treating with the chemical reagents, mostly focusing on alkali and reducing agents (Palmqvist et al. 2000). Commonly, $Ca(OH)_2$ adjustment of pH is preferred over NaOH because of better fermentability than NaOH adjustment due to the precipitation of ‘toxic compounds’ (Van et al. 1988). A large precipitate will be formed after the overliming treatment (pH 10), which results in higher ethanol productivity per unit time. The mechanism

of overliming treatment has been demonstrated by the fact of precipitation of toxic components and the instability of some inhibitors at high pH (Jonsson et al. 1998). The activated charcoal could adsorb inhibitory compounds and after its adsorption saturation, it could be reactivated or regenerated by heating the mixture of used activated charcoal and water (Converti et al. 2000). However, during the adsorption saturation, there would be a loss of glucose adsorbed by activated charcoal as well as the inhibitory compounds. Using ion exchange resin is another useful detoxification method, which could use different polymeric adsorbents (resin) and different sized columns (Joseph et al. 2002). It is effective to remove the inhibitors inside the prehydrolysate; especially it could completely remove the Hibbert's ketones, the related inhibitory compounds of which could be one important key compound inside the softwood prehydrolysate (Tengborg et al. 1998). The results of all the detoxification methods used in this study were compared with control group through the change of the amount of sugars and inhibitors and the fermentation results.

Methods

Prehydrolysate

The raw wood chips were collected from a local mill, the shapes of wood chips are around 3×3×2 cm, and the chemical compositions were determined and shown in Table 3.1. The pretreatment was prepared in a Parr reactor with the 4415 controller, the load of the dry woody biomass is around 400 grams, while the liquid solid ratio is controlled to 7:1 volume by weight. The temperature was set at 180 ^{o}C, the process time was 30 minutes. After the pretreatment reaction, the liquid phase and solid phase were separated by the filter; the liquid phase was the prehydrolysate and it was collected and stored at -5 ^{o}C for the future research.

Detoxification

Control group

All the control groups were directly taken from the liquid phase stored in the fridge; no treatment was applied to the control groups. The chemical compositions of control group are shown in Table 4.1 below.

Table 4.1: The chemical compositions of the prehydrolysate

Chemical composition	Amount (g/L)	STDEV
Glucose	5.32	0.08
Xylose	8.80	0.11
Galactose	1.80	0.04
Arabinose	1.46	0.05
Mannose	14.37	0.14
Acetic acid	3.74	0.04
Levulinic acid	3.23	0.03
Furfural	1.06	0.01
HMF	1.08	0.01

Treatments with alkali

After the prehydrolysate was taken out from the fridge, treatments were performed by adding prepared 20%(w/v), 10% (w/v) and 5% (w/v) $Ca(OH)_2$ and 5% (w/v), 10 (w/v) NaOH solution into the prehydrolysate until the pH of prehydrolysate reach 10, the treated prehydrolysate was then put into the shaker for 1 hour under the temperature of 30 ^{o}C, the prehydrolysate liquid were centrifuged at the speed of 2000 rpm and then filtered by No. 541 Whatman filter paper. Then the liquid was preserved into fridge and processed for the following steps. Treated liquid (2 ml) was quantified by chemical methods below to analyze the useful chemical compounds in this study.

Treatment with activated charcoal

The dry weight of 2g activated charcoal powder was added into 40 ml liquid and then the detoxification process was performed in the shaker with the speed of 150 rpm and the temperature of 50 ^{o}C. After 1 hour, the liquid was centrifuged at the speed of 2000 rpm, filtered by No.541 Whatman filter paper and preserved into 5 ^{o}C fridge for the following process. Treated liquid (2mL) was quantified by chemical methods below to analyze the useful chemical compounds.

Treatments with anion exchange resins

Four resins were selected to process the detoxification, they were bought from Sigma chemical company, the names of them were as follows: Amberlite IRA 410, Dowex 1×4, Dowex 22 Cl and Dowex 50wx4. The first three resins were activated by the following process: The resins were firstly washed by DI water and then washed by saturated NaCl for 30 min and at last the resins were activated by 1 mol/L NaOH for 30 min. After these processes, they were washed by DI water until the pH of flowing water was stable. The Dowex 50w ×4 resin was activated by the following process: The resin was washed by DI water and then washed by saturated NaCl for 30min. And finally it was activated by 1 mol HCL for 1 hour, they were washed by DI water until the pH of flow water was stable. After this, 2g dry weight of resin was treated with 40 ml of prehydrolysate liquid, the detoxification condition was performed in a shaker with the conditions of 150 rpm, 50 ^{o}C for 1 hour. After the detoxification, the liquid was centrifuged and filtered to be preserved under 5 ^{o}C for the processes of following steps. Treated liquid (2mL) were quantified by chemical methods below to analyze the useful chemical compounds in this study.

Microorganism

Saccharomyces cerevisiae strain was maintained in the solid YPG mediums (yeast extract, peptone, glucose) containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose and 15 g/L agar. Colony of *Saccharomyces cerevisiae* was cultured in the liquid YPG containing 10 g/L yeast extract, 20 g/L peptone, 20 g/L glucose. After the yeast was cultured at 30 ^{o}C and 150 rpm for 6 hours, The liquid YPG with yeast inside was centrifuged and washed by sterile DI water for 3 times, then the dry weight of the concentrated *Saccharomyces cerevisiae* was measured as175 g/l.

Fermentation

The concentrated *Saccharomyces cerevisiae* (0.23 mL) was added into each serum bottle with hydrolysate above to make the initial *S. cerevisiae* concentration of each sample 2 g/L. The serum bottles were then sealed with rubber septa, vented by a needle. Bottles were incubated in an orbital shaker at 30 ^{o}C and 150 rpm for 48 h. Samples of fermentation liquid were taken out at 0 h, 6 h, 12 h, 24 h and 48 h. 0.2 ml of the aliquots mixed with *S. cerevisiae* were transferred into a 1.5 ml centrifuge tube and centrifuged at 12000 rpm for 3 minutes. After the centrifuge, 0.1 ml of the supernatant was withdrawn and mixed with 0.9 ml DI water to be filtered and analyzed chemical analysis.

