CHAPTER II
THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Lignocellulosic-Biomass Materials

Long-term economic and environmental concerns have resulted in a great amount of research in the past couple of decades on renewable sources of liquid fuels to replace fossil fuels. Burning fossil fuels such as coal and oil releases CO_2, which is a major cause of global warming (Yat, 2008).

Conversion of abundant lignocellulosic biomass to biofuels as transportation fuels presents a viable option for improving energy security and reducing greenhouse emissions. Unlike fossil fuels, which come from plants that grew millions of years ago, biofuels are produced from plants grown today. They are cleaner-burning than fossil fuels, and the short cycle of growing plants and burning fuel made from them does not add CO_2 to the atmosphere. It has been reported that cellulosic ethanol and ethanol produced from other biomass resources have the potential to cut greenhouse gas emissions by 86 %. Lignocellulosic materials such as agricultural residues (e.g., wheat straw, sugarcane bagasse, corn stover), forest products (hardwood and softwood), and dedicated crops (switchgrass, salix) are renewable sources of energy. As a result, lignocellulosic biomass has been identified as the most suitable feedstock for biofuel production due to it is sufficiently abundant and generates very low net greenhouse emissions, which makes it a promising feedstock for global bioethanol production (Kumar *et al.*, 2009; Harun *et al.*, 2010).

Currently, bioprocessing of lignocellulosics is focused on enzymatic hydrolysis of the cellulose fraction to glucose, followed by fermentation to fuel-grade ethanol (Mussatto *et al.*, 2008). Table 2.1 shows the advantages of biofuels.

Table 2.1 Major benefits of biofuels (Balet, 2011)

Economic impacts	Sustainability
	Fuel diversity
	Increased number of rural manufacturing jobs
	Increased income taxes
	Increased investments in the plant and equipment
	Agricultural development
	International competitiveness
	Reducing the dependency on imported petroleum
Environmental impacts	Greenhouse gas reductions
	Reducing of air pollution
	Biodegradability
	Higher combustion efficiency
	Improved land and waste use
	Carbon sequestration
Energy security	Domestic targets
	Supply reliability
	Reducing use of fossil fuels
	Ready availability
	Domestic distribution
	Renewability

2.2 Chemical Structure and Basic Components of Lignocellulosic Materials

Lignocellulose is the primary building block of plant cell walls. Plant biomass is mainly composed of cellulose, hemicellulose, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash. The major constituents of lignocellulose are cellulose, hemicellulose, and lignin, polymers that are closely associated with each other constituting the cellular complex of the vegetal biomass (Kumar *et al.*, 2009; Mussatto and Teixeira, 2010). Table 2.2 shows the approximate composition of various biomass materials or agriculture wastes. Basically, cellulose forms a skeleton, which is surrounded by hemicellulose and lignin (Figure 2.1).

Table 2.2 Approximate composition (as a percentage) of various biomass materials or agriculture waste products (Van Dyk and Pletschke, 2012)

Biomass	Cellulose (wt%)	Hemiceluose (wt%)	Lignin (wt%)
Corncobs	45	35	15
Corn stover	39	19.1	15.1
Wheat straw	36.6	24.8	14.5
Rice straw	41	21.5	9.9
Bagasse	38.1	26.9	18.4
Melon shells	35	19	30
Coconut fiber	17.7	2.2	34
Coffee pulp	24	8.9	19.4
Rice hulls	36.1	19.7	19.4

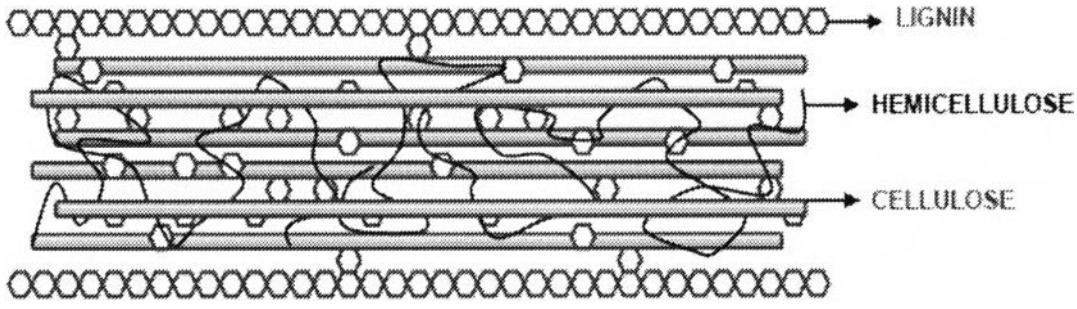


Figure 2.1 Representation of lignocellulosic structure showing cellulose, hemicelluloses, and lignin fraction (Mussatto and Teixeira, 2010).

2.2.1 Cellulose

The major component of plant biomass (30-60 % of total feed stock dry matter) is cellulose. Cellulose is the main structural constituent in plant cell walls and is found in an organized fibrous structure. The structure of cellulose is shown in Figure 2.2. This linear polymer consists of D-glucose subunits linked to each other by β-(1,4)-glycosidic bonds. Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains. The long-chain cellulose polymers are linked together by hydrogen and van der Waals bonds, which cause the cellulose to be packed into microfibrils. Cellulose in biomass is present in both crystalline and amorphous forms. Crystalline cellulose comprises the major proportion of cellulose, whereas a small percentage of unorganized cellulose chains form amorphous cellulose. Cellulose is more susceptible to enzymatic degradation in its amorphous form (Kumar *et al.*, 2009; Balet, 2011).

