Studies on the cellulose hydrolysis and hemicellulose monosaccharide degradation in concentrated hydrochloric acid

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ABSTRACT

Given the volatile, generally high price of crude oil, as well as environmental concerns associated with its use as a fuel, development of alternative energy sources is currently of considerable interest. Lignocellulose-derived energy has the potential to supplant traditional fossil fuels in the future because of its economic and environmental advantages. Lignocellulosic biomass is abundant and renewable. Lignocellulose is primarily composed of cellulose, hemicellulose and lignin, which can be converted by acid hydrolysis to simple sugars used in fermentation to produce biofuels.

In this study, hemicellulose was hydrolyzed with different concentrations of hydrochloric acid at different temperatures. The resulting components were analyzed by high performance liquid chromatography (HPLC). The hydrolysis of cellulose was similarly characterized, with two additional parameters, the degree of polymerization (DP) and the crystallinity index (CrI), which were analyzed by Ubbelohde viscometer and X-ray diffraction respectively. The experimental results indicate that the hydrolysis rate of hemicellulose and the generation rate of furfural and 5-hydroxymethylfurfural (HMF) increased with increasing hydrochloric acid concentrations and reaction temperatures. In the selected five monosaccharides, xylose, glucose, mannose, arabinose and galactose, xylose has the highest hydrolysis rate and the accumulation of furfural during xylose hydrolysis is also the highest. Moreover, the hydrolysis rate of cellulose and the generation rate of glucose also increased with increasing hydrochloric acid concentrations and reaction temperatures. DP and CrI, both decreased when the cellulose was treated in concentrated hydrochloric acid. The rate of change of DP increased with the concentrations of acid and the reaction temperatures. The change rate of CrI increases

by increasing concentration of acid and the temperature when it is above 0℃, while the CrI index decrease sharply when the reaction temperature was kept below 0℃. Experimental results also show that the hydrolysis rate of cellulose is much lower than that of hemicellulose.

RÉSUMÉ

Étant donné le prix volatil et généralement élevé du pétrole, ainsi que l'impact environnemental associé à son utilisation comme carburant, le développement de nouvelles sources d'énergie est d'un intérêt considérable. Dû à ses avantages économiques et environnementaux, l'énergie produite de biomasse lignocellulosique pourrait un jour remplacer les carburants à base de pétrole. La biomasse lignocellulosique, est abondante, renouvelable, et est composée principalement d'hémicellulose, de cellulose et de lignine, qui peuvent être convertis en monosaccharides qui peuvent à leur tour être convertis en biocarburants par fermentation.

Dans cette étude, de l'hémicellulose fut hydrolysée par différentes concentrations d'acide hydrochlorique à différentes températures. Les saccharides libérés furent analysés par chromatographie liquide à haute performance. La même approche fut utilisée pour l'étude de l'hydrolyse de la cellulose, et deux paramètres supplémentaires, l'index de cristallinité (ICr) et le degré de polymérisation (DP), furent mesurés à l'aide d'un viscomètre Ubbelohde et par diffraction à rayon-X respectivement. Les résultats indiquent que le taux d'hydrolyse de l'hémicellulose et le taux de rendement du furfural et du 5-hydroxymethylefurfural (HMF) augmentent lorsque la concentration d'acide hydrochlorique et la température de réaction sont augmentées. Des cinq monosaccharides étudiés, le taux de production de xylose était le plus élevé, et l'accumulation de furfural était beaucoup plus grande que celle d'HMF. Le taux d'hydrolyse de cellulose et son rendement de glucose augmentent aussi lorsque la concentration d'acide hydrochlorique et la température de réaction sont augmentées. Le DP et l'ICr diminuent tous deux lorsque la cellulose fut traitée par de l'acide hydrochlorique

concentré. Le taux de changement du DP augmente à mesure que la concentration d'acide et la température augmentent. Le taux de changement de l'ICr augmente avec la concentration d'acide lorsque la température de réaction est maintenue au-dessus de 0°C, mais diminue rapidement à des températures au-dessous de 0°C. Les résultats démontrent aussi que le taux d'hydrolyse de cellulose est beaucoup plus bas que celui d'hémicellulose.

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NOMENCLATURE

A_{am}	area of amorphous region of cellulose (dimensionless)
A_{cr}	area of crystalline region of cellulose (dimensionless)
A_{peak}	area of peak which obtained from HPLC (dimensionless)
C	concentration of solutions (mol/L)
C_{HCl}	concentration of hydrochloric acid (w/w%)
C_{sam}	concentration of samples (mg/mL)
D	distance of molecule (nm)
DP	degree of polymerization (dimensionless)
DP_n	number average degree of polymerization (dimensionless)
DP_v	viscosity average degree of polymerization (dimensionless)
DP_w	weight average degree of polymerization (dimensionless)
L	crystallite size (nm)
I_{002}	intensity of 002 lattice plane of cellulose (dimensionless)
I_{am}	intensity of amorphous regions of cellulose (dimensionless)
I_{cr}	intensity of crystalline regions of cellulose (dimensionless)
I_{α}	integral area of amorphous area of cellulose (dimensionless)
M_v	viscosity average molecular weight (dimensionless)
m	mass of powder (g)
R	scanning rate of x-ray diffraction (°/min)
S_p	integral area of crystalline area of cellulose (dimensionless)
$S_{\delta 84}$	integral area of the chemical shift at 84ppm (dimensionless)
$S_{\delta 89}$	integral area of the chemical shift at 89ppm (dimensionless)

T	temperature (°C)
t	time when the solution flow through the viscometer (second)
t_0	time when the solvent flow through the viscomter (second)
t_r	reaction time (min)
V	volume of containers (mL)

Greek symbols

α	crystallinity index (dimensionless)
δ	signal intensity (ppm)
η	viscosity of solution (Pa/s)
η_0	viscosity of solvent (Pa/s)
η_r	relative viscosity (dimensionless)
η_{sp}	specific viscosity (dimensionless)
$[\eta]$	intrinsic viscosity (dL/g)
2θ	scanning angle (°)
λ	wavelength of CuKα source (nm)
ω	rotation speed (rpm)

CHAPTER 1

Introduction

1.1 Background

Fossil energy is the main energy in nowadays however it also shows lots of problems. High price of crude oil, high pollution, shortage of resource and global warming lead human to discover new energy technologies.

It is meaningful to produce carbohydrates such as ethanol from biomass resource by fermentation. Ethanol is a significant product in the fuel market. Its production grew from less than 1 billion liters in 1970s to more than 60 billion liters in 2012. Less than 4% of the ethanol is synthesized by fossil resource, a large amount of ethanol produced by fermentation from biomass. As a potential product, ethanol can replace the fuel market for gasoline however the feedstock which used for produce ethanol is limited. The feedstock for ethanol production is monosaccharide, but as an important raw material of human foods, the price of sugar is expensive. The competition is leading to increasing the price of the ethanol.

Lignocellulosic materials are considered as abundant, inexpensive and renewable resources. They are mainly made up of cellulose, hemicellulose and lignin and they are the most abundant biomass resource in the world. Unlike fossil energy, they are carbon neutral material and it can highly reduce the effect of global warming. Compare with sugar, the price of lignocellulose is relatively cheap because it can be obtained from agriculture residue such

as sawdust, sugar cane and corn cobs. It could convert into monosaccharide under certain conditions. The degradation processes are called hydrolysis and the pretreatment and hydrolysis of lignocellulosic biomass can be classified in chemical hydrolysis and enzymatic hydrolysis and the most applied method of chemical hydrolysis is acid hydrolysis. Acid hydrolysis has been described at least since 1819 and it may be divided into two approaches, concentrated acid at low temperatures (38%-41% concentrated acid, 15℃-100℃) and dilute acid at high temperatures(3%-5% dilute acid, 200℃-240℃), which can be hydrolyzed with different acids, such as sulfuric, nitric, hydrochloric, hydrofluoric, sulfurous acid.

The first scientist using concentrated acid to hydrolyze lignocellulose is Arena Peluse. He is a France chemist and he developed a process to degrade woods in concentrated sulfuric acid to produce ethanol in 1854 and the first ethanol-produce factory was built in the next year. Another French chemist, A Bechamp reported produce ethanol from biomass by fuming hydrochloric acid in 1856. After that, several researches have been reported on lignocellulose hydrolysis in concentrated hydrochloric acid and the process is known as the Bergius-Rheinau process. Dr. Bergius is Germany and he developed a process using 41% HCl hydrolysis stage with a 3:1 acid wood ratio. He obtained Nobel prize in Chemistry in 1931 and German used his method to produce food and energy from woods successful in Second World War. Nevertheless, Bergius-Rheinau process is low economic efficiency which makes it difficult to industrialize. Recently, an Israelis company has developed an HCl recovery process, recovering HCl in gaseous form directly from aqueous solution by an immiscible extractan, which makes the modified Bergius-Rheinau process economy and clean.

Another reason which makes the process unprofitable is byproducts are formed when the lignocellulose hydrolyze to monosaccharide. The byproducts such as furfural and 5-hydroxymethylfurfural not only consume the amount of monosaccharide but also poison the bacteria in fermentation process. The byproducts are converted by monosaccharide in concentrated acid and this process always be ignored. To reduce the amount of byproducts have a further meaningful to increase the economic efficiency of the Bergius-Rheinau process.

1.2 Objectives

The main objective of this work, whose results are applicable to lignocellulosic biofuel production, is the comparison of the formations of furfural and HMF at different conditions, including different concentrations of hydrochloric acid and reaction temperatures. During the hydrolysis experiment of cellulose, the liquid phase is analyzed by high performance liquid chromatography, and the solid phase is by X-ray diffraction and with a Ubberlohde viscometer.

1.3 Outline

The thesis is composed of four chapters. Chapter 1 gives a brief introduction about the work and its background. Chapter 2 is a literature review relevant to lignocellulosic hydrolysis. It contains an introduction to biorefineries and biomass-derived biofuels, and a summary of analytical methods used for characterization of such processes. Chapter 3 describes the experimental work performed, including all relevant methodologies, and discusses all experimental results related to the hydrolysis rates of hemicellulose and cellulose in different concentrations of hydrochloric acid at various temperatures. Finally, Chapter 4 presents conclusions and recommendations for future works.

CHAPTER 2

Introduction to biomass and lignocellulose hydrolysis process - A review

2.1 Biomass

2.1.1 Introduction

Biomass is an organic material which is produced over time by photosynthesis with CO_2, water and soil. It includes all kinds of plant, microorganism, animal which eating the plant and microorganism, and wastes that they produce. Representative biomass streams are crops, agricultural residues, woods, animal manure etc. Biomass energy is a renewable energy source and it is widely distributed. It is also associated with lower levels of pollution than other energy sources such as fossil fuels [1].

As a kind of solar energy because it is produced through photosynthesis, biomass energy is abundant. Through photosynthesis, biomass is effectively able to absorb and store solar energy in significant quantities. As a result, biomass energy is a kind of solar energy, stored as chemical energy. It is estimated that solar energy has been consumed by all plants on earth accounts for 0.2% of the total amount of radiation. The represents a significant proportion since it is 40 times than the total amount of solar energy due to human consumption and 15-20 times than that of fossil energy.

Plants can convert CO_2 and H_2O to glucose with the help of chlorophyll by photosynthesis and store the energy. Synthesized glucose can also be converted to compounds constituting plant body such as starch, cellulose, hemicellulose and lignin. Moreover, biomass energy can help reducing global warming because CO_2 releasing from burning biomass can be used by the plants' photosynthesis. The use of biomass energy will not increase the amount of CO_2 in atmosphere. Biomass energy is thus carbon neutral and cleaner energy than fossil energy.

Lignocellulose is a significant biomass material and it is composed by cellulose, hemicellulose and lignin which are the main cell wall compounds and the proportion of these polymeric compounds account for 97%-99% of the content of woods. The proportions of hemicellulose and lignin are different in softwoods and hardwoods, however cellulose is a uniform composition of all woods. The cellulose content of cotton is 90%, wood is 40–50% and hemp is 45% [2].

2.1.2 Cellulose

2.1.2.1 Introduction

Cellulose was discovered in 1838 by Anselme Payen, a French chemist, who separated it from a plant and determined its chemical formula [3, 4]. It was used to produce celluloid, the first successful thermoplastic polymer, by the Hyatt Manufacturing Company in 1870. The polymer structure was determined by Hermann Staudinger in 1920 and was chemically synthesized by Kobayashi and Shoda in 1992 [5].

Cellulose is an organic compound with a chemical formula of $(C_6H_{10}O_5)_n$, and is a polysaccharide consisting of a linear chain of containing several hundreds to over ten thousands linked D-glucose units [6]. Cellulose is the most significant structural component of cell wall of plants and algae, and is also the most abundant renewable polymer resource and biomass in the world [7]. It has been estimated that 10^{11} to 10^{12} tons are produced annually by photosynthesis. Commercial cellulose production comes from wood which is harvested sources or cotton which is naturally sources and further cellulose-containing materials include agriculture residues, grasses, water plant etc.

2.1.2.2 Structures

Cellulose is derived from D-glucopyranose units, linking through β(1-4)-glycosidic bonds. The kind of bond is different from α(1-4)-glycosidic bonds presenting in starch, glycogen, and other carbohydrates. Cellulose can be considered as an isotactic polymer of cellobiose.

Each glucose unit carries hydroxyl groups at positions C-2, C-3 and C-6, the terminal hydroxyl group at C-4 is also like an aliphatic hydroxyl. However, the hydroxyl at C-1 shows different behavior. The C-1 end has a reducing character and the C-4 hydroxyl group is non-reducing. The conformation of the anhydroglucose unit in cellulose is that of a chair of $^4C_1$. The free hydroxyl groups are positioned equatorially and the hydrogen atoms are positioned axially (Fig. 2.1) [8].

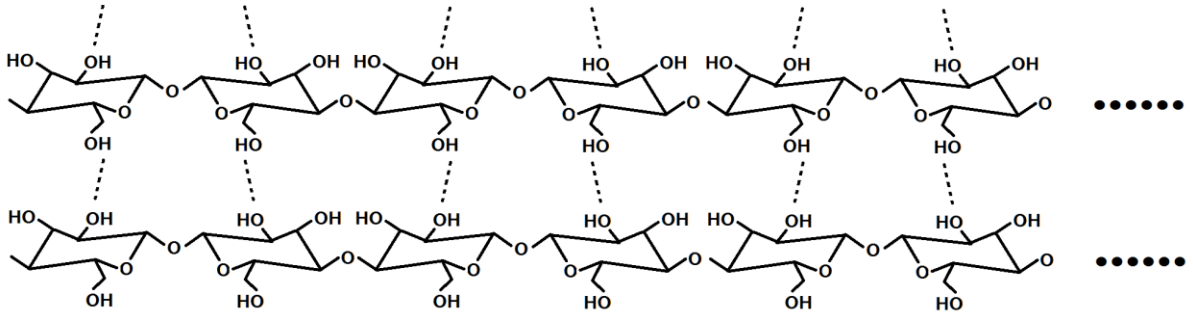

Fig. 2.1 The molecular structure of cellulose

Cellulose is a straight chain polymer where no coiling or branching occurs, and the molecule adopts an extended and rather stiff rod-like conformation, aided by the equatorial conformation of the glucose residues. The hydroxyl groups on the glucose from one chain connect with oxygen atoms on the same or on a neighbor chain by hydrogen bonds, holding the chains firmly together side-by-side and forming micro-fibrils with high tensile strength. These bonds generate tensile strength in cell walls, where cellulose micro fibrils are meshed into a polysaccharide matrix.

2.1.2.3 Supra-molecular Structures

Cellulose is crystalline and it requires a temperature of 320 ℃ and pressure of 25 MPa to become amorphous in water [9]. Several different crystalline structures of cellulose are known, corresponding to the location of hydrogen bonds. The various types of intermolecular hydrogen bonds result in a complex organization. The fundamental studies of cellulose crystal structure were proposed in the 1930s [10]. Natural cellulose is cellulose I, consisting of rod-like crystalline microfibrils containing parallel chains with a two-fold screw symmetry along the chain axes. There are two phases co-existing within native cellulose I, with the different structure I_α and I_β. Cellulose produced by bacteria and algae is enriched in I_α while

cellulose in high plants consists mainly of I_β. Cellulose in regenerated cellulose fibers is cellulose II which is more stable allomorph. Conversion of cellulose I to cellulose II is irreversible because cellulose I is metastable and cellulose II is stable. With various chemical treatments, it is possible to produce cellulose III and cellulose IV from cellulose I [11].

2.1.2.4 Properties

Cellulose has no taste, is odorless, is hydrophilic with the contact angle of 20-30, is insoluble in water and most organic solvents, is chiral and is biodegradable. As such, cellulose is stable in room temperature, because of hydrogen bonds existing between the molecules inside. Under certain conditions, cellulose can react with water. In the reaction, oxygen bonds break while water molecule participates to cause long chain cellulose breakage into short chain celluloses. At the end, cellulose converts to glucose when all oxygen bonds rupture. Cellulose can be broken down chemically into its glucose units by treating it with concentrated acid or alkali solution [12]. The reaction has three steps. Firstly, water molecule may cause cellulose limited swelling. After that, certain acid solution or alkaline solutions may permeate into the crystalline region of cellulose. As a result, the acid or alkaline solution could cause cellulose unlimited swelling and result in cellulose decomposition. It also has a chemical reaction with oxidants and produces a series of compounds which have different structure compared to the original cellulose. Cellulose is soluble in cupriethylenediamine (CED), cadmiumethylenediamine (Cadoxen), N-methylmorpholine N-oxide and lithium chloride/dimethylformamide [13].

Many properties of cellulose depend on its chain length or degree of polymerization (DP), which is the number of glucose units that make up one polymer molecule. Cellulose from wood pulp has typical chain lengths between 300 and 1700 units; cotton and other plant fibers as well as bacterial cellulose have chain lengths ranging from 800 to 10,000 units [14]. Molecules with very small chain length result from breakdown of cellulose into cellodextrins; and, in comparison to long chain cellulose, cellodextrins are typically soluble in water and organic solvents.

Plant-derived cellulose is usually found in a mixture with hemicellulose, lignin, pectin and other substances, while microbial cellulose is quite pure, has much higher water content, and consists of long chains.

Cellulose has low flexibility because of its polar molecules and its strong intermolecular forces. The structure of glucopyranose also makes the molecule difficult to rotate. Another reason for this lack of flexibility is that the hydrogen bonds exist intermolecularly and intramolecularly, which greatly increases the flexibility of the resulting cellulose.

2.1.2.5 Synthesis and decomposition

2.1.2.5.1 Cellulose Synthesis

In vascular plants, cellulose is synthesized at plasma membrane by rosette terminal complexes (RTCs). The RTCs are hexameric protein structures, approximately 25 nm in diameter, that contain cellulose synthase enzymes that synthesize individual cellulose chains [15]. Each RTC floats in the plasma membrane of the cell and spins a micro-fibril into the cell wall.

RTCs contain at least three different cellulose synthases, encoded by CesA genes, in an unknown stoichiometry [16]. Separate sets of CesA genes are involved in primary and secondary cell wall biosynthesis.

Cellulose synthesis requires two separate processes: chain initiation and elongation. CesA glucosyltransferase initiates cellulose polymerization using a steroid primer, sitosterol-beta-glucoside, and UDP-glucose [17]. Cellulose synthase utilizes UDP-D-glucose precursors to elongate the growing cellulose chain. A cellulase may function to cleave the primer from the mature chain.

Cellulose is also synthesized by animals, particularly in ascidians, and is also a minor component of mammalian connective tissue [18].

2.1.2.5.2 Cellulose detection methods

Cellulose can be assayed using a method described by Updegraff in 1969 [6]. The fiber is dissolved in acetic and nitric acid to remove lignin, hemicellulose, and xylosans and the resulting cellulose is allowed to react with anthrone in sulfuric acid. The resulting colored compound is assayed spectrophotometrically at a wavelength of approximately 635 nm.

2.1.2.5.3 Cellulose degradation

The process of breaking down cellulose into smaller polysaccharides or glucose units is called cellulolysis, and is a hydrolysis reaction. Because cellulose molecules bind strongly to each

other, cellulolysis is relatively difficult compared to the breakdown of other polysaccharides [19].

The enzymes utilized to cleave the glycosidic linkage in cellulose are glycoside hydrolases including endo-acting cellulases and exo-acting glucosidases. Such enzymes are usually secreted as part of multienzyme complexes that may include dockerins and carbohydrate-binding modules [20].

Most mammals have only very limited ability to digest cellulose. Some animals, particularly ruminants and termites, can digest cellulose with the help of symbiotic micro-organisms that live in their guts [21]. Humans can digest cellulose to some extent, however cellulose mainly acts as a hydrophilic bulking agent for feces and is often referred to as "dietary fiber" [22, 23]. Fungi, in nature are responsible for recycling of nutrients, are also able to break down cellulose.

2.1.2.6 Cellulose-derived products

The main use of cellulose is to produce paper. Cellulose is the major component of paper, paperboard and textiles made from cotton, linen, and other plant fibers. Smaller quantities are converted into cellophane and rayon, which are kinds of derivative products. Cellophane is a thin transparent film. Rayon is an important fiber that has been used for textiles since the beginning of the 20th century. Both cellophane and rayon are known as "regenerated cellulose fibers" and have the same chemical structure as cellulose. They are usually made by dissolving pulp via viscose. A more recent and environmentally friendly method to produce rayon is

Lyocell process [24]. Cellulose is the raw material to produce nitrocellulose. Nitrocellulose is usually adopted in smokeless gunpowder and as the basic material for celluloid which is used for photographic and movie films until the mid-1930s.

Cellulose contained in energy crops can be converted to biofuels and materials such as cellulosic ethanol- as alternative fuel sources. Cellulose for industrial use is mainly obtained from wood pulp and cotton.

Moreover, water-soluble adhesives and binders can be made from cellulose. For instance, methyl cellulose and carboxymethyl are products made from cellulose and are used in wall paper paste. Other products from cellulose such as microcrystalline cellulose (E460i) and powdered cellulose (E460ii) are used as inactive fillers in tablets and as thickeners and stabilizers in processed foods [25].

Furthermore, cellulose consisting of crystalline and amorphous regions and amorphous regions can be broken down by using strong acid. It is possible to produce a novel material based on nanocrystalline cellulose, which itself has many desirable properties. Recently, nanocrystalline cellulose has been used as the filler phase in bio-based polymer matrices to produce nanocomposites with superior thermal and mechanical properties [26].

Finally, cellulose is also used as a stationary phase for laboratory uses of thin layer chromatography [27]. Cellulose fibers are also used in liquid filtration to create a filter bed of inert material by combination with diatomaceous earth or other filtration media. Cellulose is further used to make hydrophilic and highly absorbent sponges. Cellulose insulation made

from recycled paper is also becoming popular as an environmentally preferable material for building insulation.

2.1.3 Hemicelluloses

2.1.3.1 Introduction

Hemicelluloses are heteropolysaccharides, presenting along with cellulose in almost all plant cell walls. They are different from cellulose because they contain several branched sugar units and short chains with a degree of polymerization (DP) of 50-200. Hemicelluloses have a random, amorphous structure with little strength while cellulose is crystalline, strong, and resistant to hydrolysis. Hemicelluloses are easily hydrolyzed by dilute acid or base as well as myriad hemicellulase enzymes.

2.1.3.2 Structures

Hemicelluloses are a series of polysaccharides which include xylan, glucuronoxylan, arabinoxylan, glucomannan, and xyloglucan. These polysaccharides contain many different sugar monomers. Hemicelluloses include xylose, mannose, galactose, rhamnose, and arabinose while cellulose contains only anhydrous glucose. As shown in Fig. 2.2. Hemicelluloses contain most of D-pentose sugars, and occasionally small amounts of L-sugars as well. Regular sugars as well as their acidified forms such as glucuronic acid and galacturonic acid can be found in hemicellulose.

There are significant differences between softwoods and hardwoods in relation to content and type of hemicelluloses in the wood cell walls. It contributes 30-32% in softwoods while it

accounts for 15%-35% in hardwoods. And it is also known that softwoods have a higher proportion of mannose units and more galactose units than hardwoods, while hardwoods have a high proportion of xylose units and more aceyl groups than softwoods [8].

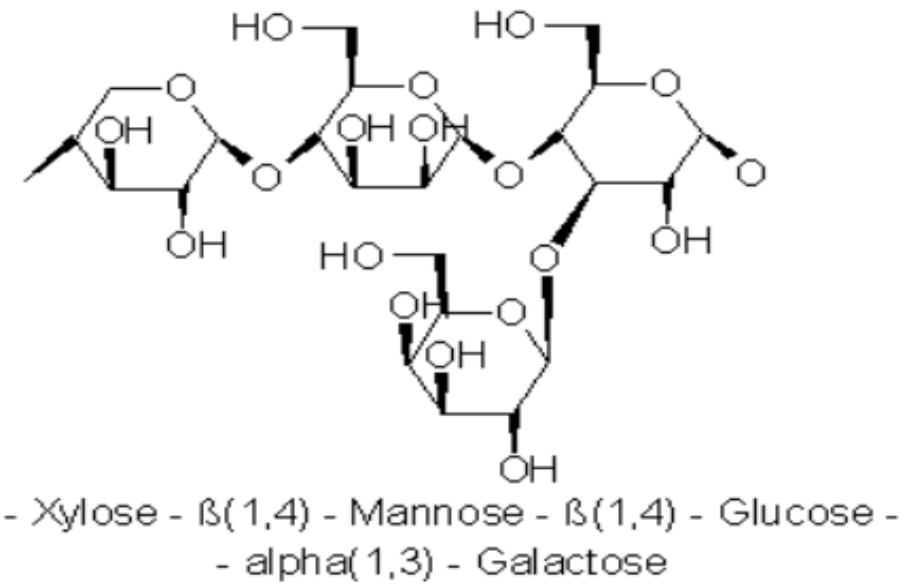

Fig. 2.2 Structure of hemicelluloses [28]

2.1.3.3 Properties and functions

Unlike cellulose, hemicellulose has hydrophilic property- which causes swelling of cell wall and increases elasticity of fiber. In treatment process of pulp industry, adding hemicelluloses is advantage for fiber construction and increasing binding force between fibers. It improves surface adsorption of fiber and has an effect on strength of produced paper. Adding hemicellulose is also beneficial for pulp and paper processes because hemicelluloses are much better agents to make paper swelling than cellulose and also enhance flexibility of pulp. As a result, adding hemicellulose will not only decrease energy consumption during pulping process, but also keep gaining desired strength of paper.

