CHAPTER II
LITERATURE REVIEW

2.1 Biomass-based fuels

Biomass-based fuels or biofuels are one kind of alternative energy made from bio-based material through thermochemical process such as pyrolysis, gasification, liquefaction, supercritical fluid extraction, supercritical water liquefaction, and biochemical (Balat, 2011). Biofuels include bioethanol, biomethanol, vegetable oils, biodiesel, biogas, bio-syngas, biooil, and biochar. The production of biomass energy can be made from the biomass residues obtained from wood processing industry (sawdust, cut-offs, bark), agriculture industry (sugar cane bagasse, corn stover, wheat straw), food processing industry (organic waste animal manure and residues), waste water and landfill municipal sewage (Susta *et al.*, 2003; Ramirez *et al.*, 2007). The usage of biomass energy can be divided into two types.

- Traditional uses (e.g., firing for cooking and heating)
- Modern uses (e.g., producing electricity, stream, and liquid biofuels)

The usage of biofuels offers many advantages over petroleum-based fuels. For examples, they are available from common biomass sources, representing a CO_2-cycle in combustion, and considering as environmental friendly and sustainable fuel. The major benefits of biofuel are shown in Table 2.1.

Table 2.1 Major benefits of biofuel (Balat, 2011)

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

The benefits of biofuels large-scale production give the opportunity for developing countries to reduce their demand for oil import, thus it is good for the economics of these countries.

2.2 Bioethanol

Bioethanol is the most dominant biofuel because it is the alternative liquid transportation fuels with powerful economic, environmental, and strategic attributes (Bai *et al.*, 2008). It is widely used in fuel oxygenate or gasohol to partial replace gasoline in transportation in US since 1980s. Gasohol is most commonly blended 10% bioethanol with 90% gasoline, known as E10, and bioethanol can be used at higher levels like E85 with engine modification.

Many research groups confirmed the benefit of bioethanol fuel. Shapouri *et al.* (1995) concluded that the ethanol energy content was higher than the energy for ethanol production while Kim and Dale (2002) estimated that the ethanol could be used as a liquid transportation fuel reducing fossil fuel consumption. Wang *et al.* (1999) showed that the use of ethanol-blended fuel for automobiles could reduce petroleum use and greenhouse gas (GHG) emission that is good for environment.

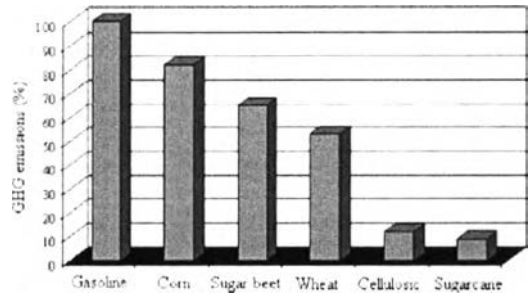


Figure 2.1 Demonstration of the lower GHC emissions, resulting from the use of biofuels, as compared to gasoline on a life cycle basis. (Philippidis G., 2009)

In addition, ethanol can partially replace methyl tertiary butyl ether (MTBE), the toxic additive chemical compound used to provide cleaner combustion (McCarthy and Tiemann, 1988).

Although the bioethanol shows many advantages against the petroleum source fuel, the cost of bioethanol is relatively high as compared to fossil fuel. Moreover, the rapid increasing human population and ethanol fuel demand lead to insufficient raw material for ethanol production because the current technology produces ethanol from the starch based raw materials, such as corn and sugar cane. Both are limited by agricultural land need for food and feed production. Alternatively, a potentially abundant source for low cost ethanol production is to utilize lignocellulosic material.

