CHAPTER II

THEORETICAL BACKGROUND AND LITERATURE REVIEW

2.1 Biomass-based Fuel

An alternative fuel is biomass-based fuel as known as biofuel. Biomass is classified into 2 categories, waste products and dedicated energy crops. Waste products include wood waste material (e.g. saw dust, wood chips, etc.), crop residues (e.g. corn husks, wheat chaff, etc.), and municipal, animal and industrial wastes (e.g. sewage sludge, manure, etc.). Dedicated energy crops, including short-rotation woody crops like hard wood trees and herbaceous crops like switchgrass, are agricultural crops that are solely grown for use as biomass fuels. These crops have very fast growth rates and can therefore be used as a regular supply of fuel (Sami *et al.,* 2001).

Biomass-based fuel made from bio-based materials through thermochemical process such as pyrolysis, gasification, liquefaction, supercritical fluid extraction, supercritical water liquefaction and biochemical (Balat, 2011). Conversion of biomass to biofuels presents an 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 (Kumar *et al.,* 2009).

Biomass, especially woody biomass and energy crops, is already an important energy carrier contributing substantially to cover energy demands in many parts of the world. This energy carrier has the potential to contribute even more to provide energy to replace the use of fossil fuel energy, especially in industrial countries as well as in developing countries. International biofuel trade is going to be an important factor in the future. These facts are beneficial when considering production of biofuels based on biomass (Parikka, 2004).

2.2 Bioethanol

Alcohols can be used as transportation fuels such as methanol (CH_3OH), ethanol (C_2H_5OH), propanol (C_3H_7OH), butanol (C_4H_9OH). However, only methanol and ethanol fuels are technically and economically suitable for internal combustion engines (Demirbas *et al.*, 2009).

Bioethanol is used in variety of ways; the major widely used bioethanol is an oxygenated fuel additive (Wheals *et. al.* 1999). Bioethanol and bioethanol/ gasoline blends have a long history as alternative transportation fuels. It has been used as internal combustion engines in Germany and France since 1894 (Demirbas and Karslioglu, 2007). Brazil has utilized bioethanol as a fuel since 1925. By that time, the bioethanol production was 70 times higher than petrol production (Lang *et al.*, 2001). The bioethanol is widely used as a fuel in Europe and US since 1900s. Mostly, bioethanol is produced by fermentation of corn glucose in the US or sucrose in Brazil. The major raw material in each country is shown as Table 2.1.

Table 2.1 The major raw material in each country (Balat, 2011)

Country	*Energy Crop*	*Bioethanol Yield (l/ha)*
Brazil	Sugarcane, 100%	6641
USA	Corn, 98%	3770
	Sweet sorghum, 2%	1365
China	Corn, 70%	2011
	Wheat, 30%	1730
EU-27	Wheat, 48%	1702
	Sugar beet, 29%	5145
Canada	Corn, 70%	3460
	Wheat, 30%	1075

Nevertheless, the major raw materials of bioethanol production in present are main food crop. Therefore technology for ethanol production from nonfood-plant source has been interested to large-scale production. Agronomic residues, such as corn stover (corn cobs and stalks), sugar cane waste, wheat or rice straw, forestry, and paper mill discards, the paper portion of municipal waste and dedicated energy crops can be converted into fuel ethanol (Lin and Tanaka, 2006).

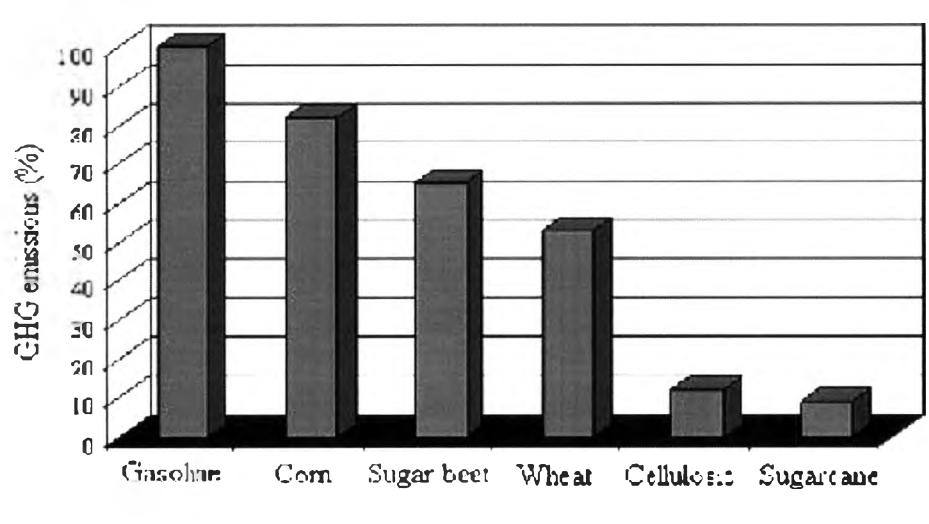

Figure 2.1 Reduction in GHG emissions by bioethanol produced from a variety of feedstocks on a life-cycle basis compared to gasoline (Philippidis and Smith, 1995).

Using ethanol fuel for automobile can be utilized as oxygenate of gasoline elevating its oxygen content, allowing a better oxidation of hydrocarbons and reducing the amounts of greenhouse gas emission into the atmosphere (Hill *et al.*, 2006)

Furthermore, ethanol is attractive alternative for replace to methyl tertiary butyl ether (MTBE), the common oxygenated additive to gasoline intend to improve the combustion process and more specifically, to significantly reduce CO emission. MTBE is a toxic chemical compound that was considered as a potential human carcinogen (Belpoggi *et al.*, 1995).

Although, bioethanol production has been improved by technologies, there are still challenges to achieve lower costs, thus the supply of cheap raw materials is

necessity (Galbe and Zacchi, 2002). Lignocellulosic raw material has been considered as feedstock because of its renewable, abundance, and low cost (Saha *et al.*, 2005).

2.3 Lignocellulosic Biomass

The ethanol fuel production from lignocellulosic biomass has become an important process for producing environmentally friendly renewable energy (Girio *et al.*, 2010), especially from lignocellulosic biomass in which reduces competition with food demand. Lignocellulosic biomass represents the major fraction of plant matter. Generally, lignocellulosic materials for ethanol fuel production can be divided into 6 groups (Verma *et al.*, 2011).

i) Crop residues (cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum bagasse, olive stones, and pulp)

ii) Hardwood (aspen, poplar)

iii) Softwood (pine, spruce)

iv) Cellulose wastes (newsprint, waste office paper, recycled paper sludge)

v) Herbaceous biomass (alfalfa hay, switchgrass, reed canary grass, coastal bermudagrass, thimothy grass, miscanthus grass)

vi) Municipal solid wastes

Lignocellulosic biomass composes of a mixture of carbohydrate polymer (cellulose and hemicelluloses) and lignin. The carbohydrate polymers are tightly boundary to lignin by hydrogen bond and some covalent bonds. The conversion of lignocellulose to ethanol fuel thus requires delignification to discharge carbohydrate polymer from lignin, depolymerization of the carbohydrate polymer to produce sugar, fermentation of monomeric sugar to ethanol (Lin and Tanaka, 2006), thus increasing cost of ethanol fuel produced from lignocellulosic biomass. However, ethanol fuel is still interesting to research to achieve lower cost for production (Yu and Zhang, 2004).

2.4 The Composition of Lignocellulosic Biomass

Significance of the ethanol conversion from lignocellulosic biomass is chemical composition of which the structure and chemical composition are extremely variable because of genetic and environmental influences and their interactions (Lee *et al.*, 2008). Lignocelluloses are mainly composed of cellulose, hemicelluloses, and lignin, along with small amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jorgensen *et al.*, 2007).

The presence of lignin in lignocelluloses leads to a protective barrier that prevents plant cell destruction by yeast or bacteria for conversion cellulose and hemicellulose to fuel (Broder *et al.*, 1995). The structure of lignocellulosic biomass is shown in Figure 2.2.