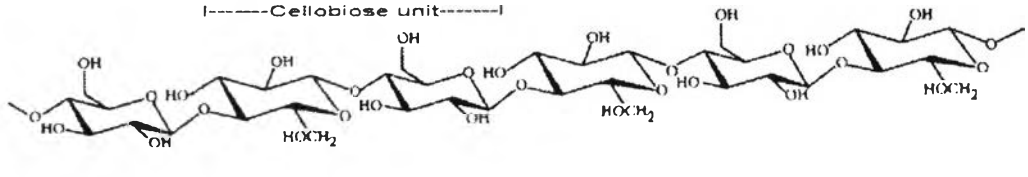


Figure 2.2 Schematic representation of a cellulose chain (Mousdale, 2008).

2.2.2 Hemicellulose

The main feature that differentiates hemicellulose from cellulose is that hemicellulose (20-40 % of total feedstock dry matter) has branches with short lateral chains consisting of different sugars. These monosaccharides, shown in Figure 2.3, include pentoses (xylose, rhamnose, and arabinose), hexoses (glucose, mannose, and galactose), and uronic acids (e.g., 4-*o*-methylglucuronic, D-glucuronic, and D-galactouronic acids). The backbone of hemicellulose is either a homopolymer or a heteropolymer with short branches linked by *β*-(1,4)-glycosidic bonds and occasionally *β*-(1,3)-glycosidic bonds. Also, hemicelluloses can have some degree of acetylation, for example, in heteroxylan. In contrast to cellulose, the polymers present in hemicelluloses are easily hydrolyzable. These polymers do not aggregate, even when they cocrystallize with cellulose chains (Kumar *et al.*, 2009; Balat, 2011).

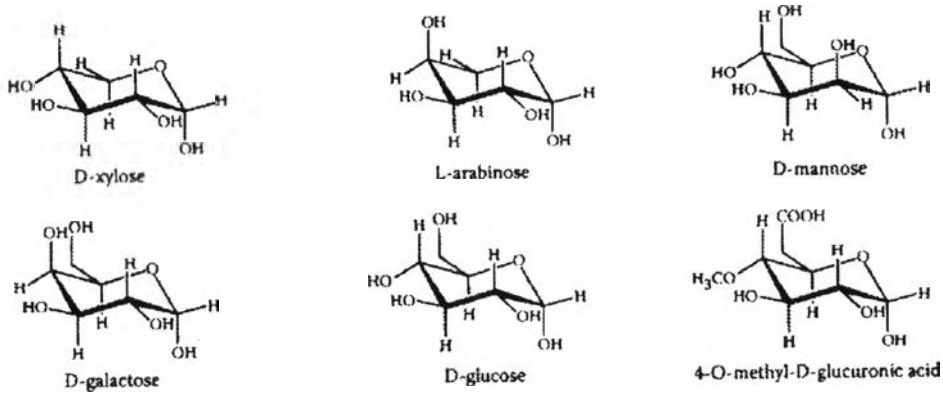
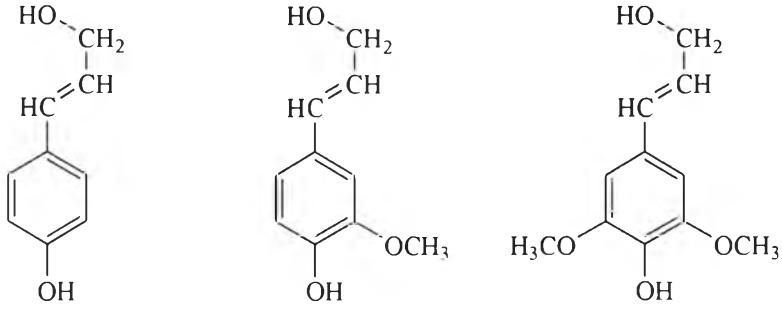


Figure 2.3 Schematic of the basic structure of hemicellulose. A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; X, xylose (Mousdale, 2008).

2.2.3 Lignin

Lignin (15–25 % of total feedstock dry matter) is an aromatic polymer synthesised from phenylpropanoid precursors. The basic chemical phenylpropane units of lignin (primarily syringyl, guaiacyl and p-hydroxy phenol) are bonded together by a set of linkages to form a very complex matrix. This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl and carbonyl, which imparts a high polarity to the lignin macromolecule. Softwood and hardwood lignins belong to the first and second category, respectively. Softwoods generally contain

more lignin than hardwoods. Lignin is one of the drawbacks of using lignocellulosic-biomass materials in fermentation, as it makes lignocellulose resistant to chemical and biological degradation (Balat, 2011). Figure 2.4 shows lignin building blocks.



p-Coumaryl alcohol Coniferyl alcohol Sinapyl alcohol

Figure 2.4 Lignin building blocks.