Chemical analysis

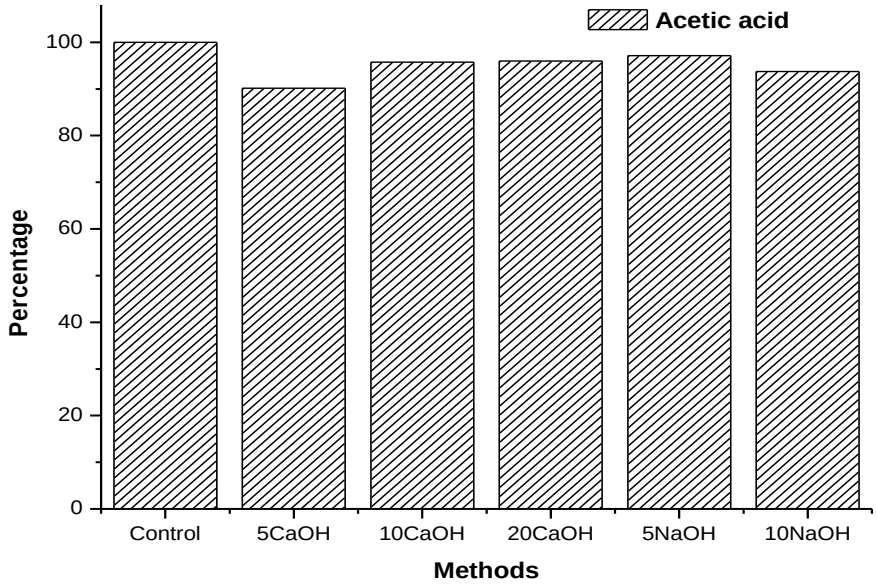
The carbohydrates of samples above were quantified on a SHIMADZU high performance high performance liquid chromatography equipped with a Bio-Rad Aminex® hpx-87p column. The mobile phase and gradient elution was described previously by Raymond Ruiz and Tina Ehrman 2. Ethanol furfural and hydroxymethyl furfural (HMF) were quantified on

another SHIMADZU high performance high performance liquid chromatography equipped with a Bio-Rad Aminex® hpx-87H column. The conditions were controlled at 60 ^{o}C with 5mM H_2SO_4 as the mobile phase, the flow rate was 0.6 mL/min.

Results and Discussion

The change of key compounds after detoxification

Some of the key compounds were selected to reveal the level of toxic compounds; these compounds were quantified like the sugars in the beginning of detoxification and the following steps were taken. The compounds used in this research were acetic acid, furfural and HMF, all the compounds were considered as the inhibitors in the history (Kumar et al. 2009, Nilvebrant et al. 2011, Chandel et al. 2007), in this study, because of the complicated compositions of the prehydrolysate, these three compounds were considered as the indicators of the level of toxic compounds. The change of acetic acid, furfural and HMF were recorded and summarized in the Figure 4.1, Figure 4.2 and Figure 4.3.



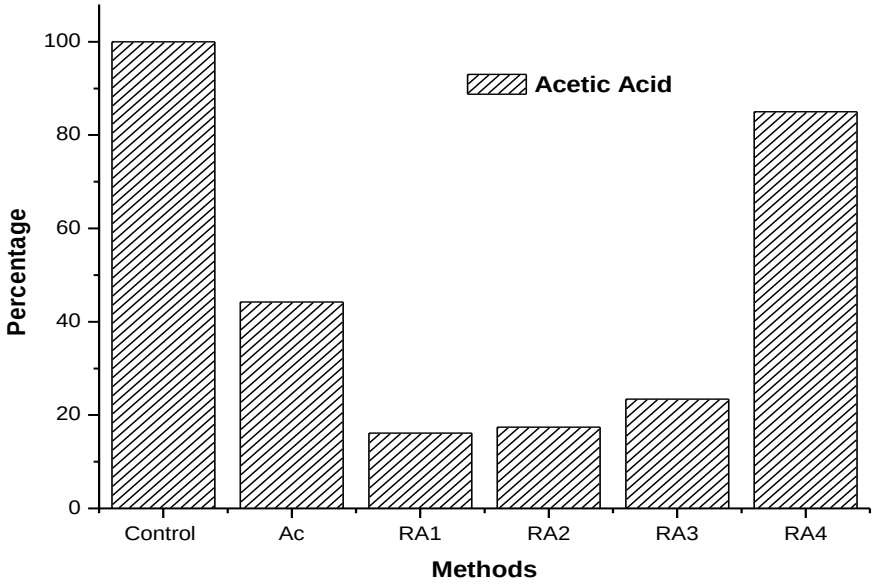
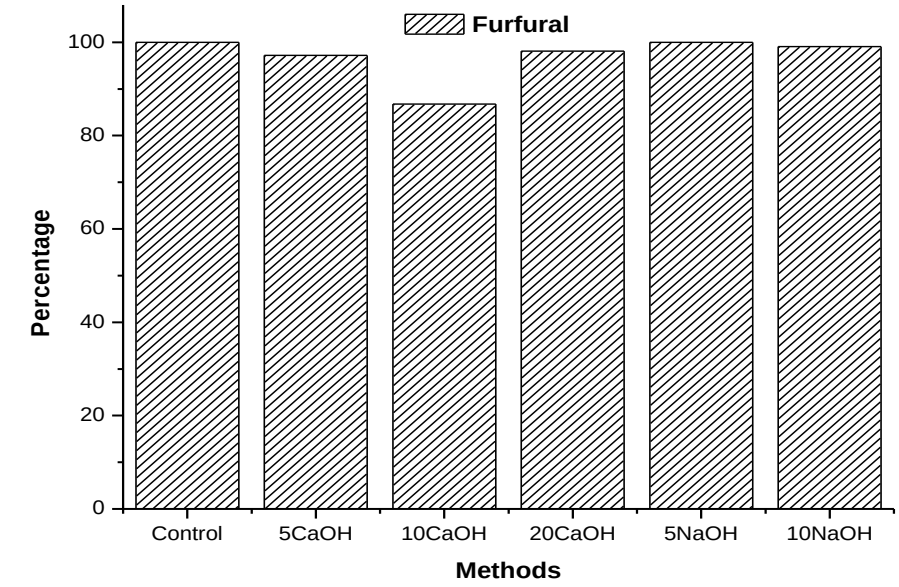


