CHAPTER II
LITERATURE REVIEW

2.1 Pathways to Biofuel Production

There are several pathways (Sun *et al.*, 2002) to producing biofuel from biomass (Figure 2.1). Two major platforms are defined based on the nature of the conversion process: the gasification platform and the carbohydrate platform.

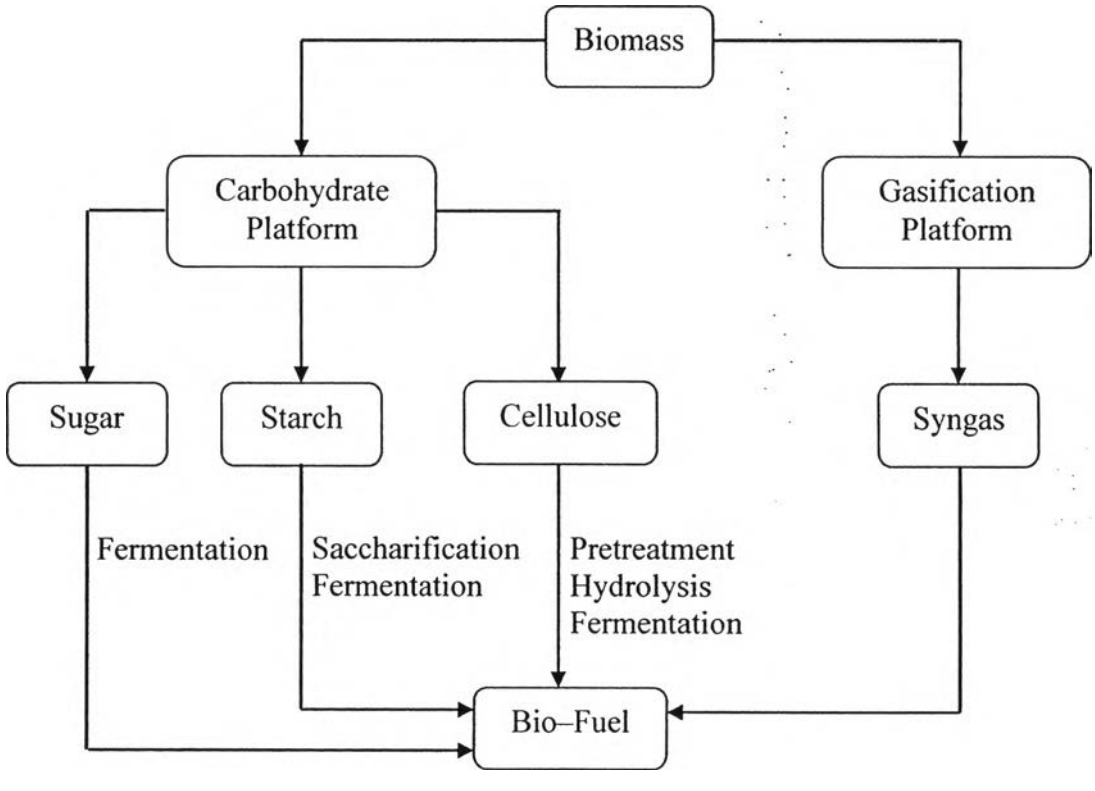

Figure 2.1 Different pathways for the production of biofuel from lignocellulosic biomass.

The latter can be sub–divided into three pathways based on the specific types of carbohydrates at the starting point of the process: lignocellulose pathway, starch pathway and sugar pathway.

2.1.1 Gasification Platform

The gasification platform involves the production of syngas from biomass. Syngas is a mixture of carbon dioxide (CO_2), carbon monoxide (CO), and hydrogen (H_2). There resulting gaseous mixture is then fermented into ethanol by microorganisms. Although syngas can be converted into ethanol and other value–added products using non–biological catalysts, microbial fermentation offers specific advantages. Some of these include high specificity of the biocatalyst, lower energy costs, and the ability to handle varying ratios of components in syngas compiled a list of mesophilic microorganisms capable of producing ethanol from syngas.

