CHAPTER II
LITERATURE REVIEW

2.1 Lignocellulosic Biomass

The fourth largest source of energy in the world after coal, petroleum, and natural gas is biomass, which is 14 % of the world's primary energy consumption (Saxena *et al.*, 2009). Biomass can meet a variety of energy requirements, including generating electricity, fueling vehicles, and providing process heat for industries. Among all the renewable sources of energy, biomass is unique as it effectively stores solar energy. Biomass is the only renewable source of carbon that can be converted into convenient solid, liquid, and gaseous fuels through different conversion processes, as shown in Figure 2.1.

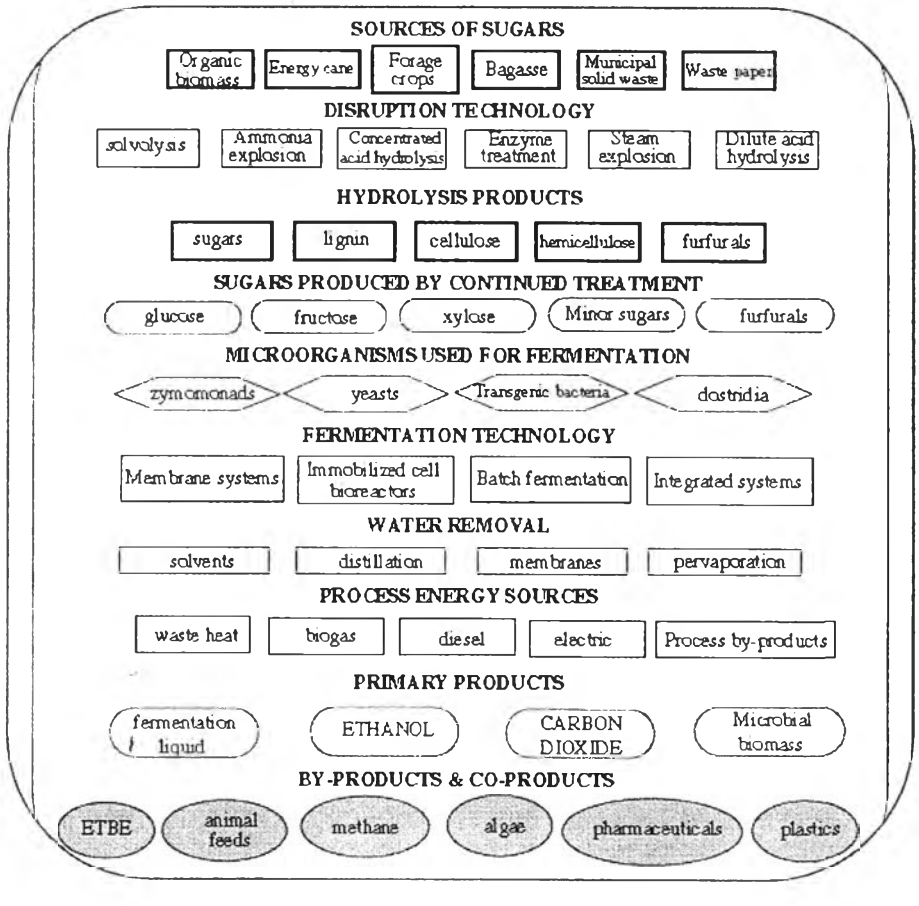

Figure 2.1 Different steps of biomass to ethanol (Saxena *et al.*, 2009).

The renewable agricultural residues, which are abundant, inexpensive, sustainable, and readily available sources of renewable lignocellulosic biomass and their utilizations are being considered. There is an increasing interest around all over the world, particularly for the production of novel materials for environmentally friendly industrial utilizations after chemical modification (Liu *et al.*, 2007).

Table 2.1 Composition of various lignocellulosic residues (Aita and Salvi, 2010)

Biomass	Cellulose	Hemicellulose	Lignin	Other*
	percent dry biomass			
Corn stover	36.4	21.4	17.2	25.0
Wheat straw	38.2	24.7	23.2	13.9
Rice straw	34.2	24.5	23.0	18.3
Miscanthus	31.0	24.4	17.6	27.0
Poplar sawdust	49.9	20.4	18.1	11.6
Sugarcane bagasse	40.2	21.5	24.2	14.1
Sorghum	44.5	27.7	22	5.8

*Protein, ash, acetyl, uronic acids, nonstructural sugars

Lignocellulosic wastes come in many different types. They can be grouped into four main categories: (1) wood residues (including sawmills and paper mill discards), (2) municipal paper waste, (3) agricultural residues (including corn stover and sugarcane bagasse), and (4) dedicated energy crops (mostly composed of fast growing tall, woody grasses). Table 2.1 shows the composition of some lignocellulosic residues.

2.2 Sugarcane Bagasse

Sugarcane is original to tropical South Asia and Southeast Asia. In 2009, Thailand was ranked as the fourth largest sugarcane producer. Sugarcane has stout, jointed, and fibrous stalks that are rich in sugar. The fibrous residue remaining after sugarcane stalks are crushed to extract their juice is called sugarcane bagasse. As 10

tonnes of sugarcane crushed, nearly 3 tonnes of wet bagasse are produced in a sugar factory. This means that the quantity of production in each country is in line with the quantity of sugarcane produced.

Sugarcane bagasse is one of the most extensively used agricultural residues, which is usually used to generate heat and power to run sugar milling process. Moreover, they are currently used as a renewable resource in the manufacture of pulp and paper products and building materials. Remainder bagasse will be stockpiled for future use. However, stockpiled bagasse cause an environmental problem to sugar mill and surrounding communities, especially for extended period stockpile, due to the risk of spontaneous combustion occurring within the pile (Lavarack *et al.*, 2002).

As bagasse fiber consists, mainly, of lignocellulosic material, considerable research effort has been extended on investigating hydrolysis to cleave the intrachain linkages in cellulose and hemicellulose chains. Sugarcane bagasse basically consists of 40–50 % cellulose, 20–30 % hemicellulose, 20–25 % lignin, and 1.5–3.0 % ash (Saxena *et al*, 2009).