Figure 4.1. The acetic acid change in the prehydrolysate after the detoxification. (Control indicates the original acetic acid content in prehydrolysate, AC indicates activated charcoal; RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)



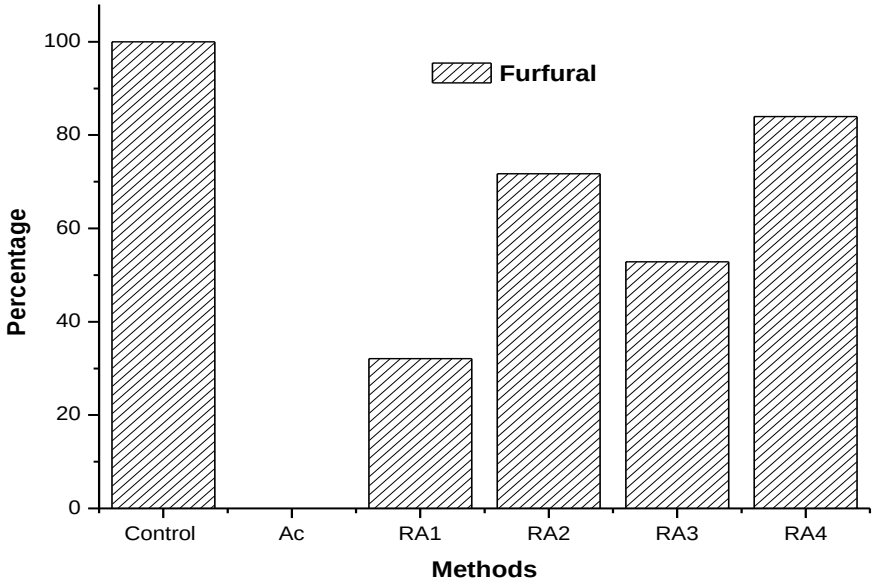
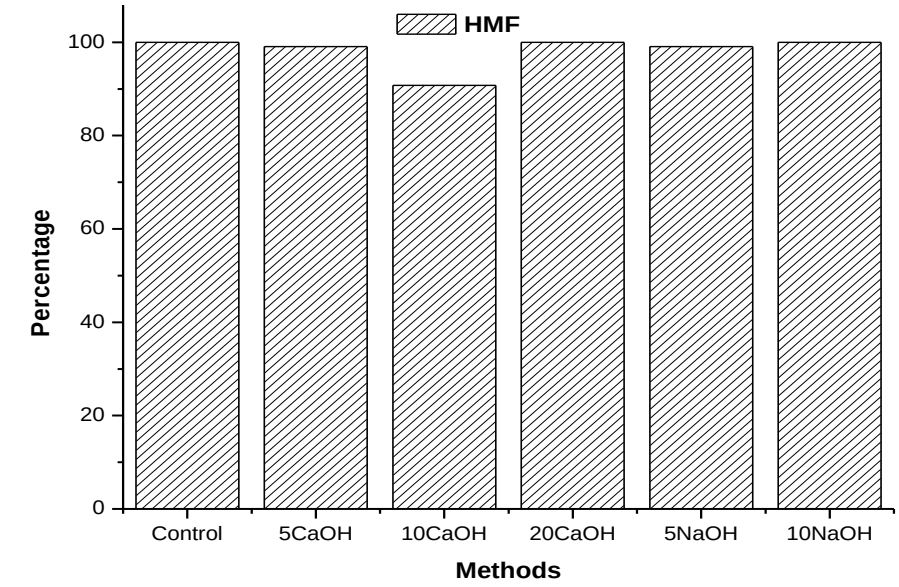


Figure 4.2. The furfural change in the prehydrolysate after the detoxification.

(Control indicates the original furfural content in prehydrolysate, AC indicates activated charcoal; RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)



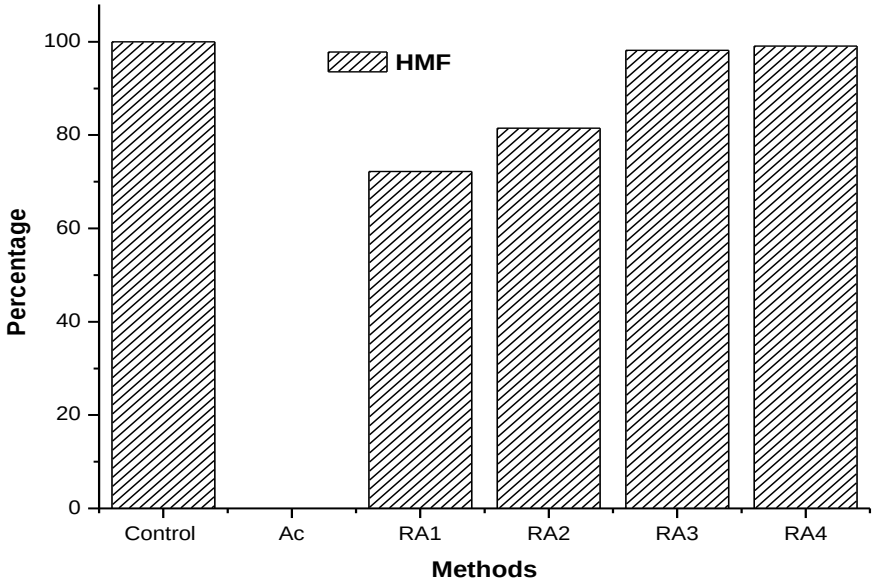


Figure 4.3. The HMF change in the prehydrolysate after the detoxification.