2.1.2 Carbohydrate Platform

The carbohydrate platform involves the extraction of carbohydrates from biomass and the subsequent fermentation into biofuels. The sugar pathway is the simplest approach and involves extraction of readily fermentable six–carbon and five sugars presented in biomass such as sugarcane, sugar beet, and sweet sorghum.

The starch pathway is currently the primary means of biofuel production in the carbohydrates in the form of starch are present in large quantities in biomass such as corn, potato, and sweet potato. Corn is the dominant starch feedstock for ethanol production and the industry is quite mature. Unlike the sugar pathway, starches firstly have to be saccharified into simple sugars using hydrolytic enzymes (amylases). These sugars are then fermented into ethanol. Starch is a homopolymer of α–D–glucose, a six–carbon sugar, and exists in two forms: amylose and amylopectin. Individual glucose units are linked via α–1–4 and α–1–6 glycosidic bonds. The nature of these bonds creates polymeric structures that have low crystallinity and are easily hydrolyzed by low cost amylase enzymes.

The lignocellulose pathway is considered a viable long–term option for bioethanol production. Lignocellulosic biomass includes woody biomass, logging residues, dedicated energy crops like switchgrass, miscanthis and poplar, agricultural residue such as wheat straw, corn stover and bagasse, residual pulp from paper mill, municipal solid waste, and wastes from food processing industries. The components of each lignocellulosic biomass are shown in Table 2.1

Table 2.1 Composition of some agricultural lignocellulosic biomass (Keshwani *et al.*, 2007)

	Composition (%, dry basis)		
	Cellulose	**Hemicellulose**	**Lignin**
Corn fiber	15	35	8
Corn cob	45	35	15
Corn stover	40	25	17
Rice straw	35	25	12
Wheat straw	30	50	20
Sugarcane bagasse	40	24	25
Switchgrass	45	30	12
Coastal Bermuda gras	25	35	6

There are several steps to produce biofuels from lignocellulosic biomass. Unlike the starch platform, the carbohydrates in lignocelluloses are not easily accessible for enzymatic hydrolysis. This recalcitrance is primarily due to the composition of lignocellulosic biomass and the step specific components interact with each other. Therefore, the first step is the pretreatment of lignocellulosic biomass to reduce biomass recalcitrance, thereby improving the yield of fermentable sugars. The second step used of hydrolytic enzymes in the lignocellulose pathway. The final step is the fermentation of six–carbon sugars and five–carbon sugars, which can account for 70–80% and 20–30% of the carbohydrate fraction of lignocellulose, respectively.

2.2 The Composition of Lignocellulosic Biomass

Lignocellulose provides structure to plants and is found in roots, stalks and leaves. As shown in Figure 2.2, it is composed of three major components: cellulose (38–50%), lignin (15–30%), and hemicellulose (23–32%) (Keshwani *et al.*, 2007).

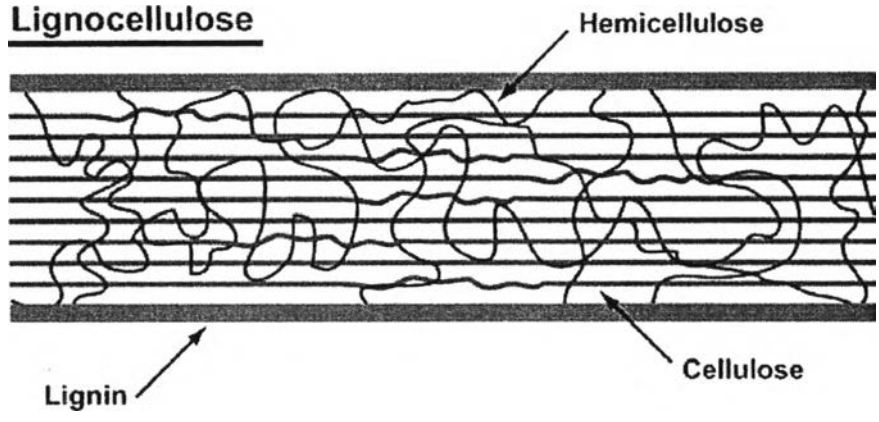

Figure 2.2 Lignocellulose consists of cellulose, hemicellulose and lignin (http://www.sfi.mtu. edu/FutureFuelfromForest/LignocellulosicBiomass.htm).