2.3 Glucose

Glucose is a simple sugar, which is an important carbohydrate in biology. Cells utilize glucose as a source of energy and a metabolic intermediate. One of the main products of photosynthesis is glucose, which starts cellular respiration. However, glucose is useful not only biologically but also chemically. Glucose can be used as the starting raw material to produce a wide variety of chemicals and fuels. This is usually carried out with the help of microorganisms, such as fermentation of glucose to ethanol and conversion of glucose into solvents, e.g. acetone and butanol by *Clostridium acetobutylicum*. Because of the overwhelming quantity of cellulose and the renewable resource, the world will depend on it more heavily for food, fuel, chemical supplies, and raw materials in the future. There is great potential of alleviating the need for petroleum, which is fast decreasing on supply (Wang, 2009).

2.4 Sugar Production from Lignocellulosic Materials

There are several possible ways to hydrolyze lignocelluloses, as shown in Figure 2.5. The most commonly applied methods can be classified into two groups: chemical hydrolysis and enzymatic hydrolysis. In addition, there are some other hydrolysis methods, in which no chemicals or enzymes are applied. For instance, lignocelluloses may be hydrolyzed by ray or electron-beam irradiation, or microwave irradiation. However, those processes are commercially unimportant (Balat, 2011). Several products can result from hydrolysis of lignocellulosic material (Taherzadeh and Karimi, 2007; Demirbas, 2008).

2.4.1 Concentrated Acid Hydrolysis

This process involves an acid (dilute or concentrated) pretreatment to liberate the hemicellulosic sugars, while the subsequent stage requires the biomass to be dried followed by the addition of concentrated sulfuric acid (70-90 %). The acid concentration used in concentrated acid hydrolysis process is in the range of 10–30 %. Reaction times are typically much longer than for dilute acid process. This process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to five-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and cost effectively recovers the acid for recycling (Hayes, 2009; Demirbas, 2008).

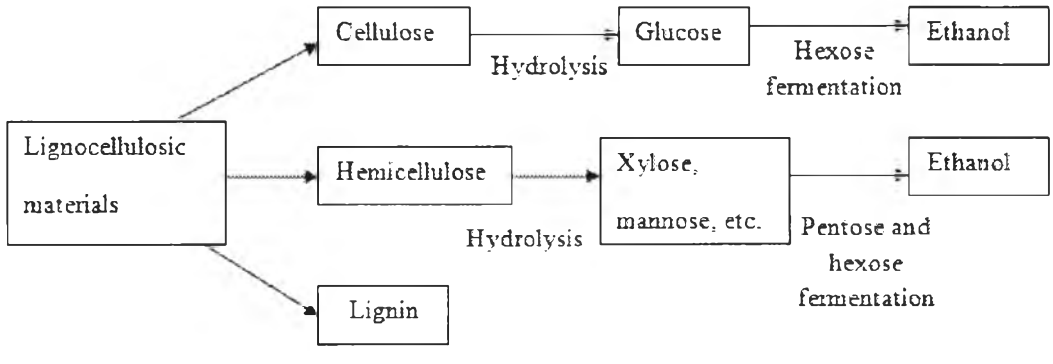


Figure 2.5 Overall view of sugar and ethanol productions from lignocellulosic materials (Eourarekullart, 2011).

2.4.2 Dilute Acid Hydrolysis

Dilute acid hydrolysis is the oldest technology for converting cellulose biomass to bioethanol. This process is conducted under high temperature and pressure, and has a reaction time in the range of seconds or minutes. which facilitates continuous processing. Dilute acid process involves a solution of about 1 % H_2SO_4 concentration in a continuous flow reactor at a high temperature (about 488 K). Most dilute acid processes are limited to a sugar recovery efficiency of around 50 %. The combination of acid and high temperature and pressure dictates special reactor materials, which can make the reactor expensive. The first reaction converts the cellulosic materials to sugar and the second reaction converts the sugars to other chemicals (Balat, 2009). Figure 2.6 shows a flow sheet for dilute acid hydrolysis.

2.4.3 Enzymatic Hydrolysis

Enzymatic hydrolysis has attracted increasing attention as an alternative to concentrated acid hydrolysis because the process is highly specific, can be performed under milder reaction conditions (pH around 5 and temperature less than 50 ^{o}C) with lower energy consumption and lower environmental impact. In addition, it does not present corrosion problems, and gives high yield of pure glucose with low formation of by-products that is favorable for the subsequent hydrolysate use in fermentation processes. Enzymatic hydrolysis of natural lignocellulosic materials is a very slow process because cellulose hydrolysis is hindered by structural parameters of the substrate, such as lignin and hemicellulose content, surface area, and cellulose crystallinity. A large variety of enzymes with different specificities are required to degrade all components of lignocellulosic (Mussatto and Teixeira, 2010; Balat, 2011; Van Dyk and Pletschke, 2012). Table 2.3 shows a comparison of process conditions and performance of three cellulose hydrolysis process. Table 2.4 shows a brief overview of the types of enzymes that are required to degrade complex lignocellulose substrates.