In addition, hemicelluloses are also significant components of the cell wall. Hemicelluloses are cross-linked together and combine with the surface of the cellulose-microfibrils, Lignins assist and strengthen the attachment of hemicelluloses to microfibrils. They connect together and form hard fibers interconnected networks of cells. (Fig 2.3)

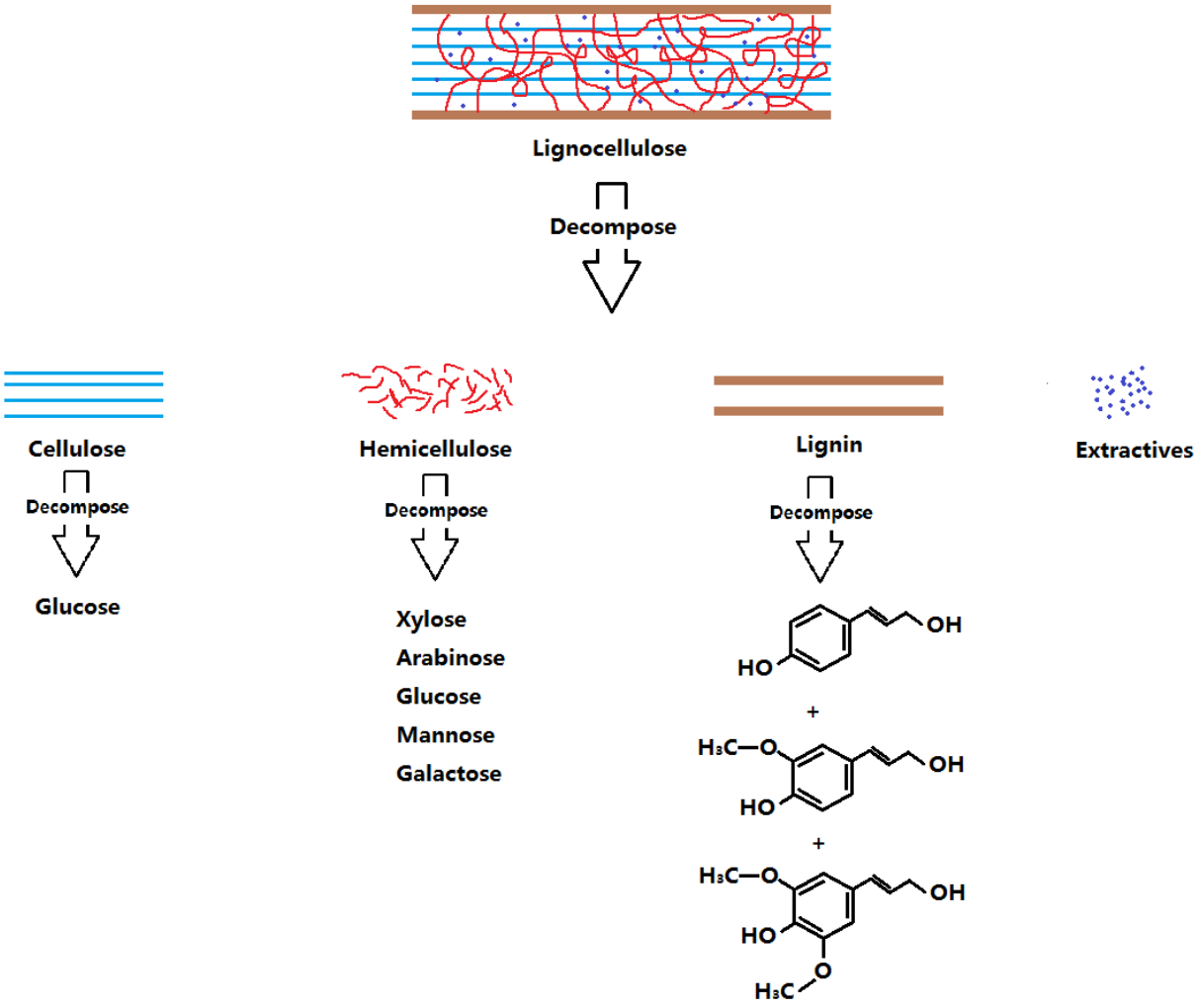

Fig. 2.3 The composition of lignocellulosic biomass

2.1.4 Lignin

Lignin is a complex chemical compound which is commonly derived from wood and is a part of secondary cell walls of plants and algae [30]. Next to cellulose, it is the most abundant polymeric substance in plants. It is a characteristic chemical and morphological component of the tissue of plants. It accounts for 30% of non-fossil organic carbon [31]. The amount of lignin is different in various plants. For instance, lignin content ranges from 20 to 40% in woods. The main function of lignin in plants is for liquid transport and to improve mechanical strength of the woods [32].

2.1.5 Extractive

Apart from the above-montioned components including hemicelluloses, lignin, biomass also contains a large number of other compounds known as extractives. These compounds can be extracted with organic solvents or aqueous solutions. Although they are classified as waste products of plant metabolism, they influence chemical, biological, physical, and optical properties of the biomass. For instance, lipophilic resins as extractives may improve stability and durability of wood, so that swelling and biodegradability may be significantly reduced in pulp processes.

The extractives content in wood accounts for 2-5% weight. Concentration and composition of extractives in wood depend on wood species and different parts of woods. There are also variations depending on geographical site and seasons [8].

2.2 Bio-refineries

2.2.1 Introduction

For the reason of great increasing in petroleum price and shortage of fossil energy resource, people focus on exploring alternative energy to replace petroleum. From the perspective of using biomass products as a major resource, forests can be used to produce logs, wood pulp, solvents and building materials while biomass derived from agriculture can produce large quantities of crops, starch, sugar cane and other products which can be converted to useful chemicals and materials. These abundant renewable resources from agricultural and forested sources could be used to replace fossil energy. However, bio-refineries and their associated technological developments are still in the early stages of implementation [33].

Sugar products play a significant role in the biorefinery industry because it is demonstrated that sugars can be converted to useful chemicals such as ethanol by fermentation with bacteria using sugar as the main carbon source. The products attained from sugar fermentation have been considered to hold considerable potential for future production. Cellulosic biomass such as agricultural residues may be converted to monosaccharides by hydrolysis with acid or enzyme at economically viable costs. Therefore, conversion of cellulosic biomass into fermentable sugars is an important topic in which further research is warranted [34, 35].

2.2.2 Lignocellulosic biorefinery

2.2.2.1 Introduction

Hardship for the business is caused by the high price of crude oil in recent years and will be more and more seriously in the future. From this perspective, investigation and

implementation of alternative forms of energy possesses are beneficial for both economy and environment [36, 37].

2.2.2.2 Strategy

The strategy for relieving energy crisis is to reduce the position of petroleum as the most essential source of energy and raw materials for chemical production. The biomass carbon resources which are derived from plants are the best replacement source because of their abundance in nature. Biorefineries will, in ideality, be capable of producing the same or functionally similar chemicals derived from petroleum refineries. Moreover, biomass resources are renewable and largely derived from materials currently regarded as waste, so that biomass would be the best dominant hydrocarbon source to replace petroleum [38].

A significant consideration made in the replacement of petroleum with biomass is that biomass constitutes a "two-use" source in that everything which grows or is derived from organic sources has at least two uses. For example, agricultural residues, used tires and plastics, human and animal wastes, are converted into new chemicals or fuels by biorefineries and municipal solid wastes are collected and recycled to the biorefinery. In general, carbon is recycled in biorefineries so biomass is renewable energy.

Another important reason to use biomass as a replacement for petroleum is that it would have immediate and far-reaching environmental benefits. First and foremost, biomass is a kind of renewable resource. The carbon as carbon dioxide produced from burning of biomass and released into the atmosphere would be recycled into new plants. This cycle is significant for

improving air quality and reducing the impacts of global warming. Secondly, biomass resources could be found domestically and can be derived largely through the implementation of the two-use ethic. Lastly, the production and the conversion of biomass resources to liquid fuels and organic chemicals implies the use of chemical processes with materials of less toxicity than the products traditionally produced from petrochemical processes [38-40]. However, the current cost for the implementation and the operation of biorefineries is relatively high. The estimated operation cost of a-1000 to 2000 tons per day-product plant is approximately $ 0.5 billion. Further development of biorefiner-process technique is meaningful [41].

2.2.2.3 Various Biofuel production processes

Biomass materials are different from petroleum, because they contain mainly solid components. In general, initial treatment of biomass includes drying and physical size reduction. The chemistry of a lignocellulosic biorefinery is quite different from petroleum refinery processes. The process chemistries used for the depolymerization of lignocellulose biomass include: pyrolysis, gasification, thermochemical liquefaction, hydrolytic liquefaction, fermentation and chemical synthesis.

Figure 2.4 presents common-used chemical and biochemical conversion processes of biomass as an energy source. Pyrolysis is a treatment at mild temperatures (300-600℃) in the absence of oxygen to cause depolymerization of biomass [42]. The product at slow heating rates yields volatile gases, organic acids and aldehydes, mixed phenols etc. High heating rates tend to minimize liquid production and maximize gas production. On the other hand,

gasification treatment occurs under high temperatures (＞700℃) and involves the reaction in the absent oxygen with addition of steam to convert the biomass to synthesis gas, a mixture gas of hydrogen, carbon monoxide, carbon dioxide and methane. Synthesis gas is a kind of fuel or chemical intermediate in the production of methanol, organic acids and synthetic gasoline. Furthermore, thermochemical liquefaction is pyrolytic process involved the addition of hydrogen, carbon monoxide, carbon dioxide and selected catalysts to convert the biomass into hydrocarbons, mixed phenols and light gases [43]. Hydrolytic liquefaction processing usually involves water as solvent with/without catalyst such as acids, alkalis and enzymes to depolymerize polysaccharides into monosaccarides. This process is used for further processing in aqueous phase [44, 45]. Additionally, fermentation is a biochemical process which use microorganisms and enzymatic reactions to convert a fermentable substrate into recoverable products. It is performed in aqueous solution.

Chemical synthesis uses chemical reagents to convert biomass into valuable products. For instance, xylose from hemicellulose by hydrolytic depolymerization process can be reacted in the same process to make furfural (Fig. 2.4) [46, 47].

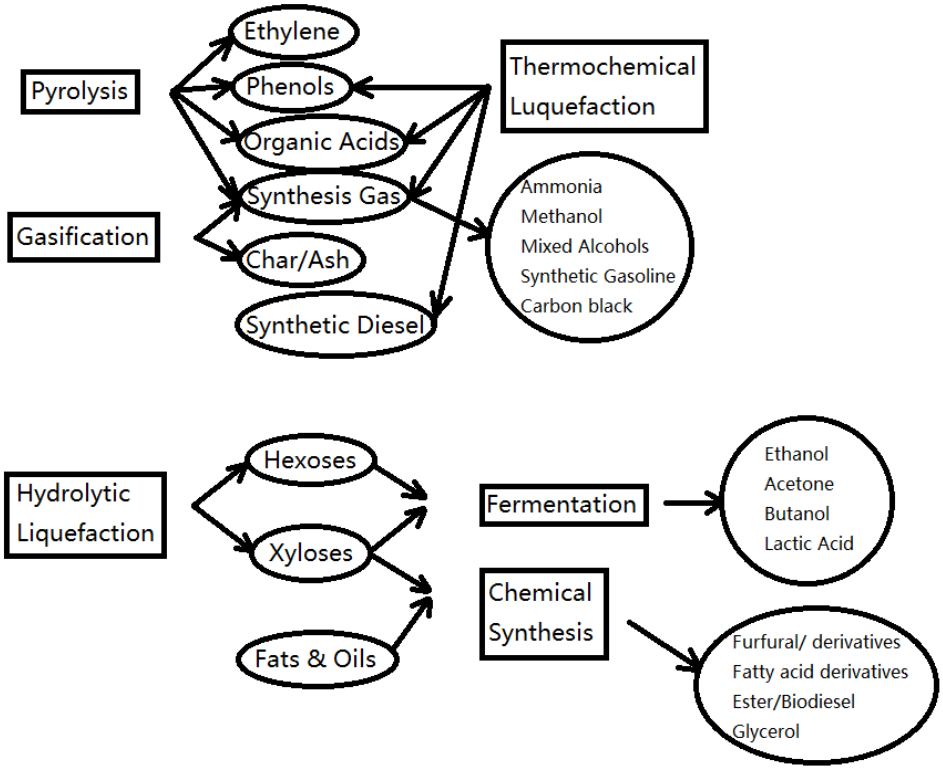

Fig. 2.4 Scheme of chemical and biochemical conversion of biomass to biofuel or valuable chemicals

2.2.2.4 Products

Products derived from biomass have several kinds and functions. Saccharides and polysaccharides are the significant lignocellulosic biomass. The basic saccharide structure is glucose and the polysaccharides are represented by molecules such as cellulose, starch, hemicellulose and lignin. Current industrial uses of starch and hexose sugars are for ethanol and other fermentation products, and the uses of starch derivatives are for polymers, absorbents and adhesives. The glucose from cellulose and hemicellulose could be used for the production of furfural. Moreover, lignin is a polymer which is linked with cellulose and

hemicellulose as the structure of the cell walls in plants and the three polymers make up lignocellulose. The major utilization of lignocellulose is for pulp and paper products. Triacylglycerides and lipids such as vegetable oils and animal fats are also a component of the biomass products. Oils and fats play a significant role in the human diet, although derivatives of fats and oils have extensive use in non-food applications. The use of triglyceride derivatives ranges from latex paints, high performance lubricants and polymers, to biodiesel fuel in recent years. Triglyceride contributes the largest aspect of biomass resources application for chemical industry today. Finally, proteins are long-chain polymer based on amino acids, and the current usages of protein are for non-food uses such as leather products, protein glues and personal care products.

2.2.2.5 An example of cellulosic biorefineries

Using lignocellulosic feedstocks(LCF) has been proposed in the United States. An approach is to use chemical and enzyme treatments to depolymerize lignocellulose to produce sugars and lignin [48]. The main product of the process is ethanol. The process is shown on Fig 2.5 and involving reactions are as follows [33]:

Lignocellulose + Water Xyloses + Cellulose + Lignin (Acid Process) (1)

Cellulose + Water → Glucose (Enzyme Process) (2)

Glucose Fermentation → Ethanol + CO_2 + Biomass (3)

Xylose Fermentation → Ethanol + CO_2 + Biomass (4)

Lignin + Biomass → Heat + Steam (5)

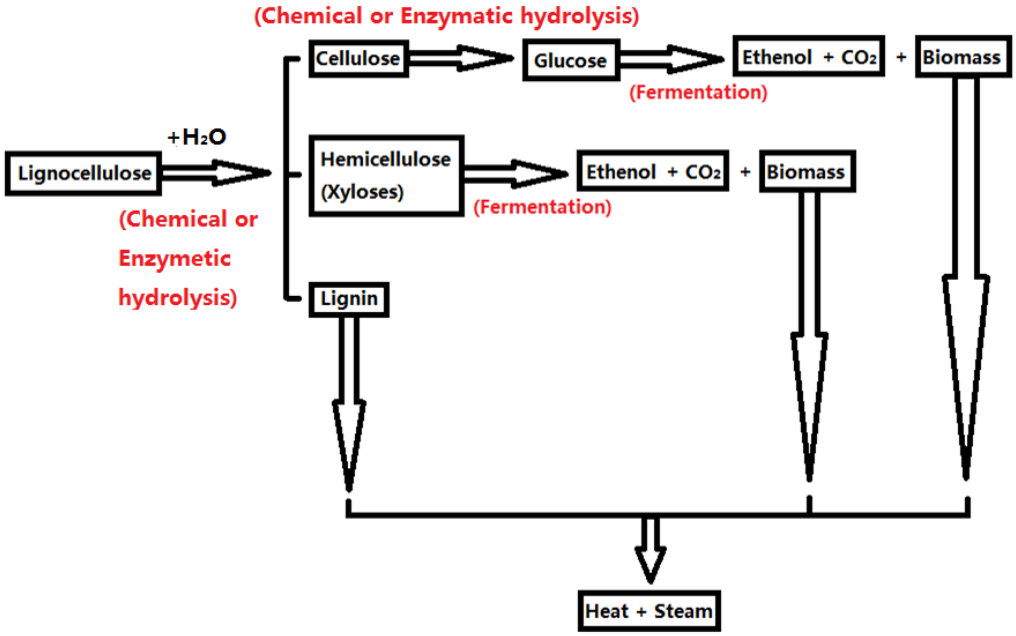

Fig. 2.5 Industry processes of lignoncellulose hydrolysis

However, because ethanol is the main product, the process is not profitable. Xyloses fermentation process would produce a kind of highly versatile chemical intermediate - furfural, but the capacities of production are much lower than the optimum capacity. The process is unprofitable either because of the high plant capital investment and the high cost of operation. The costs of assembling sufficient feed stock are also too high, and represent another reason that these processes are currently unprofitable. Nevertheless, the process has potential to produce highly useful chemicals in the future [49].

2.2.3 Hydrolysis of lignocellulose

Lignocellulose is a potential resource because it could be used to produce fuels and chemicals to reduce dependence on petroleum. Hydrolysis processes involves depolymerization of

polysaccharides (cellulose, hemicellulose) by the use of acids, alkalis and enzymes. Biomass hydrolysis technology began in the early 1900s [50-52].

2.2.3.1 Acid hydrolysis process

Polysaccharides can be catalytically converted to monosaccharides by acids, such as sulfuric, hydrochloric, hydrofluoric and nitric acids [53-56]. The process can be expressed by the following formulas:

$$\text{Cellulose} \rightarrow \text{glucose} \rightarrow \text{HMF} \rightarrow \text{tars} \quad (6)$$

$$\text{Hemicellulose} \rightarrow \text{monosaccharide} \rightarrow \text{furfural} + \text{HMF} \rightarrow \text{tars} \quad (7)$$

The processes are widely used in biomass treating plants. Biomass has a reaction with dilute sulfuric acid solution and steam at temperatures ranging from 140-260℃. At higher temperature, biomass is quickly converted to furfural and 5-hydroxymethylfurfural [57, 58]. Concentrated acid also plays a significant role in the acid hydrolysis process of lignocellulose at lower temperatures (100-120℃). Using acid to hydrolyze biomass is an attractive option because the acids are relatively cheap, improving the process economics.

2.2.3.2 Enzymatic hydrolysis process

Although the acid hydrolysis process is economically attractive, sugars are also converted to degradation products by acids [59, 60]. Compared to acids, cellulase, a multi-component enzyme system, would convert cellulose to the target product (glucose) without degradation. The enzymes are produced by microorganisms. For instance, the fungus *Trichoderma reesei* is

commonly used to produce enzymes [61]. Nevertheless, enzymes do not have a high efficiency, for the ability of the enzyme to access the cellulosic substrate affects the conversion rates. Biomass would be therefore be required to be treated by chemical or physical methods to break the structure of cellulose in order to increase the accessibility of the substrate for the enzymes. The cost of enzymetic conversion is relatively higher than acid hydrolysis due to the price of enzymes and the retreatment processes [62].

2.3 Hydrolysis of lignocellulose

Lignocellulose is abundant in nature and the annual production is larger than other sources of biomass. Lignocellulose tends to be more productive and require less energy to produce ethanol. The lignocellulose industry is not commercially viable because the hydrolysis of cellulose is more difficult than that of other polysaccharides such as starch. This is due to the D-anhydroglucopyranose unit which is the basic unit of cellulose being linked by β-(1,4)-glycosidic bond, which is not easily broken. Therefore, the degradation of cellulose is much more energy-intensive than the other polysaccharides [63].

2.3.1 Hemicellulose hydrolysis

The electrophilic hydrogen atoms of water molecule attack the glycosidic oxygen of the D-anhydroglucopyranse unit resulting in breakage of the polysaccharides chain in the protonation reaction [63]. The process conditions for treatment of hemicellulose polysaccharides are not severe because the degree of polymerization of hemicellulose is lower and the intermolecular bonding in most hemicellulose is less than that observed in lignocellulose.

Many papers published on the hydrolysis of hemicellulose by acid are presented, however the hydrolysis of hemicellulose monosaccharide by hydrochloric acid has been investigated to a lesser extent. For example, a study explored the hydrolysis of hemicellulose and lignin in acid solution which contains acetic acid solution and low concentration hydrochloric acid [64]. The results examined the effects of treatment time and concentration of hydrochloric acid. The kinetics of hemicellulose monosaccharide hydrolysis was modelled assuming first-order and irreversible reaction. Zhuang et al. [65] described a system of hydrolysis of wheat straw hemicellulose in a mixed solution of hydrochloric acid and formic acid and analyzed the reaction products by HPLC. The results showed the hydrolysis was strongly affected by temperature, time, concentration of hydrochloric acid and the ratio of solid to liquid. It presented the optimal conditions for the conversion of hemicellulose and reported the yields of xylose, glucose, and arabinose obtained. Another study was reported which explored a kind of combined production of hemicellulose carbohydrates and wheat straw residue using dilute hydrochloric acid for hydrolysis at mild temperature [66]. The authors compared the hydrolysis rate of dilute HCl solution with dilute $FeCl_3$ solution at temperatures of 100 and 120℃. The results showed that even small concentrations of hydrochloric acid affect the hydrolysis of hemicellulose at 100℃ to 120℃, where the recovery of hemicellulose derived carbohydrates was nearly 100% and the main product were xylose and arabinose. Dilute $FeCl_3$ solution was thought to act only indirectly on the hemicellulose hydrolyze, however it was presented as an option for mineral acid replacement. Another research [67] explored the hydrolysis rate with different acids, hydrochloric acid, sulfuric acid and sulfurous acid. The degradation rate was expressed by a second order reaction rate which was constant with substrate and concentrations of the acids but the hydrolysis rate differs from the different acids

even if in the same conditions. It was shown that the degradation of monosaccharides in sulfurous acid was much lower than the other two acids and the second-order reaction rate of the monosaccharide dependents on the type of acid. In addition, another study explored the different hydrolysis rates of sugar cane bagasse under various concentrations of hydrochloric acid, reaction durations and temperatures [68]. They showed that xylose, glucose, arabinose and glucose were obtained as sugars and furfural and acetic acid were determined to be the degradation products. The authors developed kinetic models and parameters based on the Saeman model and the two-fraction model for predicting the compounds in the hydrolysates and reported the optimal conditions for sugar cane hydrolysis in dilute hydrochloric acid.

2.3.2 Cellulose hydrolysis

Mechanism of cellulose hydrolysis is also protonation of the glycosidic oxygen however it is a very slow reaction. The H^+ ions in water molecule will attack the β-(1, 4)-glycosidic bond but the resistance of cellulose hydrolysis is much higher than hemicellulose hydrolysis. Several methods can be used to increase hydrolysis rate such as promoting temperatures and pressures, acids (concentrated or dilute) or highly selective enzymes [63]. Mechanism of acid-catalyzed hydrolysis of cellulose is that H^+ ions equilibrate oxygen atoms of water molecule and glycoside in the system. After that, an equilibrium concentration of protonated glycoside is formed and the equilibrium tends towards the protonated glycoside when system temperature is increased. The protonated conjugate acid then slowly breaks down the cyclic carbonium ion which forms the chair conformation. Finally, free glucose is liberated by rapidly adding water.

Acid concentration affects degradation rate. Lower acid concentrations require more extreme conditions such as higher temperature and higher pressure and longer hydrolysis time for conversion of cellulose. The concentrated acid and higher-temperature and pressure vessels may reduce the incurred of the costs, however the cost of equipment corrosion and acid loss may be expensive. The hydrolysis rate of cellulose is also associated with the degree of the polymerization and the degree of crystallinity of the cellulose [63].

Many researchers investigated hydrolysis of cellulose with dilute hydrochloric acid. For example, hydrolysis of cotton cellulose in hydrochloric acid in benzene was reported [69]. The study investigated rate and site of hydrolysis as a function of amount of water present, and found HCl concentration to dominate the conversion. The results also indicated that hydrolysis tends to be confined to the ends of the cellulose chain with little water present. Another research [70] presented hydrolysis of cellulose in hydrochloric acid and aqueous sulfur dioxide and determined the hydrolysis rate of these two acids by the calculation of first-order reaction constants. The rates were compared with sulfuric and phosphoric acids and the results showed the hydrolysis rate of hydrochloric acid occurred at about the same rate as sulfuric acid, however sulfur dioxide was only one-seventh the rate of sulfuric acid. Smia et al. [71] presented the hydrolysis rate of cellulose I, II and III by 0.5N hydrochloric acid solution under mild conditions.

Although several researches have presented about hydrolysis of cellulose with hydrochloric acid, few papers explored hydrolysis of cellulose under concentrated or superconcentrated hydrochloric acid. In one of these papers, Mannhein et al. [72] investigated the wood hydrolysis with superconcentrated hydrochloric acid in Germany during the Second World

War. Goldstein et al. [73] explored the hydrolysis of cellulose in concentrated hydrochloric acid in 1983. The author inferred that the decrystallization process of the cellulose was the essential first step in the hydrolysis of cellulose by superconcentrated hydrochloric acid at moderate temperatures. The paper indicated higher liquid to solid ratios, smaller particle size and presence of certain agitation may increase hydrolysis rate.

2.3.3 Measurement and analysis method

2.3.3.1 Concentration measurement

The hemicellulose is hydrolyzed to monosaccharide and they will be converted to furfural and HMF in concentrated hydrochloric acid while cellulose is degraded into oligosaccharides and glucose. In order to analyze the change of monosaccharide-hydrolysis rate and cellulose-hydrolysis rate, concentration of monosaccharide should be measured because concentration of monosaccharide represents the hydrolysis rate of the monosaccharide hydrolysis. Similar with monosaccharide, the concentration of glucose, furfural and HMF represents the generation rate.