(Control indicates the original HMF content in prehydrolysate, AC indicates activated charcoal; RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)

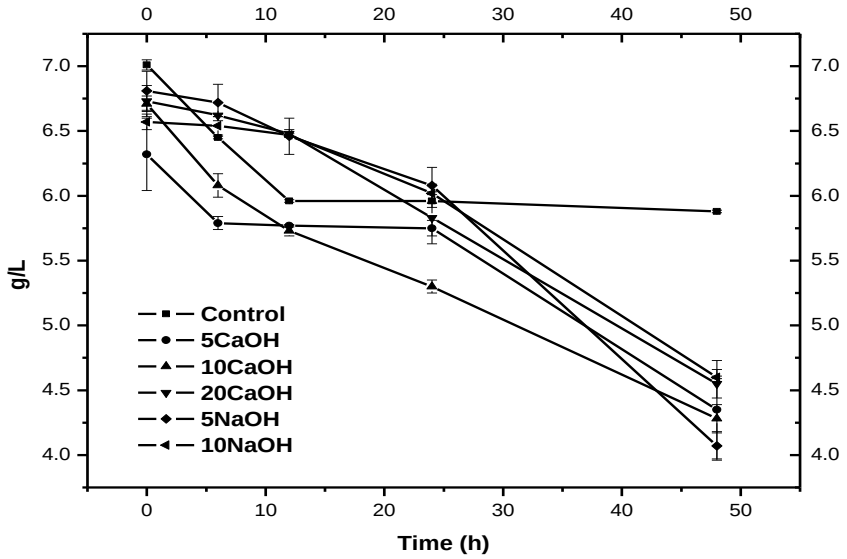
From the figures above, the three selected inhibitors changed after being applied to the detoxification methods. The chemical methods changed less inhibitors compared with activated charcoal and activated resins. For the acetic acid, due to its physical characteristics, it is a small molecule of organic acid which has low molecular weight. For the two groups of detoxification methods, the chemical methods basically did not remove more than ten percentage of acetic acid concentration, but for the activated charcoal and resins group, except the resin 4, most of these methods could remove more than half of acetic acid, the reason for this could be explained as the activated charcoal has strong affinity to any compounds in the aqueous solution, while the first three resin were activated in the strong base solution (NaOH), they could have even stronger affinity to the acid like acetic acid, so they could remove most of the acetic acid in the prehydrolysate. But for the resin 4, it is difficult for an acid activated resin to have strong affinity to another resin, due to the ion-exchange resins are based on crosslinked polymer, they have certain ability to absorb the compounds in the prehydrolysate, which explained the weak absorbance of acetic acid by resin 4.

For the furfural and HMF, they have very similar structure and have similar physical and chemical characteristics, the chemical methods did not show great ability to remove furfural and HMF, this is because the base groups could hardly have chemical reactions with furfural and HMF, but in the second group, most methods showed certain ability to remove furfural and HMF, especially, the group treated with activated charcoal showed the maxim performance in removing furfural and HMF, the reasons for this could also be explained by the structure of resins and activated charcoal make them have affinity to the small chemical

compounds. Though HMF and furfural have very similar characteristics, the HMF has an extra hydroxyl group, which make HMF have stronger affinity to water and harder to be removed in the aqueous solution. These three compounds changed after the detoxification, however, because the real toxic compounds or the detoxification mechanisms were not figured out, these three compounds could hardly be considered as the compounds that will have significant influence to the fermentation. So the standard for the good detoxification effect is the following fermentation rate, including both ethanol yield and sugars consumption.

The change of key compounds in the fermentation

As indicated above, the key compounds selected probably are not the major toxic compounds; however, they could be used as the indicator of the level of the toxic compounds, so in the fermentation step, the level of these three compounds were determined in the samples of fermentation, they were shown in Figure 4.4, Figure 4.5 and Figure 4.6.



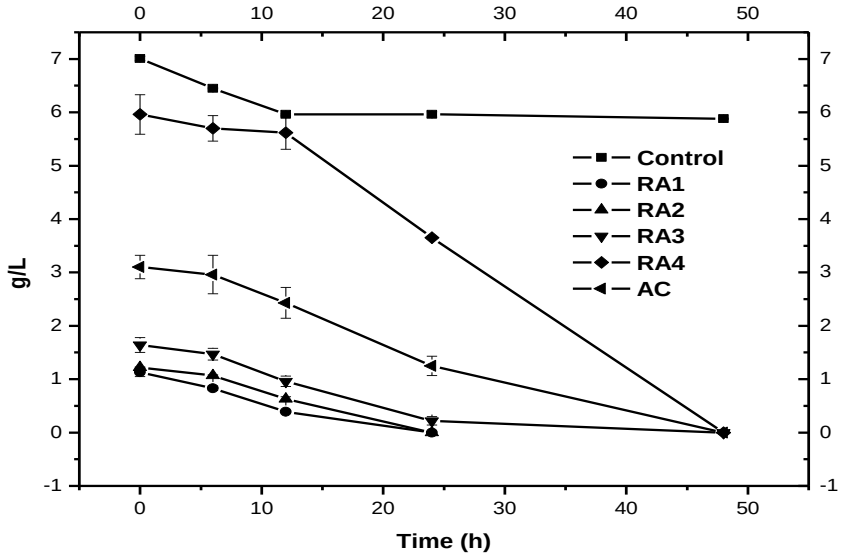


Figure 4.4. Acetic acid concentration change in 48h fermentation.

(Control indicates the acetic acid change in control group, AC indicates activated charcoal; RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)