2.3 Lignocellulosic Biomass

Lignocellulosic biomass is an attractive feedstock for fuel ethanol production because it is available in large quantities and low cost (Cardona and Sanchez,

2007; Cheng *et al.*, 2008). Lignocellulosic biomass for ethanol production can be divided into 6 groups (Jin-Suk Lee *et al.*)

i) Crop residues (e.g. cane bagasse, corn stover, wheat straw, rice straw, rice hulls, barley straw, sweet sorghum, olive stones and pulp)
ii) Hard wood (e.g. aspen, poplar)
iii) Soft wood (e.g. pine, spruce)
iv) Cellulose wastes (e.g. newsprint, waste office paper, recycled paper sludge)
v) Herbaceous biomass (e.g. alfalfa hay, switch grass, reed canary grass, coatal Bermuda grass, thimothy grass, miscanthus grass)
vi) Municipal solid waste (MSW)

Although lignocellulosic biomass is the good choice to solve the limit of ethanol production from starch based raw material, the ethanol production from lignocellulosic biomass is still being developed. Today, the ethanol production cost from lignocellulosic biomass is too high, thus it needs to reduce the production cost to about at least the same level as oil and diesel for promoting ethanol from lignocellulosic material as a large-scale transportation fuel.

To utilize lignocellulosic biomass in large-scale production, numerous researchers try to develop the ethanol production from lignocellulosic biomass, but the major limiting factor is the nature and composition of the feedstock, contributing to the higher complexity in processing of the feedstock.

In lignocellulosic biomass for ethanol production, one needs to break cellulose and hemicelluloses into fermentable sugars in order to be converted into ethanol or other valuable products (e.g. xylans, xylitol, hydrogen, and enzymes).This degradation process is complicated, incomplete developed, and energy consuming (Cardona and Sanchez., 2008). The advent of modern genetic technologies and other tool can reduce the ethanol production cost from the lignicellilosic biomass in the future, so it is possible for this fuel to become a widely used fuel.

The composition of lignocellulosic biomass

Chemical composition of lignocellulosic material is a key factor affecting efficiency of biofuel production during conversion process. Lignocellulose, the primary building block of plant cell walls, is mainly composed of cellulose, hemicelluloses, and lignin, along with smaller amounts of pectin, protein, extractives (soluble nonstructural materials such as nonstructural sugars, nitrogenous material, chlorophyll, and waxes), and ash (Jorgensen *et al.*, 2007).Cellulose, hemicelluloses, and lignin, polymers are closely associatedwith each other constituting the cellular complex of the lignocellulosic biomass. Basically, cellulose forms a skeleton which issurrounded by hemicellulose and lignin (Fig. 2.2).

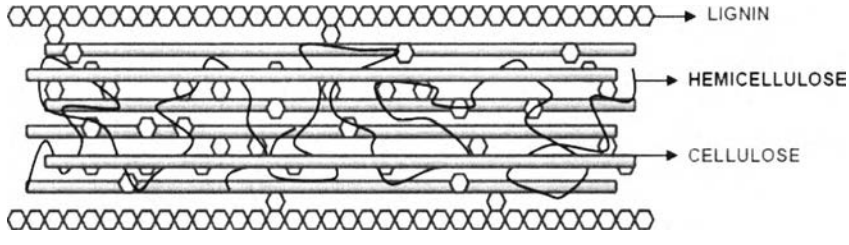


Figure 2.2 Representation of lignocelluloses structure, showing cellulose, hemicel luloses, and lignin fractions (Fengel *et al.*, 1983)

The portion of lignocellulose constituents varies between species, and there dintinct differences between hardwood and softwood. Cellulose and hemicelluloses contents are more in hardwoods (78.8%) than softwoods (70.3%), but lignin is more in softwoods (29.2%) than hardwoods (21.7%) (Balat M, 2009).A typical chemical composition of lignocellulosic materials is 48 wt.% C, 6 wt.% H, and 45 wt.% O, the inorganic matter being a minor component (Molina-Sabio *et al.*, 2004). The structural composition of various types of lignocellulosic biomass materials are given in (Table 2.2).

Table 2.2 The contents of cellulose, hemicelluloses, and lignin in common agricultural residues and wastes. (Source: Reshamwala *et al.*(1995), Cheung and Anderson (1997), Boopathy (1998) and Dewes and Hunsche (1998).)