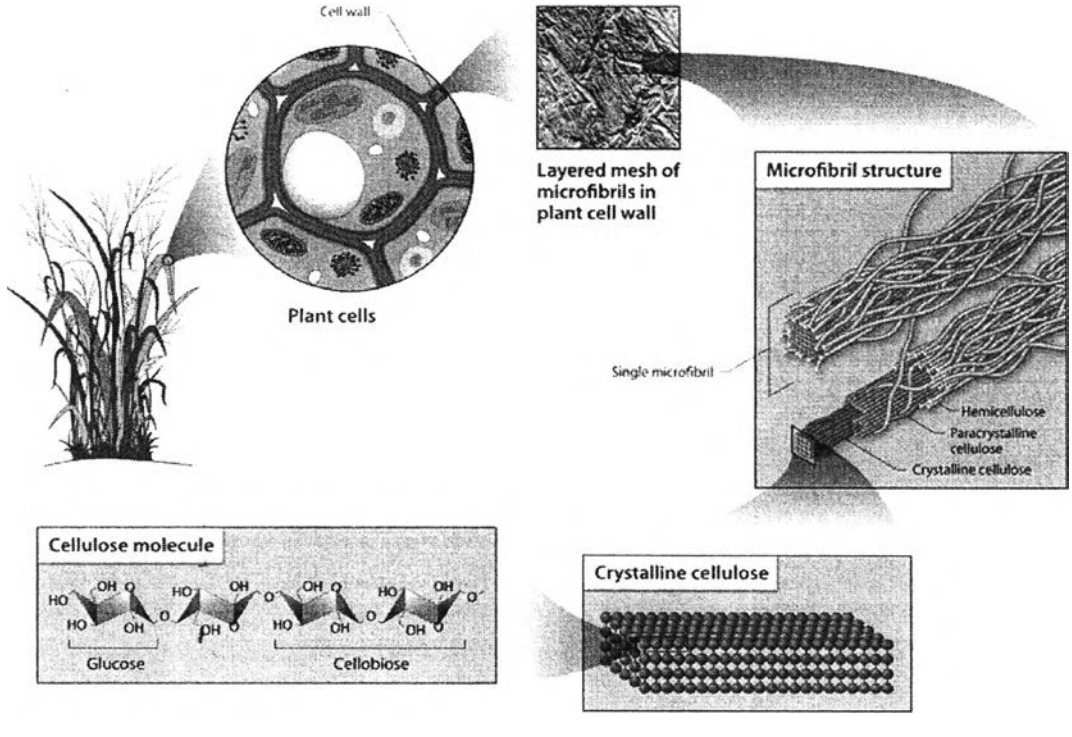

Figure 2.2 Arrangement of microfibrils, cellulose, and hemicellulose in cell walls (http://genomicscience.energy.gov/benefits/cellulosestructure.shtml).

2.2.1 Cellulose

Cellulose is a linear polymerized chain of glucose molecules, linked by β-1,4 glycosidic bonds, which is the primary structural component of plants. Both inter and intra-molecular hydrogen bondings exist in cellulose chains. Cellulose in a plant consists of parts with highly organized crystalline microfibrils, and parts with not well-organized, amorphous matrices. Because of the β-1,4 bonds, cellulose is highly crystalline and closely packed, resulting in very resistant to biological attack property. The cellulose chains are bundled together and form cellulose bundles or cellulose fibrils as shown in Figure 2.2. Cellulose fibrils are mostly independent and weakly bound through hydrogen bonding (Hendriks and Zeeman, 2009). The difference of glucose linkage between starch and cellulose makes it impossible for the starch-digesting enzymes, e.g. alpha-amylase, to digest cellulose.

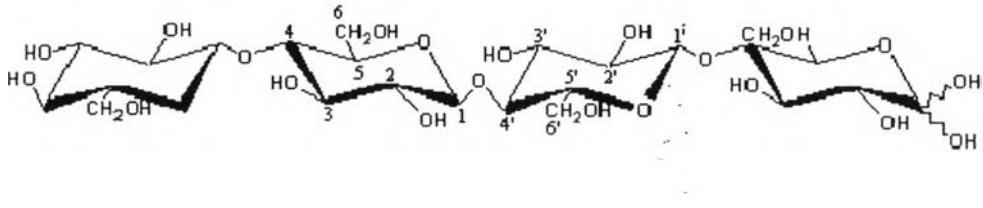

Figure 2.3 Schematic representation of a cellulose chain (Pérez and Mackie, 2001).

Cellulose is biodegradable, unscented, tasteless, chiral, hydrophilic, and insoluble in water and most organic solvents. Other properties of cellulose depend on length of polymerized chain, degree of polymerization, or number of glucose units for one polymer molecule (Zhang *et al.*, 2006). Figure 2.3 shows structure of cellulose chain.

2.2.2 Hemicellulose

Hemicellulose is a complex polysaccharide structure that contains different carbohydrate polymers like pentoses (e.g. xylose and arabinose) and hexoses (e.g. mannose, glucose, and galactose), and sugar acids. The major component of hemicellulose from hardwood and agricultural plants, such as grasses and straw, is xylan, and that from softwood is glucomannan. Hemicellulose normally exists in association with cellulose.

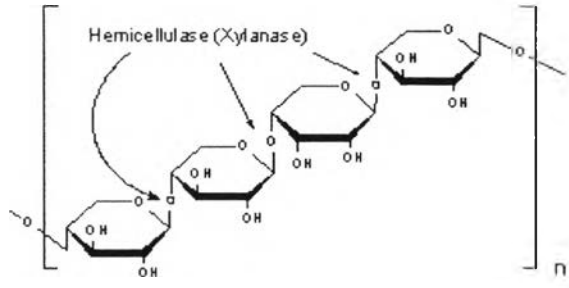

Figure 2.4 Basic structure of hemicellulose (http://blogs.princeton.edu/chm333/f2006/biomass/bio_oil/02_chemistryprocessing_the_basics/01_chemistry/).

Molecular weight of hemicellulose is lower than cellulose. Hemicellulose has branches with short lateral chains that are composed of different sugars, which can be easily hydrolyzed. Hemicellulose functions as a connection between lignin and cellulose fibers and makes the whole cellulose–hemicellulose–lignin network more rigidity. The tightness and complexity of lignocellulose causes much more difficult than starch to enzymatically degrade to fermentable sugars. Figure 2.4 shows basic structure of hemicellulose, and the hemicellulose composition of various lignocellulosic materials is given in Table 2.2.

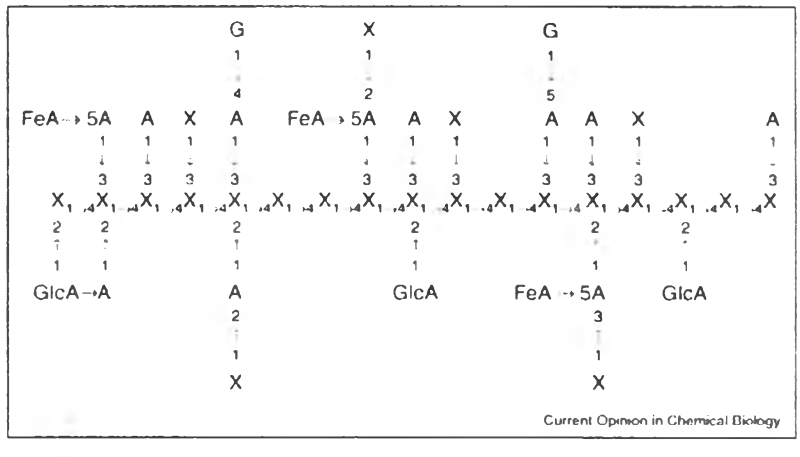

Figure 2.5 Schematic of the structure of hemicellulose: A, arabinose; FeA, ferulic acid; G, galactose; Glc, glucuronic acid; and X, xylose (Gray *et al.*, 2006).