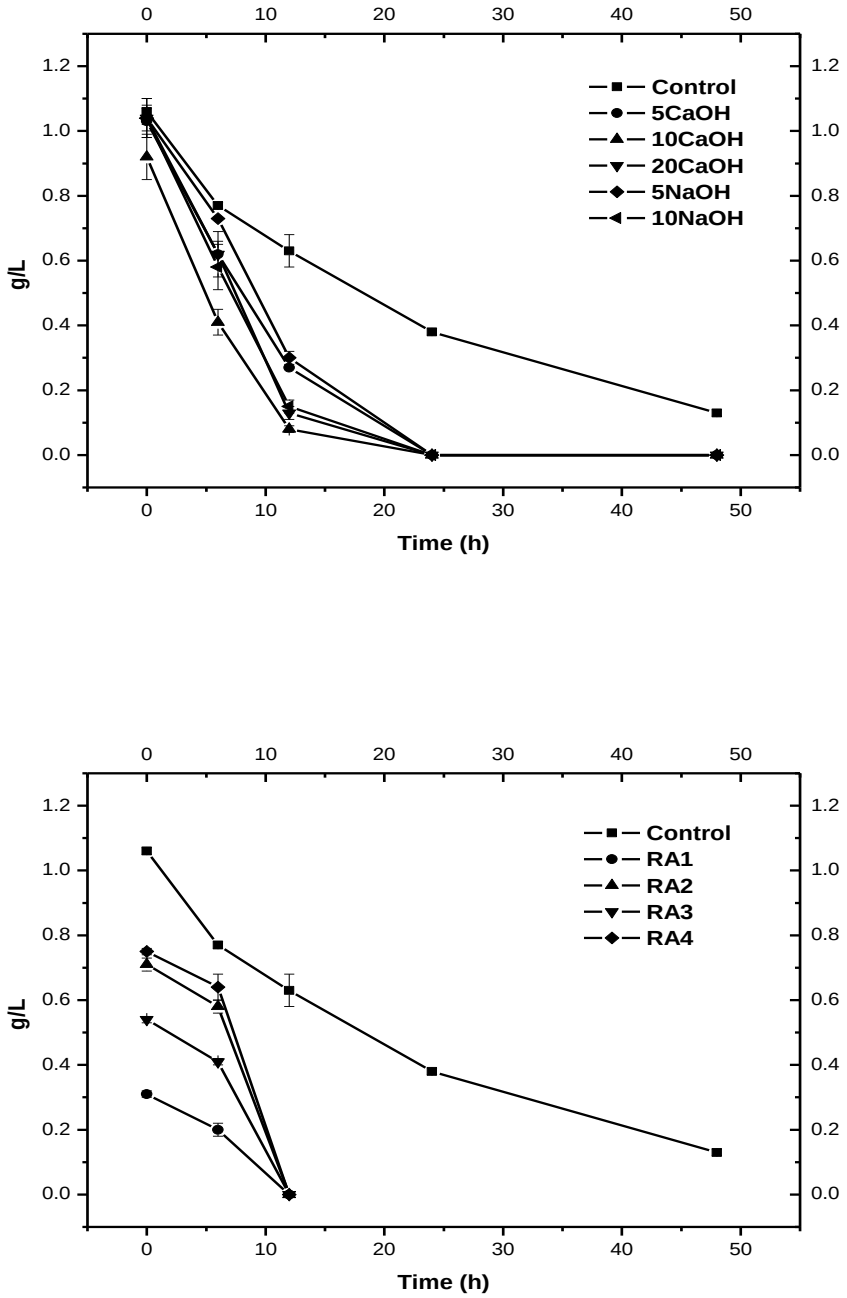


Figure 4.5. Furfural concentration change in 48h fermentation.

(Control indicates the furfural change in control group, AC indicates activated charcoal;

RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)

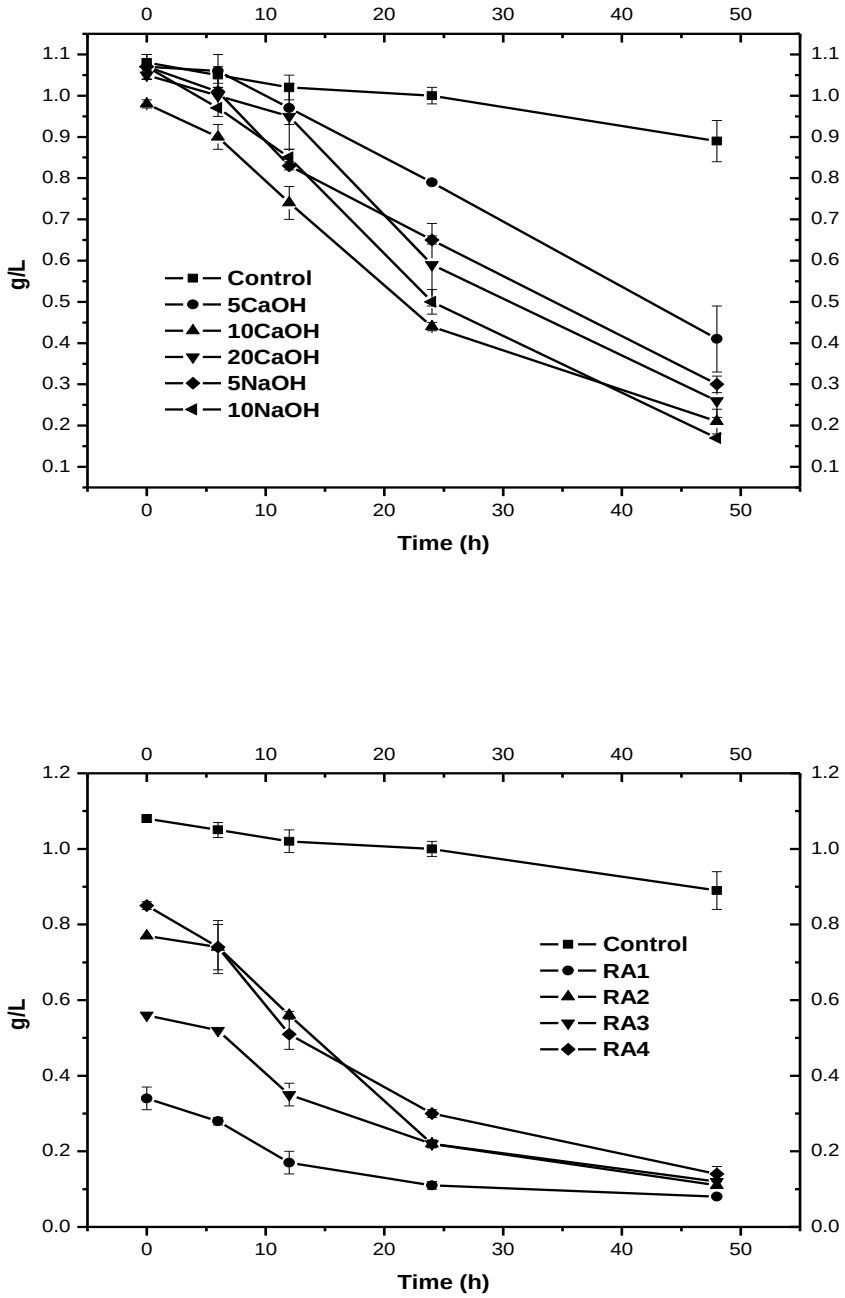


Figure 4.6. HMF concentration change in 48h fermentation.