Lignocellulosic materials	**Cellulose (%)**	**Hemicelluloses (%)**	**Lignin(%)**
Hardwoods stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Grasses	25-40	35-50	10-30
Paper	85-99	0	0-15
Wheat straw	30	50	15
Sorted refuse	60	20	20
Leaves	15-20	80-85	0
Cotton seed hairs	80-95	5-20	0
Newspaper	40-55	25-40	18-30
Waste papers from chemical pulps	60-70	10-20	5-10
Primary wastewater solids	8-15	-	24-29
Swine waste	6.0	28	-
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Coastal Bermuda grass	25	35.7	6.4
Switch grass	45	31.4	12.0

Cellulose

Cellulose, the main component in plant cell wall (30-60% of total feedstock dry matter), is a high molecular weight linear homopolymer of D-glucose. The repeating unit of cellulose is called cellobiose, two D-glucopyranose monomer units

bound by β-1-4 glycosidic linkages, and the degree of polymerization of cellulose varies between 7,000 and 15,000 depending on material.

In natural cellulose, the long chain cellulose are connected by hydrogen bond from hydrophilic functional group in molecule and Van der Walls force. This interactions cause the alignment and packing of cellulose to from microstructure, whether in crystalline or amorphous structures. More ordered or crystalline cellulose is less soluable and less degradable (Zhang *et al.*, 2004, Taherzadeh *et al.*, 2008). The chemical structure of cellulose is shown in (Figure 2.3).

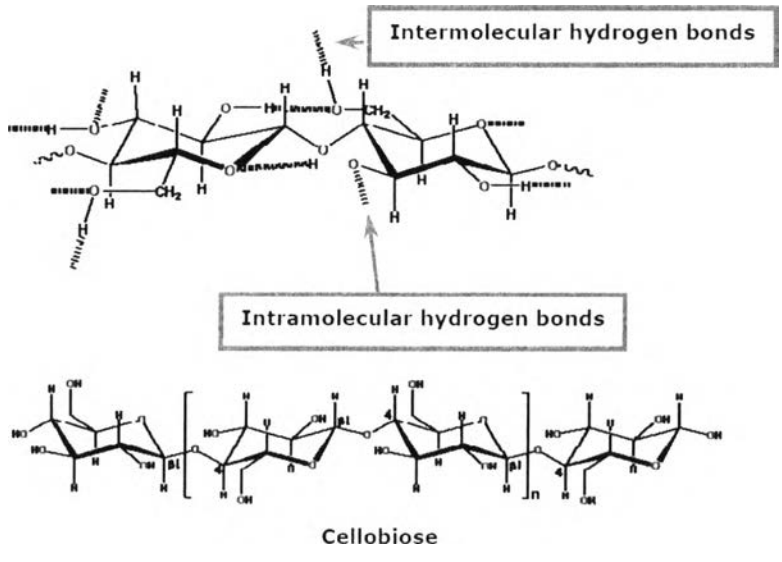


Figure 2.3 Chemical structure of cellulose (Coughlan, 1985)

With this orientation of cellulose, the cellulose structure is rigid and difficult to break. In cellulose hydrolysis, the polysaccharide is broken down to free sugar molecule by addition of water (Hamelinck, 2005). This process is called saccharification, and the six-carbon sugar products, glucose, are formed.

Hemicelluloses

Hemicelluloses (20-40% of total feedstock dry matter) are closely related with cellulose in plant tissues and together with cellulose. Hemicelluloses are a linear and branched heterogeneous class of polymer typically made up of the mixture of pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, α-D-

galactose), and/or uronic acid (α-D-glucoronic, α-D-4-O-methylgalacturonic and α-D-galacturonic acids). It may contain other sugar such as α-L-rhamnose and α-L-fucose in small amount and the hydroxyl group of sugar can be partially substituted with acetyl groups. The hemicelluloses backbone can be a homopolymer general consisting of single sugar repeating units or a heteropolymer (mixture of different sugar).