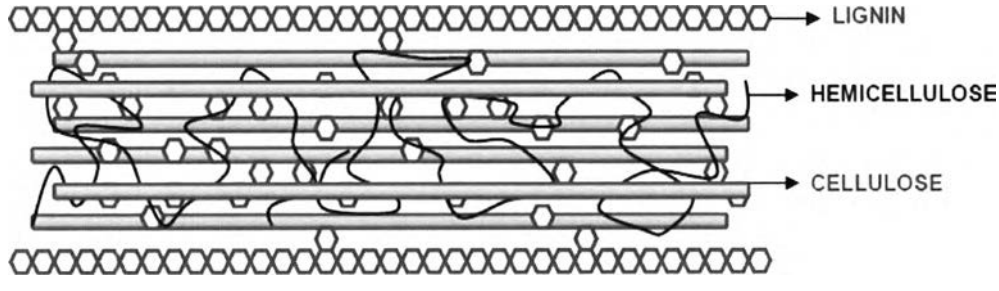

Figure 2.2 Representation of lignocelluloses structure showing cellulose, hemicellulose and lignin fractions (Fengel and Wegener, 1984).

The major fraction of lignocellulose, typically of 35–50 %, is a polymer of glucose known as cellulose. The next largest fraction, of 20–35 %, is hemicellulose. Hemicellulose is also a polymer of sugars, consisting of xylose, arabinose, mannose, galactose, rhamnose, glucurono-pyranose, galacturono-pyranose. However, the types and distributions of these sugars are varied, depending on the biomass source. For many types of lignocellulosic biomass, the five carbon sugar xylose represents the predominant fraction of the hemicellulose component. The third largest fraction is typically lignin (15–25 %), a phenyl-propene polymer of complex composition that

cannot be broken down to form sugar molecules (Wyman, 1994). The typical chemical constituents vary by groups of lignocelluloses, as shown in Figure 2.3.

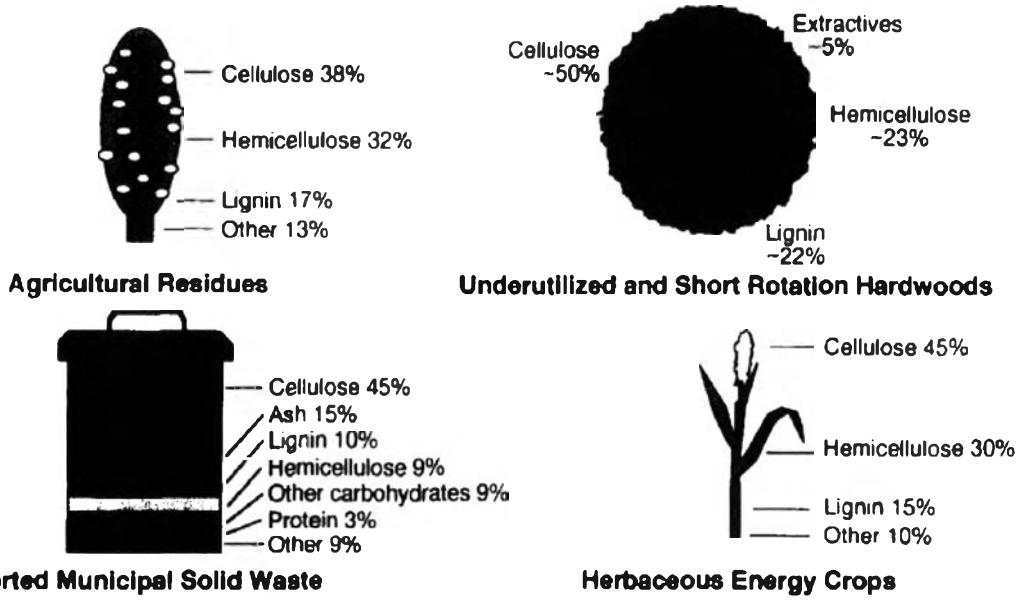

Figure 2.3 The chemical composition ratio of lignocellulose (Wyman, 1994).

Moreover, there are several researches studying the chemical composition of different lignocelluloses biomasses, as shown in Table 2.2. Both Figure 2.3 and Table 2.2 show the potential of lignocelluloses biomass for conversion to ethanol biofuel.

Cellulose

Cellulose is the main structural constituent in cell walls of plant, particularly in stalks, stems, trunks and all woody portions of plant tissue. An unbranched homopolysaccharide, cellulose is composed of β-D-glucopyranose units linked by 1,4 glycosidic bonds (Dudley *et al.*, 1983). These pyranose rings have been found to be in the chair conformation with the hydroxyl groups in an equatorial position, as shown in Figure 2.4.

Table 2.2 Composition of various types of lignocellulosic-biomass material (Reshamwala et *al.*, 1995; Boopathy, 1998; Demirbas, 2004; Jorgensen *et al.*, 2007)

Lignocellulosic Material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Barley straw	33.8	21.9	13.8
Coastal bermudagrass	25	35.7	6.4
Corn cobs	45	35	15
Corn fiber	15	35	8
Corn stalks	35	16.8	7
Corn stover	40	25	17
Cotton seed hairs	80-95	5-20	-
Cotton stalks	58.5	14.4	21.5
Grasses	25-40	35-50	10-30
Hardwood stems	40-55	24-40	18-25
Leaves	15-20	80-85	-
Newspaper	40-55	25-40	18-30
Nut shells	25-30	25-30	30-40
Oat straw	39.4	27.1	17.5
Paper	85-99	-	0-15
Primary wastewater solids	8-15	-	-
Rice straw	36.2	19	9.9
Rye straw	37.6	30.5	19
Softwood stems	45-50	25-35	25-35
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Sorted refuse	60	20	20
Soya stalks	34.5	24.8	19.8
Sugarcane bagasse	40	27	10
Sunflower stalks	42.1	29.7	13.4
Swine waste	6	28	Na
Switchgrass	45	31.4	12
Waste papers from chemical pulps	60-70	10-20	5-10
Wheat straw	30	50	15

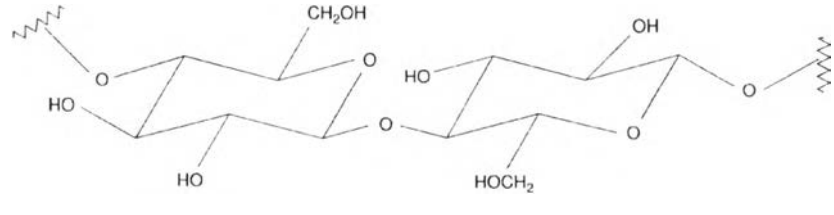

Figure 2.4 Fragment (repeating unit) of a cellulose chain (Antoinette, 1997).

Cellobiose is the repeat unit established through this linkage, and it constitutes cellulose chains, as shown in Figure 2.5. Naturally, cellulose chains have a degree of polymerization (DP) of approximately 10,000 cellobiose units in wood cellulose and 15,000 in native cotton cellulose (SjostrÖm *et al.*, 1981). The long-chain cellulose polymers are linked together by hydrogen and Van der Waals bonds, which cause the cellulose to be packed into microfibrils (Beguin and Aubert, 1994).

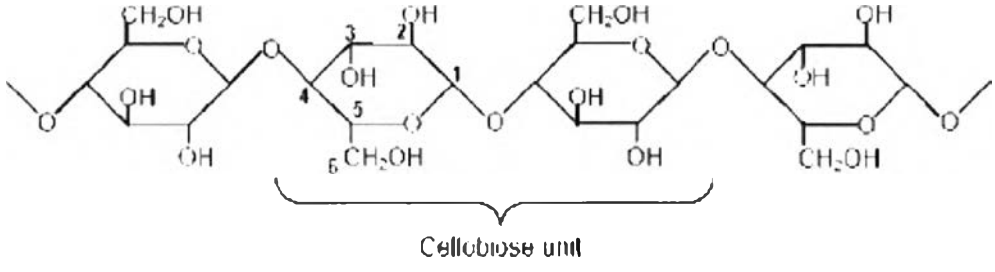

Figure 2.5 Illustration of a cellulose chain (Kumar *et al.*, 2009).