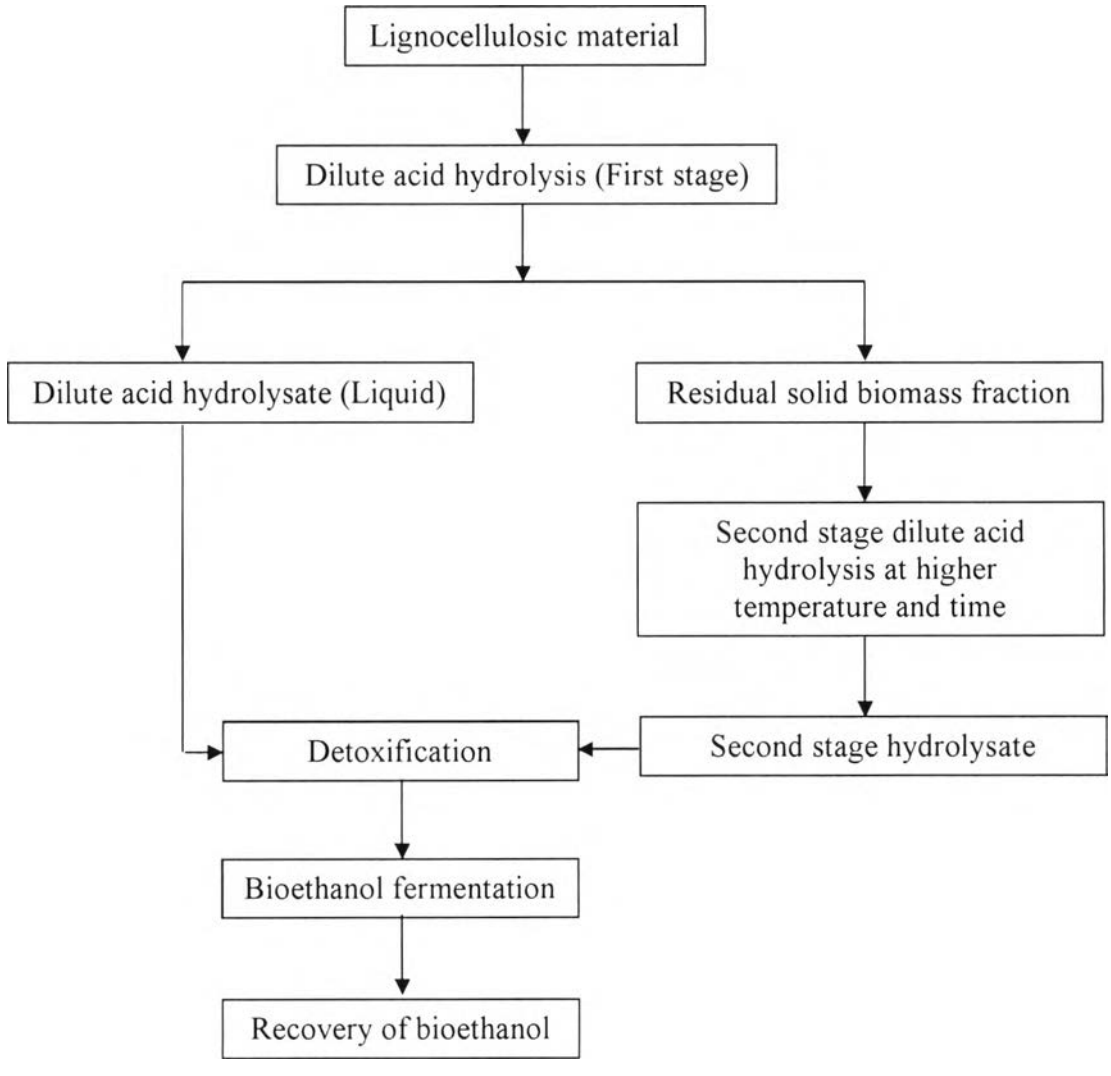


Figure 2.6 Dilute acid hydrolysis (Balat, 2009).

Table 2.3 Comparison of process conditions and performance of three hydrolysis processes (Hamelinck *et al.*, 2005)

	Consumables	Temperature (K)	Time	Glucose yield (%)
Dilute acid	<1 % H_2SO_4	488	3mins	50-70
Concentrated acid	30-70 % H_2SO_4	313	2-6 h	90
Enzymatic	Cellulase	323	1.5days	75-90

Table 2.4 Some of main enzymes required to degrade lignocelluloses to monomers (Van Dyk and Pletschke, 2012)

Lignin	Laccase, Manganese peroxidases, Lignin peroxidase
Pectin	Pectin methyl esterase, pectate lyase, polygalacturonase, rhamnogalacturonan lyase
Hemicellulose	Endo-xylanase, acetyl xylan esterase, β-xylosidase, endo-mannanase, β-mannosidase, α-L-arabinofuranosidase, α-glucuronidase, ferulic acid esterase, α-galactosidase, p-coumaric acid esterase
Cellulose	Cellobiohydrolase, endoglucanase, β-glucosidase

2.5 Cellulase Enzymes

Cellulase is a group of enzymes that synergistically hydrolyzes cellulose, as shown in Figure 2.7. These enzymes are produced by several microorganisms, commonly by bacteria and fungi. These microorganisms can be aerobic or anaerobic, mesophilic or thermophilic. Three major groups of cellulases are involved in the hydrolysis process (Sun and Cheng, 2002; Balat, 2011).

(1) Endoglucanases (EG, endo-1,4-β-D-glucanses, or EC 3.2.1.3.), which hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains randomly to produce new chain ends.