2.2.1 Cellulose

Cellulose is a homopolymer of β–D–glucose units that are linked via β–1–4 glycosidic bonds. The basic repeat unit of cellulose is cellobiose, which consists of two glucose molecules. The nature of β–1–4 bonds result in the formation of a linear chain of glucose molecules (Figure 2.3). This linearity results in an ordered packing of cellulose chains that interact via inter–molecular and intra–molecular hydrogen bonds involving hydroxyl groups and hydrogen atoms of neighboring glucose units. Consequently, cellulose exists as crystalline fibers with occasional amorphous regions. The crystallinity of cellulose fibers is a major hurdle for efficient enzymatic hydrolysis.

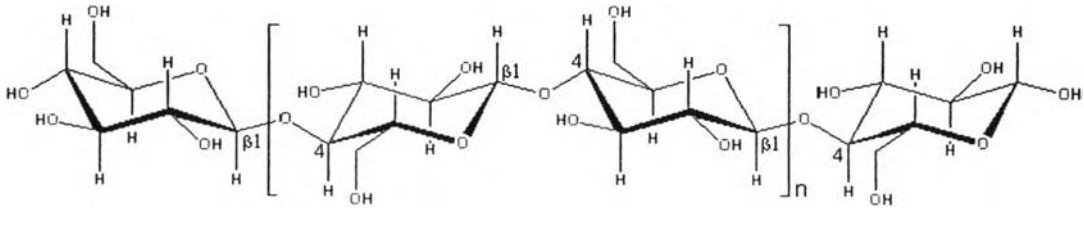

Figure 2.3 Structure of cellulose chain (http://www.thaigoodview.com/library/contest2552/type2/science04/28/P_Untitled–11.html).

2.2.2 Hemicellulose

In contrast to cellulose, hemicelluloses are heteropolymers that are made up of five–carbon sugars such as xylose and arabinose, and six–carbon sugars such as galactose and mannose. While the structure of cellulose is the same for all lignocellulosic biomass, the structure and composition of hemicelluloses can vary. Grasses such as switchgrass contain two types of hemicelluloses. The major hemicellulose is arabinoxylan, which consists of a xylan backbone made up of β–1,4–linked D–xylose units with frequent arabinose side chains (Figure 2.4). Although the backbone xylan structure is similar to cellulose, the presence of arabinose side chains minimizes hydrogen bonding. As a result, hemicellulose has low crystallinity. The minor hemicellulose is glucomannan, which is a copolymeric chain of glucose and mannose units (Figure 2.5). Occasional branching in glucomannan also contributes to the low crystallinity of hemicellulose.

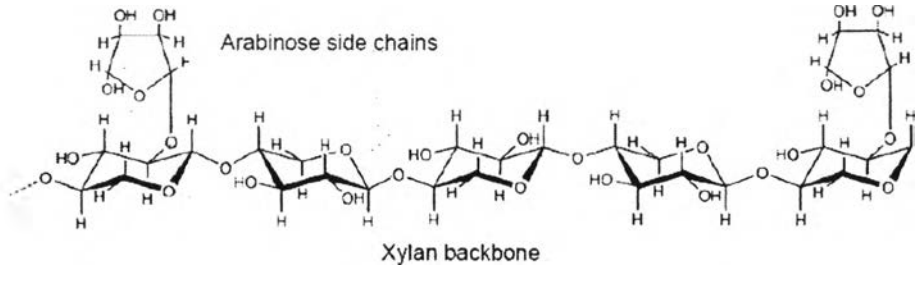

Figure 2.4 Structure of arabinoxylan (Keshwani *et al.*, 2007).