By forming hydrogen and Van der waal bonds, the chains of cellulose tend to arrange in parallel and form a crystalline structure. Therefore, cellulose microfibrils have both highly crystalline regions and less amorphous regions. More ordered or crystalline cellulose is less soluble and less degradable (Zhang and Lynd, 2004; Taherzadeh and Karimi, 2007). The crystalline cellulose makes the polymer rigid and difficult to break. In polysaccharide hydrolysis is broken down cellulose to free sugar molecules by addition of water. The product is a six-carbon sugar as known as glucose (Hamelinck *et al.*, 2005).

Hemicellulose

Hemicellulose is a heterogeneous class of polymers representing 15-35 % of plant biomass. Hemicellulose is a short, partially linear and highly branched polymer which may compose different types of sugar for example pentose (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). Other sugars such as α-L-rhamnose and α-L-fucose may also be present in small amounts and other components, such as acetic, glucuronic, and ferulic acids (Girio *et al.*,

2010). The monomer of hemicelluloses is shown in Figure 2.6. The backbone of the chains of hemicelluloses can be generally consisting of single sugar repeat unit as homopolymer or a mixture of different sugars as heteropolymer (Mussatto *et al.*, 2010).

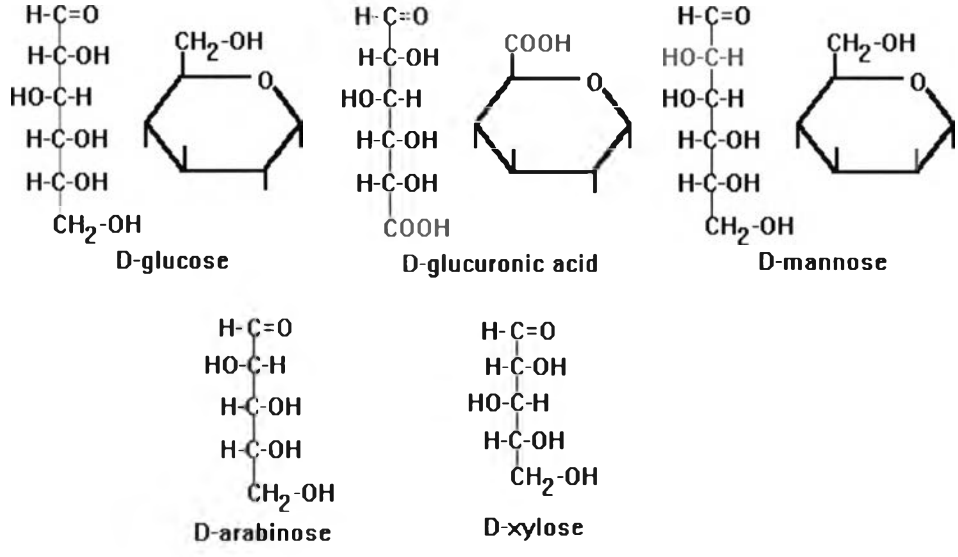

Figure 2.6 The monomer of hemicelluloses (Girio *et al.*, 2010).

Xylans are the main hemicelluloses components, having about 20–30 % of hardwood biomass and herbaceous plants. In some tissues of grasses and cereals xylans can be up to 50 % (Ebringerová *et al.*, 2005). Xylans are usually available in large amounts as herbaceous biomass, by-products of forest, agriculture, agro-industries, wood and pulp and paper industries. The degree of polymerization of hardwood xylans, 150–200, is higher than that of softwoods, 70–130 (Saha *et al.*, 2005). In contrast to cellulose, the polymers present in hemicelluloses are shorter chain, branching of main chain molecules, mostly amorphous which was easily hydrolyzed (Fengel and Wegener, 1984).

Lignin

Lignin is a large complex molecule, containing cross-linked polymers of phenolic monomers (Perez *et al.*, 2002). Lignin associated with cellulose and hemicelluloses in the plant cell and its function is to provide rigidity and cohesion to the material cell wall, to confer water impermeability to xylem vessels, and to form a physic-chemical barrier against microbial attack (Fengel and Wegener, 1984).

Unlike most natural polymers, which consist of a single intermonomeric linkage, lignin is a network polymer made up of many carbon-to-carbon and ether linkages. The tight physical binding and chemical linkages between lignin and cell-wall polysaccharides also practically prevents its isolation in an unaltered form. This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl and carbonyl, which impart a high polarity to the lignin macromolecule (Fengel and Wegener, 1984).

The lignin structure consists of a cross-linked polymer of glyceryl methoxyphenol units. These units are commonly referred to as guaiacyl (the most abundant in gymnospermous wood), syringyl, and p-hydroxyphenyl units. The major type of linkage between these monomers is the β-O-4 linkage, where the β carbon atom of the side chain is linked with the oxygen atom connected to the carbon atom. In natural lignin, dimmers formed by a β-O-4 linkage occur in two diastereomeric forms threo and erythro (Akiyama *et al.*, 2003).

Softwood and hardwood lignin belongs to the first and second category, respectively. Softwoods generally contain more lignin than hardwoods (Demirbas, 2008). Lignin contents on a dry basis in both softwoods and hardwoods generally range from 20 % to 40 % by weight and from 10 % to 40 % by weight in various herbaceous species, such as bagasse, corncobs, peanut shells, rice hulls and straw (Yaman, 2004). Lignin is one of drawbacks of using lignocellulosic-biomass material in conversion to ethanol fuel due to its molecular configuration and its extreme resistance to chemical and enzymatic degradation (Palmqvist and Hahn-Hagerdal, 2000).

2.5 Ethanol Conversion Process

Ethanol production process comprises of five stages: pretreatment (first hydrolysis), saccharification (second hydrolysis), detoxification, fermentation and separation (Balat, 2011). Biochemical conversion of lignocellulosic materials through saccharification and fermentation is a major pathway for bioethanol production from biomass. The ethanol production process is shown in Figure 2.7.

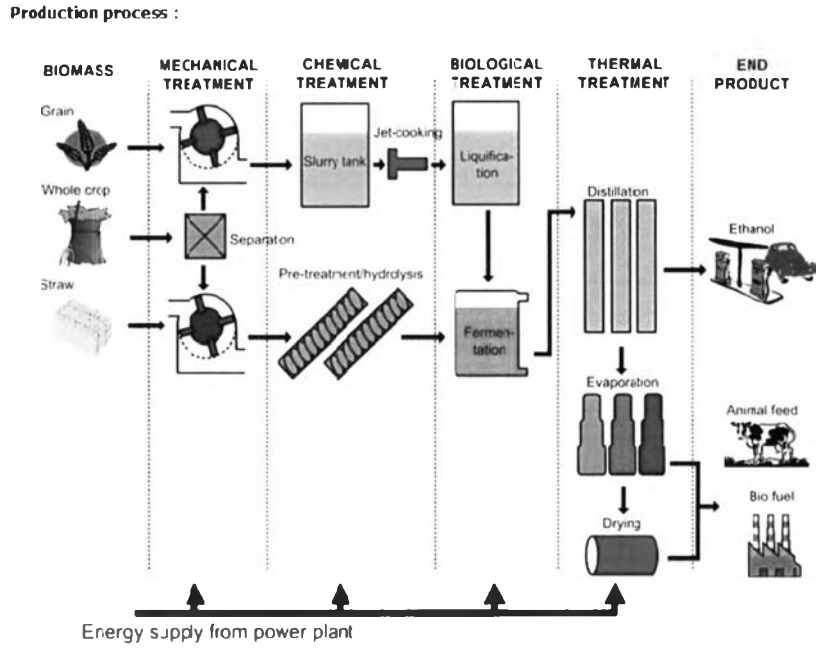

Figure 2.7 The ethanol production process (Galbe and Zacchi, 2002).

Bioconversion of lignocellulosic biomass to bioethanol is rather difficult due to the resistant nature of biomass to breakdown, the variety of sugars which are released when the hemicelluloses and cellulose polymers are broken, and the need to find or genetically engineer organisms to efficiently ferment these sugars, costs for collection and storage of low density lignocellulosic materials (Balat, 2011).