(2) Exoglucanases or cellobiohydrolases (CBH, 1,4- β-D-glucan cellobiohydrolase, or EC 3.2.1.91.), which processively cleave cellulose chains at the ends to release soluble cellobiose or glucose.

(3) β-Glucosidases (BGL, cellobiases or EC 3.2.1.21), which hydrolyze cellobiose to glucose in order to eliminate cellobiose inhibition.

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

Cheng, 2002). A schematic representation of the cellulose enzymes over the cellulose structure is shown in Figure 2.8.

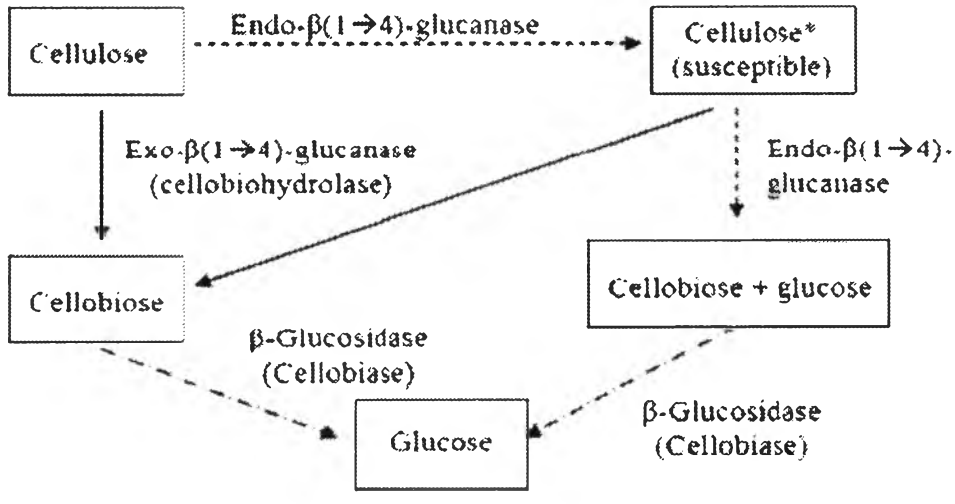


Figure 2.7 Mode of action of cellulolytic enzymes (Balat, 2011).

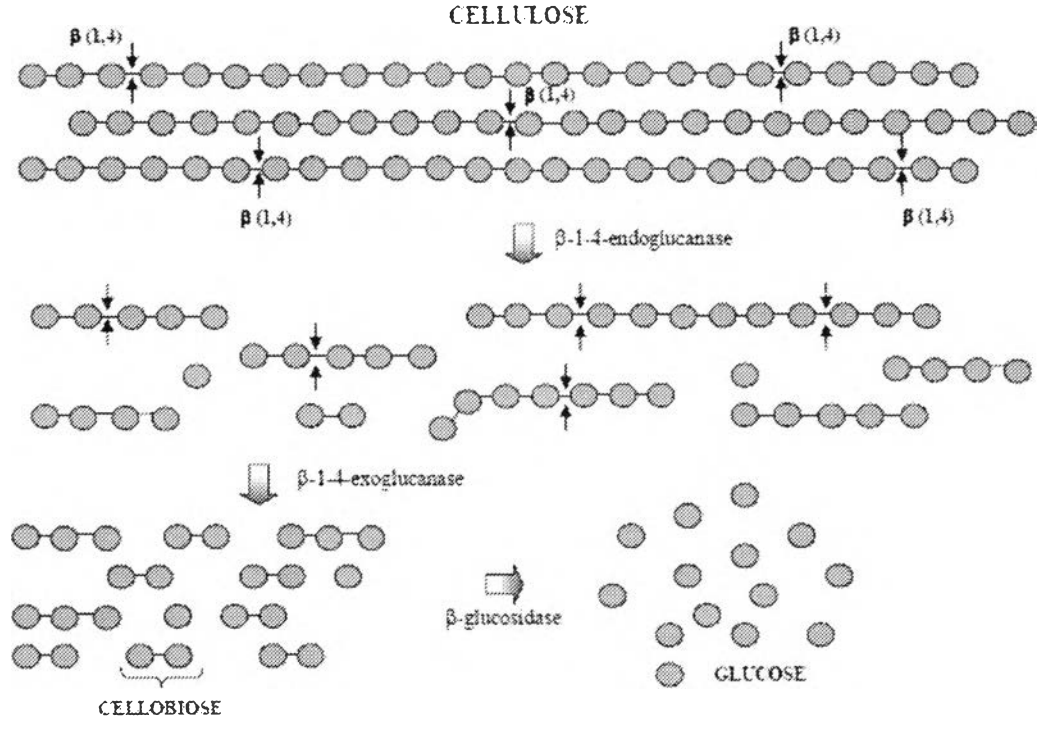


Figure 2.8 Schematic representation of the cellulose enzymes over the cellulose structure (Mussatto and Teixeira, 2010).