(Control indicates the HMF change in control group, AC indicates activated charcoal;

RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)

From the acetic acid results shown above, the control group basically has no decreasing of acetic acid in 48 hours, for the chemical detoxification methods, they have more decreasing of acetic acid than control group; but in general, they did not decrease too much in the concentration (no more than 3 g/L). This could be explained as the absorbance of acetic acid by *Saccharomyces cerevisiae* , during the 48 hours fermentation, the absorbance of acetic acid increased because the growth of the number of *S. cerevisiae*. To the second group detoxified by the activated charcoal and ion resins, because the acetic acid was decreased during the detoxification process, so for most methods in the second group, the acetic acid was decreased quickly in 48 hours fermentation.

The change of sugars after detoxification

The process of detoxification needed to utilize the chemicals which could have reactions with sugars in the prehydrolysate; besides, the physical structures of activated charcoal and ion resins make them have affinity to all the chemicals in the prehydrolysate, which indicated the concentration of five sugars: glucose, mannose, xylose, galactose and arabinose will change more or less after the detoxification. Because these five sugars are reducing sugars and non-reducing sugars, they also have different physical and chemical structures, so the reactions in the detoxification process could be different but the main sugars used for fermentation were the glucose and mannose, so the change of these two sugars are significant to evaluate the performance of detoxification. They are presented in Figure 4.7 and 4.8, respectively.

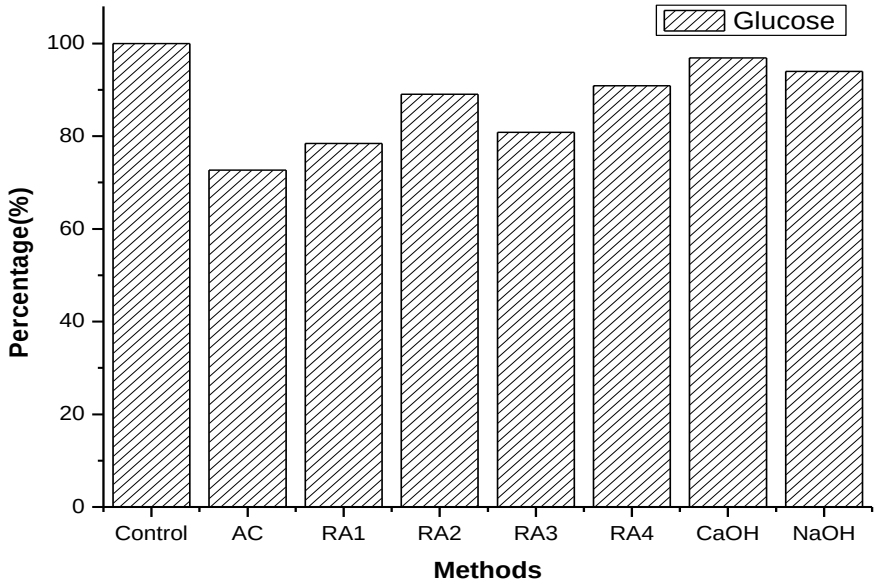


Figure 4.7. The glucose change in the prehydrolysate after the detoxification.

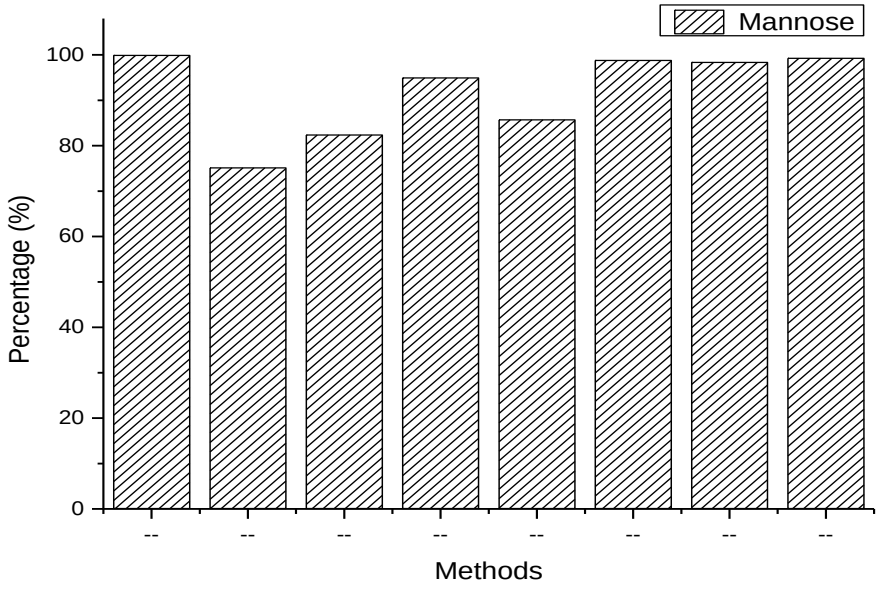


Figure 4.8. The mannose change in the prehydrolysate after the detoxification.

From the results shown above, the sugars content are less after the detoxification, to the glucose; the chemical methods preserved most of glucose, because after the detoxification,

the five chemical methods had preserved more than 90% glucose, but for the other group, the perseverance of glucose was worse, the reason for this is both ion resins and activated charcoal were absorbing the compounds in the prehydrolysate. The chemical methods can hardly have reaction with glucose, but because the addition of these chemicals, the volume of hydrolysate increased and leaded to the decreasing of glucose concentration. For the trend of mannose, basically, we could use the same explanation as for the glucose. For the rest three sugars, they have similar trend for chemical methods group and activated charcoal and resin groups. While the galactose and arabinose showed stronger affinity than with the resins and activated charcoal than that with the glucose and mannose.

The consumption of sugars

The sugars in the fermentation process were determined to understand the consumption rate of fermentation after different detoxification methods. Because the xylose, arabinose and galactose can hardly be utilized in the fermentation, they were not reported. Only glucose and mannose change were shown in this study, they were shown as Figure 4.9 and Figure 4.10.