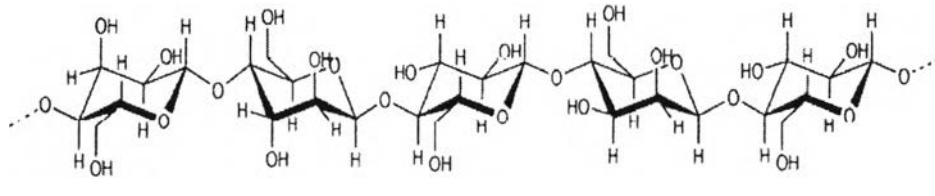

Figure 2.5 Structure of glucomannan (Keshwani *et al.*, 2007).

2.2.3 Lignin

In contrast to cellulose and hemicellulose, the structure of lignin is difficult to depict. Lignin is a highly complex polymer made up of three types of phenolic acids: p–coumaryl alcohol, coniferyl alcohol and synapyl alcohol. These phenolic acids are called monolignols (Figure 2.6) and their proportions vary based on the type of lignocellulosic material. In general, grasses such as switchgrass typically contain equal amounts of all three monolignols. Numerous types of carbon–carbon and ether bonds between individual monolignols result in the formation of dimers, trimers and tetramers that form random linkages with each other resulting in the complex structure of lignin. The carbon–carbon bonds are the strongest and are primarily responsible for the barrier nature of lignin.

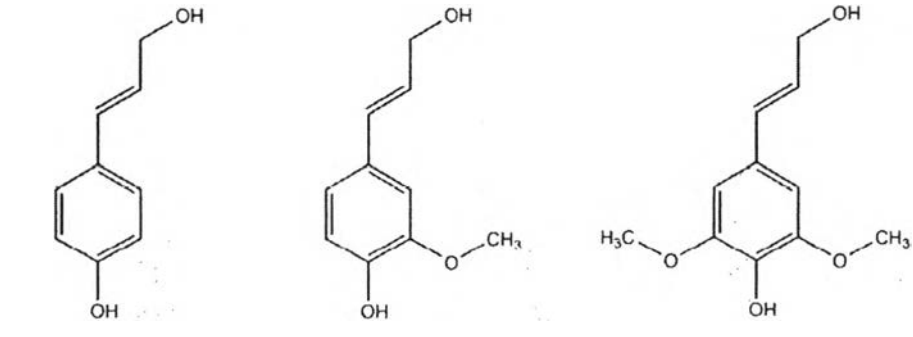

Figure 2.6 Structures of monolignols (Keshwani *et al.*, 2007).

2.3 Pretreatment of Lignocellulosic Biomass

The purpose of the pretreatment is to remove hemicellulose and lignin, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements (as shown in Figure 2.7): (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost–effective (Sun *et al.*, 2002).

The material and energy balance of several pretreatment processes (as shown in Figure 2.8) can be divided into 2 parts: First, raw materials of the process are pretreatment additives, biomass and energy mechanical heat. Second, main product is solid residue consisting of cellulose hemicellulose and lignin. Other products are form of vapor and liquid phase.

Physical, physic–chemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials but this thesis concentrates on chemical pretreatment of corn by dilute acid and enzymatic hydrolysis.

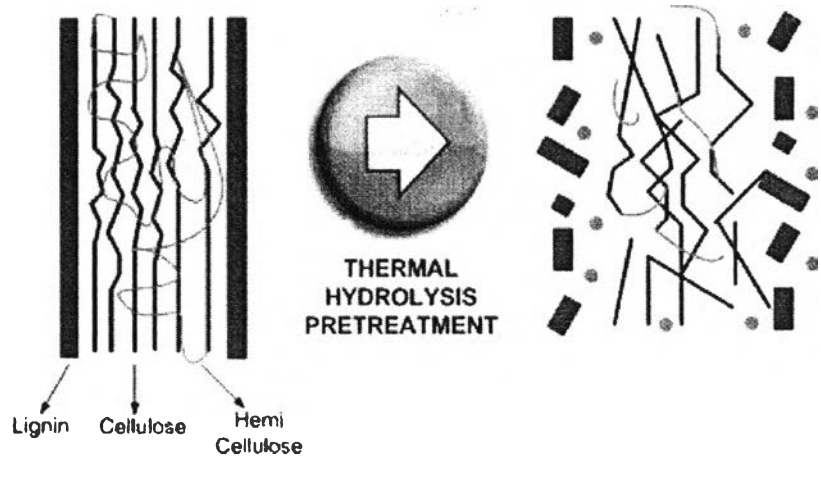

Figure 2.7 Schematic of goals of pretreatment on lignocellulosic material (http://www.hrs–heatexchangers.com/en/applications/bioethanol.aspx).