Without a profitable utilization of the hemicellulose fraction, biofuel is too expensive to compete in commercial markets. Therefore, to support the commercial production of lignocellulosic biofuel, bioconversion of the hemicellulose into fermentable sugars is very necessary. The most capability method for hydrolysis of polysaccharides to fermentable sugars is by use of enzymes, i.e. cellulase and hemicellulase. Moreover, hemicellulase eases cellulose hydrolysis by exposing the cellulose fibres, thus making them more accessible.

Table 2.2 Hemicellulose composition of various lignocellulosic materials (Gírio *et al*, 2010)

Raw material	Xyl	Ara	Man	Gal	Rha	UA	AcG
Softwoods							
Douglas fir	6.0	3.0		3.7			
Pine	5.3 10.6	2.0 4.2	5.6 13.3	1.9 3.8		2.5 6.0	1.2 1.9
Spruce	5.3 10.2	1.0 1.2	9.4 15.0	1.9 4.3	0.3	1.8 5.8	1.2 2.4
Hardwoods							
Aspen	18 27.3	0.7 4.0	0.9 2.4	0.6 1.5	0.5	4.8 5.9	4.3
Birch	18.5 24.9	0.3 0.5	1.8 3.2	0.7 1.3	0.6	3.6 6.3	3.7 3.9
Black locust	16.7 18.4	0.4 0.5	1.1 2.2	0.8		4.7	2.7 3.8
Eucalypt	14 19.1	0.6 1	1 2.0	1 1.9	0.3 1	2	3 3.6
Maple	18.1 19.4	0.8 1	1.3 3.3	1.0		4.9	3.6 3.9
Oak	21.7	1.0	2.3	1.9		3	3.5
Poplar	17.7 21.2	0.9 1.4	3.3 3.5	1.1		2.3 3.7	0.5 3.9
Sweet gum	19.9	0.5	0.4	0.3		2.6	2.3
Sycamore	18.5	0.7	1.0				3.6
Willow	11.7 17.0	2.1	1.8 3.3	1.6 2.3			
Agricultural and agro industrial materials							
Almond shells	34.3	2.5	1.9	0.6			
Barley straw	15	4.0					
Brewery's spent grain	15	8	0	1	0	2	0.8
Cardoon	26.0	2.5	3.7	1.4	0.9		
Corn cobs	28 35.3	3.2 5.0		1 1.2	1	3	1.9 3.8
Corn fibre	21.6	11.4		4.4			
Corn stalks	25.7	4.1	<3.0	<2.5			
Corn stover	14.8 25.2	2 3.6	0.3 0.4	0.8 2.2			1.7 1.9
Olive stones	2.0 3.7	1.1 1.2	0.2 0.3	0.5 0.7	0.3 0.5	1.2 2.2	
Rice husks	17.7	1.9					1.62
Rice straw	14.8 23	2.7 4.5	1.8	0.4			
Sugar cane bagasse	20.5 25.6	2.3 6.3	0.5 0.6	1.6			
Wheat bran	16	9	0	1	0	2	0.4
Wheat straw	19.2 21.0	2.4 3.8	0 0.8	1.7 2.4			

[a] Non-glycosidic units.
[b] Expressed as g/100 g of dry material.
[c] The percentages of thoses were, in some cases, calculated from the corresponding "polymers". Xyl, xylose; Ara, arabinose; Man, mannose; Gal, galactose; Rha, rhamnose; UA, uronic acids; AcG, acetyl groups.

As seen in Figure 2.5, hemicellulose in bagasse is a xylan polymer backbone, onto which other groups are bonded, mainly glucuronic acid and arabinose (Lavarack *et al.*, 2002).

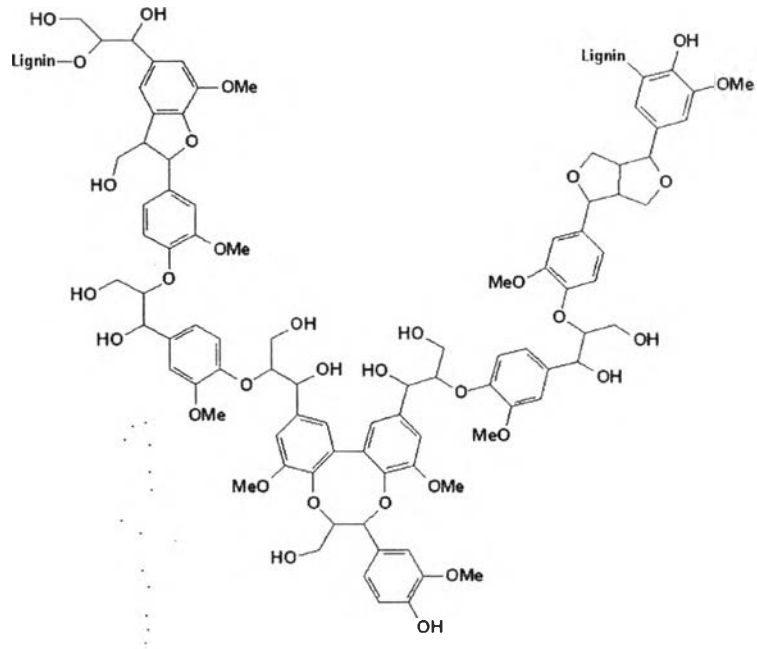

Figure 2.6 Schematic structure of lignin (http://en.wikipedia.org/wiki/File:Lignin Structure.jpg).

2.2.3 Lignin

One of the most abundant polymers in nature is lignin, which is present in the cellular wall. Lignin is an amorphous heteropolymer consisting of three different phenylpropane units (p-coumaryl, coniferyl, and sinapyl alcohol), which are held together by different kinds of linkages as shown in Figure 2.6. The main purpose of having lignin is to provide structural support, impermeability, and resistance against microbial attack and oxidative stress to the plant. Amorphous heteropolymer is also non-water soluble and optically inactive; all of these make lignin very tough to degradation. The solubility of the lignin in acid, neutral, or alkaline environments is based, however, on the precursor (p-coumaryl, coniferyl, sinapyl alcohol, or combinations of them) of the lignin (Hendriks and Zeeman, 2009).