A number of pretreatments, such as concentrated acid hydrolysis, dilute acid hydrolysis, alkali treatment, sodium sulfite treatment, sodium chlorite treatment, steam explosion, ammonia fiber explosion, lime treatment have been used to remove lignin and to improve the saccharification of the cell wall carbohydrates. The

pretreatment is necessary to increase the rate of production and the total yield of monomeric sugars in hydrolysis step (Quintero *et al.*, 2011). Hydrolysis of biomass is significant for generation of fermentable sugars which then converted to ethanol. Acid and enzymatic approaches are primarily employed for biomass hydrolysis with varying efficiencies depending on treatment conditions, type of biomass, and the properties of the hydrolytic agents.

Detoxification is a previous step before fermentation to remove furfural and other inhibitors like soluble lignin compounds, giving a problem for the fermentation step because such compounds can inhibit, or even stop the fermentation (Laser *et al.*, 2002).

The produced monomeric hexoses, six carbon sugars, can be fermented to ethanol well easily, while the fermentation of pentoses, five carbon sugars, is only done by a few strains. Volatile products are also not easily fermented to ethanol. A problem occurring during the fermentation is that the formed product ethanol is an inhibitor for the yeasts/ bacteria. This makes a limit to the concentration of fermentable sugars. (Hendriks and Zeeman, 2009).

After fermentation, the ethanol has to be recovered from the fermentation broth by separation (Mosier *et al.*, 2005). Generic block diagram of bioethanol production from lignocelluloses material is given in Figure 2.8.

Today the production cost of bioethanol from lignocelluloses is still too high. Significant growth of bioethanol industry has to improve economy and efficience of production processes that convert cellulosic biomass from lignocellulose into ethanol (Woodson and Jablonowski, 2008).

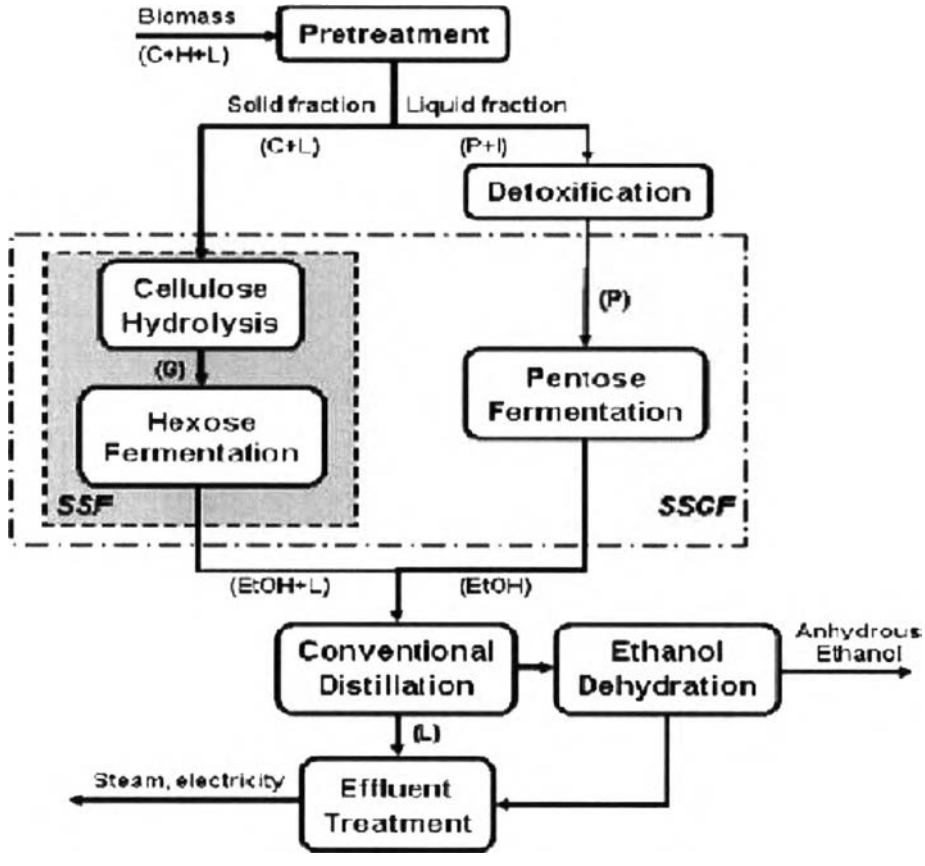

Figure 2.8 Generic block diagram of bioethanol production from lignocelluloses biomass. Possibilities for reaction-reaction integration are shown inside the shaded boxes: SSF-simultaneous saccharification and fermentation; SSFC-simultaneous saccharification and co-fermentation. Main stream components are: C-cellulose; H-hemicellulose; L-lignin, G-glucose; P-pentose; I-inhibitors; EtOH-ethanol (Cardona Alzate and Sanchez, 2006).

2.6 Pretreatment of Lignocellulosic Biomass

The recalcitrance of lignocelluloses is one of the major barriers to the bioethanol economical production. The technical approach to overcome recalcitrance has been pretreatment of biomass feedstock to remove the barriers and make cellulose more accessible to hydrolytic enzymes for conversion to glucose (Zhu *et*

al., 2008). The objectives of pretreatment on lignocellulosic material are described in Figure 2.9.

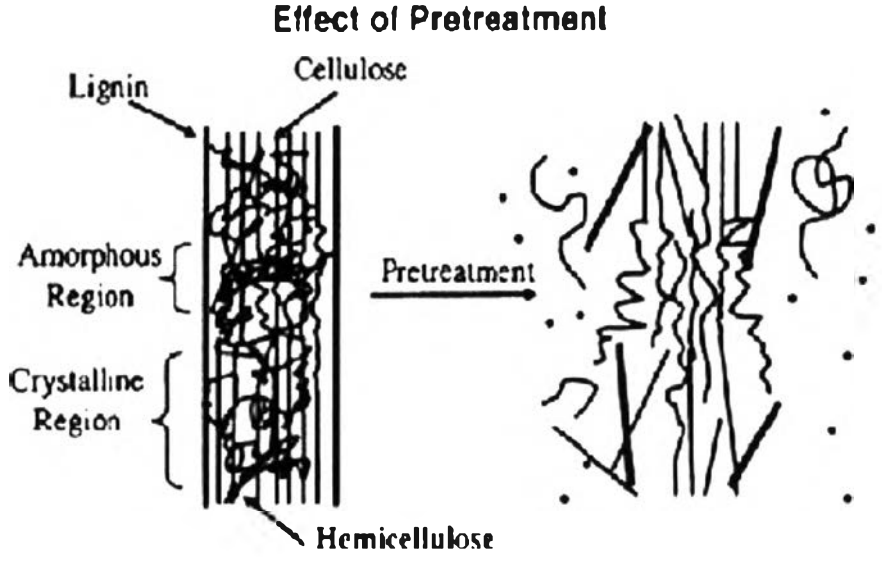

Figure 2.9 Schematic of goals of pretreatment on lignocellulosic material (Hsu *et al.*, 2010).

If the pretreatment is not efficient enough, the resultant residue is not easily hydrolysable by cellulase enzyme and if it is more severe, result is the production of toxic compounds which inhibit the microbial metabolism (Kodali and Pogaku, 2006).

The prerequisites for ideal lignocelluloses pretreatment (Taherzadeh and Karimi, 2007) are

i) Production of reactive cellulosic fiber for enzymatic attack

ii) Avoiding destruction of hemicelluloses and celluloses

iii) Avoiding formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms

iv) Minimizing the energy demand

v) Reducing the cost of size reduction for feedstocks

vi) Reducing the cost of material for construction of pretreatment reactors

vii) Producing less residues

viii) Consumption of little or no chemical and using a cheap chemical

Pretreatment is crucial for ensuring good ultimate yields of sugars from both polysaccharides. Hydrolysis without preceding pretreatment yields typically less than 20 % whereas yields after pretreatment often exceed more than 90 % (Hamelinck *et al.*, 2005). Pretreatment method can be divided into 4 groups.