The most widely used method for monosaccharide detection is high performance liquid chromatography (HPLC) analysis [74]. Monosaccharides, such as glucose, mannose, galactose, arabinose, xylose, which are hydrolyzed by hemicellulose are soluble in water solutions. Joung et al. indicated the contents of monosaccharide in the hydrolysates could be measured and detected by HPLC with refractive index detection (RID) [75]. Zhang, et al. explored an HPLC method for analysis the monosaccharide composition. From this study, the method was shown to be accurate and could be used to measure the concentration of the

monosaccharides of fucoidans [76].

Another study explored a new micro-extraction method to analyze hemicellulose and the ratio of cellulose and lignin to hemicelluloses in different tissues of 28 plant species by HPLC-RID [77]. All these studies demonstrated HPLC analysis for monosaccharide measurement was sensitive and accurate. Similar with monosaccharides, some kinds of oligosaccharides, such as cellobiose (G2), cellotriose (G3), cellotetrose (G4), cellopentase (G5) and cellohexaose (G6), which are degraded by cellulose are also soluble in water solutions [78]. The soluble sugars are in liquid phase and they could be measured and quantified by HPLC-RID. Liang, et al. [79] used HPLC analysis to measure the concentration of cello-oligosaccharides with pYBGA1 yeast and Peng et al. [80] discovered the chemical degradations of highly-purified cellotriose, cellotetraose, and cellopentaose in H_2O_2 and NaOH media and analyzed by HPLC, FTIR, and GC-MS techniques. The other degradation products such as furfural and HMF, which are hydrolyzed by monosaccharides could also be analyzed by RID as they are soluble in water solution and can be detected by HPLC. Xu et al. presents a sensitive and selective analysis method for simultaneously quantifying furfural and HMF by HPLC [81].

2.3.3.2 Crystallinity index (CrI) measurement

Crystillinity index (CrI) is related to the chemical and physical properties of the cellulose. Cellulose is polymorphism and contains crystalline regions and amorphous regions. In crystalline regions, arrangement of the cellulose molecule is completely regular. There are five different crystal structures of cellulose, namely: cellulose I, cellulose II, cellulose III,

cellulose IV and cellulose V. Among these constructions, cellulose I is natural cellulose and it contains two different crystalline constructions, cellulose I_α (triclinic) and I_β (monoclinic). The ratio of the constructions differs from types of the materials and pre-treatment methods and the crystal structure affect properties of cellulose [82]. The connecting regions between crystalline regions are called amorphous regions, and the edge of crystalline and amorphous regions is not clear. The ratio of these two regions is different from materials and completeness of cellulose. CrI index which stands for the ratio of crystal structure in whole cellulose structure is a key factor of the cellulose-supramolecular construction, because properties of cellulose are of relevance of CrI index. Measurement of CrI plays a significant role in cellulose industries such as wood chemicals industries and adhesive fiber industries. Evans et al. and Hattula et al. [83-85] indicated that CrI is increased with the removal of amorphous regions in pulp process with sulphates.

Major measurement of CrI of cellulose is via X-ray diffraction (XRD) method, Fourier Transform infrared spectroscopy (FTIR) method and Cross Polarization /Magic Angle Spinning (CP/MAS ^{13}C-NMR) method [86], which will be discussed in the following sections.

2.3.3.2.1 XRD method

XRD method is essential and the most direct method to measure the crystallinity index of the cellulose. XRD method can be used to obtain unit cell size of cellulose crystal in long molecular chain and CrI index by intensity and position of the strongest diffraction point. CrI index depends on purity of samples and method of data collection. There are different

calculations available [87]. Three calculations are presented here. The first one was reported by Segal et al [88] involved the calculation according to the empirical formula and it is a rapid method to determine the value of CrI by X-ray diffraction spectrum, however the deviation was observed comparatively large than the other calculations. The equation is as follows:

$$\mathrm{CrI} = \frac{I_{002} - I_{am}}{I_{002}} \times 100\% \quad (8)$$

Where I_{002} stands for the intensity of 002 lattice plane, $2\theta=22.5°$

I_{am} Stands for the intensity of the amorphous regions, $2\theta=18°$

The second calculation as shown in Eq. 9 assumes that structures of cellulose only have two phases presenting, crystalline phases and amorphous phase, and there is an imaginary line which between the two lowest values of the diffraction intensity to separate a crystalline phase and an amorphous phase [89, 90].

$$\mathrm{CrI} = \frac{A_{cr}}{A_{cr} + A_{am}} \times 100\% \quad (9)$$

Where A_{cr} is area of crystalline region and A_{am} is area of amorphous region.

The third calculation is by peaks separation. In the method, the diffraction curve is analyzed by a peaks separation process by Lorentzian function. Except peaks of crystalline regions, at lattice planes 101, 10$\underline{1}$, 002, the maximum of amorphous peak equals to the value of the wave trough between the lattice plane 101 and 002. The equation is as follows:

$$\mathrm{CrI} = \left(1 - \frac{I_a}{I_a + S_p}\right) \times 100\% \quad (10)$$

Where I_a is the integral area of amorphous area while S_p is the integral area of crystalline area which is in lattice plane 101, 10$\underline{1}$, 002.

With development of data processing, MDI JADE software has been used to calculate the integral area of crystalline peaks and amorphous peaks. However, the method possesses some disadvantages, for example, the coverage of crystalline regions and amorphous regions and the existence of the hemicellulose and lignin are hard to separate from cellulose. As a result, the method is difficult to obtain an accurate value from XRD measurement [91].

2.3.3.2.2 FTIR method

Fourier Transform Infrared Spectroscopy (FTIR) Analysis consists of two main methods for analysis of CrI index, Deuterium substitution method and Nelson & O'Connor method. The Deuterium substitution method is based on a deuterium substitution mechanism. It works by treating cellulose with deuterium substitution and making OH change to OD of amorphous regions while OH in crystalline regions does not react. Reflected in the infrared spectroscopy, a band intensity of 3400 cm^{-1} is decreased while the peak appears on the band intensity of 2530 cm^{-1}. The ratio of band intensity 3400 cm^{-1} and 2530 cm^{-1} is the crystallinity index (CrI). The advantage of the method is that detection is accurate. However, the procedure of deuterium substitution is complex, so it is not widely used to measure CrI. The second method, presented by O'Connor [92] in 1958, indicated that band intensity of 1429 cm^{-1} is

decreased while the one of 839 cm^{-1} is increased when in the cellulose-grinding process. The CrI is calculated by the ratio of the band intensity of 1429 cm^{-1} and 839 cm^{-1} and it is called O'KI, However, this method is only used for the CrI calculation of Cellulose I. O'Connor and Nelson [93] developed change of CrI of cellulose I and Cellulose II, in relation to the bending vibration energy of C-H bond and the band intensity at 1372 cm^{-1}. The equation of CrI index is described as the ratio of band intensity 1372 cm^{-1} and 2900 cm^{-1} and it is written as N.O'KI. The study also demonstrated that CrI and N.O'KI possess a linear relationship while CrI and O'KI have a parabolic relationship.

2.3.3.2.3 CP/MAS ^{13}C-NMR method

Cross polarization/magic angle spinning (CP/MAS) ^{13}C nuclear magnetic resonance (NMR) is widely used to analyze the component composition of plant materials such as cellulose. It is based upon the high-resolution solid-state NMR, which can distinguish the signal of cellulose-crystalline regions and cellulose-amorphous regions [94].

In the process of grinding cellulose, the signal intensity δ of the chemical shift at 89ppm decreased whereas it increased at 84ppm. The lattice relaxation time also indicated that the molecular movement at 84ppm was more intense than that at 89ppm. This was explained by the difference of the chemical shift of the glucoside between the amorphous region (δ=84ppm) and the crystalline regions (δ=89ppm) of the cellulose [95, 96].

Using the convolution method to analyze the signal of crystalline and amorphous regions by NMR spectrum, the NMR CrI index value (CNMR) can be obtained.

$$C_{NMR} = \frac{S_{\delta 89}}{S_{\delta 89} + S_{\delta 84}} \times 100\% \quad (11)$$

Where $S_{\delta 89}$ and $S_{\delta 84}$ are the integral areas of the chemical shift at 89ppm and 84ppm, respectively.

The experiment indicated that the method above had a good agreement with XRD-CrI for cellulose I. The method can also be applied for high-purity cellulose, although it is not an ideal method to calculate CrI index for the cellulose which contains higher content of hemicellulose and lignin. The C4 peak of cellulose should be covered by the hemicelluloses when δ = 84ppm and the side chains of lignin also affect the C4 peak [97]. As a result, a large deviation should appear when using the calculation for cellulose with a significant moisture component [98].

The above-mentioned three analysis methods for determination of CrI index are always used together to determine change of crystallinity. However, in the literature the CrI value of cellulose was found to vary greatly depending on measurement techniques, calculation approaches, and sample drying conditions [99].

2.3.3.3 The degree of polymerization (DP) measurement

The degree of polymerization (DP) is a characteristic of chain length of cellulose, and may be defined as number average DP (DPn), weight average DP (DPw) and viscosity average DP (DPv) [100]. In order to measure the DP of cellulose, the cellulose should be dissolved in a solution while the technique does not change the chain length. Several solutions have

been used such as Bis(ethylenediamine)copper(ii) hydroxide solution (cuen solution) [101-103] and N,N-dimethylacetamide (DMAc)/LiCl [104].

In these measurements, viscosity average degree of polymerization (DPv) of the cellulose is the most convenient and accurate measurement. When cellulose dissolves into the solution which can maintain the chain length of the cellulose, the DP value can be obtained by measuring the viscosity of the solution by viscometers. A few papers explored the calculations between DPv and intrinsic viscosity. [105, 106] A recent study investigated the relationship between the viscosity and the DP value [107]. Cellulose was dissolved into Cuen solution (it is also called CED solution) and (DMAc)/LiCl solution first and determined the intrinsic viscosity by capillary viscometer. It provided an updated Mark-Houwink-Sakurada (MHS) equation for cellulose, enabling determination of the true viscosity average degree of polymerization (DP*v*) based on intrinsic viscosity measurements in Cuen with corrections for cellulose degradation, and SEC-MALLS in 0.5% DMAc/LiCl. The study also reported the relationship between DPv and DPw.

The MHS equation for DPv calculation is:

$$[\eta] = 2.45DP_v^{0.07} = 0.070M_v^{0.07} \quad (12)$$

Where [η] is intrinsic viscosity and can be calculated by relative viscosity measured by viscometer. DPv is viscosity average degree of polymerization and Mv is viscosity average molecular weight.

Number average degree of polymerization (DPn) can be measured by membrane or vapor pressure osmometry, cryoscopy, ebullioscopy, determination of reducing end concentration,

or electron microscopy [108]. A study presented a rapid and accurate method for determining the DPn [109]. It was established by a chemical method and DPn of the insoluble cellulose and soluble cellodextrins can be determined. The calculation is the ratio of glucosyl monomer concentration and the reducing-end concentration, where the glucosyl monomer concentration determined by the phenol-sulfuric acid method and the reducing-end concentration determined by a modified 2, 2'-bicinchoninate (BCA) method.

Weight average degree of polymerization (DPw) can be measured by light scattering, sedimentation equilibrium, and X-ray small angle scattering [100]. The distribution of DPs among a population of cellulose molecules can be measured by size exclusion chromatography [110]. Among the methods of DP measurement, DPv and DPw show a good relationship with polymer properties [111] while DPn is appropriate for the description of cellulose hydrolysis [108, 111].

References

[1] McKendry P., 2002. Energy production from biomass (part 1): overview of biomass. Bioresource technology, 83(1), 37-46.

[2] Sjostrom, E., 1993. Wood Chemistry. Fundamentals and Applications.

[3] Crawford, R. L., 1981. Lignin biodegradation and transformation. John Wiley and Sons, New York.

[4] Young, Raymond, 1986. Cellulose structure modification and hydrolysis. Wiley, New York.

[5] Kobayashi, Shiro, et al., 1992. Enzymatic polymerization: the first in vitro synthesis of cellulose via nonbiosynthetic path catalyzed by cellulase. Marcomolecular Symposia, 54-55(1), 509-518.

[6] Updegraff D. M., 1969. Semimicro determination of cellulose in biological materials. Analytical Biochemistry, 32 (3), 420-424.

[7] Klemm et al., 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. ChemInform, 36(36).

[8] Herbert Sixta, 2006. Handbook of pulp. ISBN-10: 3-527-30999-3.

[9] Deguchi S, Tsujii K, Horikoshi K., 2006. Cooking cellulose in hot and compressed water. Chem. Commun., (31), 3293-3295.

[10] Meyer K. H., Misch I., 1937. Helv. Chim, Avta, 20, 232.

[11] Serge Pérez, William Mackie, 2001. Structure and morphology of cellulose, CERMAV-CNRS, Chapter IV.

[12] Kamide K., Okajima K., Kowsaka K., 1992. Dissolution of natural cellulose into aqueous alkali solution: role of super-molecular structure of cellulose. Polym J, 24(1), 71-86.

[13] Stenius Per, 2000. Forest Products Chemistry. Papermaking Science and Technology, 35.

[14] Klemm Dieter, Heublein Brigitte, et al., 2005. Cellulose: Fascinating Biopolymer and Sustainable Raw Material. ChemInform, 36 (36).

[15] Kimura S, Laosinchai W, et al, 1999. Immunogold labeling of rosette terminal cellulose-synthesizing complexes in the vascular plant vigna angularis. The Plant cell, 11 (11), 2075–86.

[16] Taylor N. G., 2003. Interactions among three distinct CesA proteins essential for cellulose synthesis. Proceedings of the National Academy of Sciences, 100 (3), 1450.

[17] Peng L, Kawagoe Y, Hogan P, Delmer D, 2002. Sitosterol-beta-glucoside as prim, or cellulose synthesis in plants. Science, 295 (5552), 147–50.

[18] Endean, 1961. The Test of the Ascidian, Phallusia mammillata. Quarterly Journal of Microscopical Science, 102-1, 107-117.

[19] David G. Barkalow, Roy L. Whistler, 2008. Cellulose. AccessScience.

[20] Brás, Natércia, N. M. F. S. A. Cerqueira, P. A. Fernandes, M. J. Ramos, 2008. Carbohydrate Binding Modules from family 11: Understanding the binding mode of polysaccharides. International Journal of Quantum Chemistry, 108 (11), 2030–2040.

[21] Tokuda, G, Watanabe, H, 2007. Hidden cellulases in termites: revision of an old hypothesis. Biology Letters, 3 (3), 336–339.

[22] Slavin JL, Brauer PM, Marlett JA, 1981. Neutral detergent fiber, hemicellulose and cellulose digestibility in human subjects. The Journal of Nutrition, 111 (2), 287–97.

[23] Joshi S, Agte V, 1995. Digestibility of dietary fiber components in vegetarian men. Plant foods for human nutrition, 48 (1), 39–44.

[24] Rosenau T, Potthast A, Sixta H, et al., 2001. The chemistry of side reactions and byproduct formation in the system NMMO/cellulose (Lyocell process). Progress in Polymer Science, 26(9), 1763-1837.

[25] Weiner Myra L., Lois A. Kotkoskie, 2000. Excipient Toxicity and Safety. New York.

[26] Lawrence Pranger, Rina Tannenbaum, 2008. Biobased nanocomposites prepared by in situ polymerization of furfuryl alcohol with cellulose whiskers or montmorillonite clay. Macromolecules, 41, 8682.

[27] Cellulose. 2008. Encyclopædia Britannica. Retrieved January 11, 2008, from Encyclopædia Britannica Online.

[28] Wikipedia, 2013. Hemicellulose. http://en.wikipedia.org/wiki/Hemicellulose.

[29] Ronald Asturias, Jeannelou Altez et al., 2008. Opportunities and challenges involved in the production of cellulose-based alcohol. University of the Philippines.

[30] Lebo, Stuart E. Jr., et al., 2001. Lignin. Kirk Othmer Encyclopedia of Chemical Technology.

[31] Boerjan W., Ralph J., 2003. Lignin bios.Plant Biol, 54 (1), 519–549.

[32] Fengel D., Wegener G., 1989. Wood- Chemistry, ultrastructure, reactions. Walter de Gruyter.

[33] Birgit Kamm, Patrick R. Gruber, et al., 2006. Biorefineries- Industrial Processes and Products. Chapter 3.

[34] Mitchinson Colin, 2004. Improved cellulases for the biorefinery: a review of genencor's progress in the DOE subcontract for cellulose cost reduction for bioethanol. Stanford GCEP biomass energy workshop.

[35] Michael Knauf, Mohammed Moniruzzaman, 2004. Lignocellulosic biomass processing: a perspective. International Sugar Journal, 106, 1263, 147-150.

[36] Chotani G., Dodge T., et al., 2000. Commercial production of chemicals using pathway engineering. Biochemica Biophysica Acta, 1543(2), 434-455.

[37] Manoj Kumar, Jeff Pucci et al., 2004. Biocatalytic conversion of renewable feedstock to industrial chemicals. In Lignocellulose Biodegradation, Oxford Univ. Press, 363-376.

[38] Birgit Kamm, Patrick R. Gruber, Michael Kamm, 2006. Biorefineries-Industrial Processes and products. 1, Chapter 5, 115-117.

[39] Morris D., Ahmed I., 1992. The carbohydrate economy: Making chemicals and Industrial materials from plant matter. The institute for local self reliance.

[40] Wiltsee G., 1998. Urban waste grease resource assessment.

[41] Van Dyne D.L., Blaise M.G., et al., 1999. A strategy for returning agriculture and rural america to long term full enployment using biosmass refineries. Perspectives on new crops and new uses.

[42] Szmant H. H., 1986. Industrial Utilization of renewable resources. Technomic pulishing company.

[43] Johnson R. W., Fritz E., 1989. Fatty acids in Industry. Marcel Dekker.

[44] Donaldson T. L., Culberson O. L., 1983. Chemicals from biomass: an assessment of the potential for production of chemical feedstocks from renewable resources.

[45] Wise D. L., 1983. Organic chemicals from biomass. The benjamin/cummings publishing company.

[46] Clements L. D., Beck S. R., Heintz C., 1983. Chemicals from biomass feedstocks. Chem. Engr. Prog., 79, 56-62.

[47] Lowenstein, M. Z., 1985. Energy applications of biomass. Elsevier applied sciences publishers.

[48] Sherrard E., KressmanF., 1945. Ind. Eng. Chem., 37, 5.

[49] Katzen R., Othmer D., 1942. Ind. Eng. Chem., 34, 314.

[50] Wright J., Power A., 1984. Evaluation of concentrated halogen acid hydrolysis processes for alcohol fuel production. Solar Energy research institute.

[51] Sherrard E., 1923. Ind. Eng. Chem., 15, 1164.

[52] Harris E., Berlinger E., Hajny G., et al., 1945. Ind. Eng. Chem., 37, 12.

[53] Burton R., 1983. Process of the Royal Soc. Of Canada Internat, Symp. On ethanol from biomass. Royal society of Canada, 247.

[54] Lawford R., Charley R., et al., 1984. Fifth Canadian Bioenergy R&D Seminar. National Research Council of Canada, 503.

[55] Wright J., Bergeron P., Werdene P., 1987. Ind. Eng. Chem. Res., 26, 299.

[56] Bulls M., Watson J., et al., 1991. Energy from Biomass and Waste XIV. Institute of gas technology, 1167.

[57] Lee Y., Zhang W., et al., 2000. Biresource Technol.71, 29.

[58] Yamada T., Fitigati M., Zhang M., 2002. App. Biochem. Biotechnol., 899, 98-100.

[59] Kottke R., 1988. Kick- Othmer Encyclopedia of chemical Technol. Supplement to 4th ed., John Wiley & Sons, 155.

[60] Katzen R., Monceaux D., 1995. App. Biochem. Biotechnol. 585, 51-52.

[61] Hayn M., Steiner W., et al., 1993. Bioconversion of forest and Agricultural Plant residues. CAB International, 33.

[62] Mosier N., Wyman C., et al., 2005. Bioresource Technol., 96, 673.

[63] Birgit Kamm, Prtrick R. Gruber, Michael Kamm, 2006. Biorefineries-Industrial Processes and products.

[64] Parajo J. C., Alonso J. L., et al., 1993. On the behaviour of lignin and hemicelluloses during the acetosolv processing of wood. Bioresource technology, 233-240.

[65] Junping Zhuang, Ying Liu, Zhen Wu, 2009. Hydrolysis of wheat straw hemicellulose and detoxification of the hydrolysate for xylitol production. BioResources, 4(2), 674-686

[66] Marcotullio G., Krisanti E., Giuntoli J., Jong W., 2011. Selective production of hemicellulose-derived carbohydrates from wheat straw using dilute HCl or $FeCl_3$ solutions under mild conditions. X-ray and thermo-gravimetric analysis of the solid residues. Bioresource Technology, 102, 5917–5923.

[67] Yan shi, Tomoya Yokoyama, et al., 2012. Degradation kinetics of monosaccharides in hydrochloric, sulfuric and sulfurous acid. Bioresources, 7(3), 4085-4097.

[68] Guadalupe Bustos, Jose Alverto Ramirez, Gil Garrote, et al., 2003. Modeling of the Hydrolysis of Sugar Cane Bagasse with Hydrochloric Acid. Applied Biochemistry and Biotechnology, 104, 51-69.

[69] Thomas P., William R. Upton, 1976. The hydrolysis of cotton cellulose by hydrochloric acid in benzene. Carbonhydrate Research, 49, 163-174.

[70] Elwin E. Harris, Albera A. Kline, 1948. Hydrolysis of wood cellulose with hydrochloric acid and sulfur dioxide and the decomposition of its hydrolytic products.

[71] Samia A. Helmy, M. Amine Abou-state, 1993. Studies on the acid degradation of cellolusic fibers II effect of pulp characteristics on the course of degradation in hydrochloric acid-zinc chloride solution. Polymer degradation and stability, 41, 245-251.

[72] Wenzi H. F. J., 1970. The chemical techmology of wood. Chapter 4.

[73] Irving S. Goldstein, Helena Pereira, et al., 1983. The hydrolysis of cellulose with superconcentrated hydrochloric acid. Biotechnology and bioengineering symp., 13, 17-25.

[74] Sluiter A., Hames B., Ruiz R., et al., 2008. Determination of sugars, byproducts, and degradation products in liquid fraction process samples, Technical report NREL.

[75] Joung Ho Ko, Huazi Huang, Gyoung Won Kang, et al., 2005. Simultaneous quantitative determination of monosaccharides including fructose in hydrolysates of yogurt and orange juice products by derivatization of monosaccharides with p-Aminobenzoic acid ethyl ester followed by HPLC. Bull. Korean Chem. Soc. 26, 10, 1533.

[76] Zhang J., Zhang Q., et al., 2009. Analysis of the monosaccharide composition of fucoidan by precolumn derivation HPLC. Chinese Journal of Oceanology and Limnology, 27, 3, 578-582.

[77] Christina Scha¨del, Andreas Blo¨chl, et al., 2010. Quantification and monosaccharide composition of hemicelluloses from different plant functional types. Plant Physiology and Biochemistry, 48, 1-8.

[78] Yi-Heng Percival Zhang, Lee R. Lynd, 2003. Cellodextrin preparation by mixed-acid hydrolysis and chromatographic separation. Analytical Biochemistry, 322, 225–232.

[79] Xianxiang Liang, Takashi Yoshida, Toshiyuki Uryu, 2013. Direct saccharification and ethanol fermentation of cello-oligosaccharides with recombinant yeast. Carbohydrate Polymers, 91, 157–161.

[80] Hong Peng, Yong Sun, Junhua Zhang, et al., 2010. Degradation of celloolignosaccharides in oxidative mediun and alkaline medium: HPLC, FTIR, and GC-MS analysis. Bioresources, 5(2), 616-633.

[81] Jun Xu, Jie Wua, Ling-Ying Zhu, et al., 2012. Simultaneous determination of iridoid glycosides, phenethylalcohol glycosides and furfural derivatives in Rehmanniae Radix by high performance liquid chromatography coupled with triple-quadrupole mass spectrometry. Food chemistry, 135, 2227-2286.

[82] Hult E. L., Iversen T., Sugiyama J., 2003. Characterization of the supermolecular structure of cellulose in wood pulp fiber. Cellulose, 10, 103-110.

[83] Lindergen T., Edlund U., 1995. A multivariate characterization of crystal transformations of cellulose. Cellulose, 2, 273-288.

[84] Evans R., Newman R. H., Roick U. C., et al., 1995. Changes in cellulose crystallinity during kraft pulping. Comparison of infrared, X-ray diffraction and solid state NMR results. Holzforschung, 49, 498-504.

[85] Hattula T., 1986. Effect of kraft cooking on the ultrastructure of wood cellulose. Paperi ja Puu, 26, 926-931.

[86] Ma Xiaojuan, Huang Liulian, Chen Lihui, et al., 2012. Determination methods for crystallinity of cellulose. Paper Science and Technology, 31, 2.

[87] Atalla R. H., Vanderhart D. L., 1984. Native cellulose: a composite of two distinct crystalline forms. Science, 223, 283-284.

[88] Segal L., Creely J. J., Martin Jr A. E., et al., 1959. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. Textile Research Journal, 29, 786-794.

[89] Focher B., Palma M. T., 2001. Structural diferences between nonwood plant celluloses: evidence from solid state NMR, vibrational spectroscopy and X-ray difractometry. Industrial Crops and Products, 13, 193-208.

[90] Zavadskii A. E., 2004. The X-ray diffraction method of determining the polymorphous composition of cellulose for textured materials. Fibre Chemistry, 36(2), 425-430.

[91] Wang Y. P., Wang G., Cheng H. T., et al., 2010. Structures of bamboo fiber for textiles. Textile Research Journal, 80(4), 334-343.

[92] Oconnor R. T., Dupre E. F., Mitchum D., 1958. Applications of infrared absorption spectroscopy to investigations of cotton and modified cotton. Textile Research Journal, 28, 38.

[93] Nelson M., O'Connor R., 1964. Relation of certain infrared bands to cellulose crystallinity and crystal latticed type. Part I. Spectra of lattice types I, II, III and of amorphous cellulose. J Appl Polym Sci, 8(3), 1311–1324.

[94] Atalla R. H., Gast J. C., Sindorf D. W., Bartuska V. J., 1980. The 13C NMR spectra of cellulose polymorphs. Journal of the American Chemical Society, 102, 3249.