Hemicelluloses can be classified by the main sugar residue in the backbone e.g. xylans, mannans, glucans, glucuronoxylans, arabinoxylans,glucomannans, galactomannans, galacto glucomannans, β-glucans, and xyloglucans.The dominant hemicelluloses types are xylan and mannan.

Xylan, the main hemicelluloses component of secondary cell walls of hardwoods and herbaceous plant, are heteropolysaccharide with homopolymeric backbone chains of 1,4-linked β-D-xylopyranose units. Apart from xylose, xylans may contain arabinose, glucuronic acid or its 4-O-methyl ether, andacetic, ferulic, and p-coumaric acids. The compositions of branches are dependent on the source (Aspinall 1980). Xylan can be classified as linear homoxylan, arabinoxylan, glucuronoxylan, and glucurono arabinoxylan depending on substituent groups.

Corn fiberxylan is one of the complex heteroxylans, containing β-(1,4)-linked xylose residues (Saha *et al.*, 1999). About 80% of the xylan backboneis highly substituted with monomeric side-chains of arabinose or glucuronic acid linked to O-2 and/or O-3 of xylose residues, and also by oligomeric side chains containing arabinose, xylose, and sometimes galactose residues (Fig. 2.4) (Saha *et al.*, 2003). The model for the corn fiber cell wall is shown the heteroxylans, which are highly cross-linked by diferulic bridge (Fig. 2.5).

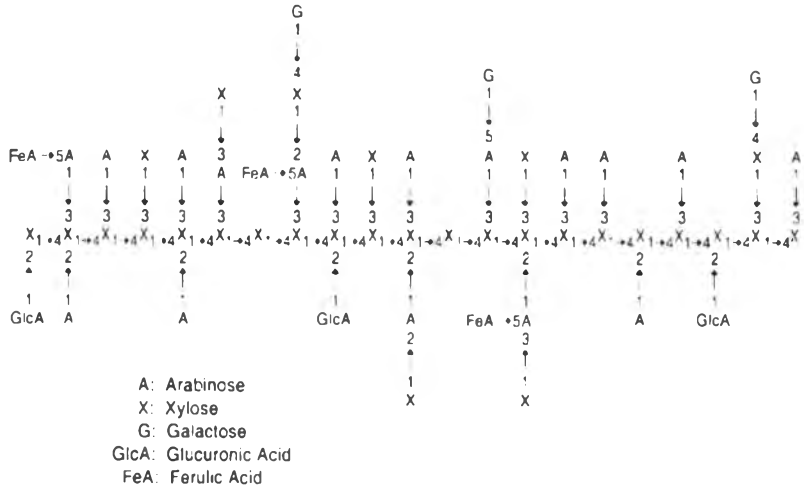


Figure 2.4 Schematic structure of corn fiber heteroxylan (Saha *et al.*, 2003).

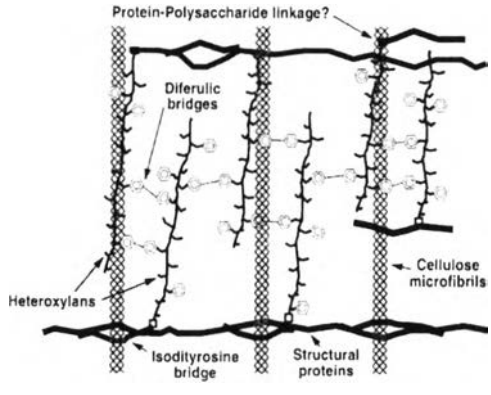


Figure 2.5 Model for corn fiber cell walls (Saha *et al.*, 2003).

Mannan is the main hemicelluloses component of secondary cell walls of softwoods, like glucomannans and galactomanans, with a few amounts in hardwood. The differences of hemicelluloses depend on their biological origin (Table 2.3).