2.6.1 Physical Pretreatment

2.6.1.1 Mechanical Pretreatment

Lignocellulosic materials can be comminuted by a combination of chipping, grinding, and milling to reduce particle size and cellulose crystallinity (Leustean, 2009). The reduction in particle size leads to an increase of available specific surface and a reduction of the degree of polymerization. Power requirements of mechanical comminution depend on the final particle size and the characteristic of lignocellulosic biomass (Cadoche *et al.*, 1989). Power requirements increase rapidly with decreasing particle size, as shown in Figure 2.10.

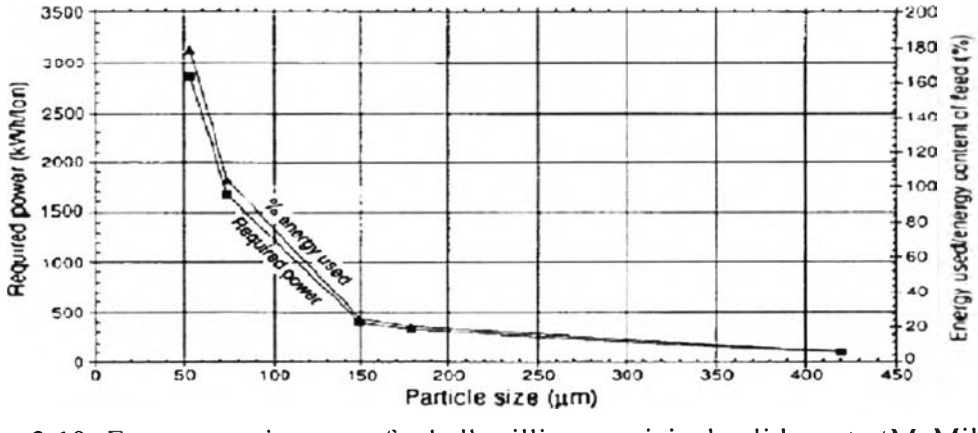

Figure 2.10 Energy requirements for ball milling municipal solid waste (McMillan, 1994).

2.6.1.2 Thermal Treatment (Pyrolysis)

During this pretreatment, the lignocellulosic biomass is heated. When the temperature increase above 150–180 °C, firstly the hemicelluloses starts to solubilize after that the solubilization of lignin starts at above 160 °C which

produces phenolic compounds. It is an inhibitory or toxic effect on yeast and bacteria in fermentation step. If the lignocellulosic biomass is treated at temperature greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char (Sun and Cheng, 2002). The thermal pretreatment improves the conversion of cellulose to glucose yield from hydrolysis step (Leustean, 2009).

2.6.2 Physico-chemical Pretreatment

2.6.2.1 Steam Explosion (Autohydrolysis)

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic biomass (McMillan, 1994). Steam explosion treatment increases crystallinity of cellulose by promoting crystallization of the amorphous portion. Hemicellulose is easily hydrolyzed and steam explosion promotes delignification. In this method, chipped biomass is treated with high pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decomposition. Steam explosion is considered the most cost effective option for hardwood and agriculture residues, but is less effective for softwood (Prasad *et al.*, 2007).

2.6.2.2 Ammonia Fiber Explosion

Ammonia fiber explosion (AFEX) is one of the alkaline physic-chemical pretreatment processes. In this process, the material is subjected to liquid ammonia at high temperature and pressure which make a subsequent fast decompression. Similar to the steam explosion, Ammonia fiber explosion causes a fast saccharification of lignocellulosic material (Abril and Abril, 2009). The effective parameters in the ammonia fiber explosion processes are ammonia loading, temperature, water loading, pressure, time, and number of treatments (Taherzadeh and Karimi, 2007). Ammonia fiber explosion works only moderately and is not attractive for the biomass with high lignin content (Balan *et al.*, 2009).

2.6.2.3 Liquid Hot-water Pretreatment

Biomass is treated with water at high temperature and pressure during a fixed period and it presents elevated recovery rates for pentoses and generates low amount of inhibitors (Tomas-Pejo *et al.*, 2008). This pretreatment process has involved temperatures of 200–230 °C for up to 15 min, around 40–60%

of the total mass is dissolved, with 4–22% of the cellulose, 35–60% of the lignin and all of the hemicelluloses being removed (Hu *et al.*, 2008). If the pH is maintained between 4 and 7, the degradation of monosaccharide sugars can be minimized (Hayes, 2009).

2.6.3 Chemical Pretreatment

2.6.3.1 Ozonolysis

Ozonolysis involves in using ozone gas to breakdown the lignin and hemicellulose and increase the biodegradability of the cellulose. The pretreatment is usually carried out at room temperature and is effective at lignin removal without the formation of toxic by-products (Vidal *et al.*, 2011). Ozonation has been widely used to reduce the lignin content of both agricultural and forestry wastes (Neeley, 1984). Disadvantage of ozonolysis is that a large amount of ozone is required, which can make the process expensive (Kumar *et al.*, 2009).

2.6.3.2 Alkaline Pretreatment

Alkaline pretreatment refer to the application of alkaline solutions to remove lignin and various uronic acid substitutions on hemicelluloses that lower the accessibility of enzyme to the hemicelluloses and celluloses (Silverstein *et al.*, 2008; Han *et al.*, 2009). These processes utilize lower temperature and pressure compared to other pretreatment technologies. Alkali pretreatment may be carried out at ambient conditions, but pretreatment time is required in term of hours or days. Sodium, potassium, calcium and ammonium hydroxide are appropriate chemicals for pretreatment. Dilute NaOH treatment of lignocellulosic biomass cause swelling, leading to an increase in the internal surface area, a decrease in crystallinity, separation of structural linkages between lignin and carbohydrates, and disruption of the lignin structure (Mosier *et al.*, 2005).

2.6.3.3 Acid Pretreatment

Acid pretreatment aims for high yields of sugars from lignocellulosic materials. Acid pretreatment involves the use of sulfuric, nitric, or hydrochloric acids to remove hemicelluloses components and lignin and expose cellulose for enzymatic digestion (Silverstein *et al.*, 2008). The acid pretreatment can operate either under high temperature and low acid concentration or under low

temperature and high acid concentration (Taherzadeh and Karimi, 2007). Dilute acid hydrolysis has been developed for pretreatment of lignocellulosic biomass. The dilute acid pretreatment works fairly well on agricultural feedstock (Zhu *et al.*, 2008). While dilute acid pretreatment is known to improve enzymatic hydrolysis, their cost is relatively high compared to physico-chemical pretreatment (Keshwani and Cheng, 2009). This pretreatment method gives high reaction rates and significantly improves cellulose hydrolysis (Karimi *et al.*, 2006). However, dilute acid has some drawbacks, for example corrosion, need of neutralization before fermentation, formation of degradation and inhibitor (Zheng *et al.*, 2009).

2.6.3.4 Organosolv Pretreatment

In the organosolv process, an organic or aqueous organic solvent mixture with inorganic acid catalysts (HCL or H_2SO_4) is used to break the internal lignin and hemicelluloses bonds. The organic solvents used in the process include methanol, ethanol, acetone, ethylene glycol, triethylene glycol and tetrahydrofurfuryl alcohol (Chum *et al.*, 1988; Thring *et al.*, 1990). Organic acids such as oxalic, acetylsalicylic and salicylic acid can also be used as catalysts in the organosolv process (Sarkanen, 1980). At the high temperature (Above 185 °C), the addition of catalyst was unnecessary for satisfactory delignification (Aziz and Sarkanen, 1989). Solvents used in the process need to be drained from the reactor, evaporated, condensed and recycled to reduce the cost. Removal of solvents from the system is necessary because the solvents may be inhibitor to fermentation process.

2.6.4 Biological Pretreatment

Biological pretreatment involves microorganisms, such as brown-, white-, and soft-rot fungi that are used to degrade lignin and solubilize hemicellulose. White-rot fungi are the most effective biological pretreatment of lignocellulosic biomass. The advantages of this method include low energy requirement and friendly with environmental. However, the rate of hydrolysis is very low (Sun and Cheng, 2002).

Summary of advantages and disadvantage of each pretreatment is shown in Table 2.3.