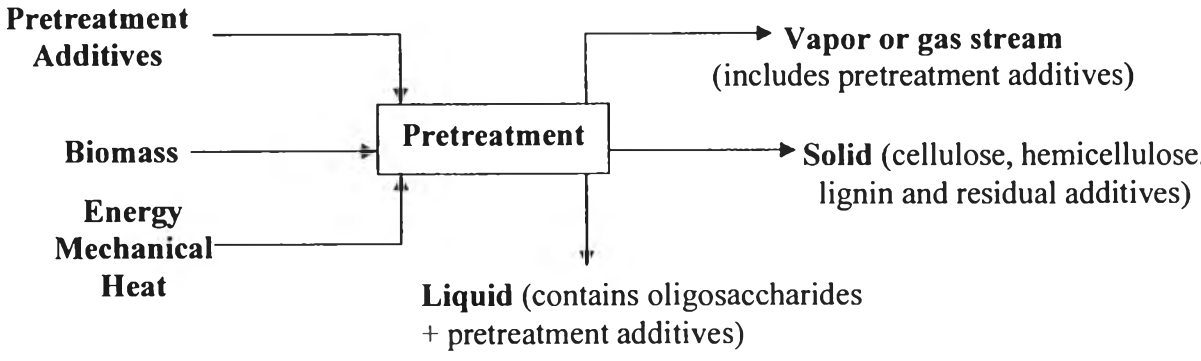

Figure 2.8 Schematic of pretreatment process (Alvira *et al.*, 2009).

2.3.1 Dilute Acid

Dilute or concentrate acids break down the cellulose and hemicelluloses polymers in lignocellulosic biomass to form individual sugar molecules which can be fermented into biofuel. It is important to note that hemicellulose is more easily hydrolysed than cellulose (Lenihan *et al.*, 2010).

The main objective (Alvira *et al.*, 2009) of the acid pretreatments is to solubilize the hemicellulosic fraction of the biomass and to make the cellulose more accessible to enzymes. This type of pretreatments can be performed with concentrated or dilute acid but utilization of concentrated acid is less attractive for ethanol production due to the formation of inhibiting compounds. Furthermore, equipment corrosion problems and acid recovery are important drawbacks when using concentrated acid pretreatments. The high operational and maintenance costs reduce the interest of applying the concentrated acid pretreatment at commercial scale.

Dilute acid pretreatment appears as more favourable method for industrial applications and have been studied for pretreating wide range of lignocellulosic biomass. Different types of reactors such as percolation, plug flow, shrinking–bed, batch and countercurrent reactors have been applied for pretreatment of lignocellulosic materials. It can be performed at high temperature (e.g. 180 °C) during a short period of time; or at lower temperature (e.g. 120 °C) for longer retention time (30–90 min). It presents the advantage of solubilizing hemicellulose, mainly xylan, but also converting solubilized hemicellulose to fermentable sugars.

Nevertheless, depending on the process temperature, some sugar degradation compounds such as furfural, 5–hydroxymethylfurfural (HMF) and aromatic lignin degradation compounds are detected, and affect the microorganism metabolism in the fermentation step. Anyhow, this pretreatment generates lower degradation products than concentrated acid pretreatments.

2.3.2 Factors for an Effective Pretreatment of Lignocellulosic Biomass

There are several key properties to take into consideration for low–cost and advanced pretreatment process (Alvira *et al.*, 2009).