Table 2.3 Main types of polysaccharides present in hemicelluloses (information based mainly on Alen, 2000; Carpita and Gibeaut, 1993; de Vries and Visser, 2001; Ebringerova *et al.*, 2005; Pereira *et al.*, 2003)

Polysaccharide type	Biological origen	Abbreviation	Amount[a]	Units			DP[b]	Schematic representation
				Backbone	Side chains	Linkage		
Arabinogalactan	Softwoods	AG	1–3.35[c]	β-D-Galp	β-D-Galp α-L-Araf β-L-Arap	β-(1 → 6) α-(1 → 3) β-(1 → 3)	100–600	
Xyloglucan	Hardwoods, grasses	XG	2–25	β-D-Glcp β-D-Xylp	β-D-Xylp β-D-Galp α-L-Araf α-L-Fucp Acetyl	β-(1 → 4) α-(1 → 3) β-(1 → 2) α-(1 → 2) α-(1 → 2)		
Galactoglucomannan	Softwoods	GGM	10–25	β-D-Manp β-D-Glcp	β-D-Galp Acetyl	α-(1 → 6)	40–100	
Glucomannan	Softwoods and hardwoods	GM	2–5	β-D-Manp β-D-Glcp			40–70	
Glucuronoxylan	Hardwoods	GX	15–30	β-D-Xylp	4-O-Me-α-D-GlcpA Acetyl	α-(1 → 2)	100–200	
Arabinoglucuronoxylan	Grasses and cereals, softwoods	AGX	5–10	β-D-Xylp	4-O-Me-α-D-GlcpA[b]-α-L-Araf	α-(1 → 2) α-(1 → 3)	50–185	
Arabinoxylans	Cereals	AX	0.15–30	β-D-Xylp	α-L-Araf[Feruloy	α-(1 → 2) α-(1 → 3)		
Glucuronoarabinoxylans	Grasses and cereals	GAX	15–30	β-D-Xylp	α-L-Araf 4-O-Me-α-D-GlcpA Acetyl	α-(1 → 2) α-(1 → 3)		
Homoxylans	Algae	X		β-D-Xylp				

[a] % dry biomass.
[b] Degree of polymerization.
[c] May also present β-(1 → 3) linkages on the backbone.
[d] up to in the heartwood of larches. ○ β-D-Galp; ● β-D-Glcp; ○ β-D-Manp; ● β-D-Xylp; ○ β-L-Araf; ○ β-L-Arap; ○ α-L-Fucp; ● 4-O-Me-α-D-GlcpA; ▼ Acetyl; ◆ Feruloyl

The varieties of bindings and ramifications, just as the presence of different monomeric units, contribute to the hemicellulose structure complexity and its different conformations (Jacobsen, 2000 and Malburg *et al.*, 1992). Figure 2.6 shows the structures of hemicelluloses of angiosperm (A) and gymnosperm (B), of which the principal linear chains are constituted of xylans.