2.2.4 Extractives

Other compounds that have been found in lignocellulosic biomass are known as extractives. Extractives include resins, fats and fatty acids, phenolics, phytosterols, salts, minerals, and other compounds.

2.2.5 Ash

3–10% of total feedstock dry matter of biomass is ash. Ash is the residue remaining after ignition of herbaceous biomass by dry oxidation at 575 ± 25°C. Ash can be composed of minerals such as silicon, aluminum, calcium, magnesium, potassium, and sodium.

2.3 Sugars

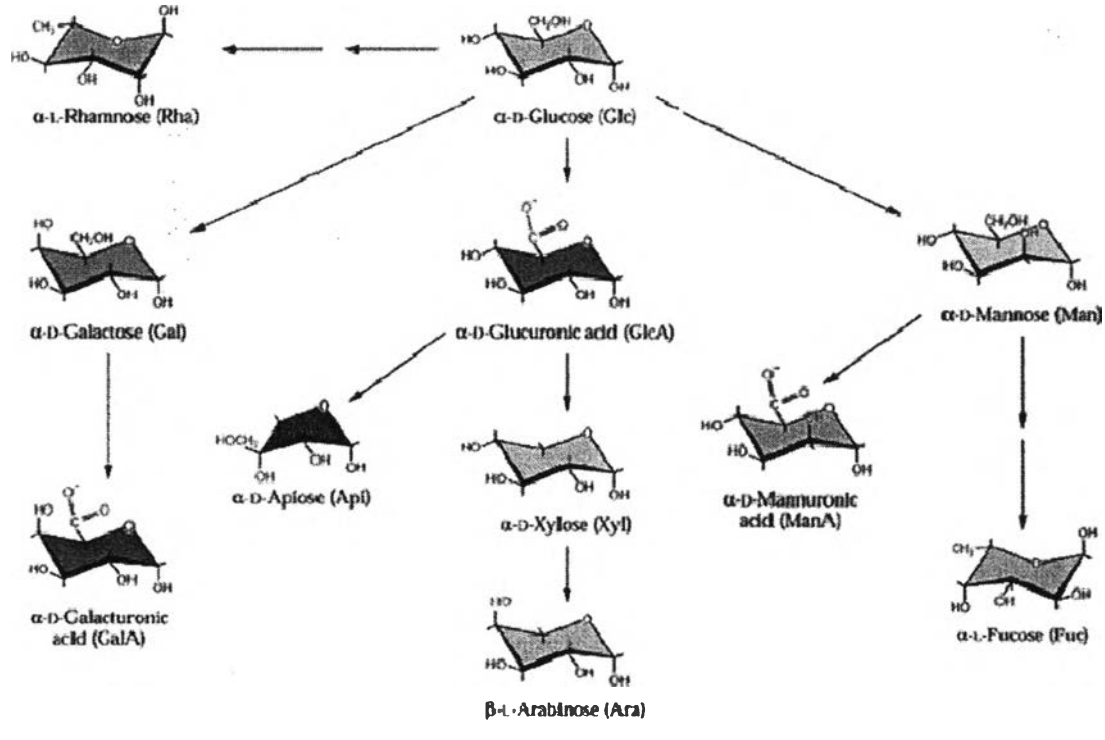

Figure 2.7 Structure of some important pentose and hexose (http://www.uky.edu/~dhild/biochem/11B/lect11B.html).

Conversion of sugarcane bagasse into fermentable sugars is feasible through chemical, thermal, or enzymatic hydrolysis (Hernández-Salas *et al.*, 2009). Fermentable sugars that can be derived from sugarcane bagasse are glucose, xylose, arabinose, mannose, and galactose (Gírio *et al.*, 2010). Figure 2.7 shows structure of each sugar.

2.3.1 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).

2.3.2 Xylose

Xylose is a sugar, which is first isolated from wood and classified as a monosaccharide of the aldopentose type. It contains five carbon atoms with an aldehyde functional group. It can be the precursor to hemicellulose, which is one of the main constituents of biomass and adopt several structures depending on conditions as most sugars can. Cause of its free carbonyl group, it is a reducing sugar. Xylose cannot be metabolised by the human it is completely absorbed and as such secreted from the kidney. For animal medicine, the test for malabsorption by administration in water to the patient after fasting performs by using xylose. If there is any xylose detected in blood and/or urine within the next few hours, it has been absorbed by the intestines (http://en.wikipedia.org/wiki/Monosaccharide).

2.3.3 Arabinose

Arabinose is an aldopentose or a monosaccharide containing five carbon atoms with an aldehyde (CHO) functional group. In fact, L-arabinose is more common than D-arabinose in nature, which is found as a component of biopolymers such as hemicellulose and pectin. The L-arabinose operon is a greatly significant operon in molecular biology and bioengineering. A formal method to organic synthesising of arabinose from glucose is the Wohl degradation (http://en.wiki pedia.org/wiki/Monosaccharide).

2.3.4 Mannose

Mannose does not significantly enter the human carbohydrate metabolism because it is not well metabolized in humans. Hence, if orally taken, traces of exogeneously introduced mannose have been detected in all body tissues by using radioactive markers and 90% of mannose ingested is excreted unconverted into the urine within 30 - 60 minutes. There is no significant gain in blood-glucose levels during this time (http://en.wikipedia.org/wiki/Monosaccharide).

2.3.5 Galactose

Galactose is monosaccharide, which is an epimer of glucose. But it is less sweet than glucose. Because it has food energy, it is considered a nutritive sweetener. Its name comes from the Ancient Greek word for milk, galaktos. Galactan is a polymer of the sugar galactose. It is found in hemicellulose and can be converted to galactose by hydrolysis. In the human body, glucose can be changed into galactose via hexoneogenesis to enable the mammary glands and secrete lactose. However, most galactose in breast milk is synthesized from galactose taken up from the blood. Galactose and glucose can be hydrolyzed from lactose by β-galactosidase which is enzyme produced by the lac operon in Escherichia coli (E. coli) (http://en.wikipedia.org/wiki/Monosaccharide).

2.4 Pretreatment of Lignocellulosic Biomass

Lignocellulosic biomass contains polysaccharides, such as cellulose and hemicellulose that have to be hydrolyzed to fermentable sugars. However, physical and chemical limitations, which are caused by the close linking of the main components of lignocellulosic biomass, obstruct the hydrolysis of cellulose and hemicellulose. The main purpose of pretreatment is to reduce structure constrain to increase the enzyme accessibility and reactivity to digest lignocellulose. Pretreatment will reform the biomass macroscopic and microscopic size and structure, as well as its submicroscopic chemical composition and structure. This step makes hydrolysis of the carbohydrate fraction to monosaccharide to be completed more rapidly and with greater yields (Mosier *et al.*, 2005).