There are different factors that affect the enzymatic hydrolysis of cellulose, namely, substrates, cellulase activity, reaction conditions (temperature, pH as well as other parameters), and a strong product inhibition. To improve the yield and rate of enzymatic hydrolysis, research has been focused on optimizing the hydrolysis process and enhancing the cellulase activity. The rate of enzymatic hydrolysis of cellulose is dependent upon several structural features of the cellulose. The cellulose features known to affect the rate of hydrolysis include: (1) molecular structure of cellulose, (2) crystallinity of cellulose, (3) surface area of cellulose fiber, (4) degree of swelling of cellulose fiber, (5) degree of polymerization, and (6) associated lignin or other materials. A low substrate concentration gives low yield and rate, and a high cellulase dosage may increase the costs disproportional (Sun and Cheng, 2002; Detry, 1982; Hamelinck *et al.*, 2005).

Rivers (1988) studied the effects of substrate composition, cellulose crystallinity, and particle size on the yields of enzymatic hydrolysis for two agricultural crop wastes, bagasse and rice straw. *Trichoderma reesei* QM 9414G was used to produce a full complement cellulase system consisting of endoglucanase, cellobiohydrolase, and cellobiase activities to hydrolyze cellulose. The absolute change in the crystallinity of rice straw following pretreatment did, however, appear to correlate with conversions to either glucose or ethanol. Substrate particle size was determined and found not to be a major factor in enzymatic hydrolysis within the range studied. However, modification of the lignocellulose matrix from its native state by caustic pretreatment resulted in the most significant increases in enzymatic hydrolysis reinforcing the concept that the nature of the native lignocellulose matrix is a major limiting factor in enzymatic hydrolysis of bagasse and rice straw. The data indicate that each individual lignocellulosic substrate requires a specific pretreatment in order to achieve maximum enzymatic hydrolysis.

Liming and Xueliang (2004) studied cellulase production using corncob residue from xylose manufacture as substrate was carried out by Trichoderma reesei ZU-02. It was found that on the same cellulose basis, the cellulase activity and yield produced on corncob residue were comparable with that on purified cellulose. Under batch process, the optimum concentration of substrate was 40 g/l, and the optimum C/N ratio was 8.0. In 500 ml flasks, cellulase activity reached 5.25 IU/ml (213.4 IU/g

cellulose) after seven days' cultivation. In a 30 m^3 stirred fermenter for large scale production, cellulase and cellobiase activity were 5.48 IU/ml (222.8 IU/g cellulase) and 0.25 IU/ml (10.2 IU/g cellulose), respectively, after four days' submerged fermentation. It can be concluded that the produced cellulase could effectively hydrolyze the corncob residue, and the yield of enzymatic hydrolysis reached 90.4 % on 10 % corncob residue (w/v) when the cellulase dosage was 20 IU/g substrate.

Movagharnejad and Sohrabi (2003) studied the effect of enzyme to substrate ratio on product concentration by using rice pollards, sawdust, wood particles, and used papers as raw materials. The compositions of these cellulosic waste materials are summarized in Table 2.5. The process has been studied in enzymatic hydrolysis method at 50 °C by using Celluclast L/CCN 03056 and *Novozyme* 188 DCN/85-4 enzymes, where celluclast, mainly a mixture of different endo- and exo-cellulases, is a liquid cellulase preparation made by submerged fermentation of a selected strain of the fungus *T.reesei*. The results showed that the enzyme/substrate ratio was a sensitive factor in enzymatic hydrolysis of cellulosic materials. In systems with higher ratios of enzyme/substrate, the concentration of substrate played a major role in the progress of reaction, whereas, at lower ratios of enzyme/substrate, enzyme concentration was the key factor. In such systems, increasing the concentration of substrate may not lead to a rapid increase in product concentration.

Table 2.5 Composition of cellulosic waste materials (Movagharnejad and Sohrabi 2003)

Materials	Cellulose (%)	Lignin (%)	Hemicellulose (%)
Rice pollards	49.5	19.0	31.0
Sawdust	50.0	26.0	24.0
Used paper	66.8	7.5	25.7

Chen *et al.* (2007) investigated the enzymatic hydrolysis of corncob and ethanol fermentation from cellulose hydrolysate. The cellulosic residue was pretreated by 1 % H_2SO_4 at 108 °C for 3 h before hydrolyzed by cellulase from *Trichoderma reesei* ZU-02 and the hydrolysis yield was 67.5 %. Poor cellobiase activity in T. reesei cellulase restricted the conversion of cellobiose to glucose, and the accumulation of cellobiose caused severe feedback inhibition to the activities of β-1,4-endoglucanase and β-1,4-exoglucanase in cellulase system. Adding cellobiase from *Aspergillus niger* ZU-07 greatly reduced the inhibitory effect caused by cellobiose, and the hydrolysis yield was improved to 83.9 % with enhanced cellobiase activity of 6.5 CBU g^{-1} substrate.

Martins *et al.* (2008) compared the activity of different type of enzymes; *Penicillium echinulatum* (potential cellulase producer for bioconversion processes) and *Trichoderma reesei* cellulases (Celluclast 1.5L FG, *Novozymes*). The total cellulase activity was determined against Whatmann no.1 filter paper (Sigma–Aldrich, St. Louis, MO), using the DNS method and high performance liquid chromatography (HPLC; see the following section) to quantify total reducing sugars (RS) and soluble carbohydrates, respectively. Analysis of substrate hydrolysates demonstrated that *P. echinulatum* enzymes had higher β-glucosidase activity than Celluclast 1.5L FG, while the latter appeared to have greater cellobiohydrolase activity. Unlike Celluclast 1.5L FG, *P. echinulatum* cellulases had enough β-glucosidase activity to remove most of the cellobiose produced in hydrolysis experiments. However, Celluclast 1.5L FG became more powerful than *P. echinulatum* cellulases when supplemented with exogenous β-glucosidase activity (*Novozym* 188).