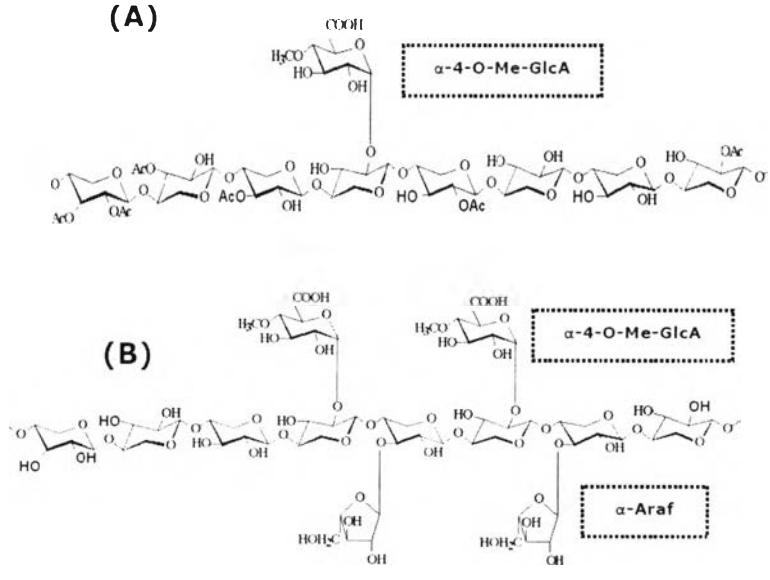


Figure 2.6 Angiosperm (A) and Gymnosperm (B) Hemicellulose Structures. Ac:acetyl group; α-Araf: α-Arabinofuranose; α-4-*O*-Me-GlcA: α-4-*O*-methylglucurinic Acid (Sunna and Antranikian., 1997)

Different from cellulose, the hemicelluloses differ by composition of sugar units, by presence of shorter chains, by a branching of main chain molecules and to be amorphous (Fengel *et al.*, 1989), which made its structure easier to hydrolyze than cellulose.

To define strategies in biomass feedstock utilization for ethanol production, the understanding in the main difference between polysaccharide component of lignocellulosic material is required (Table 2.4).

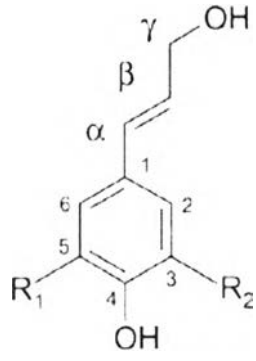
Table 2.4 Differences between Cellulose and Hemicelluloses (Nei Pereira Jr., 2008)

Cellulose	**Hemicelluloses**
Consists of glucose units	Consist of various units of pentoses and hexoses
High degree of polymerization (2,000 a 18,000)	Low degree of polymerization (50 a 300)
Forms fibrous arrangement	Do not form fibrous arrangement
Presents crystalline and amorphous regions	Present only amorphous regions
Slowly attacked by diluted inorganic acid in hot conditions	Rapidly attacked by inorganic acid diluted in hot conditions
Insoluble in alkalis	Soluble in alkalis

Lignin

Lignin (15-25% of total feedstock dry matter) is the third most abundant natural polymer presentin nature after cellulose and hemicelluloses. There are 3main groups of lignins: (1) the lignins of softwoods(gymnosperms), (2) the lignins of hardwoods (angiosperms), and (3) the lignins of grasses (non-woody or herbaceouscrops).

Lignin is a complex molecule, hydrophilic, amorphous, and cross-linked aromatic polymer of phenolic monomers. It is present in the primary cell wall, imparting structural support, impermeability, and resistance against microbial attack (Perez *et al.*, 2002). Three phenyl propionic alcohols existing as monomers of ligninare coniferyl alcohol (guaiacyl propanol), coumaryl alcohol (*p*-hydroxyphenyl propanol), and sinapyl alcohol (syringyl alcohol) (Fig 2.7), which differ from one another by possessing different substituents in their aromatic ring. The respective aromatic constituents of these alcohols in the polymer are called *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) moieties (Lewis and Yamamoto, 1990).



1. $R_1=R_2=H$
2. $R_1=OCH_3$; $R_2=H$
3. $R_1=R_2=OCH_3$

Figure 2.7 Primary precursor of lignin *p*-coumaryl (**1**),coniferyl (**2**), and sinapyl alcohols (**3**) (Anvar and Mezza., 2008)

The basic chemical phenyl propane units of lignin are bonded together by a set of linkages (Alkyl-aryl, alkyl-alkyl, and aryl-aryl ether bonds linkage) to form a very complex matrix (Demirbas, 2008). This matrix comprises a variety of functional groups, such as hydroxyl, methoxyl, and carbonyl, which impart a high polarity to the lignin macromolecule (Feldman *et al.*, 1991).

Lignin is closely bound to cellulose and hemicellulose 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 *et al.*, 1989). Due to its molecular configuration, lignins are extremely resistant to chemical and enzymatic degradation (Palmqvist *et al.*, 2000).