2.3.2.1 High Yields for Multiple Crops, Sites Ages, Harvesting Times

Various pretreatments have been shown to be better suited for specific feedstocks. For example, alkaline–based pretreatment methods such as lime, ammonia fiber explosion (AFEX), and ammonia recycling percolation (ARP), can effectively reduce the lignin content of agricultural residues but are less satisfactory for processing recalcitrant substrate as softwoods. Acid based pretreatment processes have been shown to be effective on a wide range of lignocellulose substrate, but are relatively expensive.

2.3.2.2 Highly Digestible Pretreated Solid

Cellulose from pretreatment should be highly digestible with yields higher than 90% in less than five and preferably less than 3 days with enzyme loading lower than 10 FPU/g cellulose.

2.3.2.3 No Significant Sugars Degradation

High yields close to 100% of fermentable cellulosic and hemicellulosic sugars should be achieved through pretreatment step.

2.3.2.4 Minimum Amount of Toxic Compounds

The liquid hydrolyzes from pretreatment must be fermentable following a low–cost, high yield conditioning step. Harsh conditions during pretreatment lead to a partial hemicellulose degradation and generation of toxic compounds derived from sugar decomposition that could affect the proceeding hydrolysis and fermentation steps. Toxic compounds generated and their amounts depend on raw material and harshness of pretreatment. Degradation products from pretreatment of lignocellulose materials can be divided into the following classes:

carboxylic acids, furan derivatives, and phenolic compounds. Main furan derivates are furfural and 5–hydroxymethylfurfural (HMF) derived from pentoses and hexoses degradation, respectively; Weak acids are mostly acetic and formic and levulinic acids Phenolic compounds include alcohols, aldehydes, ketones and acids.

2.3.2.5 Biomass Size Reduction not Required

Milling or grinding the raw material to small particle sizes before pretreatment is energy–intensive and costly technologies.

2.3.2.6 Operation in Reasonable Size and Moderate Cost Reactors

Pretreatment reactors should be low in cost through minimizing their volume, employing appropriate materials of construction for highly corrosive chemical environments, and keeping operating pressures reasonable.

2.3.2.7 Non–Production of Solid–Waste Residues

The chemicals formed during hydrolyzate conditioning in preparation for subsequent steps should not present processing or disposal challenges.

2.3.2.8 Effectiveness at Low Moisture Content

The use of raw materials at high dry matter content would reduce energy consumption during pretreatment.

2.3.2.9 Obtaining High Sugar Concentration

The concentration of sugars from the coupled operation of pretreatment and enzymatic hydrolysis should be above 10% to ensure an adequate ethanol concentration and to keep recovery and other downstream cost manageable.

2.3.2.10 Fermentation Compatibility

The distribution of sugar recovery between pretreatment and subsequent enzymatic hydrolysis should be compatible with the choice of an organism able to ferment pentoses (arabinose and xylose) in hemicellulose.

2.3.2.11 Lignin Recovery

Lignin and other constituents should be recovered to simplify downstream processing and for conversion into valuable coproducts.

2.3.2.12 Minimum Heat and Power Requirements

Heat and power demands for pretreatment should be low and/or compatible with the thermally integrated process.

2.3.3 Enzymatic Hydrolysis

Enzymatic hydrolysis (Sun *et al.*, 2002) of cellulose is carried out by cellulase enzymes which are highly specific. The products of the hydrolysis are usually reducing sugars including glucose. Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C) and does not have a corrosion problem. Both bacteria and fungi can produce cellulases for the hydrolysis of lignocellulosic materials. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Bacteria belonging to *Clostridium*, *Cellulomonas*, *Bacillus*, *Thermomonospora*, *Ruminococcus*, *Bacteriodes*, *Erwinia*, *Acetovibrio*, *Microbispora*, and *Streptomyces* can produce cellulases. *Cellulomonas fimi* and *Thermomonospora fusca* have been extensively studied for cellulase production. Although many cellulolytic bacteria, particularly the cellulolytic anaerobes such as *Clostridium thermocellum* and *Bacteroides cellulosolvens* produce cellulases with high specific activity, they do not produce high enzyme titres. Because the anaerobes have a very low growth rate and require anaerobic growth conditions, most research for commercial cellulase production has focused on fungi.