Table 2.3 Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Kumar *et al.*, 2009)

Pretreatment Process	Advantages	Disadvantages
Mechanical	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam Explosion	Causes hemicelluloses degradation and lignin transformation; cost-effective	Destruction of a portion of the xylan fraction; incomplete disruption of the lignin carbohydrate matrix; generation of compounds inhibitory to microorganisms
Ammonia Fiber Explosion	Increases accessible surface area, removes lignin and hemicelluloses to an extent; does not produce inhibitors for down-stream processes	Not efficient for biomass with high lignin content
CO_2 Explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduce lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid Hydrolysis	Hydrolyzes hemicelluloses to xylose and other sugars; alters lignin structure	High cost; equipment corrosion; formation of toxic substances
Alkaline Hydrolysis	Removes hemicelluloses and lignin; increases accessible surface area	Long residence times required; irrecoverable salts formed and incorporated into biomass

Table 2.3 Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Kumar *et al.*, 2009) (Con't.)

Pretreatment Process	*Advantages*	*Disadvantages*
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost
Pyrolysis	Produces gas and liquid products	High temperature; ash production
Pulsed Electrical Field	Ambient conditions; disrupts plant cells	Process needs more research
Biological	Simple equipment degrades lignin and hemicelluloses; low energy requirements	Rate of hydrolysis is very low

2.7 Hydrolysis

The carbohydrate polymers in lignocellulosic materials have to be converted to simple sugars before fermentation (Taherzadeh and Karimi, 2007). The most commonly applied methods can be classified in two groups: chemical hydrolysis (dilute and concentrated acid hydrolysis) and enzymatic hydrolysis. Several products can be resulted from hydrolysis of lignocellulosic biomass, as shown in Figure 2.11.

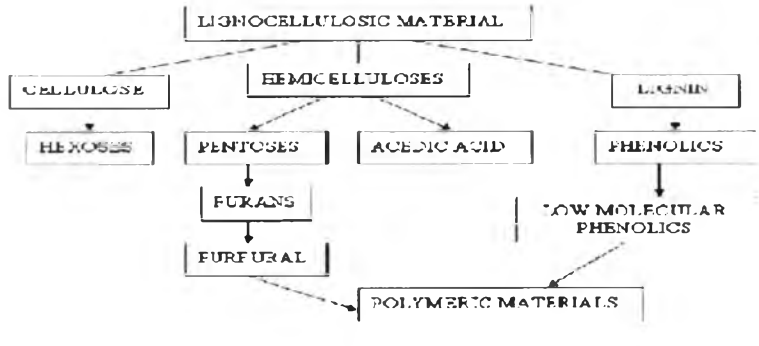

Figure 2.11 Main degradation products occurring during hydrolysis of lignocellulosic material (Demirbas, 2008).

When hemicelluloses are hydrolyzed to xylose, mannose, acetic acid, galactose (Equations 2.1 and 2.2), glucose is liberated (Gamez *et al.*, 2004).

Hemicelluloses → Xylan → Xylose → Furfural (2.1)

Acetyl group → Acetic acid (2.2)

Degradation of xylan yields eight main products: water, methanol, formic, acetic, propionic acids, hydroxyl-1-propanone, hydroxyl-1-butanone and 2-furfuraldeyde (Gullu, 2003). Under high temperature and pressure, xylose is further degraded to furfural (Dunlop, 1948). Similarly, 5-hydroxymethyl furfural is formed from hexose degradation (Ulbricht *et al.*, 1984).

Cellulose is hydrolyzed to glucose and decomposed products, as shown in Equation 2.3

Cellulose → Glucan → Glucose (2.3)

→ Decomposition products

Generally, hydrolysis process can be categorized into 2 categories.

2.7.1 Chemical Hydrolysis

Acid hydrolysis mainly produced xylose from xylan with the cellulosic and lignin fractions remaining unaltered. Xylan is more susceptible to hydrolysis by mild acid treatment because of its amorphous structure compared to cellulose, which needs severe treatment conditions for its crystalline nature (Rahman *et al.*, 2007). During acid hydrolysis, xylose is degraded rapidly to furfural and other condensation byproducts. These degradation products are inhibitory to microorganisms (Rao *et al.*, 2006). Acid-catalyzed cellulose hydrolysis is a complex heterogeneous reaction. There are two basic types of acid hydrolysis processes commonly used: dilute acid and concentrated acid (Xiang *et al.*, 2003).

2.7.1.1 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 low reaction time. Dilute acid process involves a

solution of about 1 % H_2SO_4 concentration in a continuous flow reactor at a high temperature. Most dilute acid processes are limited to a sugar recovery efficiency of around 50 %. Dilute acid hydrolysis occurs in two-stages to take advantage of the differences between hemicellulose and cellulose. The first stage is performed at low temperature to maximize the yield from the hemicelluloses, and the second stage, higher temperature is optimized for hydrolysis of the cellulose portion (Boonmanumsin *et al.*, 2012). Schematic flowsheet for dilute acid hydrolysis is shown in Figure 2.12.

2.7.1.2 Concentrated Acid Hydrolysis

The acid concentration used in the concentrated acid hydrolysis process is in the range of 10-30 % (Iranmahboob *et al.*, 2002). 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. In comparison to dilute acid hydrolysis, concentrated acid hydrolysis leads to little sugar degradation and gives sugar yields approaching 100 %. The concentrated acid process offers more potential for cost reductions than the dilute acid process. However, environment, corrosion problems, the high cost of acid consumption and recovery present major problems of concentrated acid hydrolysis (Yu *et al.*, 2008).

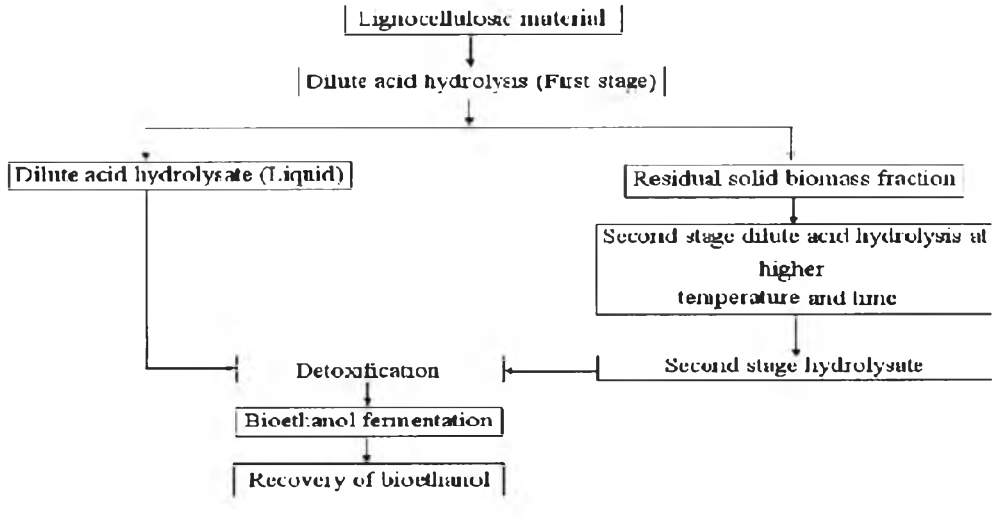

Figure 2.12 Dilute acid hydrolysis (first-stage and two-stages) and separate fermentation of pentose and hexose sugars (Chandel et al., 2009).

2.7.2 Enzymatic Hydrolysis

2.7.2.1 Enzymatic Hydrolysis of Celluloses

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 hemicelluloses content, surface area, and cellulose crystallinity X. (Pan *et al.*, 2006). Utility cost of enzymatic hydrolysis is low compared to acid hydrolysis because enzyme hydrolysis is usually conducted at mild condition (pH 4.8 and temperature 318-323 K) and does not have a corrosion problem. Enzymatic hydrolysis is attractive because it produces better yields than acid-catalyzed hydrolysis and enzyme manufacturers have recently reduced costs substantially using modern biotechnology (Pan *et al.*, 2005).