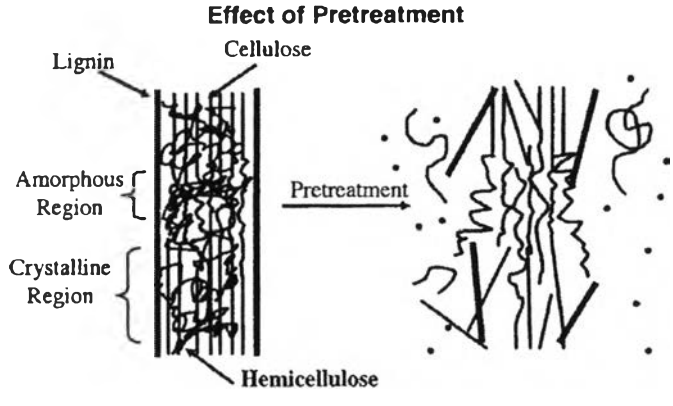

Figure 2.8 Schematic of goals of pretreatment on lignocellulosic material (Mosier *et al.*, 2005).

To break the lignin and hemicellulose, disrupt the crystalline structure of cellulose, and increase the porosity of the materials as shown in Figure 2.8, pretreatment will meet the following requirements: 1) to improve the ability and yield of sugar formation by enzymatic hydrolysis; 2) to prevent the degradation or loss of carbohydrate; 3) to avoid the formation of byproducts that will inhibit the subsequent hydrolysis and fermentation processes; and 4) to be cost-effective. The process uses pretreatment additives and/or energy to form solids that will be more

reactive than natural form, and/or to generate soluble oligo- and monosaccharides (Figure 2.9).

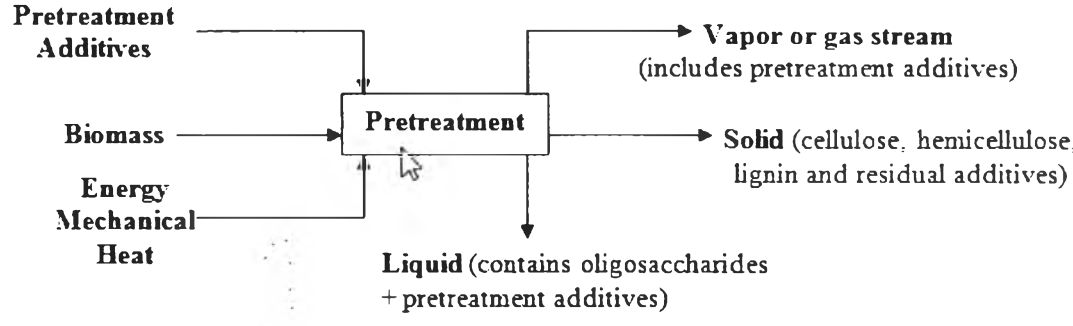

Figure 2.9 Schematic of pretreatment process (Mosier *et al.*, 2005).

During the past few decades, a number of pretreatment methods have been suggested and investigated, including physical pretreatment (e.g. milling, grinding, and irradiation), chemical pretreatment (e.g. alkali, dilute acid, organic solvent, and ionic liquid), physicochemical pretreatment (e.g. hydrothermal processes, wet oxidation, ammonia fiber explosion, and steam pretreatment/autohydrolysis), and biological pretreatment, or combinations of these. There are many interesting claims concerning the effectiveness of these pretreatment methods. However, the economic, environmental, and technological constraints limit the applicability of these known methods.

Following are examples of some attractive pretreatment technologies.

2.4.1 Mechanical Comminution

The objective of the mechanical pretreatment is to reduce particle size and crystallinity of lignocellulose for the purpose of increasing specific surface and decreasing degree of polymerization. This technique combines chipping, grinding, or milling, depending on the desired particle size of the material. Different milling processes (e.g. ball milling, two-roll milling, and vibro energy milling) can be used to improve the enzymatic hydrolysis of lignocellulosic materials. However, power requirement of this pretreatment is relatively high depending on the desired particle

size and the biomass characteristics, which make this process not economically feasible (Alvíra *et al.*, 2010).

2.4.2 Alkali Pretreatment

The effect of some bases on lignocellulosic biomass is the basis of alkaline pretreatment, which is effective depending on the lignin content in biomass. Alkali pretreatment raises digestibility of cellulose. This method can be more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal processes. Alkali pretreatment can be performed at room temperature and period ranging from seconds to days. There is less sugar degradation via alkali pretreatment than acid pretreatment. This has been shown to be more effective on agricultural residues than on wood materials. Regardless possible loss of fermentable sugars, production of inhibitory components must be considered to optimize the pretreatment conditions (Alvíra *et al.*, 2010).

2.4.3 Dilute Acid Pretreatment

Diluted acid pretreatment is more favorable method for industrial utilizations 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 used for pretreatment of lignocellulosic materials. Diluted acid pretreatment 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 period (30–90 min). There are advantages of dissolving hemicellulose, mainly xylan, and converting dissolved hemicellulose to fermentable sugars. Regardless some sugar degradation compounds, such as furfural and HMF, aromatic lignin degradation compounds are detected and affect the microorganism metabolism in the fermentation step depending on the process temperature. This pretreatment generates lower degradation products than concentrated acid pretreatments. Dilute acid pretreatment at high temperature and pressure can hydrolyze hemicellulose and remaining lignin, making cellulose more accessible. There are three different processing technologies used in the dilute acid pretreatment: countercurrent

processing, two-temperature processing, and pressurized hot washing (Alvíra *et al.*, 2010).

2.4.4 Ionic Liquid Pretreatment

Ionic liquids (ILs) can be used instead of the conventional media in fractionation processes. Ionic liquids are organic salts, which usually have melting points below 100 °C. Their high thermal stability and negligible vapor pressure allow classifying them as ''green solvents". The advantage of using ionic liquids is the possibility of a complete dissolution of wood in its native form that opens new possibilities to fractionate, derivatise, and process lignocellulosic materials (Alvíra *et al.*, 2010).