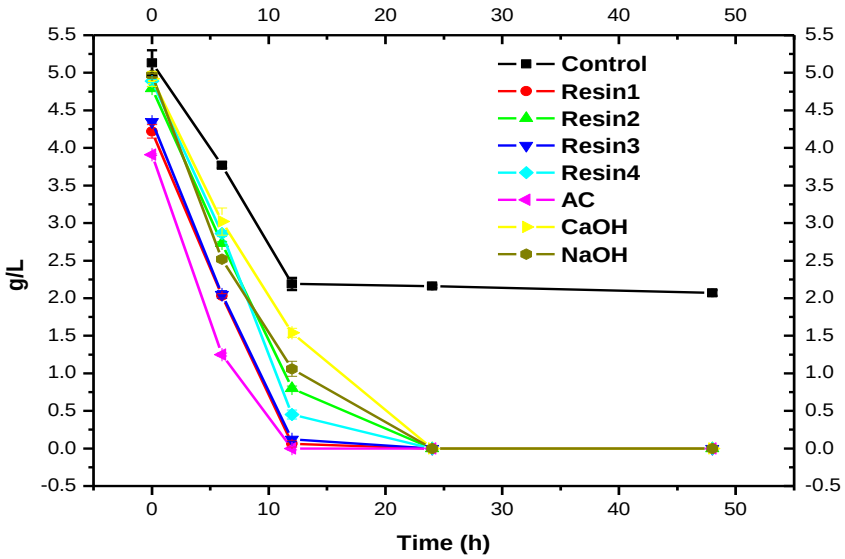


Figure 4.9. The consumption of glucose in 48 hours fermentation.

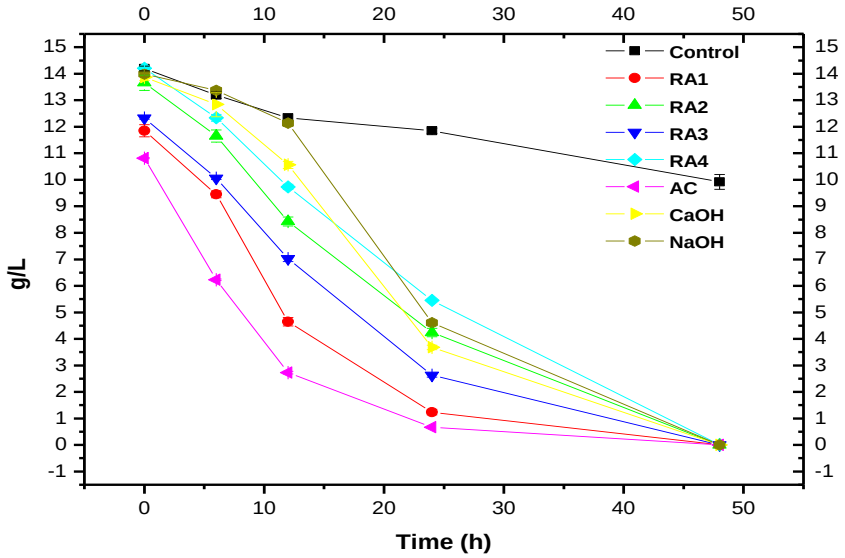


Figure 4.10. The consumption of mannose in 48 hours fermentation.

From the results above, for the glucose consumption in 48 hours fermentation, the control group can hardly consume up the glucose in 48 hours fermentation. But for other groups

treated with detoxification methods, glucose could be quickly consumed in 24 hours, for several detoxification methods in group 2, the glucose could even be converted totally in 12 hours. It is obvious that the treatment with detoxification methods could increase the fermentation of glucose. For the mannose, the similar trends were observed between control group and detoxified groups, the consumption of mannose were consumed in 48 hours fermentation, but for the control group, not all the mannose was converted in 48 hours fermentation. The reason for this is because the toxic compounds had negative effect to the fermentation in the control group.

The ethanol production

Ethanol production during the fermentation process was determined with the toxic compounds in the 48 hours fermentation. The fermentation curve is shown below in Figure 4.11 and the theoretical ethanol yield is shown in Figure 4.12.

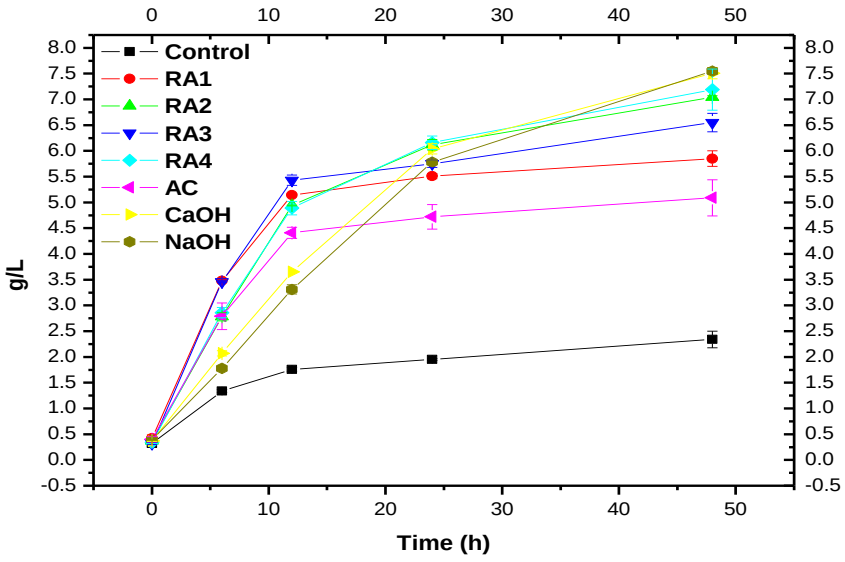
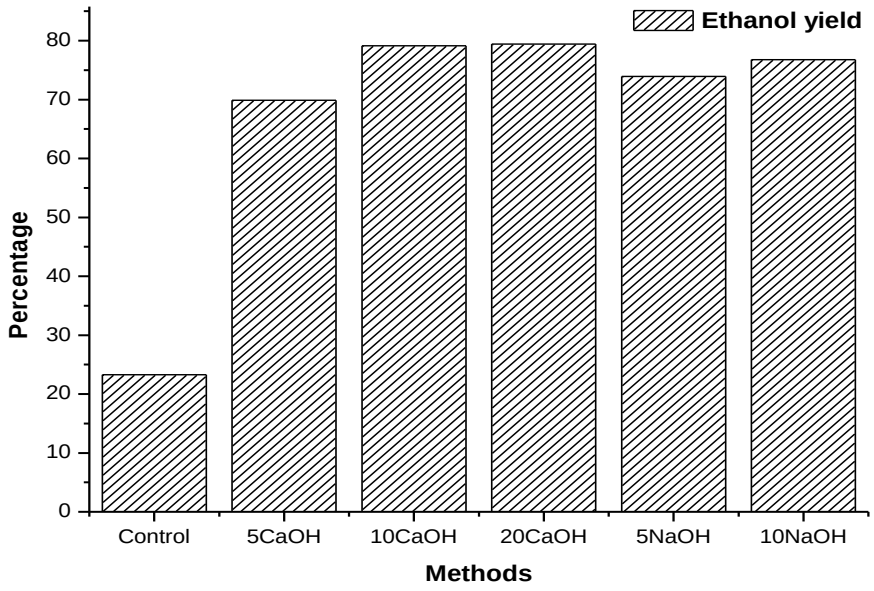