Cellulose is typically hydrolyzed by an enzyme called cellulase. These enzymes are produced by several microorgamisms 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 effectively Y. Sun and Cheng, 2002). Fungi, such as *Sclerotium rolfsii, P. chrysosporium* and species of *Trichoderma, Aspergillus, Schizophyllum* and *Penicilium* are used to produce cellulases. Mutant strains of *Trichoderma sp.* (*T. viride, T. reesei, T. longibrachiatum*) have long been considered to be the most productive and powerful destroyers of crystalline cellulose (Zhou *et al.*, 2008). Commercial products of various *T. reesei* isolated have been widely evaluated and applied in relation to bioethanol production processes. Cellulase is a group of enzymes that synergistically hydrolyzes cellulose (Kim and Dale, 2004). The mechanism for enzymatic cellulose hydrolysis involves synergistic actions by endoglucanases (endo-1,4-β-D-glucanases), exoglucanases or cellobiohydrolases (1,4-β-D-glucan cellobiohydrolases), and β-glucosidases or cellobiases. Schematic representation of the cellulase enzymes is shown in Figure 2.13 (Mussatto *et al.*, 2010). Endoglucanases hydrolyze accessible intramolecular β-1,4-glucosidic bonds of cellulose chains ramdomly to produce new chain ends; exoglucanases processively cleave cellulose chains at the ends to release soluble cellobiose or glucose; and β-glucosidases hydrolyze cellobiose to glucose in order to eliminate cellobiose

inhibition (Zhang and Lynd, 2004). β-glucosidases complete the hydrolysis process by catalyzing the hydrolysis of cellobiose to glucose. There are different factors that affect the enzymatic hydrolysis of cellulose such as substrates, cellulase activity, reaction condition, and strong a product inhibition (Sun and Cheng, 2002).

The cellulose features known to affect the rate of hydrolysis include molecular structure of cellulose, crystallinity of cellulose, surface area of cellulose fiber, degree of swelling of cellulose fiber, degree of polymerization and associated lignin or other materials (Detroy and Julian, 1982).

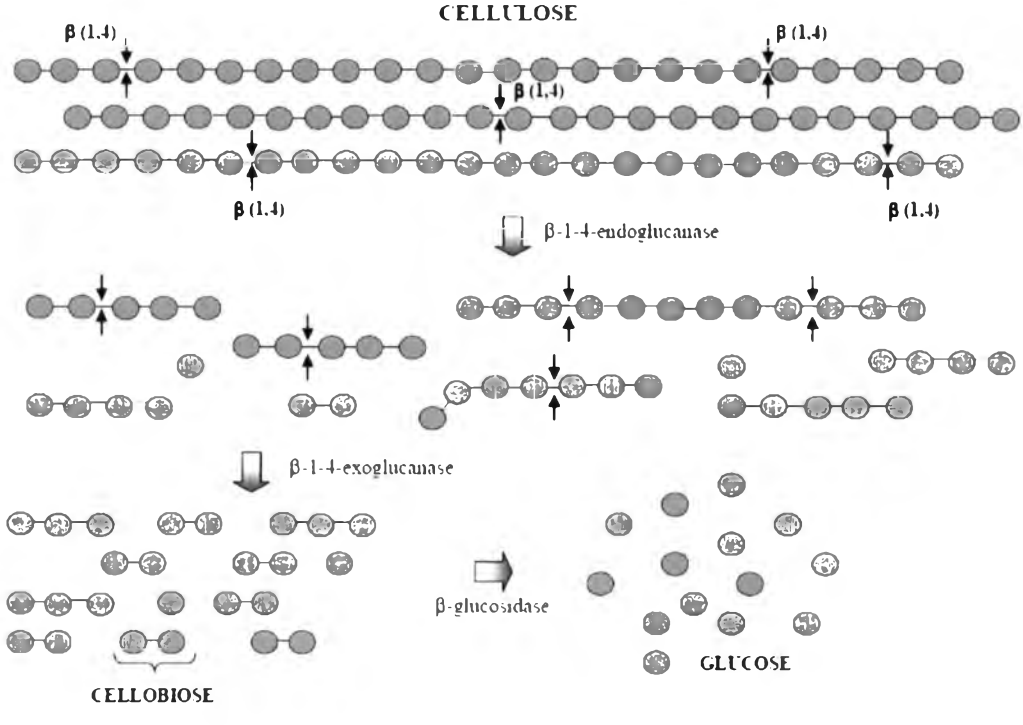

Figure 2.13 Schematic represent of the cellulase enzymes over the cellulose structure (Mussatto *et al.*, 2010).

2.7.2.2 Enzymatic Hydrolysis of Hemicelluloses

Unlike celluloses, hemicelluloses are chemically quite complex. Specific microorganisms of enzymes known as hemicellulases can promote the hemicelluloses hydrolysis. Hemicelluloses are produced by many species of bacteria and fungi. A number of hemicelluloses, including xylanases and

mannanases, have been identified in *Trichoderma reesei* (Foreman *et al.*, 2003). In xylan degradation, for instance, endo-1,4-β-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase and acetylxylan esterase all act on the different heteropolymers available in nature. In glucomannan degradation, β-mannanase, and β-mannosidase cleave the polymer backbone (Kumar *et al.*, 2009). Various enzymes responsible for the degradation of hemicelluloses is shown in Figure 2.14.

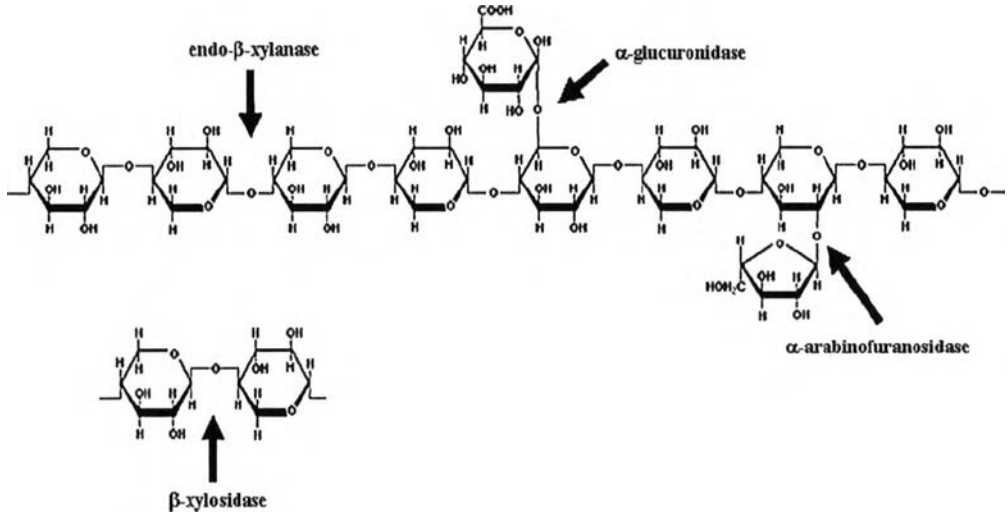

Figure 2.14 Polymeric chemical structure of hemicelluloses and targets of hydrolytic enzymes involved in hemicellulosic polymer degradation (Kumar *et al.*, 2009).

2.7.2.3 Improving Enzymatic Hydrolysis

The factors that 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 (Durand *et al.*, 1988).

Substrates

Substrate concentration is one of the main factors that affects 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 (Huang and Penner, 1991). 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. Thus, removal of lignin can dramatically increase the hydrolysis rate (Balat, 2011).

Cellulase

Increasing the dosage of cellulases in the process 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 (Converse *et al.*, 1989). Using a cellulase mixture from different microorganisms or a mixture of cellulases and other enzymes in the hydrolysis of cellulosis materials has been extensively studied (Xin *et al.*, 1993). The addition of β-glucosidases into the *T.reesei* cellulases system achieved better saccharification than the system without β-glucosidases (Beldman *et al.*, 1984). A cellulose conversion yield of 90 % was achieved in the enzymatic saccharification of 8 % alkali-treated sugar cane bagasse when a mixture of cellulases from *A.ustus* and *T.viride* was used (Mononmani and Sreekantiah, 1987). Cellulases can be recovered from the liquid supernatant or the solid residues. Enzyme recycling can effectively increase the rate and yield of the hydrolysis and lower the enzyme cost (Mes-Hartree *et al.*, 1987). The efficiency of cellulose hydrolysis decreased gradually with each recycling step (Ramos *et al.*, 1993).