Some ILs, e.g. 1-butyl-3-methylimidazolium chloride [bmim][Cl] and 1-allyl-3-methylimidazolium chloride [amim][Cl], are especially useful to dissolve cellulose. In 2002, Swatloski *et al.* reported that some imidazolium ILs can dissolve up to 25 wt% of cellulose and form very viscous solutions. They suggested that breaking the extensive hydrogen-bond network of polysaccharide by the anion of the IL causes dissolution. Another important factor stated by them is water content that was found to greatly reduce the solubility of carbohydrate via promoting the re-aggregation of the polymer's chains through the competitive hydrogen bonding. The effect of water is specifically important in case of further modification because the aggregation lowers accessibility and reactivity of the polymer. In contrast, the same feature led to easy regeneration of the already dissolved carbohydrates forming the solution, by the simple addition of water, alcohol, or acetone. ILs have been presented as very effective in cellulose solubilization; however, the solubility study of hemicellulose and lignin in ILs was reported rarely and required to be further investigated in detail (Gírio *et al.*, 2010).

2.4.5 Steam Explosion

Steam explosion can be described as a thermomechanochemical process, where the breakdown of structural components is supplied by heat from steam (thermo), shear forces from expansion of moisture (mechano), and hydrolysis of glycosidic bonds (chemical). In experiment, the material is heated using high-

pressure steam for few minutes. Then, the steam is condensed under the high pressure and wets the material. Next, the material is exploded into a pulp, when pressure in the reactor is quickly released to atmospheric pressure. The force, which is caused from decompression, leads to a desegregation of lignocellulosic matrix by breaking down inter- and intra-molecular linkages. Some of the useful compounds may be lost in the explosion stage if the temperature is too high, but the process can be catalyzed by chemical inputs, like sulfur dioxide or ammonia (Gírio *et al.*, 2010).

2.4.6 Microwave Pretreatment

Microwave-based pretreatment is considered as a physicochemical process because both thermal and non-thermal effects are involved. Microwave pretreatment is performed by immersing the biomass in dilute chemical reagents and exposing the slurry to microwave radiation for a period ranging from 5 to 20 min. Preliminary experiments identified alkalis as suitable chemical reagents for microwave-based pretreatment. There was an evaluation of different alkalis, and sodium hydroxide was found to be the most effective alkali reagent (Alvira *et al.*, 2009).

Table 2.3 Comparison of advantages and disadvantages of different pretreatment methods for lignocellulosic materials (Gírio *et al.*, 2010)

Desirable features	Concentrated acid	Dilute acid	Steam explosion	Auto-hydrolysis	Organosolv	Superacids	Alkali	Ionic liquids	Supercritical fluids
High hemicellulose solubilization	++	++	++	++	+	+	+	++	+
High hemicellulosic monosaccharide production	++	++	0		/0	+	/0	0/+	
Low hemicellulosic oligosaccharide production	+	+	0		/0	+	/0	0/+	
High cellulose recovery	++	++	++	++		++	+	+	++
High cellulose digestibility	++	++	+	+		+	++	+	0/+
High lignin quality			0/+	+	+	/0		+	+
High chemicals recycling	–		0	n.r.	–	+	/0/+	+	n.r./+
Low inhibitor formation			0	0	+	0	+		0
Low corrosion problems	–		0	0		0		/0	/0
Low need for chemicals	–		0	++	–	0/+	/0	+	++
Low neutralization requirements	–		0	n.r.	+	/0	/0	0	n.r.
Low investment costs	+	+		+	0	0	0/+	+	
Low operational costs		0	++	+		0	/0		
Low energy use	0		0	0	0	+	+	++	+

+, Advantage; –, disadvantage; 0, neutral; n.r., not relevant.

Each pretreatment technology has both advantages and disadvantages, as shown in Table 2.3. The ideally perfect pretreatment technology possibly will not exist. The most suitable pretreatment technology depends on various factors, e.g. type of material and its recalcitrance. Hence, the challenge of any lignocellulose pretreatment technology is the sufficient fractionation of hemicellulose, cellulose, and lignin, together with a minor degradation, for the purpose of maximum fermentation yields and rates. In addition, the choice of certain pretreatment has a large impact on all subsequent steps in the overall conversion scheme in terms of cellulose digestibility, generation of toxic compounds potentially inhibitory for yeast, stirring power requirements, energy demand in the downstream process, and wastewater treatment demands.

Kuo and Lee (2009) investigated the enzymatic hydrolysis of sugarcane bagasse with N-methylmorpholine-N-oxide (NMMO) monohydrate pretreatment at various conditions. Collected sugarcane bagasse was thoroughly washed by distilled water to remove residual soluble sugars, and then homogenized by using a food processor and dried at 70 °C for 2 d. Dry bagasse powder was screened with sizes between 30-mesh and 45-mesh. For pretreatment, 5 wt% of bagasse solution was prepared by mixing 0.25 g substrate with 4.75 g NMMO monohydrate in a 50 ml glass vial and heated in an oil bath at specific temperatures for various durations. After washing by additional 150 ml deionized water, 250 mg of regenerated bagasse was put in 25 ml of pH 4.8, 50 mM sodium citrate buffer supplemented with 0.02 % sodium azide. 5 FPU/g substrate of cellulase was used in the hydrolysis reactor at 37 °C under magnetic stirring, where one FPU is defined as the enzyme that releases 1 μmol of glucose equivalents per minute from Whatman No.1 filter paper. The results showed that bagasse should be pretreated in NMMO monohydrate with concentration of 20 wt% at 130 °C within 1 h period. About 95 % of the cellulose fraction was hydrolyzed into glucose after 72 h hydrolysis of the regenerated bagasse, which was at least two times higher than untreated bagasse.

2.5 Hydrolysis of Pretreated-Lignocellulosic Biomass

After lignocellulosic biomass has been exposed through a pretreatment step, various hydrolysis processes can be used to depolymerize biomass to fermentable sugars.

2.5.1 Concentrated Acid Hydrolysis

The cellulose is dried and decrystallized via a reaction with concentrated acid. The resulting gelatin is then diluted with water and heated to release sugars. In the past, the prohibitively high costs associated with large amounts of sulfuric acid have made the commercialization of concentrated acid hydrolysis rarely found. However, through separation from the sugars by use of either a membrane or a chromatographic column, the acid can be mostly recycled, and as these methods are improved, the process is becoming somewhat more economically (http://www.ef.org/documents/ce_conversion_factsheet_ef_eesi_final_1-08-07).

2.5.2 Dilute Acid Hydrolysis

This process operates on the similar principle as the dilute acid pretreatment, except that the temperature of the reaction is up to 215°C for the purpose of breaking down cellulose. There is a tradeoff between acid concentration and temperature, and using a more dilute acid requires a higher temperature. To heat the large volume of liquid that is required for hydrolysis would be very expensive (http://www.ef.org/documents/ce_conversion_factsheet_ef_eesi_final_1-08-07).