Figure 4.11. The production of ethanol in 48 hours fermentation.



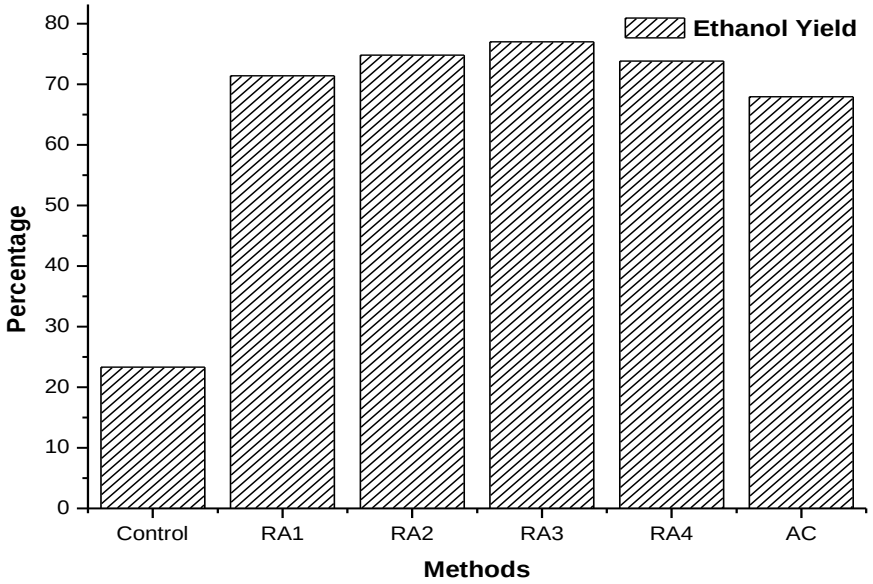


Figure 4.12. The theoretical ethanol yield.

(Control indicates the theoretical ethanol yield in control group, AC indicates activated charcoal; RA1 indicates resin 1, RA2 indicates resin 2, RA3 indicates resin 3, RA4 indicates resin 4.)

From the ethanol production curve, it can be observed that after the prehydrolysate had been treated with chemical detoxification methods, the amount of ethanol production increased significantly, the rate for ethanol production in the first 12 hours are higher than the control group, the similar trends could be found in the ethanol production curve of resin methods and activated charcoal. However, in the 12 hours fermentation, the rate of ethanol production is much higher than the chemical methods, which indicates the better fermentation results in the second group. From the ethanol yield figure, which indicates the detoxification methods could have much higher ethanol yield, though the initial sugars prepared for fermentation are much lower. The reasons for this not only because the removing of inhibitors, but also because the environment of the treated prehydrolysate, the addition of chemicals could have positive effect to the activity of *Saccharomyces cerevisiae*.

Conclusion

From this study, the original control group from the prehydrolysate can hardly be fermented. After the application of detoxification methods, the sugars content decreased and the indicators of inhibitors level decreased, inhibitors could be converted in 48 hours fermentation and the sugars could be utilized in 48 hours fermentation, both the ethanol production and sugar consumption were increased to a certain level compared with control group. The chemical methods showed advantages for the higher preservation of sugars, the ion resins and activated charcoal methods showed benefits for removing of more inhibitors and higher fermentation rate, but compared with the process of the detoxification, the chemical methods were much simpler and time-saving, which basically have the same effect as the ion resins and activated charcoal methods.

Conclusions and Future Work

In this thesis, we finished the detoxification the prehydrolysate extracting from woody biomass in the acid pretreatment. In addition, we developed the alternative methods to optimize the methods applied to hemicellulose extraction. These two works were respectively described in chapter 2 and chapter 3, the goals of this chapter are to highlight the main contributions of the thesis and concentrate on some future directions, which could be the extensions of our past and current research on the bioethanol production from hemicellulose stream.

Main Contributions

In this thesis, there are two major parts of research work. In part one, a series of work about the hemicellulose extraction was finished; the major difference compared with the previous research is the addition of surfactant under the lower concentration of sulfuric acid. Also, the generation of value added byproducts was tested under different conditions, the yield of the value added byproducts revealed the trend of the best condition to produce the byproducts. In part two, a series of work about the detoxification was studied; different kinds of methods were examined and the criterion of good results depended on the fast fermentation rate of the sugars. These research works did the contributions to the bioconversion of hemicellulose sugars to bioethanol.

1. Hemicellulose sugars could be utilized in the bioconversion to ethanol or in the hydrothermal conversion to value added byproducts.

2. Initial surfactant could have positive effect to the fermentation rate and sugars consumption rate.
3. The inhibition in the prehydrolysate from the dilute acid pretreatment could be greatly reduced by chemical detoxification methods and Anion exchange resins.

Future Work

Due to the extensive research work performed in this field, future work should focus on developmental directions. To the research in this thesis, what we can develop in related areas for future work is also complicated, but several of them are very obvious and clear. They are shown as follows:

1. Developing the additives which could help the extraction of hemicellulose in the woody biomass under the mild conditions.
2. Conducting series of experiments to figure out the mechanisms of detoxification or the major functional toxic compounds.
3. Producing the other byproducts from the hemicellulose sugars.
4. Developing effective methods to do the pretreatment of woody biomass, with the goal to extract the highest percentage of hemicellulose sugars.

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