[95] Newman R. H., Hemmingson J. A., 1990. Deternination of the degree of cellulose crystallinity in wood by carbon-13 nuclear magneticresonance spectroscopy. Holzforschung, 44, 351-355.

[96] Teeaar R., Serimaa R., Paakkarl T., 1987. Crystallinity of cellulose, as determined by CP /MAS NMR and XRD methods. Polymer Bulletin, 17, 231-237.

[97] Kolodziejski W., Frye J. S., Maciel G. E., 1982. Carbon 13 nuclear magnetic resonance spectrometry with cross polarization and magic-angle sample spinning for analysis of lodgepole pine. Wood Anal. Chem, 54, 1419-1427.

[98] Sirkka M., Tiina L., Seppo K., 2000. 13C CPMAS NMR investigations of cellulose polymorphs in different pulps. Cellulose, 7, 147-159.

[99] Noppadon Sathitsuksanoh, Zhiguang Zhu, Sungsool Wi, et al., 2010. Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. Biotechnology and bioengineering.

[100] Yi-Heng Percival Zhang, Lee R. Lynd, et al., 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems. Wiley InterScience.

[101] Kleman-Leyer K., Agosin E., Conner A. H., Kirk T. K., 1992. Changes in molecular size distribution of cellulose during attack by white rot and brown rot fungi. Appl Environ Microbiol, 58, 1266-1270.

[102] Kleman-Leyer K. M., Gilkes N. R., Miller R. C. Jr, Kirk T. K., 1994. Changes in the molecular-size distribution of insoluble celluloses by the action of recombinant cellulomonas fimi cellulases. Biochem J, 302, 463-469.

[103] Kleman-Leyer K. M., Siika-Aho M., Teeri T. T., Kirk T.K., 1996. The cellulose endoglucanase I and cellobiohydrolase II of Trichoderma reesei act synergistically to solubilize native cotton cellulose but not to decrease its molecular size. Appl Environ Microbiol, 62, 2883-2887.

[104] Striegel A. M., 1997. Theory and applications of DMAc/LiCl in the analysis of polysacharrides. Carbohydr Polymer, 34, 267-274.

[105] Evans R., Wallis A.F.A., 1989. J. Appl. Polym. Sci., 37, 2331.

[106] Immergut E. H., Ranby B. G., Mark H. F.,1953. Ind. Eng. Chem., 45, 2483.

[107] Murside Kes, Bjorn E. Christensen, 2013. A re-investigation of the Mark–Houwink–Sakurada parameters for cellulose in Cuen: A study based on size-exclusion

chromatography combined with multi-angle light scattering and viscometry. Journal of Chromatography A, 1281, 32-37.

[108] Krassig H. A., 1993. Cellulose: structure, accessibility and reactivity. Yverdon, Switzerland: Gordon & Breach.

[109] Y.-H. Percival Zhang, Lee R. Lynd, 2005. Determination of the number-average degree of polymerization of cellodextrins and cellulose with application to enzymatic hydrolysis. Biomacromolecules, 6, 1510-1515.

[110] Yau W. W., Kirkland J. J., Bly D.D., 1979. Model size-exclusion liquid chromatography. New York: John Wiley & Sons.

[111] Klemm D., Philipp B., Heinze T., Heinze U., Wagenknecht W., 1998. Comprehensive cellulose chemistry. I. Fundamentals and analytical methods. Weinheim: Wiley-VCH.

CHAPTER 3

Degradation of monosaccharide in concentrated hydrochloric acid

3.1 Introduction

Fossil energy is the most significant fuel energy in recent years however its advantages have already affected the development of society. A new energy, biomass energy, is a kind of inexpensive, carbon neutral, renewable and abundant energy resource and it may potentially replace the fuel market for its economic and environmental advantages. Lignocellulosic material is the most abundant biomass resource and it can be converted to monosaccharide, which can in turn be used in fermentation processes to produce biofuels such as biodiesel and ethanol. The main processes for lignocellulose conversion involve acid-catalyzed or enzyme-catalyzed hydrolysis, which separates the complex polymers into their constituent monomers. Although lignocellulosic biofuels are not currently cost-competitive with more traditional petroleum-based energy sources, development of novel processes for their production may contribute to lowering their price and consequently increasing their application [1].

Although acid catalyzed hydrolysis is a matured technology, however, hydrolysis of lignocellulose, hemicellulose and monosaccharide in concentrated hydrochloric acid has not

yet been thoroughly studied. On the other hand, despite extensive studies on cellulose hydrolysis, there is still a lack of knowledge on the cellulose degradation in concentrated hydrochloric acid. There was one study [2] on hydrolysis of cellulose in super-concentrated hydrochloric acid published in 1980s, which, however, did not explore the low temperature hydrolysis and only showed very limited analysis. With recent development of chemical analysis tools, the analysis of cellulose became easier and more effective.

In the present study, the hydrolysis of hemicellulose monosaccharide catalyzed by concentrated hydrochloric acid (maximum concentration of HCl is 38%) was studied. The concentration of each component was measured by high performance liquid chromatography and detected by refractive index detector. The cellulose hydrolysis in concentrated hydrochloric acid (highest concentration of HCl is 41%) at low temperature (from -4℃ to 15℃) was explored and the concentration of glucose, degree of polymerization of cellulose, crystallinity index of cellulose were measured by HPLC, Ubbelohde viscometer and x-ray diffraction, respectively.

3.2 Experimental Section

3.2.1 Materials

All materials are reagent grade. Glucose (≥99%), mannose (≥99%), xylose (≥99%), arabinose (≥99%), galactose (≥99%), cellobiose (≥99%), cellulose (white powder),

5-(Hydroxymethyl)furfural (≥ 99%), furfural (≥ 99%), Bis(ethylenediamine)copper(II) solution (1.0M in water) are purchased from Sigma-Aldrich. Hydrochloric acid (38% w/w), sodium hydroxide (≥99%), sulfuric Acid (98% w/w), ethylene glycol (≥99%) are purchased from Fisher Scientific. Cellulose is in the form of white powder and it is derived from softwood tree pulp.

3.2.2 Equipment

3.2.2.1 Experiment equipment

The experimental setup is shown in Figure 3.1. A mixing setup, including a controlling unit (part 1), a rotator (part 2) and a PTFE stirring paddle (part 5) is used to stir the solution in a 500mL, three-necked round-bottom flask (part 4) during the acid-catalyzed hydrolysis process. A thermometer (part 3) is used to continuously monitor the temperature of the solution. A temperature control system is comprised of cooling jacket (part 6) connected to a water bath equipped with a pump (part 11), which circulates water or ethylene glycol at desired temperature to cool down or warm up the reaction solution. An exhaust treatment system must be built in order to absorb the HCl gas before emission. The system follows the reaction setup, consists of a glass condenser (part 7), an air-buffering bottle (part 8 and 9) and an acid absorption bottle filled with hydroxide solution (part 10). All parts are connected with latex tubes and the system is used in the fume hood.

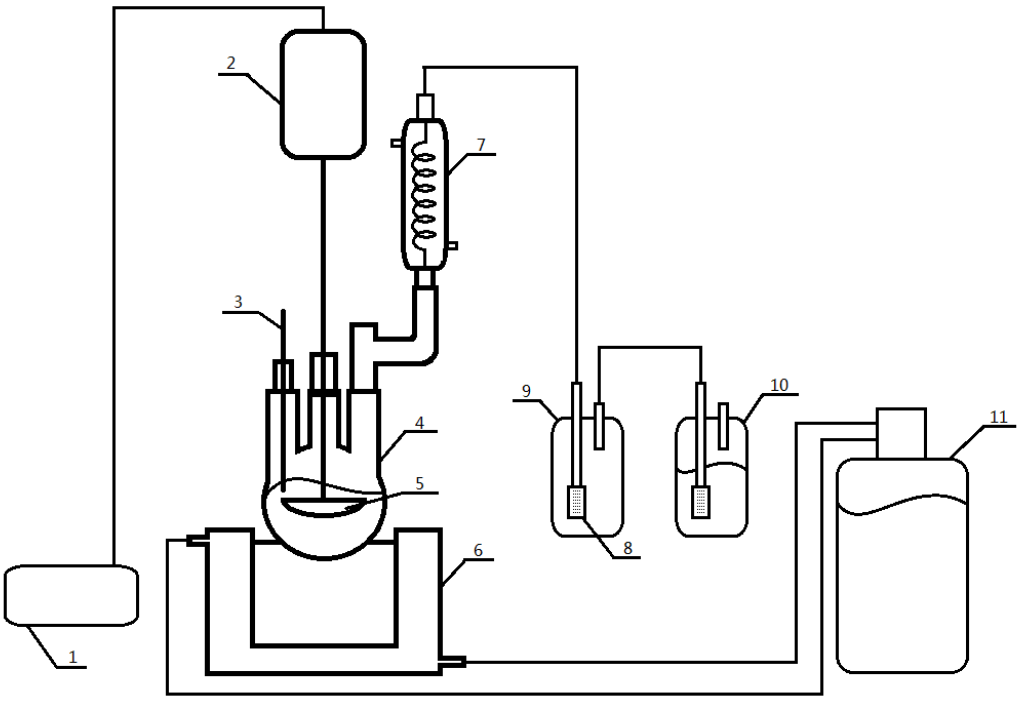

Fig. 3.1 Experimental setup for hemicellulose hydrolysis

3.2.2.2 High performance liquid chromatography (HPLC)

HPLC spectra are obtained by Agilent 1200 series high performance liquid chromatography equipped with a refractive index detector (RID). The column for HPLC analysis is Aminex HPX-87H.

3.2.2.3 X-ray diffraction (XRD)

X-ray diffraction spectra are collected using a Rigaku Ultima IV XRD with a CuKα source (λ=0.15418 nm) operating at 40 kV and 44 mA.

3.2.2.4 Ubbelohde viscometer

The Ubbelohde viscometer (Fig. 3.2) is purchased from Fisher Scientific and it was used to measure the viscosity of cellulose in Bis(ethylenediamine)copper(Ⅱ) solution in order to calculate the degree of polymerization of cellulose.

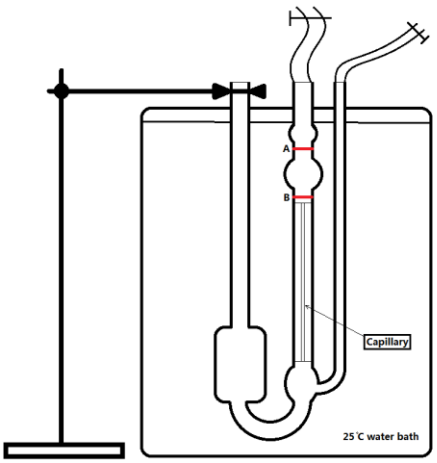

Fig. 3.2 Ubbelohde viscometer

3.2.3 Experiment methods

3.2.3.1 Acid-catalyzed hydrolysis

The experimental processes consist of two parts. The first part is the degradation of monosaccharide which is obtained from hemicellulose, and the second part is cellulose hydrolysis. Three groups of experiments have been conducted in order to identify the optimal hydrolysis condition for different monosaccharides and to separate the components which normally are not easily separated by HPLC (Shown on Fig. 3.3). Glucose and xylose are studied in the first group; galactose and arabinose are studied next (group 2) and the mannose is studied in the last group.

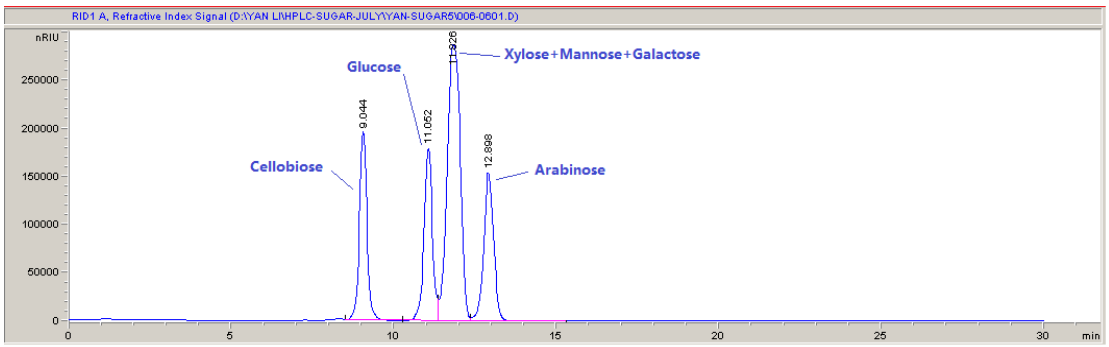

Fig. 3.3 HPLC spectra of monosaccharide

The experimental process for hemicellulose hydrolysis is as follows. First, the equipment is set-up according to Fig.3.1 and the system is switched on. . When the temperature reaches the desired value, 6g monosaccharide powder (Group 1, 2 or 3) is weighed out and put into

300mL concentrated hydrochloride acid in the 500mL flask. After that, the rotator is started to agitate the solution. The flask is purged with nitrogen for 30 seconds in order to remove the oxygen which otherwise would cause oxidation of the sugar powder. The experiment is conducted for 10 hours. When time reached 10 hours, the motor is switched off and the remaining solution is disposed into a waste container.

The same procedure is applied to the cellulose hydrolysis experiments. Because the cellulose hydrolysis requires a lower temperature (0℃ ~ -5℃), antifreeze solution (50% v/v ethylene glycol in deionized distilled water) is used instead of deionized water as the cooling liquid which circulates between the water bath and the cooling jacket. Although the temperature of the water bath can reach -5℃, some parts of the system such as tubes and cooling jacket are still exposed to the room temperature, therefore, the minimum solution temperature in the flask can only reach -3℃. The cooling jacket is placed in a polyethylene (PE) box for thermal insulation and full with ice packs for cooling.

Because HCl liquid is highly volatile and the HCl gas is irritating, all the experiments must be conducted in the fume hood.

3.2.3.2 Concentrating Set-up of Hydrochloric Acid

Because the cellulose hydrolysis requires highly concentrated hydrochloric acid (41%) and the maximum concentration of HCl that can be obtained from Fisher scientific is 37% to 38%, the HCl needs to be concentrated prior to the cellulose hydrolysis.

The HCl-concentrating setup is shown on Fig. 3.4. A simple HCl gas generation method was employed by reacting sodium chloride with concentrated sulfuric acid (98%) to avoid using HCl gas storage tank. Sulfuric acid is drop-dispensed onto the NaCl powder. The generated HCl gas flows to and gets absorbed by the 38% HCl solution in a round-bottom flask to increase the HCl concentration. The temperature of 38% HCl solution has been set to 10℃ in order to increase the solubility of hydrogen chloride gas, while the temperature of the NaCl-H_2SO_4 reaction was set to 80℃ to 90℃ because the solution form large quantity of foam when up 95℃. After 10 hours' reaction, the max concentration of hydrochloric acid could reach 41%-42%.

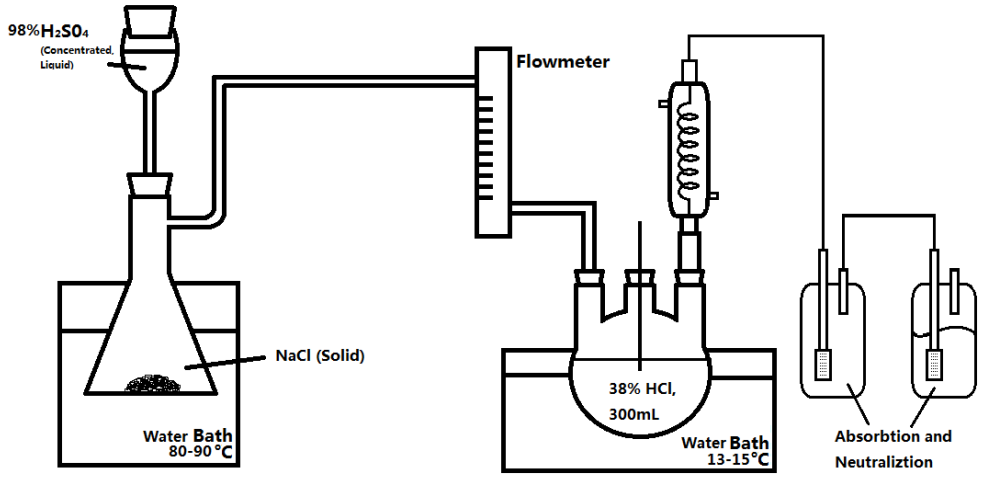

Fig. 3.4 Experimental setup for concentrating hydrochloric acid solution

3.2.3.3 Sampling

Different sampling methods are applied to monosaccharide hydrolysis and cellulose hydrolysis due to the differences in the solubility and analysis methods of monosaccharide (normally analyzed in liquid form) and cellulose (normally analyzed in solid form) (Shown on Fig 3.5). The result is the sampling procedure for cellulose hydrolysis has three separated methods (sampling A, B and C).

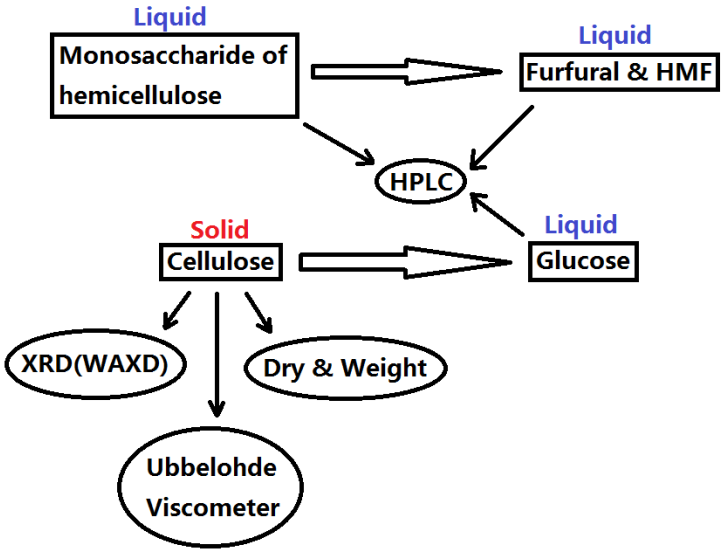

Fig. 3.5 Sampling procedure for monosaccharide and cellulose hydrolysis

3.2.3.3.1 Sampling method of hemicellulose monosaccharide hydrolysis experiment

Prior to an experiment, 1 L 1.34 mol/L NaOH solution is prepared and stored at 4 ℃. 7mL solution is added into a 10mL volumetric flask and 10 such flasks are prepared and stored at 4 ℃, which will be used later on to stop the hydrolysis reaction. Each experiment is conducted for 10 hours and totally 12 samples are collected during the experiment. The first two samples are collected immediately after sugar powders are fully dissolved in the solution, which is considered time 0. After that, 2 samples are collected every two hours (at hour 2, 4, 6 8 and 10)

When taking samples, 1mL sample is taken by 1 mL pipette. The samples are added into the flask with NaOH solution immediately after collection to stop the hydrolysis reaction by reducing the concentration of H^+ and thus the heat of neutralization. Deionized water is subsequently added till the solution reaches 10mL mark. At last, the pH of the resulting solution is tested using a pH paper. If the pH falls between 1 and 3, 1mL sample is collected, which will be used in the HPLC analysis. Fig 3.6 shows the process of sample collection and post-treatment.

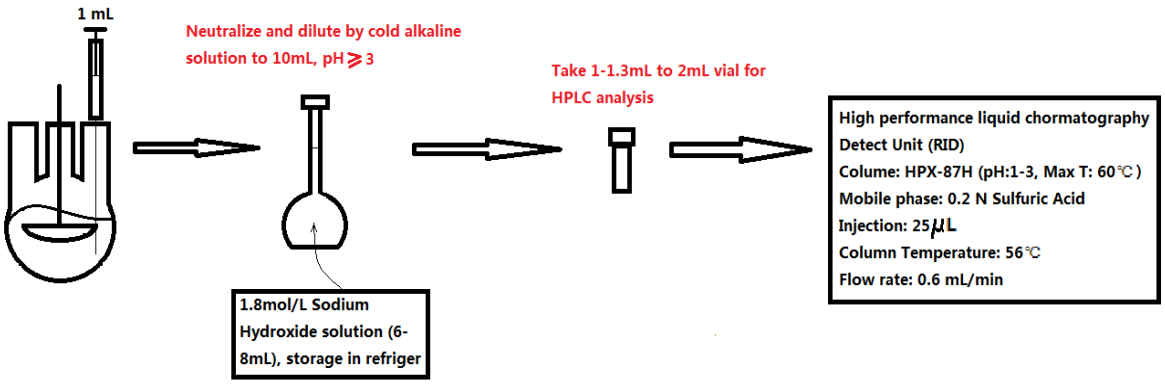

Fig. 3.6 Sampling procedure for monosaccharide hydrolysis

3.2.3.3.2 Sampling method of cellulose hydrolysis experiment

Sampling procedure A: Similar to the monosaccharide experiment, 2 samples are collected every two hours during one experiment and they are processed following the same post-treatment procedure prior to HPLC analysis (shown in Fig.3.7).

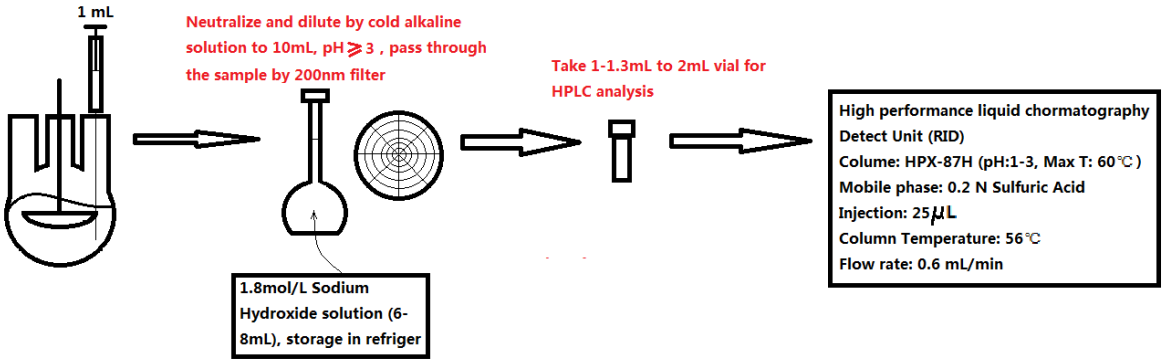

Fig. 3.7 Sampling procedure A for cellulose hydrolysis

Sampling procedure B: 4 samples (5 mL each) are collected from hour 0, 2, 6 and 10, and are diluted by deionized distillate water (DD water) to 50mL. The samples are centrifuged at 6000 rpm for 10 minutes. Supernatant is removed by pipette and the sediment is diluted by DD water to 50 mL and centrifuged again. This dilute-centrifuge cycle is repeated for three times in order to decrease the concentration of hydrochloric acid. The concentration of the HCl is diluted by 10 for 3 times, it can be assumed that the concentration of the hydrochloric acid close to zero. Subsequently, the sediment is collected in glass dishes and baked at 50 ℃ until all water is evaporated and the sediment becomes completely dry, which takes 8 to 10 hours. The resulted dry solid is collected and ground to powder for the XRD analysis of crystallinity index (CrI). The powder is stored in sealed vials in refrigerator. (The method is illustrate in Fig.3.8)

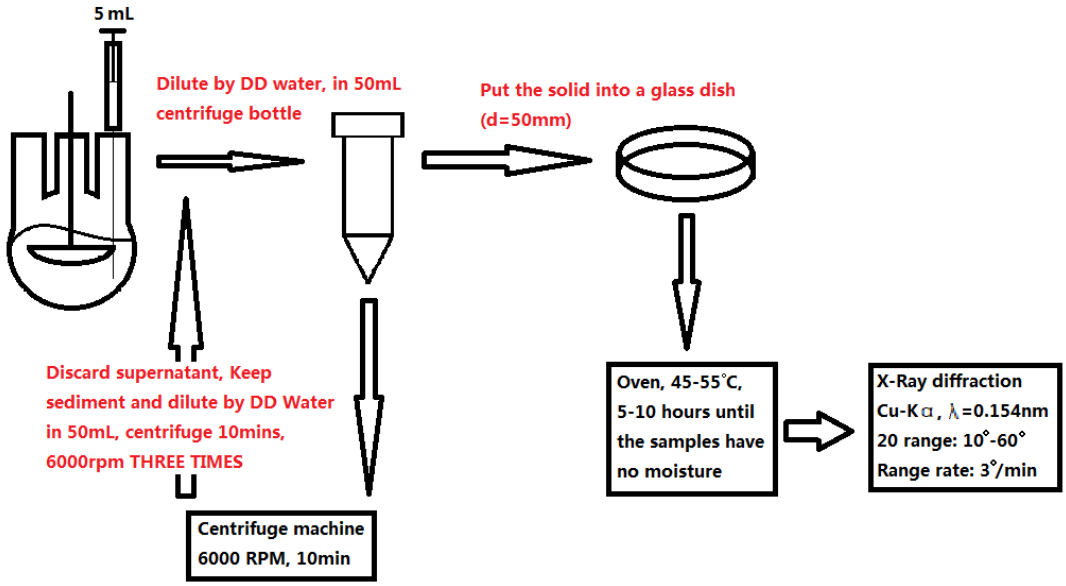

Fig. 3.8 Sampling procedure B for cellulose hydrolysis

Sampling procedure C: 1 sample (5 mL) is collected every two hours (at hour 0, 2, 4, 6, 8 and 10) and all the samples (totally 6) are processed using the same method as in procedure B before baking process. The group of samples is used for measuring viscosity which can be further used to calculate the degree of polymerization (DP). These samples are also stored in sealed containers. (The method is illustrated in Fig.3.9)

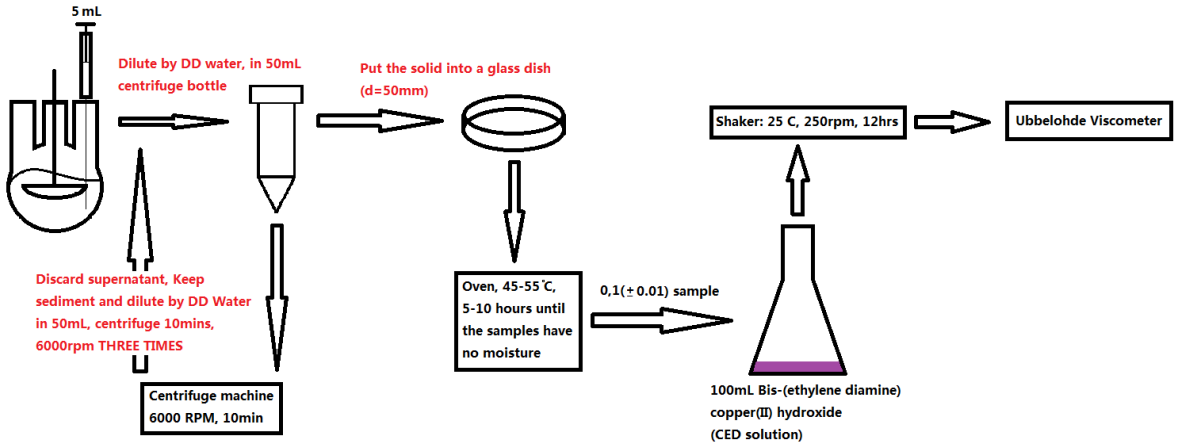

Fig. 3.9 Sampling procedure C for cellulose hydrolysis

3.2.4 Analysis Methods

3.2.4.1 HPLC analysis

The HPLC analysis in the work follows the protocol from a previous publication [3]. Aminex HPX-87H columns (Max temperature: 65 ℃, pH: 1-3) are used here and column temperature was set at 56 ℃. The mobile phase is 0.01 N sulfuric acid and the flow rate is 0.6 mL/min. Analysis time is 70 minutes for samples and 30 minutes for DD water (DD water is passed through the column after each sample analysis cycle to rinse the needle and column). The injection volume is 25 µL. The HPLC-spectra is shown on Fig. 3.10.