Fungi that have been reported to produce cellulases include *Sclerotium rolfsii*, *P. chrysosporium* and species of *Trichoderma*, *Aspergillus*, *Schizophyllum* and *Penicillium*. Of all these fungal genera, *Trichoderma* has been most extensively studied for cellulase production.

Cellulases are usually a mixture of several enzymes. At least three major groups of cellulases are involved in the hydrolysis process: (1) endoglucanase (EG, endo–1,4–D–glucanohydrolase, or EC 3.2.1.4.) which attacks regions of low crystallinity in the cellulose fiber, creating free chain–ends; (2) exoglucanase or cellobiohydrolase (CBH, 1,4–β–D–glucan cellobiohydrolase, or EC 3.2.1.91.) which degrades the molecule further by removing cellobiose units from the free chain–ends; (3) bglucosidase (EC 3.2.1.21) which hydrolyzes cellobiose to produce glucose. In addition to the three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetylesterase, xylanase, β–xylosidase, galactomannanase and glucomannanase. During the

enzymatic hydrolysis, cellulose is degraded by the cellulases to reducing sugars that can be fermented by yeasts or bacteria to ethanol.

2.3.4 Improving Enzymatic Hydrolysis

The factors (Sun *et al.*, 2002) affect the enzymatic hydrolysis of cellulose include substrates, cellulase activity, and reaction conditions (temperature, pH, as well as other parameters). To improve the yield and rate of the enzymatic hydrolysis, research has focused on optimizing the hydrolysis process and enhancing cellulase activity.

2.3.4.1 Substrates

Substrate concentration is one of the main factors that affect the yield and initial rate of enzymatic hydrolysis of cellulose. At low substrate levels, an increase of substrate concentration normally results in an increase of the yield and reaction rate of the hydrolysis. However, high substrate concentration can cause substrate inhibition, which substantially lowers the rate of the hydrolysis, and the extent of substrate inhibition depends on the ratio of total substrate to total enzyme.

The susceptibility of cellulosic substrates to cellulases depends on the structural features of the substrate including cellulose crystallinity, degree of cellulose polymerization, surface area, and content of lignin. Lignin interferes with hydrolysis by blocking access of cellulases to cellulose and by irreversibly binding hydrolytic enzymes. Therefore, removal of lignin can dramatically increase the hydrolysis rate.

2.3.4.2 Cellulase

Increasing the dosage of cellulases in the process, to a certain extent, can enhance the yield and rate of the hydrolysis, but would significantly increase the cost of the process. Enzymatic hydrolysis of cellulose consists of three steps: adsorption of cellulase enzymes onto the surface of the cellulose, the biodegradation of cellulose to fermentable sugars, and desorption of cellulase. Cellulase activity decreases during the hydrolysis. The irreversible adsorption of cellulase on cellulose is partially responsible for this deactivation. Addition of surfactants during hydrolysis is capable of modifying the cellulose surface property and minimizing the irreversible binding of cellulase on cellulose. Cellulases can be

recovered from the liquid supernatant or the solid residues and most recycled cellulases are from the liquid supernatant. Enzyme recycling can effectively increase the rate and yield of the hydrolysis and lower the enzyme cost.

2.4.3.2 End–product Inhibition of Cellulase Activity

Cellulase activity is inhibited by cellobiose and to a lesser extent by glucose. Several methods have been developed to reduce the inhibition, including the use of high concentrations of enzymes, the supplementation of β–glucosidases during hydrolysis, and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation (SSF). The SSF process has been extensively studied to reduce the inhibition of end products of hydrolysis. In the process, reducing sugars produced in cellulose hydrolysis or saccharifications are simultaneously fermented to ethanol, which greatly reduces the product inhibition to the hydrolysis.

SSF has the following advantages: (1) increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity; (2) lower enzyme requirement; (3) higher product yields; (4) lower requirements for sterile conditions since glucose is removed immediately and ethanol is produced; (5) shorter process time; and (6) less reactor volume because a single reactor is used. However, ethanol may also exhibit inhibition to the cellulase activity in the SSF process.