2.5.3 Enzymatic Hydrolysis

Enzymatic hydrolysis of cellulose is carried out by cellulose-hydrolyzing enzymes, called cellulase, to break down the cellulose after it has been pretreated. This technique of hydrolysis is generally accomplished by synergistic action of three distinct classes of cellulase enzymes that are endo-1,4-β-glucanases, exo-1,4-β-D-glucanases, and β-D-glucosidases (http://www.ef.org/documents/ce_conversion_factsheet_ef_eesi_final_1-08-07).

Hernández-Salas *et al.* (2009) studied the production of fermentable sugars through acid and enzymatic hydrolysis of agave residues from pulque industry compared with sugarcane bagasse from sugar factory. Sugarcane bagasse was ground in a small disc mill and sieved through stainless steel sieves to obtain the fractions between 0.149 and 1.68 mm. A steam pretreatment was applied to the bagasse. Then bagasse was autoclaved at 121 °C for 4 h. After the pretreatment, 2 g of bagasse samples were delignified with alkali by the using 2 wt/vol% dilute NaOH solution at a ratio of 5 ml of solution/g of bagasse and then autoclaved. The pH was adjusted with 0.25 M NaOH to get pH 5.0–7.5, depending on the optimum pH value from the enzyme manufacturer, and diluted with distilled water to 15:1 ml of liquid phase/g of bagasse. Delignified bagasse was treated with 0.4 g of the enzyme in a water bath at 55 °C for 4 h. Hydrolyzed samples were analyzed for monosaccharide composition by Refractive index detection via HPLC analysis using Shodex SC1011 column with water at 1.0 ml/min flow rate and 80 °C. Calibration curves were prepared by individual solutions of true glucose and xylose.

2.6 Cellulase Enzymes

The group of enzyme, called cellulase or cellulolytic enzyme, is enzyme that can hydrolyze β-1,4-glucosidic bond in cellulose. Mechanism of cellulase involves synergistic actions by exoglucanase, endoglucanase, and β-glucosidase, as shown in Figure 2.10. Endoglucanases randomly cut accessible intramolecular β-1,4-glucosidic bonds of cellulose chains to generate oligosaccharides of various lengths, and consequently shorter chains appear. Exoglucanases processively cleave at the ends of cellulose chains to release soluble cellobiose or glucose as major products. β-glucosidases hydrolyze produced cellobiose and cellodextrins to glucose to eliminate cellobiose inhibition. These three hydrolysis mechanism steps occur simultaneously. The depolymerization steps carried out by endoglucanases and exoglucanases are the rate-limiting steps for the whole enzymatic hydrolysis process (Zhang *et al.*, 2006).

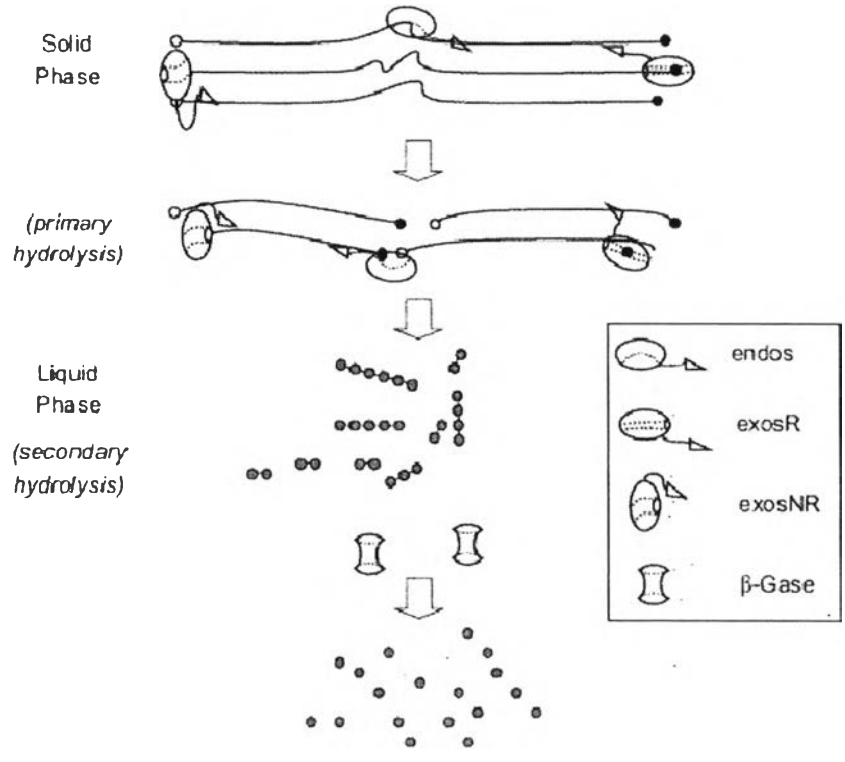

Figure 2.10 Mechanistic scheme of enzymatic cellulose hydrolysis by cellulase enzymes (Zhang *et al.*, 2006).

Taechapoempol (2009) studied the isolation of cellulase-producing bacteria from Thai higher termites for using in cellulose hydrolysis. Forty-seven cellulase-producing bacteria isolated from the termites (Figure 2.11) were identified by measuring hydrolysis capacity value and specific cellulase activity at 37°C and pH 7.2, finally resulting in the three highest HC value isolates: A 002, M 015, and F 018. Identification from the DNA base composition revealed that all effective isolates were Bacillus subtilis. Moreover, these three bacteria were tested for their toxic tolerance to [BMIM]Cl ionic liquid. All isolates were able to tolerate 0.1–1.0 vol% [BMIM]Cl, and no growth retardation in the lag phases, except A 002, which had growth retardation at 0.5–1.0 vol% [BMIM]Cl, was observed.

Higher termites,
Microcerotermes sp.

Bacillus subtilis

Figure 2.11 Higher termites, *Microcerotermes* sp. and cellulase-producing bacteria, Bacillus subtilis (Taechapoempol, 2009).

Worasamutprakarn (2010) investigated conversion of cellulose to glucose by using the above mentioned cellulase-producing bacteria isolated from the higher termites 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 in Figure 2.11 showed that strain F 018 produced the highest glucose concentration at 0.59 g/L after 4 h operation. Moreover, 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.