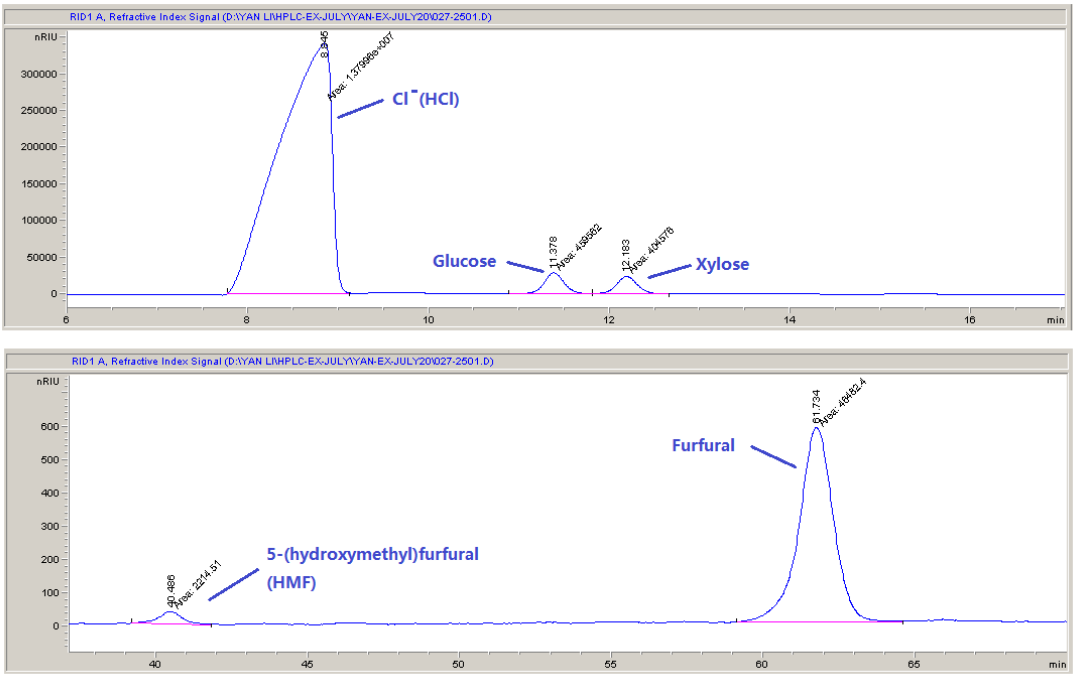

Fig. 3.10 HPLC spectra of different component

In order to distinguish the compound and measure the amount of monosaccharide in the solution by HPLC, a standard curve need to be first obtained. This standard curve establishes a relationship between the peak area and the concentration of analyte. The retention time can be used to identify the species of the compound.

To prepare the standard curve, solutions of glucose, xylose, galactose, mannose, arabinose and cellubiose at 48 mg/mL are prepared and further diluted to 24 mg/mL, 12 mg/mL, 6 mg/mL and 3 mg/mL. 1mL of each solution is taken for HPLC analysis. Measure each sample twice to get the average standard curve as shown in Fig. 3.11 and the retention time

as shown in Fig. 3.12. The standard curve for HMF and furfural also need to be drawn. The equations for different monosaccharide are as follows:

$$\text{Glucose:} \quad C_{sam} = A_{peak}/119862 \tag{1}$$

$$\text{Xylose:} \quad C_{sam} = A_{peak}/116539 \tag{2}$$

$$\text{Mannose:} \quad C_{sam} = A_{peak}/112961 \tag{3}$$

$$\text{Arabinose:} \quad C_{sam} = A_{peak}/111984 \tag{4}$$

$$\text{Galactose:} \quad C_{sam} = A_{peak}/117058 \tag{5}$$

$$\text{Cellobiose:} \quad C_{sam} = A_{peak}/114698 \tag{6}$$

$$5-(\text{hydroxymethyl})-\text{furfural:} \quad C_{sam} = A_{peak}/174764 \tag{7}$$

$$\text{Furfural:} \quad C_{sam} = A_{peak}/168096 \tag{8}$$

In the formula, A_{peak} is the area of the peak which could be obtained from HPLC and C_{sam} is the concentration of the component.

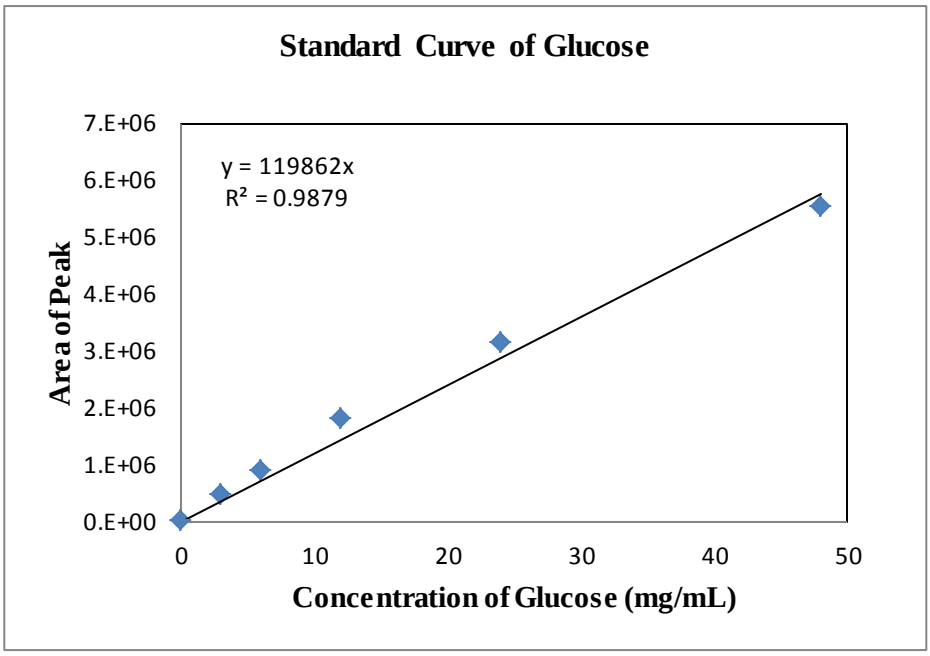

Fig. 3.11 The standard curve of Glucose

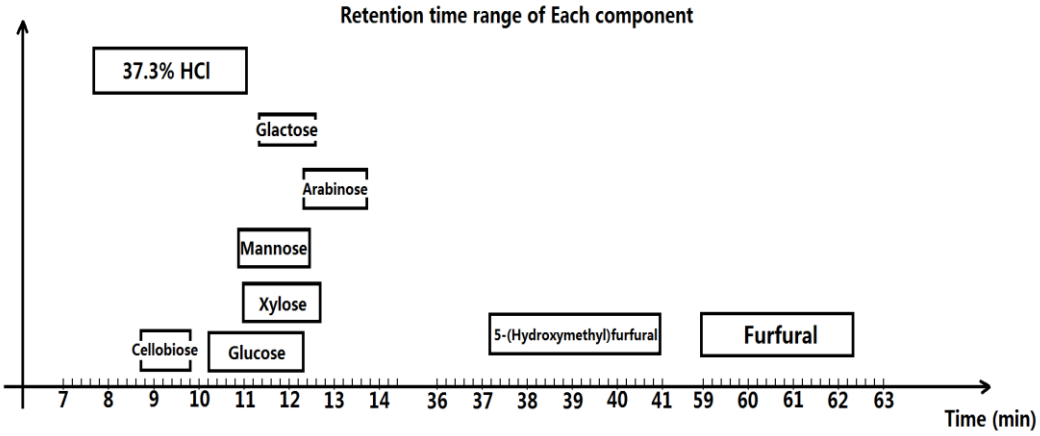

Fig. 3.12 Retention time of each component

3.2.4.2 XRD analysis

X-ray diffractograms of samples are obtained using CuKα Radiation (λ=1.54178Å=0.154nm) at a scanning rate of 4 °/min and scanning angle ranging from 10 °to 60 °.

The measurement follows previously published protocol [4]. The crystallinity index(CrI) can be calculated by equation (9) :

$$\alpha = \frac{I_{cr}}{I_{cr} + I_{am}} \times 100\% \quad (9)$$

Where I_{cr} is intensity of crystalline phase and I_{am} is the intensity of amorphous phase.

Crystallite size (L) and interplanar distance (D) are calculated by the following two equations:

$$L = k\lambda/\beta\cos\theta \quad (10)$$

$$D = \lambda/2\sin\theta \quad (11)$$

Where λ=0.154 nm, K is the Scherrer constant (0.94) and β is the full – width at half – maximum. 2θ=14.8 °, 16.3 °, 22.6 ° for crystalline phase and 2θ=18.3 ° for amorphous phase.

3.2.4.3 Viscosity analysis

The measurement of viscosity is used to derive the degree of polymerization and this method has been published previously [5].

3.2.4.3.1 Cellulose – CED solution preparation

1 M Bis(ethylenediamine)copper(II) solution (also known as Cupriethylenediamine solution, CED solution or Cuen solution) is purchased in Sigma. 0.01 g sample collected using sampling method C is placed in a 250 mL Erlenmeyer flask and 10 mL CED solution is added. The concentration of cellulose is normally 1mg/mL. Nitrogen is charged into the Flask in order to remove the oxygen which will cause a degradation of the cellulose. Copper chip is also added into the flask to prevent the degradation of the cellulose. The flasks are swirled at 250 rpm, 25 ℃ for 5 hours until the cellulose is fully dissolved in CED solution.

3.2.4.3.2 Viscosity measurement and degree of polymerization calculation

The viscosity of the cellulose – CED solution is measured by an ubbelohde viscometer. The relative viscosity can be calculated from t and t_0. The time for the solvent (CED solvent) to flow through the viscometer from one engraved line to another is t_0 while the time of the cellulose – CED solution is t. The Degree of Polymerization (DP_v) is calculated by these equations:

$$\text{Poiseuille equation:}\quad \eta_r = \frac{\eta}{\eta_0} = \frac{t}{t_0} \tag{12}$$

$$\eta_{sp} = \eta_r - 1 \tag{13}$$

$$\text{Martin equation:}\quad \lg[\eta] = \lg\frac{\eta_{sp}}{C} - K_1[\eta]C \tag{14}$$

$$\text{Mark equation:}\quad [\eta] = K_2 DP^{\alpha} \tag{15}$$

Where η_r, η_{sp}, $[\eta]$ are relative viscosity, specific viscosity and intrinsic viscosity, respectively. C is the concentration of cellulose in CED solution, mg/mL. K_1,K_2 and α are constant, and for cellulose-CED system, K_1=0.13, K_2 = 1.7g/mL, α=0.8.

3.3 Results and discussions

3.3.1 Hemicellulose monosaccharide hydrolysis

Hemicellulose can be converted to five basic monosaccharides (xylose, mannose, glucose, arabinose and galactose) by hydrolyzing in acid solution and the hydrolysis rate is much higher than that of cellulose hydrolysis since the chain length in hemicellulose is much shorter. The monosaccharide obtained from hemicellulose hydrolysis can further react with acid solution and the final product is furfural and 5-(hydroxymethyl) furfural (HMF). Xylose and arabinose are pentose so they will eventually generate furfural, while glucose, galactose and mannose will generate HMF because they are hexose (A complete hydrolysis process is shown in Fig.3.13) [6]. However, furfural and HMF are unwanted products because they are toxic to bacteria so that they will have adverse effects on monosaccharide fermentation

process. In the study, rate of hydrolysis of monosaccharide is discovered by using hydrochloric acid with different concentrations and at different temperatures. The study aims to discover the optimal condition at which both monosaccharide degradation and furfural/HMF generation are minimized [7].

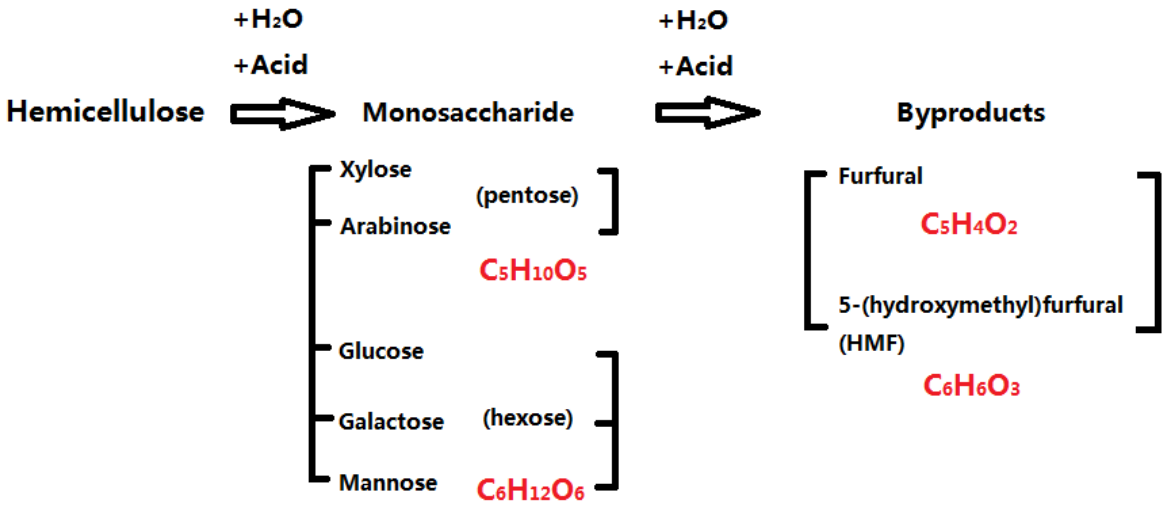

Fig. 3.13 Process of hemicellulose hydrolysis

3.3.1.1 Hydrolysis of monosaccharide

3.3.1.1.1 Effects of temperature

Hydrolysis rate of monosaccharide is highly dependent on reaction temperature which increases with increasing temperature. Fig. 3.14 shows the hydrolysis of glucose at different temperatures in 31% hydrochloric acid. In addition, the temperature dependence of hydrolysis is not affected by concentration of HCl or the species of the monosaccharide.

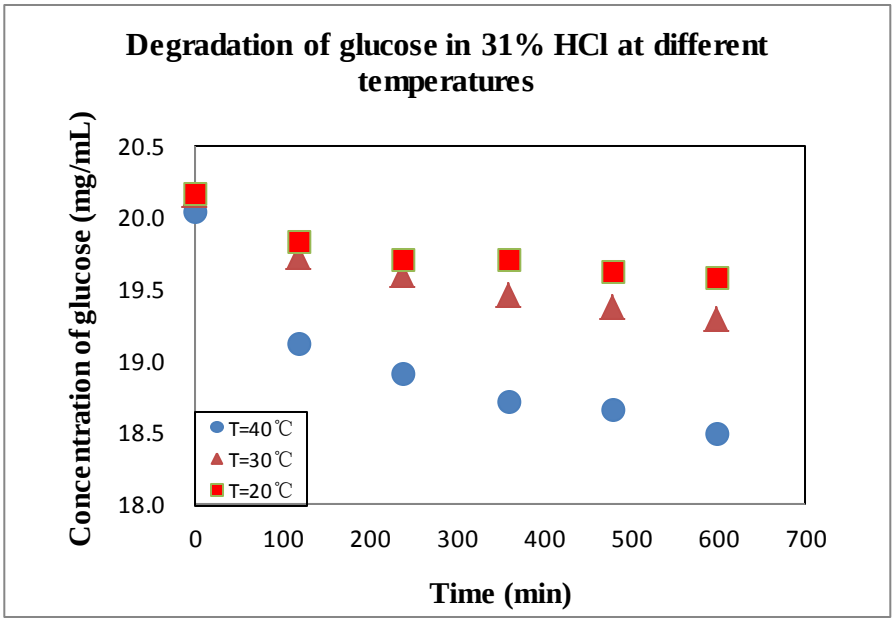

Fig. 3.14 Degradation of glucose in 31% HCl at different temperatures

The temperature dependence of hydrolysis is summarized in Table3.1 through 3.3 for 38 % HCl; Table 3.4 through 3.6 for 31% HCl; Table 3.7 and Table 3.8 for 24% HCl. The data show that the hydrolysis of all monosaccharide in 38% HCl are improved by increasing the temperature. It is also shown that there are two steps in the hemicellulose monosaccharide hydrolysis. The hydrolysis is increased greatly in the first steps in the first two hours, while the hydrolysis is leveled off in the second stage from hour 3 to hour 10. Similar conclusions can be drawn from the hydrolysis experiments conducted in 31% hydrochloric acid (shown on Table 3.4, 3.5 and 3.6) and 24% hydrochloric acid (shown on Table 3.7 and 3.8).

Table 3.1 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 38% (±0.5%) hydrochloric acid at 30°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.200	20.056	19.910	19.968	20.187	0.000	0.000	0.000	0.000	0.000
120	17.140	17.298	17.618	18.403	18.711	0.015	0.389	0.015	0.035	0.014
240	16.810	16.798	17.469	18.188	18.277	0.034	0.649	0.023	0.083	0.025
360	16.457	16.549	17.448	17.921	18.050	0.043	0.950	0.028	0.134	0.031
480	16.335	16.278	17.356	17.909	17.961	0.052	1.149	0.037	0.186	0.036
600	16.215	15.912	17.206	17.907	17.868	0.056	1.406	0.047	0.242	0.043

Table 3.2 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 38% (±0.5%) hydrochloric acid at 20°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	19.947	20.193	20.073	20.048	20.190	0.000	0.000	0.000	0.000	0.000
120	18.599	17.408	18.182	18.664	19.684	0.004	0.071	0.004	0.011	0.005
240	17.921	17.029	17.511	18.458	19.317	0.008	0.101	0.009	0.018	0.008
360	17.712	16.632	17.242	18.442	19.055	0.011	0.143	0.015	0.020	0.012
480	17.624	16.618	17.188	18.341	18.842	0.016	0.185	0.018	0.028	0.015
600	17.676	16.566	17.204	18.091	18.685	0.017	0.208	0.020	0.034	0.021

Table 3.3 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 38% (±0.5%) hydrochloric acid at 10°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.179	20.212	19.919	20.002	20.169	0.000	0.000	0.000	0.000	0.000
120	19.035	19.272	19.333	19.682	19.711	0.003	0.018	0.000	0.002	0.002
240	18.910	19.009	18.986	19.508	19.635	0.005	0.026	0.002	0.005	0.005
360	18.878	18.997	18.865	19.376	19.390	0.008	0.031	0.003	0.006	0.006
480	18.611	18.900	18.828	19.206	19.236	0.010	0.044	0.004	0.008	0.010
600	18.320	18.836	18.719	19.126	19.132	0.013	0.053	0.006	0.010	0.012

Table 3.4 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 31% (±0.5%) hydrochloric acid at 40°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.047	19.982	19.707	20.021	20.069	0.000	0.000	0.000	0.000	0.000
120	19.123	18.851	18.806	18.396	19.573	0.008	0.178	0.000	0.036	0.008
240	18.901	17.963	18.693	18.333	19.345	0.016	0.398	0.005	0.074	0.015
360	18.713	17.778	18.652	17.990	19.138	0.020	0.550	0.012	0.105	0.022
480	18.656	17.440	18.609	17.819	19.033	0.027	0.752	0.014	0.143	0.030
600	18.480	17.185	18.565	17.585	18.994	0.040	0.874	0.017	0.168	0.046

Table 3.5 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 31% (±0.5%) hydrochloric acid at 30°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.149	20.117	20.187	20.199	20.094	0.000	0.000	0.000	0.000	0.000
120	19.717	18.696	19.309	19.466	19.890	0.000	0.043	0.002	0.000	0.004
240	19.592	18.581	19.027	19.396	19.603	0.000	0.065	0.003	0.020	0.007
360	19.458	18.450	18.830	18.923	19.278	0.004	0.111	0.004	0.025	0.011
480	19.371	18.159	18.790	18.879	19.131	0.008	0.157	0.005	0.029	0.015
600	19.290	18.080	18.764	18.786	19.036	0.013	0.175	0.007	0.041	0.017

Table 3.6 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 31% (±0.5%) hydrochloric acid at 20°C

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.166	20.085	20.063	19.978	20.040	0.000	0.000	0.000	0.000	0.000
120	19.829	20.019	19.555	19.740	20.005	0.001	0.000	0.000	0.000	0.000
240	19.707	19.748	19.419	19.487	19.797	0.003	0.018	0.000	0.000	0.000
360	19.699	19.745	19.366	19.409	19.606	0.004	0.029	0.000	0.000	0.000
480	19.618	19.700	19.248	19.379	19.564	0.005	0.035	0.000	0.000	0.004
600	19.584	19.499	19.077	19.347	19.399	0.006	0.049	0.000	0.000	0.007

Table 3.7 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 24% (±0.5%) hydrochloric acid at 40℃

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.159	20.194	20.132	20.080	20.105	0.000	0.000	0.000	0.000	0.000
120	20.019	19.946	19.234	19.371	19.776	0.000	0.021	0.000	0.000	0.004
240	19.737	19.559	18.985	19.113	19.558	0.006	0.079	0.000	0.007	0.007
360	19.683	19.457	18.873	19.037	19.504	0.009	0.123	0.004	0.019	0.011
480	19.629	19.490	18.881	18.980	19.487	0.011	0.149	0.006	0.027	0.020
600	19.376	19.431	18.813	18.997	19.451	0.019	0.181	0.009	0.036	0.027

Table 3.8 Concentration (mg/mL) of the hemicellulose monosaccharide, hydrolyze with 24% (±0.5%) hydrochloric acid at 30℃

Time (min)	Glucose	Xylose	Galactose	Arabinose	Mannose	HMF from glucose	Furfural from xylose	HMF from galactose	Furfural from arabinose	HMF from mannose
0	20.012	20.048	20.156	20.198	20.008	0.000	0.000	0.000	0.000	0.000
120	19.917	19.649	19.487	19.474	19.980	0.002	0.010	0.000	0.000	0.000
240	19.890	19.416	19.308	19.364	19.779	0.003	0.022	0.000	0.003	0.000
360	19.850	19.323	18.868	19.312	19.673	0.005	0.027	0.000	0.006	0.000
480	19.838	19.290	18.785	19.263	19.584	0.007	0.037	0.000	0.010	0.000
600	19.829	19.265	18.637	19.168	19.573	0.008	0.045	0.000	0.013	0.000

3.3.1.1.2 Effects of concentration of hydrochloric acid

The hydrolysis is dependent on the concentration of the hydrochloric acid. The degradation increases by increasing the concentration of acid. The results are shown in Figure 3.15. In addition to the 30 ℃ mannose experiment shown here, the similar trends are also seen with different monosaccharide and at different temperature as shown in Table 3.1 to 3.8.

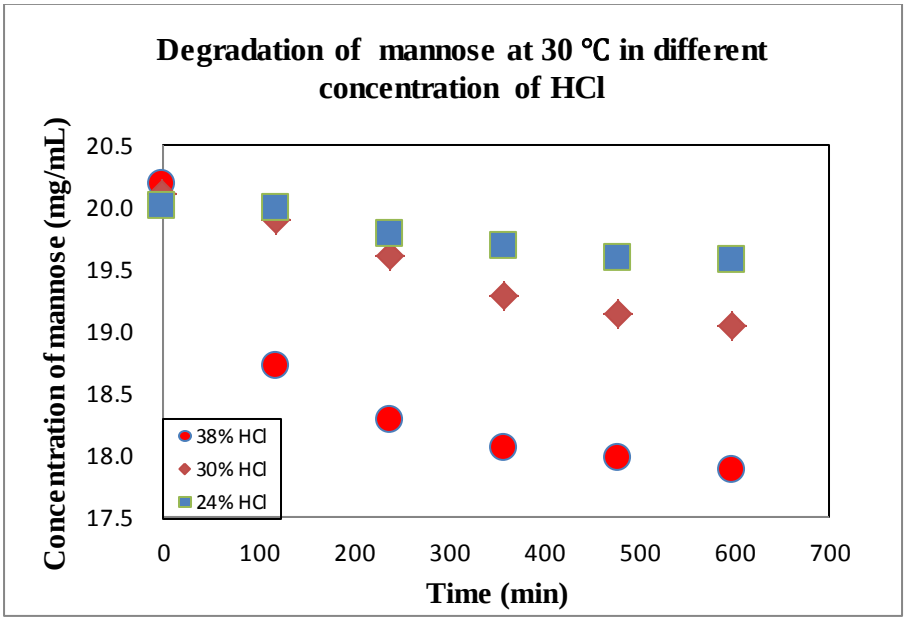

Fig. 3.15 Degradation of mannose in HCl concentration at 30 ℃

The hydrolysis of glucose, xylose, galactose, arabinose and mannose on Table 3.4 and Table 3.7 of 40 ℃, Table 3.1, 3.5 and 3.8 (30 ℃) and Table 3.2 and 3.6 (20 ℃) were presented effects of the concentration of HCl acid on the hydrolysis of hemicellulose monosaccharide. The data demonstrated that hydrolysis has a relationship with concentration of acid. The higher concentration of the acid is, the more hemeicellulose monosaccharide is hydrolyzed. The conclusions of the other temperatures are also presented the same results.