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 simultaneous saccharification and fermentation (SSF). The SSF process has been extensively studied to reduce the inhibition of end products of hydrolysis (Zheng *et al.*, 1998). In the process, reducing sugars produced in cellulose hydrolysis are simultaneously fermented to ethanol, which greatly reduces the product inhibition to the hydrolysis. The microorganisms used in the SSF are usually the fungus *T.reesei*

and yeast *S.cerevisiae*. The optimal temperature for SSF is around 38 °C, which is a compromise between the optimal temperatures for hydrolysis (45-50 °C) and fermentation (30 °C) (Philippidis and Smith, 1995).

Surfactant

Addition of surfactants may improve the enzymatic cellulose conversion into monomeric sugars (Eriksson *et al.*, 2002).Various mechanisms have been proposed for positive effect of surfactant addition on enzymatic hydrolysis. The surfactant could change or modify the nature of cellulose surface properties, reduce irreversible binding of cellulase on cellulose, prevent enzyme denaturation as well as unproductive binding of enzymes to lignin residues. Non-ionic surfactants such as Tween 20 were shown to be the most effective for enhancing of enzymatic hydrolysis (Tabka *et al.*, 2006, Kristensen *et al.*, 2007). Kristensen *et al.* (2007) investigated the effects of several non-ionic surfactants on enzymatic hydrolysis of five different surfactants. The highest increase in cellulose conversion during enzymatic hydrolysis was 70 % obtained with sulfuric acid treated straw when Berol 08 was used as surfactant. The optimum surfactant concentration was approximately 0.05 (g/g dry mass).

2.8 Fermentation

The supernatant from enzymatic hydrolysis of lignocelluloses can contain both six-carbon (hexoses) and five-carbon (pentoses) sugars (if both celluloses and hemicelluloses are hydrolyzed). Depending on the lignocelluloses source, the hydrolysate typically consists of glucose, xylose, arabinose, galactose, mannose, fucose and rhamnose (Keshwani and Cheng, 2009). Fermentation involves microorganisms that use the fermentable sugars for food and in the process produces ethyl alcohol and other byproducts. These microorganisms can typically use the 6-carbon sugars, one of the most common being glucose. Therefore, cellulosic biomass materials containing high levels of glucose or precursors to glucose are the easiest to convert to bioethanol. Microorganisms presently convert an inadequate portion of the sugars from biomass to bioethanol (Stewart and Russell, 1987). Microorganisms can

be used to ferment all lignocelluloses-derived sugars to bioethanol. Microorganisms can be classified into 3 groups.

2.8.1 Microorganisms

2.8.1.1 Bacteria

Ethanol-producing bacteria have attracted attention because their growth rate is substantially higher than that of the *Saccharomyces* which is currently used for fuel ethanol production (Dien *et al.*, 2003). Among such ethanol-producing bacteria, Z. *mobilis* is a well-known organism used historically to make alcoholic beverages (Skotnicki *et al.*, 1981). The advantages of Z. *mobilis* are its high growth rate and specific ethanol production. However, its fermentable carbohydrates are limited to glucose, fructose, and sucrose. The bacteria that is capable of yielding ethanol as the major product.

2.8.1.2 Yeast

Historically, the most commonly used microbe has been yeast, *Saccharomyces cerevisiae*, which can produce ethanol to give concentration as high as 18 % of the fermentation broth, is the preferred one for most ethanol fermentation. This yeast can grow both on sugars, such as glucose, and on disaccharide sucrose. *Saccharomyces* is also generally recognized as safe as a food additive for human consumption. Thus, it is used for producing alcoholic beverages and bread. Moreover, *Saccharomyces cerevisiae* has advantages owing to its high bioethanol production from hexoses and high tolerance to bioethanol and other inhibitory compounds in the acid hydrolysates of lignocellulosic biomass. However, because wild-type strains of this yeast cannot utilize pentoses, such as xylose and arabinose, bioethanol production from a lignocellulose hydrolysate is insufficient (Katahira *et al.*, 2006). For xylose-using *S. cerevisiae*, high bioethanol yields from xylose also require metabolic engineering strategies to enhance the xylose flux (Hahn-Hagerdal *et al.*, 2006).

2.8.1.3 Fungi

The filamentous fungus *Fausarium oxysporum* is known for its ability to produce ethanol by simultaneous saccharification and fermentation (SSF) of cellulose. However, the conversion rate is low and significant amounts of acetic acid are produced as a byproduct (Panagiotou *et al.*, 2005). A few microbial species such as *Neurospora, Monilia, Paecilomyces,* and *Fusarium* have been reported to hold the ability to ferment cellulose directly to ethanol. *F. oxysporum* produces a broad range of cellulases and xylanases (Christakopoulos *et al.*, 1996). Acetic acid was the major fermentation product of *Neocallimastix sp.*, another ethanol producting fungus (Dijkerman *et al.*, 1997).

Microorganisms for bioethanol fermentation can be described in terms of their performance parameters and other requirements such as compatibility with existing products, processes and equipment. The performance parameters of fermentation are temperature range, pH range, alcohol tolerance, growth rate, productivity, yield, genetic stability and inhibitor tolerance (Demirbas, 2004). All the recombinant strains are function best between 303 and 311 K. An organism must maintain a fairly constant balance of pH to survive. Most bacteria grow best in a narrow range of pH from 6.5 to 7.5. Yeast and fungi tolerate a range of pH 3.5-5.0. The majority of organisms cannot tolerate bioethanol concentrations above 10-15 % w/v (Aminifarshidmehr, 1996).

2.9 Fermentation Techniques

2.9.1 Separate Hydrolysis and Fermentation (SHF)

Solid fraction of pretreated lignocellulosic material undergoes hydrolysis (saccharification). This fraction contains the cellulose in an accessible form to acids or enzymes. One hydrolysis is completed. The product is fermented and converted to ethanol. One of the main features of the SHF process is that each step can be performed at its optimal operating conditions (Sanchez and Cardona, 2008).

2.9.2 Simultaneous Saccharification and Fermentation (SSF)

In this process, reducing sugars produced in cellulose hydrolysis are simultaneously fermented to ethanol, which reduces the product inhibition to the hydrolysis. However, this process operates at non optimal conditions for hydrolysis and requires higher enzyme dosage but process costs negatively. SSF has many advantages for example increase of hydrolysis rate by conversion of sugars that inhibit the cellulase activity, lower enzyme requirement, high product yield, lower requirement of detoxification because glucose is removed immediately and ethanol is produced, shorter process time and less reactor volume. However, ethanol may also exhibit inhibition to the cellulase activity in SSF process. Some disadvantages include incompatible temperature of hydrolysis and fermentation, ethanol tolerance of microbes and inhibition of enzymes by ethanol (Zheng *et al.*, 1998; Saxena *et al.*, 1992).

2.9.3 Pentoses Fermentation

Configurations involving the separate fermentation of pentoses and hexoses have been proposed. Yeast such as *Pichia stipitis*, *Candida shehatae*, and *Pachysolen tannophilus* can assimilate pentoses, but their ethanol production rate from glucose is at least five times less than that observed for *S. cerevisiae* (Claassen *et al.*, 1999).

2.9.4 Simultaneous Saccharification and Cofermentation (SSCF)

In this configuration, it is necessary that both fermenting microorganisms be compatible in terms of operating pH and temperature. A combination of C. *shehatae* and S. *cerevisiae* is suitable for this kind of process. Similarly, a system incluing the isomerization of xylose and the fermentation with S. *cerevisiae* in a simultaneous way can be utilized. Some drawbacks of this configuration are the high byproduct formation in the form of CO_2 and xylitol, poor enzyme stability, incompatible pH and temperature, and the reversibility of the enzyme transformation (Chandrakant and Bisaria, 1998).