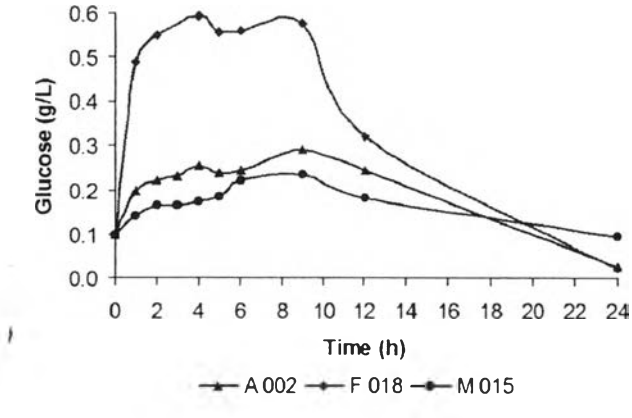

Figure 2.12 Enzymatic hydrolysis of pretreated cellulose for each strain (Worasamutprakarn, 2010).

2.7 Factors Limiting Enzymatic Hydrolysis

Factors that affect the enzymatic hydrolysis of cellulose in lignocellulosic feedstocks can be grouped in two categories: enzyme-related factor and substrate-related factor. However, many of these factors are interrelated during the hydrolysis process. Composition of the liquid fraction and solid process streams resulting from different pretreatment procedures are widely different. These differences cause a great influence on the requirements for effective enzymatic saccharification in subsequent processing steps (Alvíra *et al.*, 2010).

1) Cellulose crystallinity (crystallinity index, CrI)
2) Cellulose degree of polymerization (DP, number of glycosyl residues per cellulose chain)
3) Substrate's available surface area (pore volume)
4) Lignin barrier (content and distribution)
5) Hemicellulose content
6) Feedstock particle size
7) Porosity
8) Cell wall thickness (coarseness)
9) Change in accessibility with conversion

2.8 Components of Biomass Analysis

Li *et al.* (2004) characterize biomass raw material for fast pyrolysis in free-fall reactor to produce hydrogen-rich gas. Each component analysis of the biomass sample was done as follows. Dried biomass sample (G_0, g) was extracted with 2:1(vol) benzene/ethanol at a constant temperature for 3 h. After air-drying, the sample was dried in an oven at 105–110 °C until a constant weight was obtained, cooled to room temperature in a desiccator and weighted as G_1 (g). The residue G_1 was then added with 150 ml 20 g/l NaOH solution and boiled for 3.5 h. It was filtered and washed until no more Na^+. The residue was dried to a constant weight, cooled to room temperature in a desiccator and weighted as G_2 (g). 1 g of the residue after the extraction as G_1 was dried to a constant weight. The sample was then cooled

in a desiccator and weighed (G3, g). 30 ml of sulphuric acid (72%) was added into the sample and kept at 8–15 °C for 24 h. Then, it was diluted with 300 ml of distilled water and boiled for 1 h. After cooling and filtration, the residue was washed until no sulfate ion was left (detected by 10% barium chloride solution). The residue was dried to a constant weight, cooled to room temperature in a desiccator and weighted as G4 (g). Following equations were used to determine each component.

The extractive component, $W_1(wt\%, d) = [(G_0-G_1)/G_0] \times 100,$ (1)

The hemicellulose component, $W_2(wt\%, d) = [(G_1-G_2)/G_0] \times 100,$ (2)

The lignin component, $W_3(wt\%, d) = [G_4 \times (1-W_1)/G_3] \times 100,$ (3)

The cellulose component, $W_4(wt\%, d) = 100 - (A_d + W_1 + W_2 + W_3).$ (4)

The results showed that at the same temperature, the fast pyrolysis of biomass produced more volatile than the slow pyrolysis. The yield and composition of the hydrogen-rich gas product from fast pyrolysis corresponded to the composition of the biomass. Cellulose and hemicellulose compositions produced higher hydrogen-rich gas yield than lignin.

Ververis *et al.* (2007) examined cellulose, hemicellulose, lignin, and ash content of some organic materials and their suitability for use as paper pulp supplements. Firstly, 0.7 g of air-dried 0.5 mm-size ground algal biomass was boiled with 5 ml of 72 wt% H_2SO_4 solution for 4.5 h to hydrolyze the cellulose and hemicellulose fractions. The remaining suspension was filtered through a crucible, and the solid residue was dried at 105 °C for 24 h and weighed as W_1. Then, the residue was heated in a pre-weighed dry porcelain crucible at 600 °C for 5 h, weighed as W_2 after cooling down, and determined for ash content. Acid insoluble lignin was then calculated by $W_1 - W_2$. The filtrate from the H_2SO_4 treatment was entirely stirred and homogenized. Glucose concentration as C_1 and reducing sugar concentration as C_2 in the filtrate were determined by glucose oxidase–peroxidase assay kit (Biosis TM, Athens, Greece) and the DNS method (Miller, 1959), respectively. Then, the following equations were used.

Cellulose content (wt%) $= (0.9/0.96) \times C_1 \times (V/M) \times \alpha \times 100,$ (5)

Hemicellulose content (wt%) = (0.88/0.93) x (C_2-C_1) x (V/M) x α x 100; (6)

where 0.9 is the coefficient from the molecular weight ratio of the polymer and the monomer hexose,

0.96 is the saccharification yield,

0.88 is the coefficient from the molecular weight ratio of the polymer and the monomer pentose,

0.93 is the saccharification yield of xylane to xylose,

C_1 is the glucose concentration (g/l),

C_2 is the determined reducing sugar concentration (g/l) from the DNS method,

V is the total volume of sugar solution (liter),

M is the dry weight of the algal biomass sample (g),

α is the dilution of the sample (if any).

Lin *et al.* (2010) investigated the enzymatic hydrolysis of biomass based on cellulose, hemilellulose and lignin components. Rice straw using as the raw material of this research was washed, dried, ground and sieved with 40-mesh screen. 1 g of the biomass was soaked in 60 ml acetone at 90 °C for 2 h. After that, the residue was filtered and dried at 105 °C until a constant weight was obtained. The weight difference before and after the extraction is the extractives component. 10 ml 0.5 mol/l of sodium hydroxide solution was added into a glass containing 1 g of extractive-free biomass and held at 80 °C for 3.5 h. After that, the sample was washed to neutral pH and dried until the weight was constant. The difference between the weight before and after is the hemicellulose component. 1 g of the extractive-free biomass was hydrolyzed with 30 ml of 98 wt% sulfuric acid at ambient temperature for 24 h and boiled at 100 °C for 1 h. The sample was filtered and washed by titration of a 10% barium chloride solution until no sulfate ion was left in the filtrate. After that, the residue was dried until the weight was constant. The weight of the residue is the lignin component. By assuming that components of biomass are only extractives, hemicellulose, lignin, and cellulose, cellulose component was calculated by the total difference. Furthermore, they found that

hemicellulose and cellulose play different roles in staged hydrolysis at different time periods and the existence of free lignin had negligible effect on the enzymatic hydrolysis of cellulose and hemicellulose using the enzyme complex.