3.3.1.1.3 Different hydrolysis of hemicellulose monosaccharide

Different monosaccharide exhibits different hydrolysis. Among the five monosaccharide derived from hemicellulose, xylose has the highest hydrolysis, while mannose has the lowest. Generally, the hydrolysis of pentose (xylose and arabinose) is higher than that of hexose (glucose, galactose and mannose), which is shown in Fig.3.16.

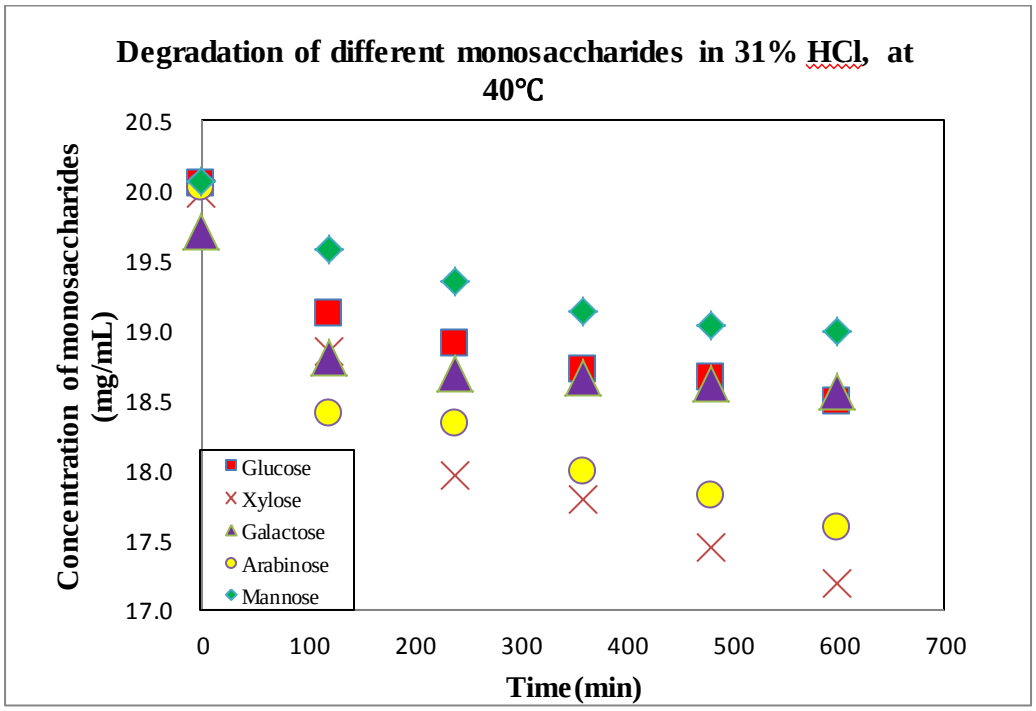

Fig. 3.16 Comparison of degradation of different monosaccharide

3.3.1.2 Generation of furfural and 5-(hydroxymethyl) furfural

The hydrolysis of hemicellulose monosaccharide results in the generation of furfural and HMF as the final products. Similar to the hydrolysis of hemicellulose monosacchardie, the generation of furfural and HMF are dependent on the temperature and concentration of hydrochloric acid. They increase with the increasing of temperature (Fig. 3.17 and 3.18) and concentration of HCl (Fig. 3.19 and 3.20).

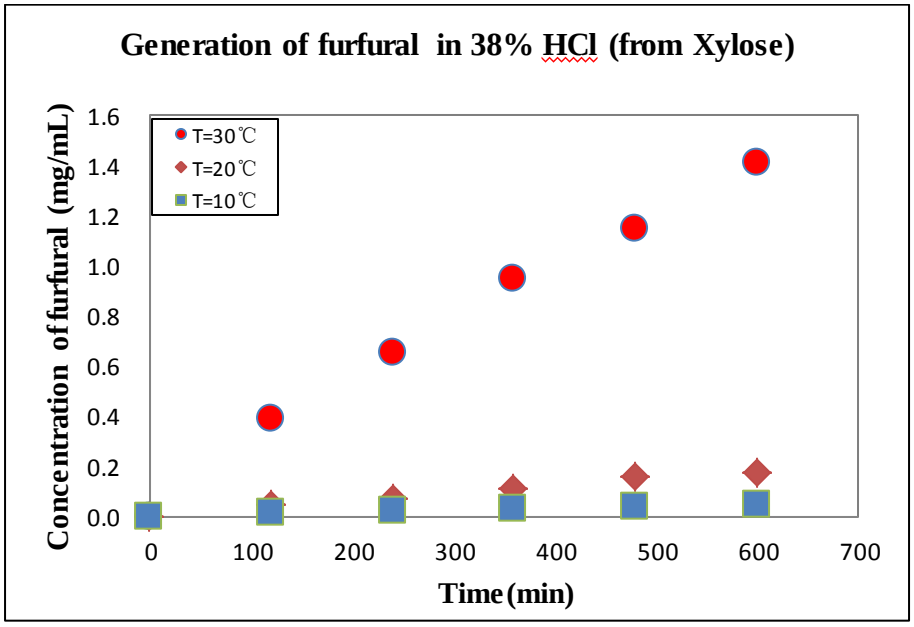

Fig. 3.17 Furfural generation at different temperature in 38% HCl

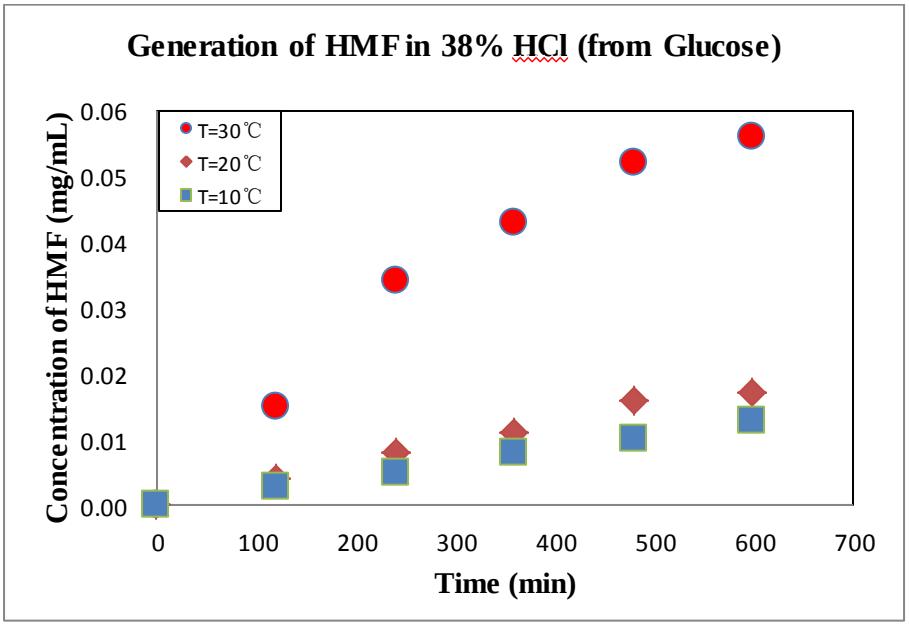

Fig. 3.18 HMF generation at different temperature in 38% HCl

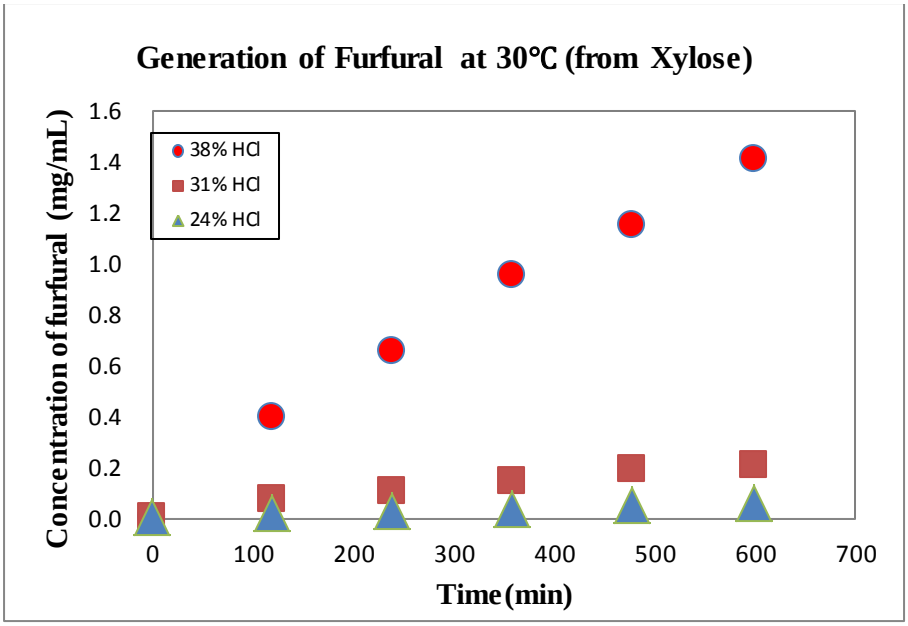

Fig. 3.19 Furfuaral generation at different temperature at 30 ℃

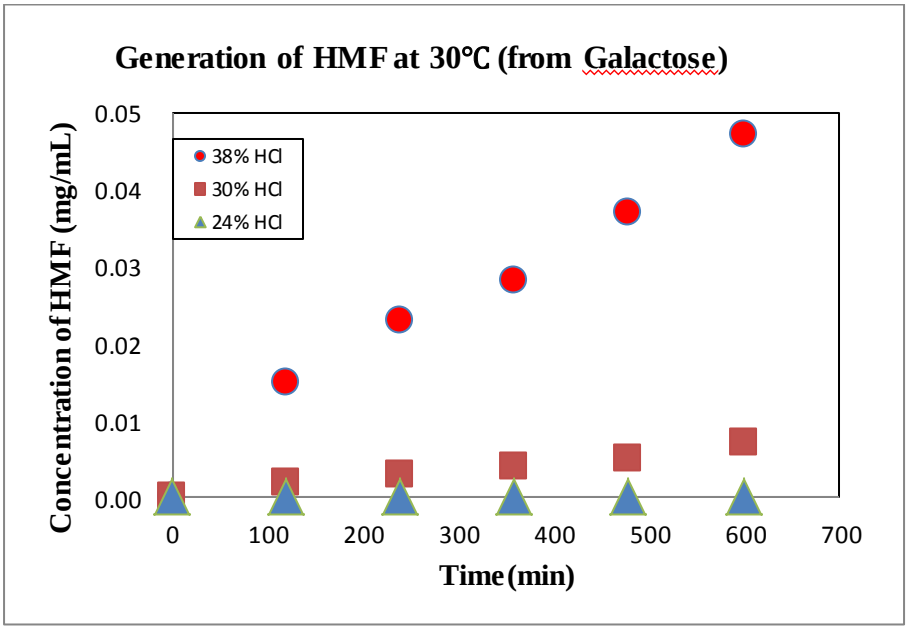

Fig. 3.20 HMF generation at different temperature at 30 ℃

Furfural is generated from the hydrolysis of xylose and arabinose while HMF is from the hydrolysis of glucose, galactose and mannose. The generation of furfural is much higher than HMF and furfural is mostly generated from xylose. Fig. 3.21 shows the result.

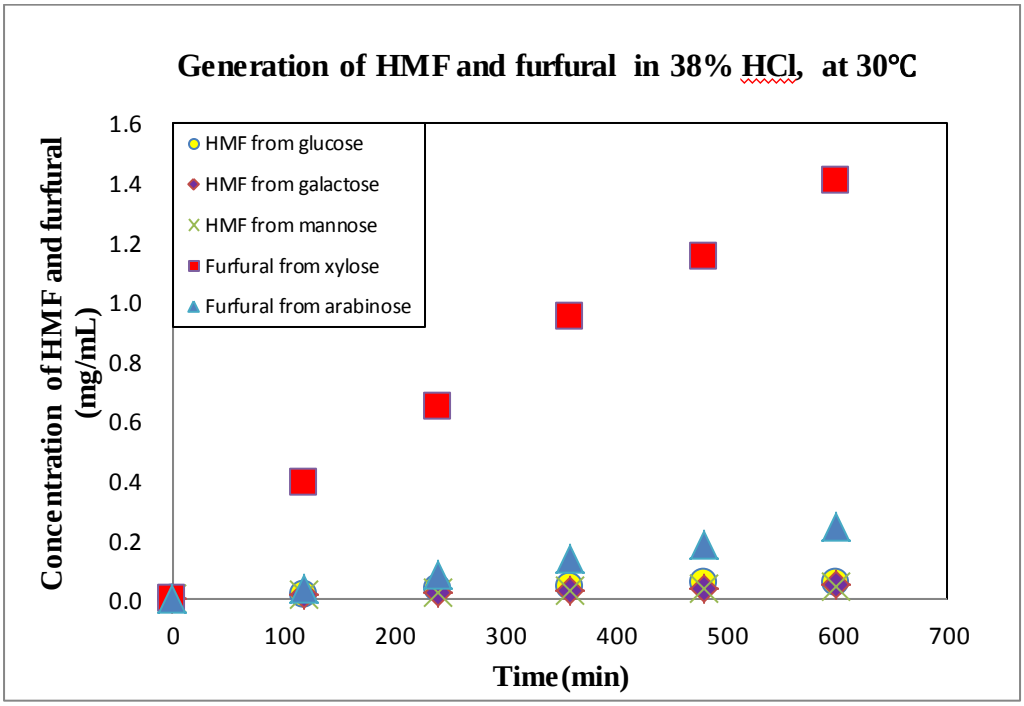

Fig. 3.21 Comparison of HMF and furfural generations

3.3.1.3 Discussions and Conclusion

The main objective of the project is to study the hydrolysis of hemicellulose and cellulose at different temperatures and concentrations of acid. Experiments have been conducted at several different temperatures, including 10°C, 20°C, 30°C and 40°C. Because the maximum concentration of hydrochloric acid at 40 °C is 37%, hydrolysis tests in 38% HCl were only conducted in lower temperatures (10 °C to 30 °C) for safety concerns. The highest concentration of HCl used in the experiments is 38% and it is the maximum concentration commercially available HCl. The other acid concentrations tested in this study are 31% and 24%. The changes of the monosaccharide concentration after 10 hours experiment at 31% HCl , 20 °C and 24% HCl, 30 °C are less than 10%. The hydrolysis is so slow that it can be considered that the hydrolysis has stopped in these two conditions.

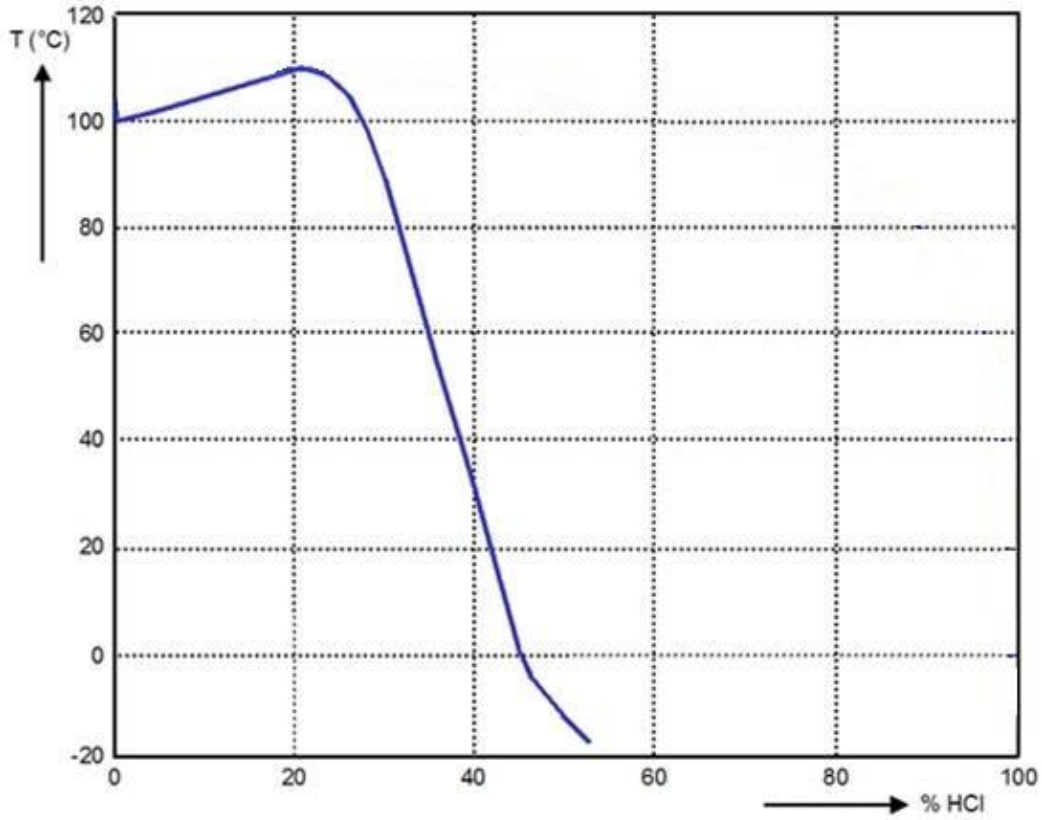

Fig. 3.22 Liquid-vapour phase diagram of binary HCl water mixtures [8]

Although the experimental results indicate that hydrolysis is dependent on the temperature and concentration of acids, they play different roles in the hydrolysis of monosaccharide. Higher temperature lowers the energy barrier to break the glycosidic bond, while higher concentration of hydrochloric acid provides more hydrogen ion to catalyze the hydrolysis reaction. In hydrolysis, temperature is described as a factor of degree however the concentration of hydrochloric acid is a factor of ability. When increasing temperature, the hydrogen bond and the gycosidic bond are more easily to break down because the system provides the enough energy. Hydrochloric acid is a strong acid so that it is fully ionized. The hydrogen ions are released into the solution to attack the glycosidic bond. The experimental results show that changing acid concentration has a stronger effect than changing temperature on hydrolysis: increasing

acid concentration by 7% improves the hydrolysis to a greater extent than elevating the temperature by 10 ℃. The generation of HMF and furfural have a similar dependence on temperature and acid concentration as monosaccharide hydrolysis.

Although the hydrolysis of the five monosaccharides derived from hemicellulose has similar temperature and acid concentration dependence, their actual hydrolysis are different. Experiments are designed to separate these five components in three groups, and each group have at most one pentose and one hexose in order to distinguish the different hydrolysis of each components. The results show that the hydrolysis of xylose is much faster than the other components which are also proved by the accumulation of the furfural which is the final product of xylose degradation. The results also showed that arabinose has the second highest degradation. It can be seen that pentose is generally easier to hydrolyze in concentrated hydrochloric acid than hexose because the solubility of pentose is higher than that of hexose. Moreover, the converting from pentose to furfural is higher than that from hexose to HMF. The ratio of the concentration (after 10 hours degradation) of furfural to HMF is 8 to 10 (Shown on Table 3.1 to Table 3.8). Although the generation of furfural is much higher than that of HMF, xylose hydrolysis has a greater contribution than arabinose hydrolysis. The data demonstrates that the hydrolysis rate of xylose is 5 to 8 times faster than that of arabinose (Shown on Table 1 to Table 8). It seems that xylose is more sensitive to the change of temperature and concentration of acid. Besides, the xylose accounts for more than 50% of all hemicellulose monosaccharide, so the results have important meanings to the hemicellulose hydrolysis industry. In conclusion, the main product of hemicellulose hydrolysis is monosaccharide and the degradation of monosaccharide generates undesired furfural and HMF. Lowing temperature and acid concentration may decrease the

generation rate of furfural and HMF and the hydrolysis rate of hemicellulose monosaccharide.

3.3.2 Cellulose hydrolysis

3.3.2.1 Degradation of cellulose

Unlike complex structural of hemicellulose, cellulose is a polysaccharide which is comprised of G-glucose unit. Cellulose can be hydrolyzed in concentrated or dilute acid solutions and the mechanism is the protonation of the glycosidic oxygen [9], which is a very slow reaction. The H+ ions in water molecule attack the β-(1, 4)-glycosidic bond but the energy barrier of cellulose hydrolysis is much higher than hemicellulose hydrolysis. The process of cellulose hydrolysis is shown in Fig 3.23. The long-chain cellulose firstly breaks into short-chain cellulose resulted from the attack of H^+ ions of HCl, and the short-chain cellulose further break into oligosaccharide. The final product of cellulose degradation is glucose, which can be further converted to HMF in the acid solution. The best way to increase cellulose hydrolysis is to increase the temperature or the concentration of acid.

The study focused on investigating the dependence of cellulose degradation on temperature and concentration of hydrochloric acid. The CrI index and DP of cellulose are obtained by XRD method and viscosity measurement, respectively, while the glucose concentration is measured by HPLC.

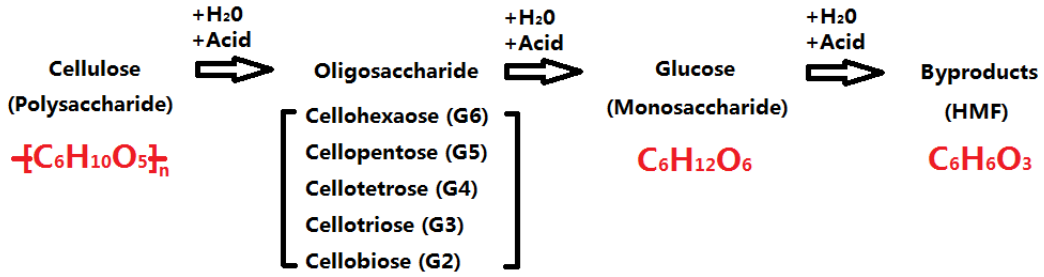

Fig. 3.23 Process of cellulose hydrolysis

3.3.2.2 Effects of Concentration - generation of glucose

Because cellulose is insoluble in water, the concentration of cellulose is difficult to measure. Instead, the dry weight of cellulose has been measured, but the deviation is extremely large. In the study, the generation rate of glucose is measured to derive the hydrolysis rate of cellulose. Glucose, cellobiose (G2), cellotriose (G3), cellotetrose (G4), cellopentose (G5), cellohexaose (G6) are soluble in water and the concentration of them can be measured by HPLC. However, since the peaks of G2 to G6 overlaps with that of hydrochloric acid (Cl^-), only the glucose concentration can be obtained from HPLC.

Although several studies on cellulose degradation have been published, the hydrolysis of cellulose at lower temperature has not yet been thoroughly studied. High concentration hydrochloric acid can be obtained in high-pressure vessels, or alternatively, by decreasing the temperature. In this study, the temperature of the experiments is set at 15 ℃, 5 ℃ and -4 ℃ in order to increase the concentration of hydrochloric acid to 41%.

3.3.2.2.1 Effects of temperature

The generation of glucose at different HCl concentration is shown in Table 3.9, 3.10 and 3.11. The effect of temperature on the generation of glucose (hydrolysis of cellulose) in

41% HCl is shown in Figure 3.24. The figure also indicates that the generation of glucose greatly reduced when the temperature decrease from 15 ℃ to 5 ℃, while the change is much less from 5 ℃ to -4 ℃. The similar results can be obtained from experiments conducted in hydrochloric acid at different concentrations. The results show that the changing temperature positively affects the hydrolysis of cellulose, which is demonstrated by the increasing generation rate of glucose when elevating the temperature. The reason for the temperature dependence is that the energy barrier for breaking the hydrogen bonds, which connects the molecular building blocks of cellulose are lowered with increasing temperature [10].