Mussatto *et al.* (2008) studied the effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain in three different forms: original (untreated), pretreated by dilute acid (cellulignin), and pretreated by a sequence of dilute acid and dilute alkali (cellulose pulp). The hydrolysis was carried out using a commercial cellulase concentrate (Celluclast 1.5L) in an enzyme/substrate ratio of 45 FPU/g, 2 % (w/v) substrate concentration, 45 C for 96 h. From the results, the cellulose hydrolysis was affected by the presence of hemicellulose and/or lignin in the sample. The cellulose conversion ratio (defined as

glucose yield + cellobiose yield) from cellulignin was 3.5-times higher than that from untreated sample, whereas from cellulose pulp such valve was 4-times higher, correspondent to 91.8 % (glucose yield of 85.6 %). It can be concluded that the lower the hemicellulose and lignin contents in the sample, the higher the efficiency of cellulose hydrolysis into glucose.

Chang *et al.* (2010) studied enzymatic conversion with freeze pretreatment of rice straw by cellulase from *Aspergillus niger* (Sigma Chemical Co.) and xylanase from *Trichoderma viride* (Sigma Chemical Co.) at -20°C. The freeze pretreatment was found to significantly increase the enzyme digestibility of rice straw from 48% to 84%. This indicated that freeze pretreatment was highly effective for enzymatic hydrolysis and low environmental impact. According to the results, enzymatic hydrolysis of untreated rice straw with 150 U cellulase and 100 U xylanase for 48 h yielded 226.77 g kg^{-1} and 93.84 g kg^{-1} substrate-reducing sugars respectively. However, the reducing sugar yields from freeze pretreatment under the same conditions were 417.27 g kg^{-1} and 138.77 g kg^{-1} substrate, respectively. In addition, hydrolyzates analysis showed that the highest glucose yield obtained during the enzymatic hydrolysis step in the present study was 371.91 g kg^{-1} of dry rice straw, following pretreatment. Roslan (2011) investigated the production of bioethanal from rice straw using cellulase by local *Aspergillus* sp. The rice straw was pretreated by few cycles of wet disc milling prior saccharified it using the crude cellulase produced from rice straw by locally isolated *Aspergillus* sp. in solid state fermentation. This crude cellulase was measured to have activity 6.3 FPU g^{-1} rice straw. The saccharification released glucose from total cellulose more than 90%. Then, the saccharified product was subjected to fermentation by yeast. The highest bioethanol yield produced from the fermentation was 0.102 g g^{-1} rice straw which isequivalent to 62.1% of theoritical buthanol yield. This sugested that the use of crude cellulase from rice straw onto rice straw can lead to a good yield of bioethaol, provided an efective pretreatment was used.

2.6 Enzyme from Termites

Schulz *et al.* (1986) studied the cellulase from the termite *Nasutitermes walker* and implied that *N. walker* consists of two enzymes. Each has broad specificity with predominantly one activity. One enzyme is an endo-α-l,4-glueanase (EC 3.2.1.4), which predominantly cleaves cellulose randomly to glucose, cellobiose and cellotriose. It hydrolyses cellotetraose to cellobiose but will not hydrolyse cellobiose or cellotriose. The second enzyme component is a β-l,4-glucosidase (EC 3.2.1.21) as its major activity is to hydrolyse cellobiose, cellotriose, and cellotetraose to glucose; it has some exoglucosidase activity as glucose is the only product produced from cellulose. They concluded that it is now well established that higher termites produce cellulolytic enzymes.

Taechapoempol *et al.* (2010) studied cellulase-producing bacteria from Thai higher termites *Microcerotermes* sp., under three different isolation conditions (aerobic anaerobic, or anaerobic/aerobic). He found that only three effective isolates of A 002, M 015, and F 018 out of forty-seven cellulase-producing bacteria isolated from the termites had the highest hydrolysis capacity value (HC value). Identification from the 16s rRNA gene sequencing method revealed that all of the effective isolates were Bacillus subtilis. The cellulase activities (F phase, endoglucanase, and β-glucosidase) of A 002, M 015, and F 018 were also tested at 37 °C and pH 7.2. The results showed that the isolate M 015 exhibited the highest endoglucanase activity whereas the isolate F 018 gave the highest FPase and β-glucosidase activities. The microbiological characteristics of the three effective isolates are summarized in Table 2.6. Furthermore, these effective isolates were tested for their toxic tolerance to [BMIM]Cl. All of the isolates were able to tolerate the [BMIM]Cl in the concentration range of 0.1 to 1.0 vol.%, and no growth retardation in the lag phases, except that the isolate A 002 had a growth retardation in the [BMIM]Cl concentration range of 0.5 to 1.0 vol.%, was observed.