2.4 Literature Review

Aguilar *et al.* (2002) reported that hydrolysis reactions of sugar polymers in a dilute–acid medium were very complex. The substrate was in a solid phase and the catalyst in a liquid phase. The mechanism of the hydrolysis reaction included (i) diffusion of protons through the wet lignocellulosic matrix; (ii) protonation of the oxygen of a heterocyclic ether bond between the sugar monomers; (iii) breaking of the ether bond; (iv) generation of a carbocation as intermediate; (v) solvation of the carbocation with water; (vi) regeneration of the proton with cogeneration of the sugar monomer, oligomer or polymer, depending on the position of the ether bond; (vii) diffusion of the reaction products in the liquid phase if it is permitted for their form and size; (viii) restarting the second step.

Silva *et al.* (2005) studied the effects of sulfuric acid loading and residence time on the composition of sugarcane bagasse hydrolyzates. They found that an increase in acid loading and residence time resulted in an increase in xylose concentration, due to synergistic effects. However, these conditions also led to increased concentrations of inhibiting byproducts such as hydroxymethyl furfural and acetic acid. In their previous study, they evaluated the effectiveness of phosphoric acid to hydrolyze sugarcane bagasse hemicellulose. It showed that an increase in acid loading did not significantly affect the concentrations of xylose, acetic acid, furfural, and hydroxymethyl furfural, likely due to the weak character of the catalyst. The increase in residence time, however, maximized not only the concentration of xylose in the hydrolyzate, but also those of inhibitors. In 2002, Aguilar *et al.* found that acetic acid in hydrolyzates was derived from the hydrolysis of the acetyl groups bound to the hemicellulosic monomers. The acid could be an inhibitor of microbial growth because it entered the cell membrane and decreased intercellular pH, thus affecting the metabolism of the microorganisms in butanol fermentation process.

Gao *et al.* (2008) investigated the influence of the acid hydrolysis reaction on the chemical structure of the silvergrass fibers by using FTIR. They found that dilute acid pretreatment with sulfuric acid would seem to cause the degradation of β–O–4 linkages or another bond rearrangement that resulted in increased carbonyl

groups within the lignin fraction. Moreover, the absorbance of the β–O–4 ether band at 1250 cm^{-1} was significantly decreased after hydrolysis. The increase in the peak at around 1205 cm^{-1} suggested an increased contribution from 2^{nd} OH groups. This was accompanied by the development of double peaks at 1034 and 1060 cm^{-1}, which were indicative of aliphatic OH groups. These results indicated that the linkages within the structure of lignin/hemicellulose complex were probably cleaved by sulfuric acid treatment. Thus, the acid hydrolysis also changed the chemical properties of the lignin, which became more hydrophilic due to the increase in carbonyl and hydroxyl groups.

Chen *et al.* (2009) used enzyme hydrolysis that cellulase was supplemented with cellobiase (2 FPU: 1 CBU) to avoid product inhibition caused by cellobiose accumulation. Batch enzymatic hydrolysis was routinely performed at 8% solid substrate concentration (grams dry weight per 100 mL) in 0.05 mol L^{-1} citrate buffer (pH 4.8), containing 40 mg L^{-1} penicillin to prevent microbial contamination. After the enzymes were added, flasks containing 100 mL of reaction mixture were incubated at 50 °C in a rotary shaker at 150 rpm. Samples were taken from the reaction mixture periodically for analysis.

Lenihan *et al.* (2010) reported the optimum conditions for acid hydrolysis of hemicellulosic biomass in the form of potato peels. The hydrolysis reaction was undertaken in a high pressure pilot batch reactor using dilute phosphoric acid. Process parameters investigated included, reactor temperature (from 135 °C to 200 °C) and acid concentration (from 2.5% (w/w) to 10% (w/w)). The results indicate that high conversion of cellulose to glucose was apparent although arabinose conversion was quite low due to thermally unstability. However, an overall sugar yield is 82.5% was achieved under optimum conditions. This optimum yield was obtained at 135 °C and 10% (w/w) acid concentration. 55.2 g sugar/100 g dry potato peel is produced after a time of 8 min.