Table 3.9 Concentration (mg/mL) of the glucose, generate with 41% HCl

	T=15 ℃	T=5 ℃	T=-4 ℃
Time(min)	Glucose(mg/mL)	Glucose(mg/mL)	Glucose(mg/mL)
0	0.048	0.059	0.071
120	0.249	0.101	0.088
240	0.644	0.148	0.098
360	1.110	0.192	0.113
480	2.057	0.250	0.123
600	2.848	0.315	0.135

Table 3.10 Concentration (mg/mL) of the glucose, generate with 38% HCl

	T=15 ℃	T=5 ℃	T=-4 ℃
Time(min)	Glucose(mg/mL)	Glucose(mg/mL)	Glucose(mg/mL)
0	0.114	0.096	0.103
120	0.235	0.149	0.110
240	0.438	0.167	0.116
360	0.720	0.202	0.117
480	1.057	0.236	0.124
600	1.472	0.266	0.128

Table 3.11 Concentration (mg/mL) of the glucose, generate with 38% HCl

	T=15 ℃	T=5 ℃
Time(min)	Glucose(mg/mL)	Glucose(mg/mL)
0	0.003	0.000
120	0.053	0.002
240	0.107	0.006
360	0.167	0.009
480	0.209	0.011
600	0.234	0.016

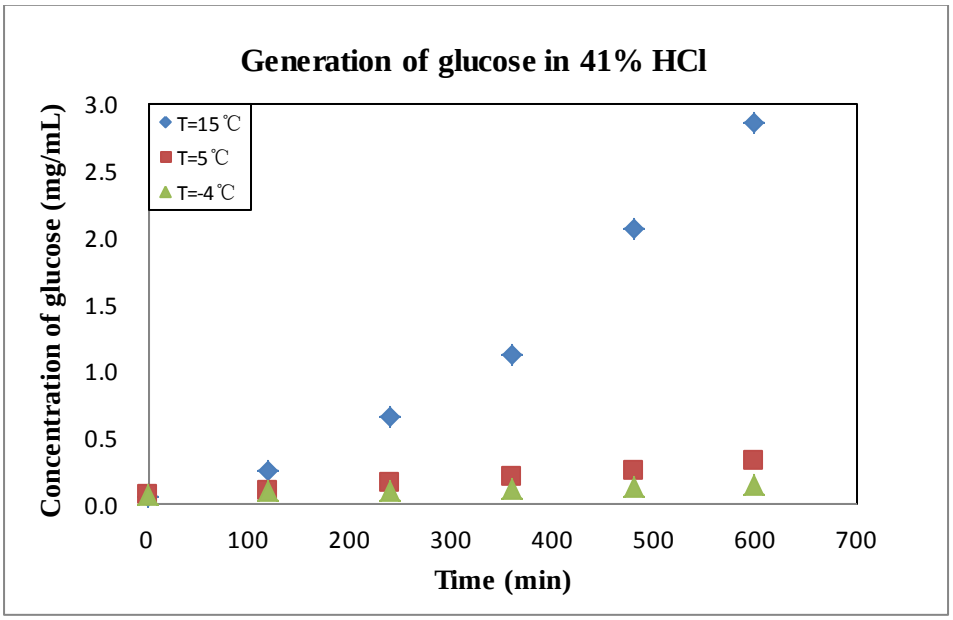

Fig. 3.24 Generation of glucose at different temperature in 41% HCl

3.3.2.2.2 Effects of concentration of HCl

Figure 3.25 presents an example of the effect of concentration of HCl on the generation of glucose (hydrolysis rate of cellulose) at 15 ℃. The generation is increased with increasing concentration of hydrochloric acid (Table 3.9, 3.10 and 3.11). It has been reported that hydrolysis of cellulose consist of three steps. The first step is the protonation of the oxygen atom on glycosidic bond by the attack of hydrogen ions (H^+). In the following step, a positive charge from the glycosidic bond gradually shifts to C1 atom

and a carbonium ion is formed with the break of C-O bond. In the last step, an OH^- is obtained from the carbonium ion by the attack of a H_2O molecule, followed by the release of a hydrogen ion and finally the glucose is generated. Similar to hemicellulose, cellulose is a polysaccharide which is constructed by β-1, 4 glyosidic bond and the glycosidic bond is sensitive to acid. The bond would break down when the reaction system has a high concentration of hydrogen ions and high temperature facilitates this reaction.

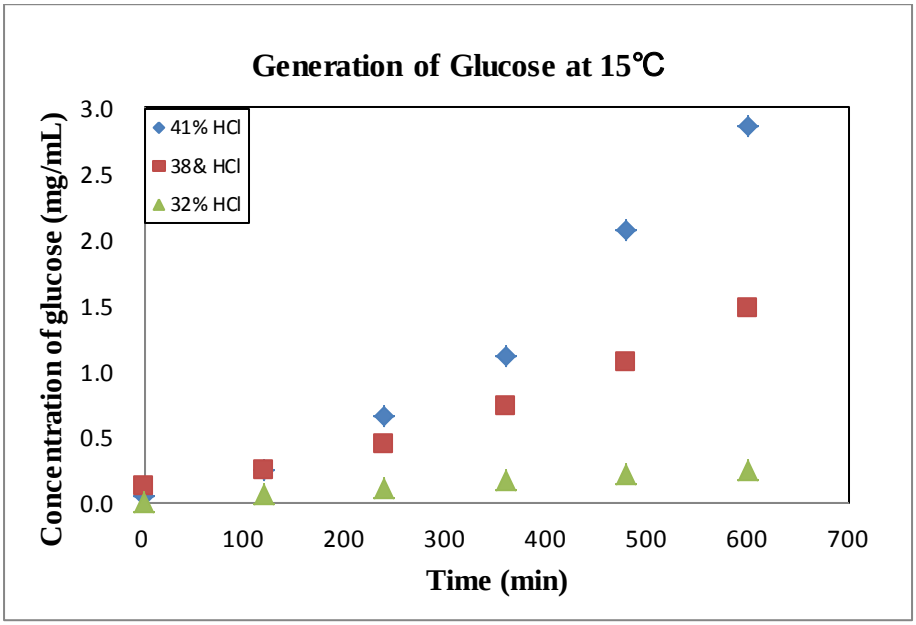

Fig. 3.25 Generation of glucose at different concentration of HCl at 15 ℃

3.3.2.2.3 Xylose generation

Because the cellulose samples contain 5% hemicellulose, xylose generation is detected from the HPLC analysis. The generation of xylose has been demonstrated to be dependent on temperature and concentration of hydrochloric acid as aforementioned. The hydrolysis increased with increasing temperature and the concentration of the hydrochloric acid. The results are shown on Fig. 3.26 and Fig. 3.27.

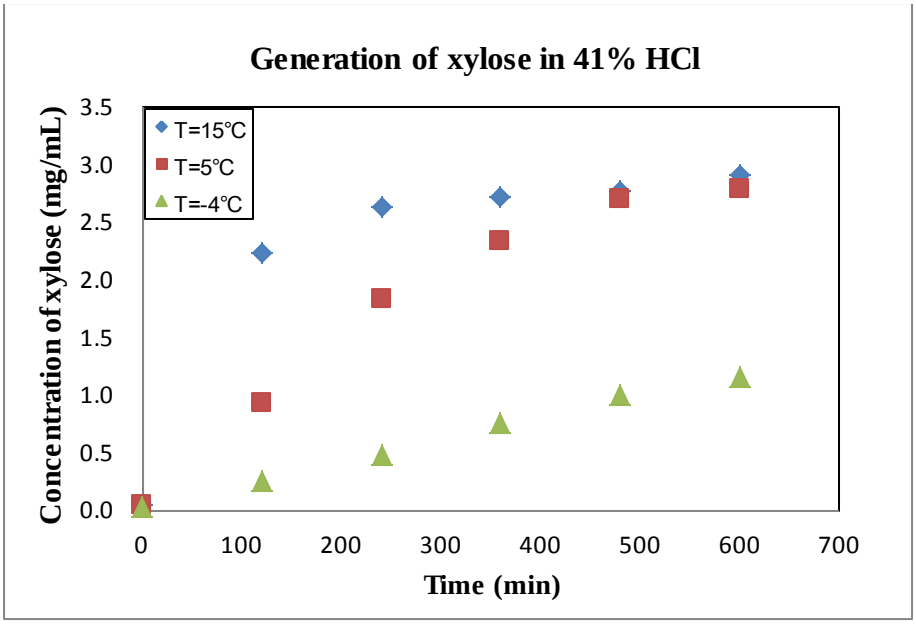

Fig. 3.26 Generation of xylose at different temperature in 41% HCl

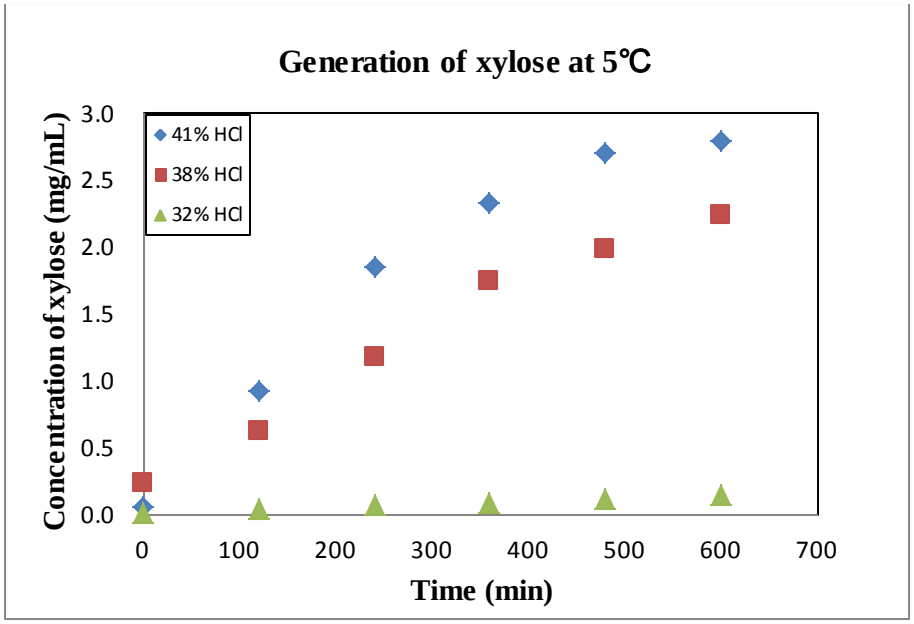

Fig. 3.27 Generation of xylose in different HCl concentration at 5 ℃

The experimental results show hemicellulose concentrations in different conditions are a same value, 3mg/mL, which presented the maximum concentration of xylose. It shows that the hemicellulose can be fully hydrolyzed to xylose, while the hydrolysis of cellulose

cannot reach the same level. Fig. 3.28 demonstrates that the generation of xylose is much faster than that of glucose. The reason for the hydrolysis difference is that the chain length of hemicellulose is much shorter and there are more amorphous regions in the hemicellulose crystalline constructions. It is more difficult for water molecule to permeate into the crystalline regions so that the resistance of cellulose to hydrolysis is much higher than that of hemicellulose.

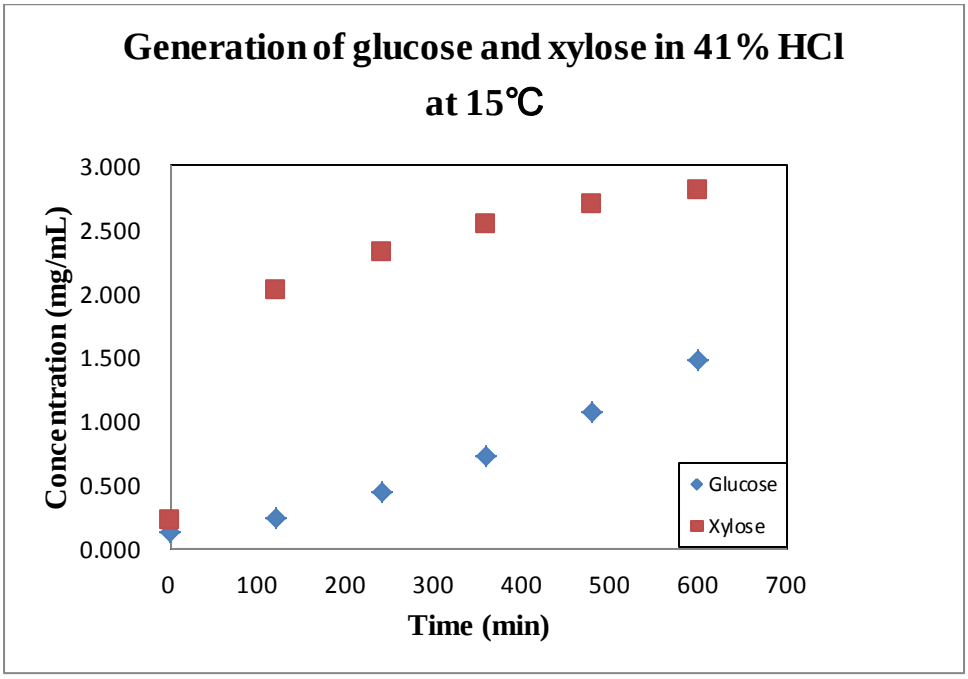

Fig. 3.28 Generation of xylose and glucose in same conditions

3.3.2.3 Change of Degree of polymerization (DP)

Unlike glucose, cellulose is insoluble in common organic and inorganic aqueous solutions, so the degree of cellulose hydrolysis has to be measured in different ways. Degree of polymerization (DP) is dependent on chain length of cellulose, which decreases when cellulose is hydrolyzed. The DP value of cellulose from different plant

origins differs. For example, DP of wood pulp cellulose is between 300 to 1700 and that of cotton and other plant fibers varies from 800 to 10000 [6].

Because cellulose is polymer, its degree of polymerization can be described by number average degree of polymerization (DP_n), weight average degree of polymerization (DP_w) and viscosity average degree of polymerization (DP_v) by different measurement methods [11]. Among these values, DP_v is the most commonly used and convenient indicator of DP and it shows a good correlation to the polymer properties. In the study, DP_v is measured by Ubberlohde viscometer.

The results showed that the DP_v value decreases during cellulose hydrolyzing in the hydrochloric acid solution. It is also shown that the decreasing rate of cellulose-DP value increased with increasing temperature and hydrochloric acid concentration. Fig. 3.29 showed the change of DP in different conditions. The original DP of the cellulose is 907-920. After ten hours hydrolysis, the final DP values of cellulose residue are 669 (41% HCl, 13 ℃), 733 (41% HCl, 5 ℃), 842 (41% HCl, -4 ℃), 774 (38% HCl, 15 ℃), 843 (38% HCl, 5 ℃), 890 (38% HCl, -3 ℃), 824 (32% HCl, 15 ℃) and 853 (32% HCl, 5 ℃) (the date is shown on Table 12).

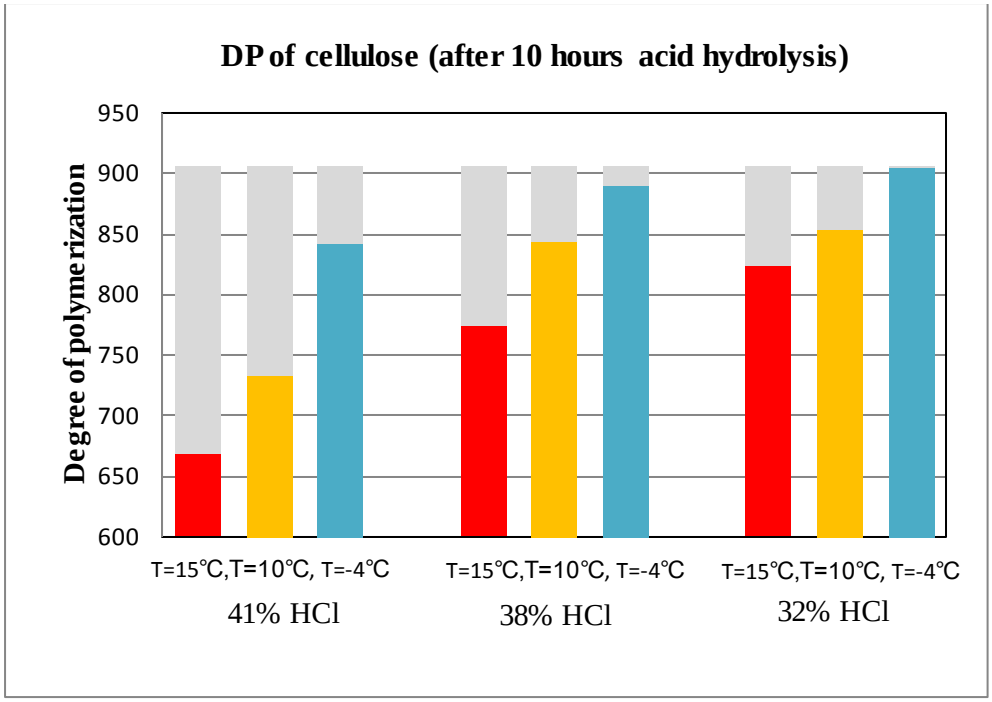

Fig. 3.29 Change of cellulose DP after being treated in hydrochloric acid for 10 hours

Table 3.12 Degree of polymerization of cellulose hydrolysis after 10 hours

Reation Conditions												
Concentration of HCl	Temperature	Time(min)	T(s)	Mass(g)	η_r	η_{sp}	C(g/mL)	η_{sp}/C	$lg(\eta_{sp}/C)$	K_1C	[η](mL/g)	DP
Original			130.37	0.0100	1.4444	0.4444	0.0010	444.3829	2.6478	0.0001	394.846	907
41	13℃	600	120.93	0.0100	1.3398	0.3398	0.0010	339.7961	2.5312	0.0001	309.711	669
41	5℃	600	123.46	0.0100	1.3678	0.3678	0.0010	367.8263	2.5656	0.0001	332.936	733
41	-4℃	600	127.79	0.0100	1.4158	0.4158	0.0010	415.7988	2.6189	0.0001	371.985	842
38	15℃	600	125.09	0.0100	1.3859	0.3859	0.0010	385.8852	2.5865	0.0001	347.738	774
38	5℃	600	127.84	0.0100	1.4164	0.4164	0.0010	416.3528	2.6195	0.0001	372.431	843
38	-3℃	600	129.70	0.0100	1.4370	0.4370	0.0010	436.9599	2.6404	0.0001	388.938	890
32	15℃	600	127.10	0.0100	1.4082	0.4082	0.0010	408.1542	2.6108	0.0001	365.820	824
32	5℃	600	128.24	0.0100	1.4208	0.4208	0.0010	420.7844	2.6241	0.0001	375.994	853

T_0(s)	V
90.26	10mL

Cellulose molecule is constructed from D-glucose building blocks through 1,4-β glycosidic bonds and straight chains are joined together by hydrogen bond. In order to hydrolyze cellulose, hydrogen bond is broken first and then the H^+ ions can permeate into the crystalline regions of the cellulose to attack the glycosidic bond. When the glycosidic bond and the hydrogen bond are destroyed, the length of the chain will decrease. As a result, temperature and concentration of H^+ ions are both significant factors which affect the change of DP value of cellulose. The cellulose hydrolysis experiment in 38% HCl and 32% HCl at -4 ℃ does not show too much DP change after 10 hours-reaction and it may be because low temperature system cannot supply enough energy to break the hydrogen bond.

3.3.2.4 Change of Crystallinity Index

Cellulose is a crystal material and it has several allomorphs. Cellulose in nature is mostly cellulose I, but two other forms, cellulose I_α and I_β also exist. Crystallinity Index (CrI) is defined as the ratio of crystalline region and amorphous region and it may be used as an indicator of the level of degradation of the crystal structure by hydrochloric acid. In this study, the cellulose sample is obtained from wood pulp so that the main crystal form is cellulose I_β and the crystallinity Index is detected by X-ray diffraction machine [12].

In the project, the CrI index decreases as the cellulose is hydrolyzed in hydrochloric acid. The changing rate of CrI is increased with increasing temperature when the temperature is above 0 ℃. However the decrease of CrI index is even faster when the temperature is below 0 ℃. The experimental results are shown in Fig. 3.30 (hydrolysis in 41% HCl) and Fig. 3.31 (hydrolysis in 38% HCl).

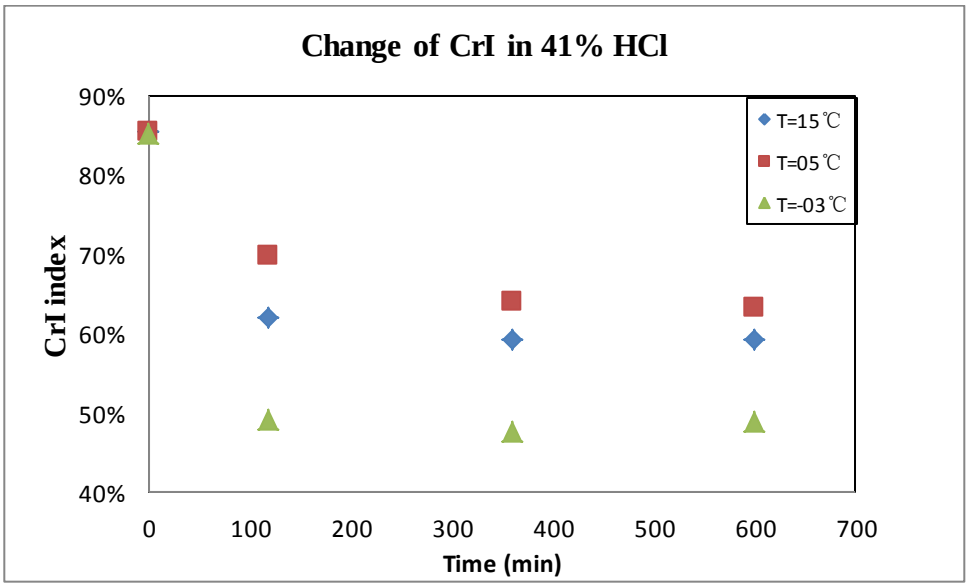

Fig. 3.30 Change of Crystallinily Index in 41% HCl

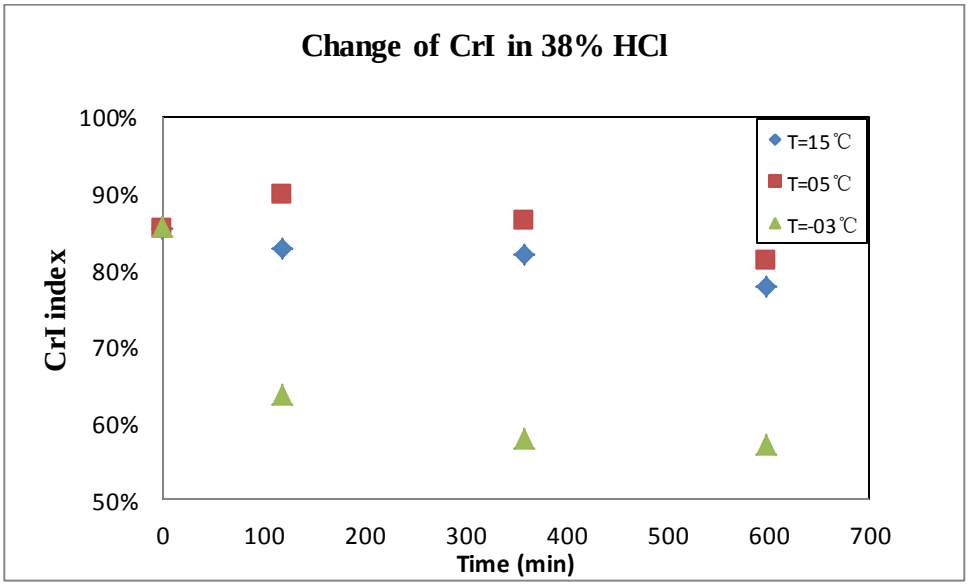

Fig. 3.31 Change of Crystallinily Index in 38% HCl

In Fig. 3.32 and Fig. 3.33, the decrease of crystallinity consists of two steps. The CrI decreases steeply in the first two hours of the experiment and then the decreasing speed

becomes slower in the next 8 hours. Interestingly, the CrI value shows an increase when the hydrolysis is conducted in 38% HCl at 5 ℃. The cellulose material has two regions, amorphous region and crystalline region, between which the amorphous region is more unstable and easily to be destroyed. When the cellulose is hydrolyzed in acid, the amorphous area is firstly damaged so that the CrI increases for a short period of time. The reason for this phenomenon is not detected in the other conditions is that the amorphous area is easily to be destroyed and the reaction time for this process is short. The first sampling time is at two hours and the amorphous region has been depleted so that it cannot be detected and recorded.

Unlike the degree of polymerization, the crystallinity index shows an increased changing rate when the temperature is below 0 ℃. One possible reason is that water is frozen below 0 ℃ and because water molecule plays an essential role in cellulose hydrolysis, the mechanism of hydrolysis may change.

The other reason for the rapid decrease of CrI index of frozen cellulose may be calculation method of CrI. The crystallinity index is calculated as the fraction of the crystalline region. The peak of lattice plane (002) appears when 2θ equals to 22.7 and it is the most significant value when calculating the CrI index. However, the data from X-ray diffraction show two different spectra when the temperature is above or below 0 ℃, which can be seen in Fig.3.29 and 3.30. The lattice plane appears when 2θ=15.7 °, 22.5 ° and 34.2 ° if the temperature is above 0 ℃. However, only one peak at 2θ=22.7° has been detected when the temperature drops below 0 ℃. Since the CrI calculation methods from previous publications was only applied to systems above 0 ℃, they couldn't be directly used to calculate the CrI of frozen

cellulose encountered here [13-15]. In the study, the differentiation of the amorphous areas and crystalline areas is depended on the value of 2θ. The CrI value of crystalline regions is collected when 2θ=14.86 °, 16.67 ° and 22.98 ° and that of the amorphous areas is collected when 2θ=19.78 °, 27.53 °and 34.50 °.