Table 2.6 Characteristics of isolates A 002, M 015, and F 018 by microbiological methods (Taechapoempol *et al.*, 2011)

Isolate	Colonial appearance	Pigmentation	Cell shape	Gram's staining	Spore forming	Oxidase test	Catalase test
A 002	Circular, flat, entire, rough, and membranous	Light brown cream	Rod	+	+	-	+
M 015	Spindle, raised, entire, glistering, and opaque	Light brown cream	Rod	+	+	-	+
F 018	Spindle, flat, filamentous, glistering, and opaque	Light brown cream	Rod	+	+	-	+

Worasamutprakarn (2010) investigated conversion of cellulose to glucose by using three effective isolates (strain A 002, M 015, and F 018), isolated from Thai higher termites Microcerotermes sp with [BMIM]Cl ionic liquid pretreatment. For the pretreatment step, it was found that [BMIM]Cl could be effectively used to decrease the crystallinity of cellulose chains with optimum conditions of 5:100 cellullose-to-[BMIM]Cl ratio and 100 °C. The crystallinity of cellulose chains decreased about 90 % after the pretreatment. However, about 5 % of [BMIM]Cl was remained in the pretreated cellulose even after washing by deionized water. The results from enzymatic hydrolysis showed that strain F 018 produced the highest glucose concentration at 0.59 g/L after 4 h operation. In addition, using no.5 Whatman filter paper with high crystalline structure gave the lowest glucose concentration. While using the no. 1, 2, and 4 Whatman filter papers with lower crystallinity gave higher glucose concentration.

Eourarekullart (2011) investigated the enzymatic hydrolysis of corncob with two effective isolates (strain A 002 and M 015), from Thai higher termites, *Microcerotermes* sp. under different particle size of corncob (40 and 60 mesh) and hydrolysis temperature (30 and 37 °C). From the results, the glucose concentration from the strain A 002 was significantly higher than that from strain M 015 and both bacteria strains hydrolyzed corncob very well in the 65 modified DSMZ broths medium 2. The maximum amount of glucose which was 1.08 g/L can be obtained from the hydrolysis reaction with strain A 002 bacteria and 60 mesh size of corncob at 37 °C.

2.7 Corncob

Corncob is one of the potential agricultural biomass feedstocks for renewable energy industries to reduce the current energy and the greenhouse gas problems. Corncob can be used for producing heat, power, gas/liquid fuels, and a wide variety of chemical products such as furfural, xylitol and activated carbon. Crofcheck and Montross (2004) found a greater yield of glucose (i.e., ethanol) from corncobs than other corn residues such as stalks or leaves plus husks.

Cobs represent about 8 to 9 % of the aboveground dry matter (grain plus residues) at grain physiological maturity. The yield of corncobs may range from 1.42 to 1.53 dry t/ha. Currently, after combining the grain, corn residues are collected as baled corn stover, which includes cobs, husks, leaves, and stalks. About 15 to 20 % (d.b) of aboveground corn residues (non-grain) is corncobs. Corncob moisture content may range from 20 to 55 % (w.b.) depending on the grain moisture content at the time of harvest. Figure 2.9 shows the picture of corncobs. Table 2.7 shows compositions of a corncob sample.

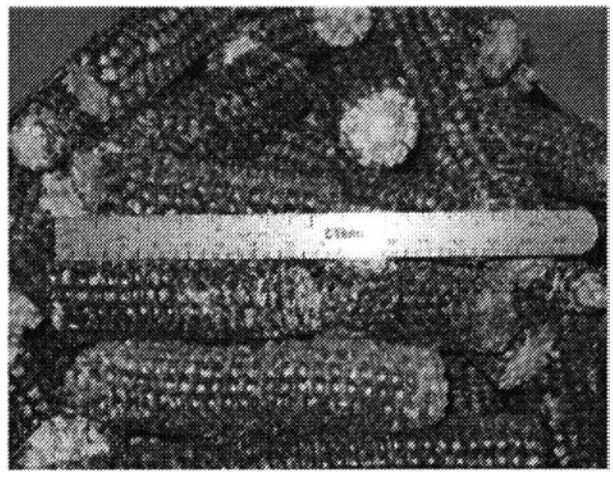


Figure 2.9 Picture of corncobs (Kaliyan and Morey, 2010)

Table 2.7 Composition of corncobs, corn stover, and switchgrass (Kaliyan and Morey, 2010)

Component	Corncobs (% of dry matter)	Corn stover (% of dry matter)	Switchgrass (% of dry matter)
Cellulose[a]	40.0	49.4	43.8
Hemicellulose[b]	41.4	26.2	28.8
Lignin[c]	5.8	8.8	9.2
Crude protein	2.5	3.6	3.9
Starch	2.1	0.4	1.0
Crude fat	0.7	0.7	0.9
Water soluble carbohydrates	1.1	7.9	2.2
Moisture content	1.7	5.4	5.7
Ash	1.8	11.2	5.0

[a] Cellulose = acid detergent fiber – lignin.

[b] Hemicellulose = neutral detergent fiber – acid detergent fiber. The hemicelluloses content is higher than cellulose content because of the approximate estimation of hemicellulose content by the difference between neutral detergent fiber and acid detergent fiber contents.

[c] Lignin values measured for the biomass materials were acid insoluble lignin contents (not total lignin contents, which would be much higher than this values).