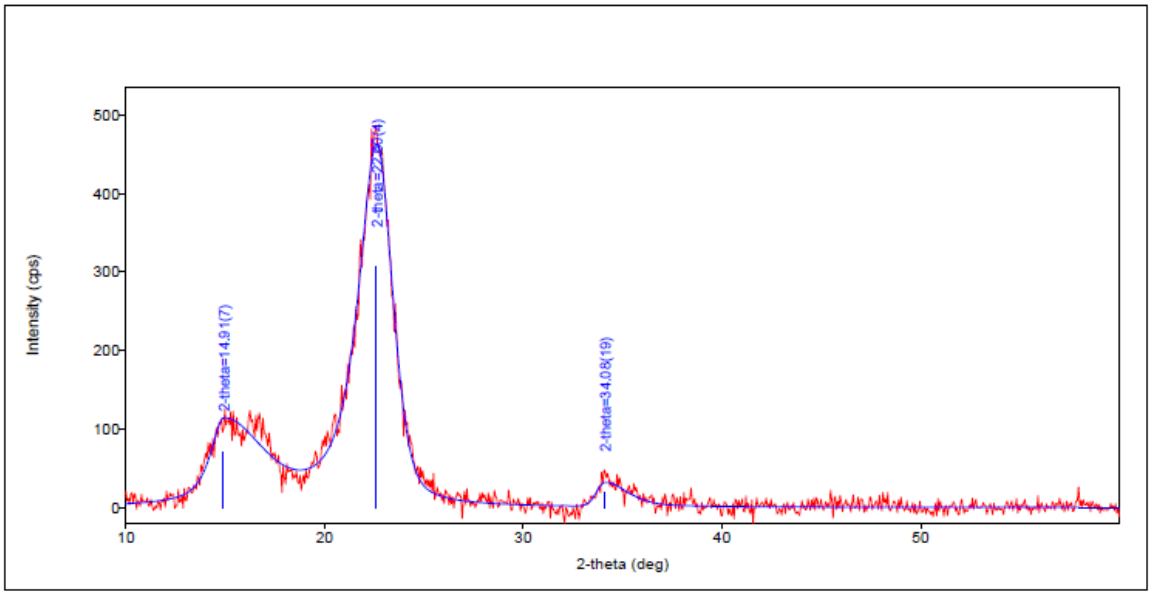

Fig. 3.32 The XRD spectrum of cellulose hydrolysis, 38% HCl, 15℃

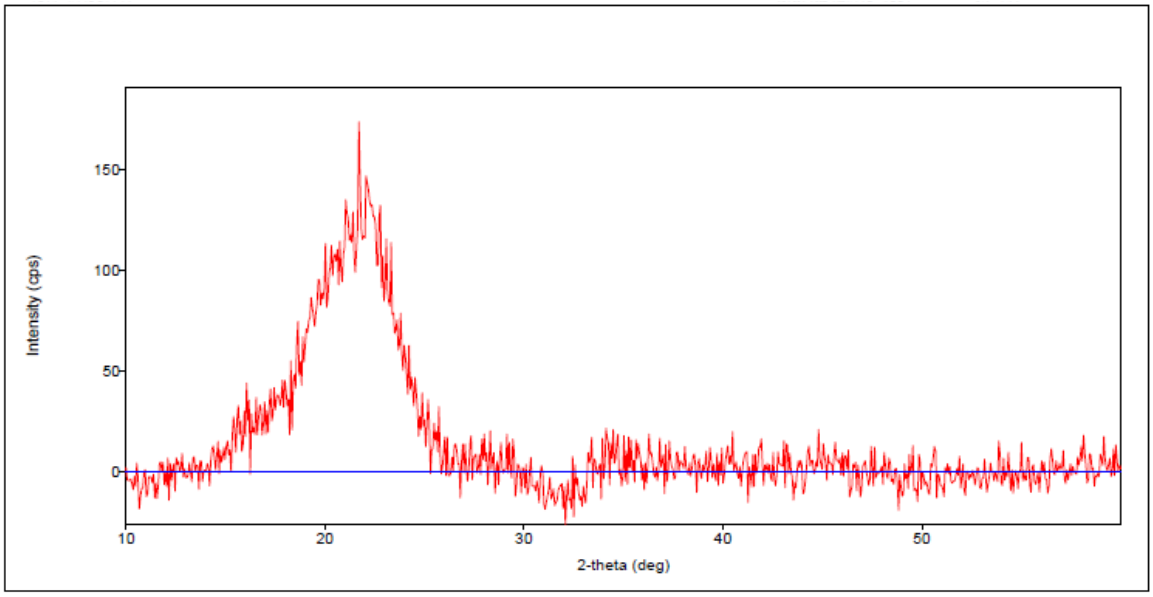

Fig. 3.33 The XRD spectrum of cellulose hydrolysis, 38% HCl, -3℃

Similar to the effect of temperature, increasing the concentration of hydrochloric may speed up the CrI change rate above 0 ℃ and a rapid decrease of cellulose CrI is observed when the temperature is below 0 ℃ (as can be seen in Fig. 3.34 and Fig. 3.35). In the figures, the use of 41% HCl leads to faster change of CrI than 38% HCl and 32% HCl. However, the difference between the CrI changing rate in 38% HCl and 32% HCl is relatively small.

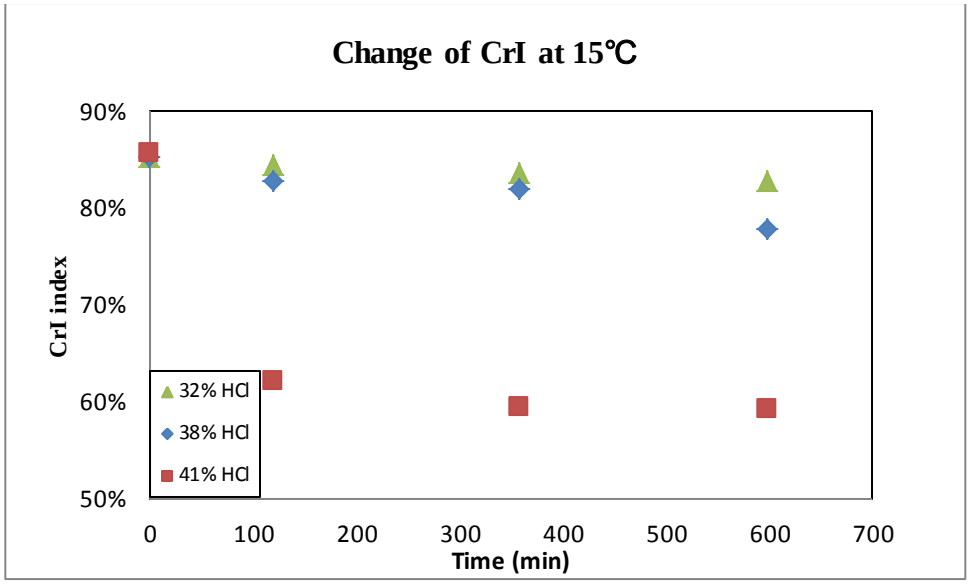

Fig. 3.34 Change of Crystallinily Index at 15 ℃

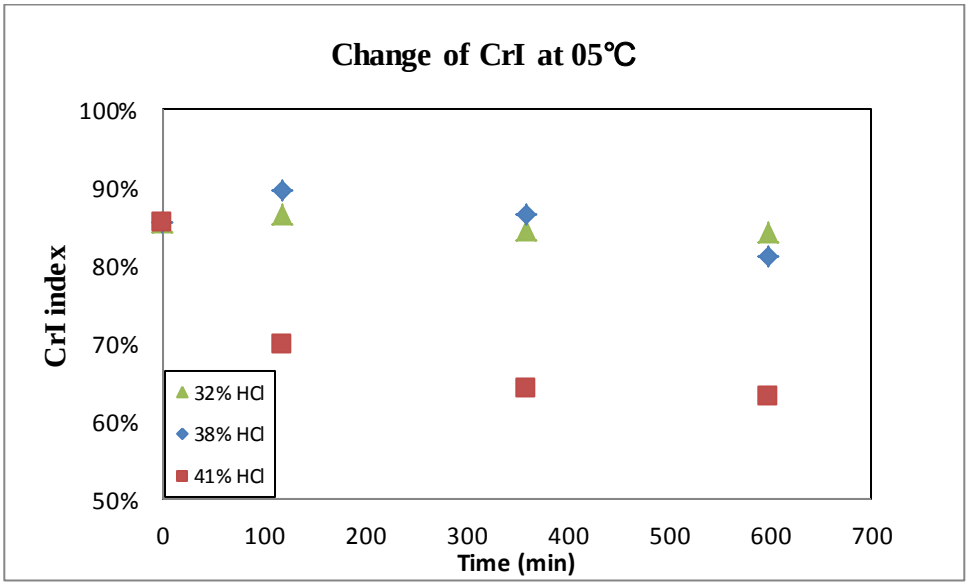

Fig. 3.35 Change of Crystallinily Index at 5 ℃

The change of the CrI demonstrates that the crystalline structure has been destroyed by acid solution and the rate of damage is increased with increasing temperature (above 0 ℃) and concentration of hydrochloric acid. When the temperature is below 0 ℃, the XRD spectrum

changes and lots of peaks disappear. The experimental result under 0 ℃ shows a rapid decrease of CrI , which may be resulted from the frozen water molecule in the acid solutions. Further investigation is required to fully understand this phenomenon.

In conclusion, the hydrolysis of cellulose and generation of glucose increases by increasing the temperature and the hydrochloric acid concentration. Although the mechanisms of these two methods are different, similar results are obtained.

3.3 Error analysis

The possible errors and their effects are discussed in this section. The measurements of this study are conducted with high performance liquid chromatography (HPLC), x-ray diffraction (XRD) and Ubbelohde viscometer. All the liquid phase HPLC analysis have been conducted twice to ensure the repeatability of the data. Because all data obtained from HPLC spectrum show the area of peaks by RID, while the value of concentration can be calculated from standard curve, the standard curve experiment is repeated three times to ensure accuracy. The results of error analysis show that the maximum deviation of standard curve is 4.5%. Figure 3.36 shows an example of glucose standard curve.

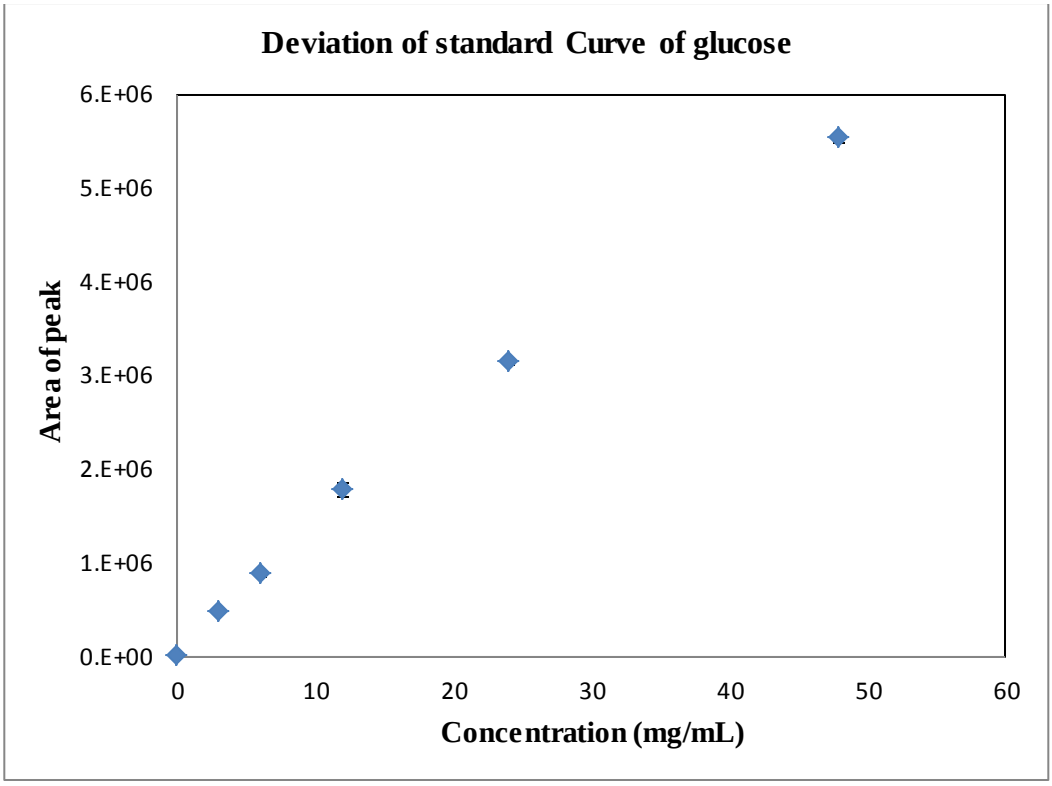

Fig. 3.36 Deviation of standard curve of glucose

One fifth of the hemicellulose monosaccharide hydrolysis experiments have been conducted twice and two samples have been collected each time for error analysis. The results show that the deviation of hemicellulose monosaccharide hydrolysis is less than 3.2%. An example of the error analysis of monosaccharide hydrolysis is shown in Fig. 3.37. The same error analysis method is also applied to cellulose hydrolysis. The results show that the maximum deviation is 10.6%. Fig. 3.38 shows an example.

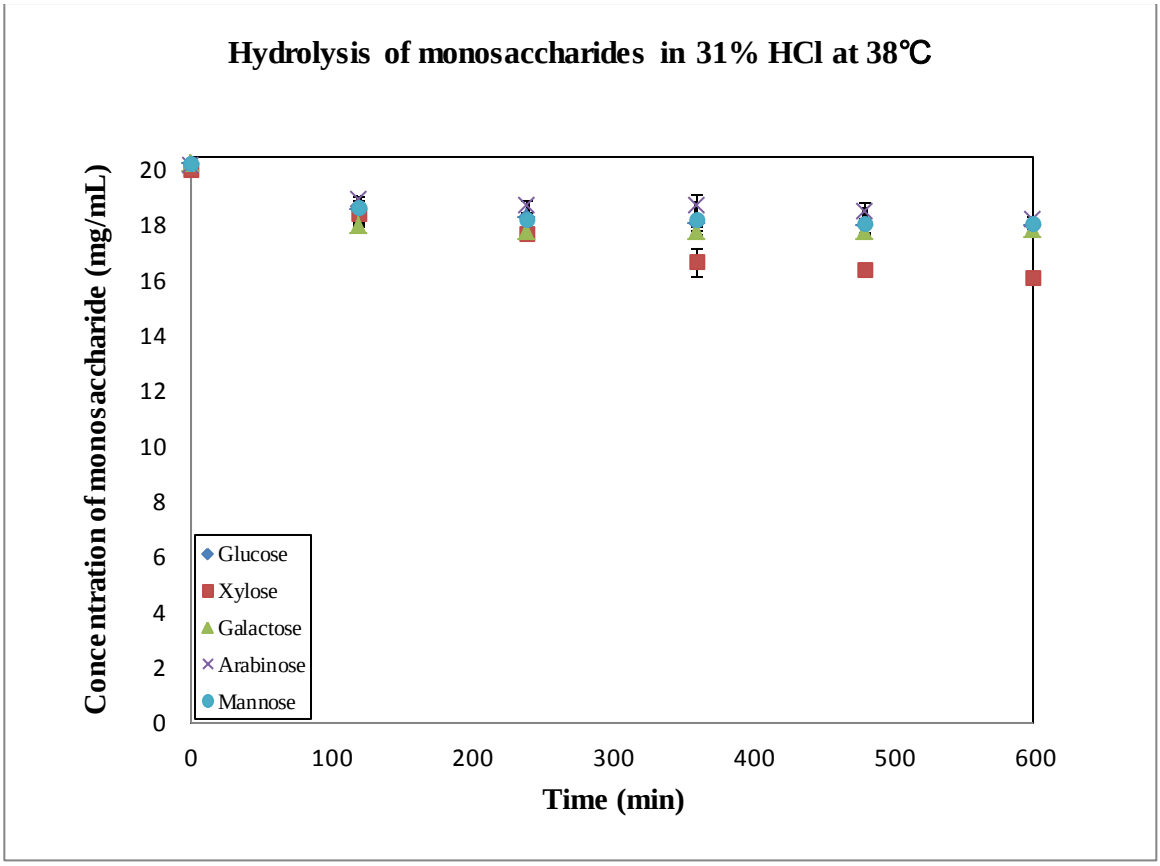

Fig. 3.37 Hydrolysis of monosaccharides in 31% HCl at 38 ℃

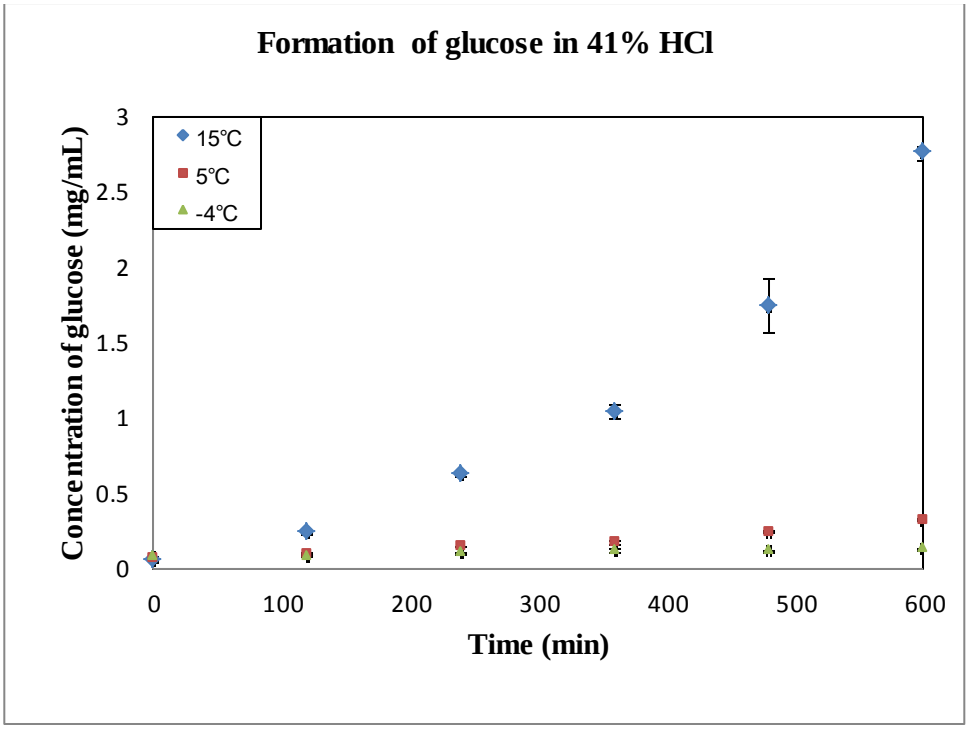

Fig. 3.38 Generation of glucose in 41% HCl at different temperatures

Because the XRD analysis is more time consuming and expensive, all XRD analysis are only conducted once. For the Ubberlohde viscometer, all samples have been tested three times and the data obtained from stop watch must be less than one second, which leads to a deviation less than 1%.

The deviation of the experimental results may be caused by one or more possible reasons. First, the insufficient cleaning of the HPLC system may affect the results of the HPLC analysis. Besides, the inaccuracy of metering tools such as pipette, volumetric flask may lead to the error when measuring samples. Lastly, the error by humans could not be ignored when obtaining the data from HPLC. Special circumstances such as one peak overlaps with

another peak or the swift of retention time could result in misinterpretation of the data and inaccurate calculation of the sample concentration.

3.4 Remarkable Summary

In the study, the hydrolysis of hemicellulose monosaccharide in hydrochloric acid is investigated. The concentration of hemicellulose monosaccharide, furfural and 5-hydroxymethyl-furfural (HMF) are measured by high performance liquid chromatography equipped with RID, from which the hydrolysis rate of monosaccharide and the generation rate of furfural and HMF are calculated. The results show that the hydrolysis rate of hemicellulose monosaccharide and generation rate of furfural and HMF are increased by increasing the temperature and the concentration of hydrochloric acid. The mechanism is that higher temperature may facilitate the break-down of hydrogen bond between cellulose molecules and higher concentration of acid may provide larger number of hydrogen ions which attack the glycosidic bond to degrade monosaccharide. Because furfural and HMF is undesired for monosaccharide fermentation, the optimal condition for minimizing the monosaccharide hydrolysis is to set temperature and concentration of hydrochloric acid as low as possible. However the low temperature and concentration of acid may also decrease the form of monosaccharide, the best method is decrease one of the parameter, temperature or concentration. Compare with the two conditions, concentrated acid at lower temperature and dilute acid at higher temperature, the first one have higher economic efficiency. So that lignocellulose hydrolysis in concentrated acid at low temperature should be the most appropriate condition to reduce the amount of inhibitors.

The hydrolysis of cellulose in concentrated hydrochloric acid at low temperatures is also studied in the work. In the liquid phase, the concentration of glucose which is the main hydrolysis product of cellulose is measured by HPLC with RID. The generation rate of glucose shows a dependence on temperature and concentration of hydrochloric acid. The generation rate is increased with increasing temperature and concentration of hydrochloric acid. In the solid phase, two main factors which can describe the degree of hydrolysis of cellulose are studied, including the degree of polymerization (DP) and the crystallinity index (CrI). The DP and CrI are measured by Ubbelohde viscometer and x-ray diffraction machine, respectively. DP indicates the average chain length of cellulose molecules and it decreases when the cellulose is hydrolyzed in HCl. The change rate of DP increased by the elevating the temperature and concentration of hydrochloric acid. CrI measures the degree of crystallization of cellulose material. CrI is also decreased when cellulose is degraded in acid solutions and the change rate of CrI increased with increasing hydrochloric acid concentration and temperature above 0℃. When the hydrolysis temperature is below 0 ℃, the change rate of CrI is much higher than that at above 0 ℃. This phenomenon is possibly caused by the different mechanism of cellulose hydrolysis below 0 ℃ or the inaccurate calculation method of CrI.

Because the cellulose samples contain hemicellulose, the product of hemicellulose hydrolysis is also discovered. For the cellulose samples, which contain 5% hemicellulose are derived from wood pulp, the degradation product of hemicellulose is mainly xylose and the hydrolysis rate is also increased with increasing temperature and concentration of the hydrochloric acid. The results show that the hydrolysis rate of hemicellulose is much higher

than that of cellulose. Last but not the least, as an unwanted byproduct, HMF did not detect in all the cellulose hydrolysis experiment showed glucose almost not degrade to HMF at low temperature in high concentrated hydrochloric acid.

Reference

[1] Birgit Kamm, Patrick R. Gruber, et al., 2006. Biorefineries- Industrial Processes and Products. Chapter 3.

[2] Irving S. Goldstein, Helena Pereira, et al., 1983. The hydrolysis of cellulose with superconcentrated hydrochloric acid. Biotechnology and bioengineering symp., 13, 17-25.

[3] A. Sluiter, B. Hammes, et al., Determination of sugars, byproducts, and degradation products in liquid fraction process samples, National renewable energy laboratoty, 2008. NREL/TP-510-42623.

[4] Chen Y., Wang Y., Wan J, Ma Y, Crystal and pore structure of wheat straw cellulose fiber during recycling. Cellulose, 2010, 17, 329-338.

[5] Murside Kes, Bjorn E. Christensen, 2013. A re-investigation of the Mark–Houwink–Sakurada parameters for cellulose in Cuen: A study based on size-exclusion chromatography combined with multi-angle light scattering and viscometry. Journal of Chromatography A, 1281, 32-37

[6] Herbert Sixta, 2006. Handbook of pulp. ISBN-10: 3-527-30999-3.

[7] Birgit Kamm, Patrick R. Gruber, et al., 2006. Biorefineries- Industrial Processes and Products. Chapter 6.

[8] Gemilin L., 1981. Gemelin handbook of inorganic and organometallic chemistry.

[9] Fengel D., Wegener G., 1989. Wood- Chemistry, ultrastructure, reactions. Walter de Gruyter

[10] Ma Xiaojuan, Huang Liulian, Chen Lihui, et al., 2012. Determination methods for crystallinity of cellulose. Paper Science and Technology, 31, 2.

[11] Yi-Heng Percival Zhang, Lee R. Lynd, 2004. Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulose systems. Wiley InterScience.

[12] Noppadon Sathitsukasanoh, Zhiguang Zhu, et al., 2010. Cellulose solvent-based biomass pretreatment breaks highly ordered hydrogen bonds in cellulose fibers of switchgrass. Biotechmology and bioengineering.

[13] Joseph A. Rollin, Zhiguang Zhu, et al., 2011. Increasing cellulose accessibility is more important than removing lignin: a comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia. Biotechnology and bioengineering, 108: 22-30.

[14] Su-jin Kim, Jinho Jang, 2013. Effect of degree of polymerization on the mechanical properties of regenerated cellulose fibers using synthesized 1-allyl-3-methylimidazolium chloride. Fibers and polymers, 14,6,909-914.

[15] Yangmei Chen, Yan Wang, et al., 2010. Crystal and pore structure of wheat straw cellulose fiber during recycling. Cellulose, 17:329-338.

CHAPTER 4

Conclusions and Future Work

4.1 Conclusions

The increasing environmental and economic concerns associated with the use of fossil fuels emphasize the need for the development of novel, cost-effective, and renewable carbon-neutral energy sources. Biomass-derived fuels, although not yet cost-feasible on the required scale, are currently one of the best candidates to achieve these objectives. Lignocellulose, the most abundant type of biomass, can be decomposed to cellulose, lignin and hemicellulose, its constituent components, which in turn are hydrolyzed into simple sugar monomers, used in fermentation processes for the production of such fuels.

In the current work, the formation of byproducts (furfural and HMF) and glucose by the hydrolysis of hemicellulose monosaccharide and cellulose in concentrated hydrochloric acid is studied. The experimental results indicate that the hydrolysis of hemicellulose monosaccharide and the formation of furfural and HMF increase with the increasing of reaction temperatures and hydrochloric acid concentrations. At higher temperatures, inter-molecular hydrogen bonds are less stable, allowing for the higher hydrogen ion concentrations available at high acid concentrations to improve the hydrolysis rates. The different monosaccharide also have different hydrolysis rate.

The hydrolysis of cellulose in concentrated hydrochloric acid at low temperatures is also studied. The glucose formation rate increases with the increasing of temperatures and

hydrochloric acid concentrations. Two items of the solid state cellulose: the degree of polymerization (DP) and the crystallinity index (CrI) were studied. DP represents the length of cellulose chains, and it will decreases when cellulose is hydrolyzed. The change of rate of DP is higher when increasing temperatures and hydrochloric acid concentrations. CrI is the level of crystallinity of crystalline cellulose, which decreases in acid solutions. The change of rate of CrI is higher when increasing the hydrochloric acid concentrations and temperatures above 0 ℃. However, when the hydrolysis conditions were kept below 0 ℃, the rate of change of the CrI was much higher than that at higher temperatures. This may be due to the different mechanism of cellulose hydrolysis above and below 0 ℃, or the calculation method for CrI at those temperatures. Moreover, the xylose formation rate increases with the increasing of temperatures and hydrochloric acid concentrations. Results indicate that the rate of hemicellulose hydrolysis is much higher than that of cellulose.

In conclusion, as an expected product, a high yield rate of glucose and other monosaccharide (xylose, mannose, galactose, arabinose) should increase with the elevating of the temperature and concentration of acid. However the increasing generation rate of monosaccharide in lignocellulose hydrolysis may also promote the formation of byproducts (furfural and HMF). Furfural and HMF are unexpected byproducts of monosaccharide fermentation because they poison the bacteria, an optimal condition which not only increase the amount of monosaccharide but also decrease the amount of byproducts need to be figured out. However the difference of composition of different cellulosic biomass and hydrolysis rate of each component made it difficult to be found. In this study, the hydrolysis of each component in certain conditions has been researched and it can be used to calculate complex biomass

hydrolysis rate. It contributes the generation of academic knowledge and the development of new technologies.

4.2 Suggestions for future work

Experiment of Hemicellulose hydrolysis at lower temperatures and use of super-concentrated hydrochloric acid are suggested. Regarding cellulose hydrolysis, the mechanism of hydrolysis below 0 ℃ should be further characterized. This work used pure components to study the hydrolytic processes, which may not be perfectly represent the more complex actual biomass. The findings should therefore be further validated using actual biomass samples.