Normally, the molecule of lignin is always involved in carbohydrate (especially, hemicelluloses)via covalent bonds at two sites: α-carbon and C-4 in the benzenering. This relation is called lignin–carbohydrate complex (LCC) structure.

In herbaceous plant, LCC are formed by attaching hydroxycynnamic acids (*p*-coumaric and ferulic acids) to lignin and hemicelluloses via ester and ether bonds as bridges between them, forming lignin/phenolics–carbohydrate complexes (Baucher *et al.*, 1998; Sun *et al.*, 2002) (Fig 2.8). The LCC from herbaceous crops are structurally different from those from woods and contain ferulic bridges between lignin and carbohydrates (arabionxylans) via ester-linked ferulic acids (Himmelsbach, 1993; Lapierre and Monties, 1989).

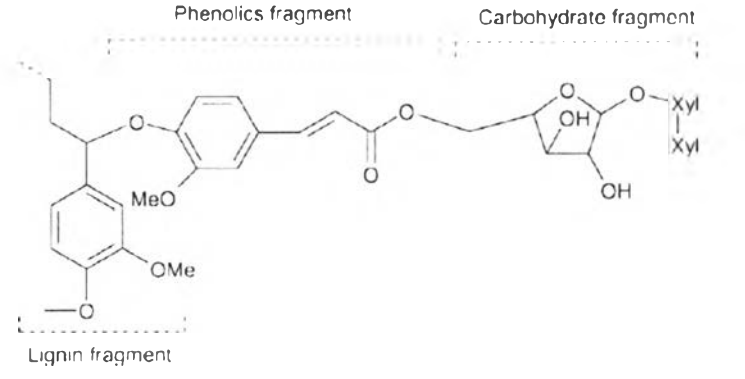


Figure 2.8 Lignin/phenolics–carbohydrate complex in wheat straw (adapted from Sun *et al.*, 1997).

The content of lignin in each groups of lignocellulosic material should be considered in bioethanol production. Softwood and hardwood lignins belong 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. Herbaceous plants such as grass generally have the lowest contents of lignin, while softwoods have the highest lignin contents. The lignin content of herbaceous plants generally range from 10% to 40% by weight in various herbaceous species, such as bagasse, corncobs, peanut shells, rice hulls and straws (Yaman, 2004). Lignin is an obstacle for using lignocellulosic biomass in fermentation process, so the pretreatment processes are required.

Besides these three main components, there are others in minor proportions, such as resins, tannin, fat acids etc. Nitrogen compounds are found in small quantities, in general in the form of proteins. Amongst the mineral salts, the salts of calcium, potassium and magnesium are the most frequently found compounds (D'Almeida, 1988 and Wayman & Parekh, 1990).

2.4 Ethanol conversion process overview

To utilize the lignocellulosic material as a bioethanol production feedstock, many researchers extensively study on the conversion of lignocellulosic materials to fuels, especially ethanol in the past few decades. The conversion includes the hydrolysis of lignocellulosic material to fermentable reducing sugar, followed by fer-

mentative reducing sugar to fuels, such as ethanol and butanol. The ethanol conversion processes of lignocellulosic material follow 4 major unit operations: pretreatment, hydrolysis (saccharification), fermentation, and product separation/purification (Fig 2.9).

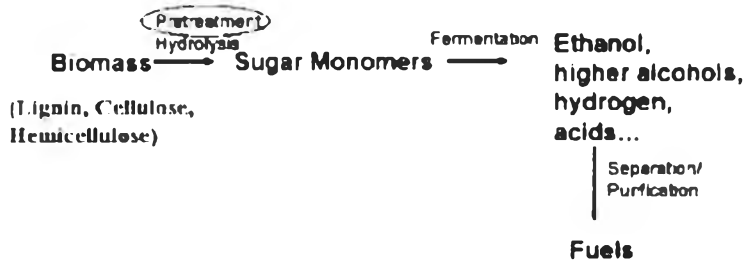


Figure 2.9 Schematic of the conversion of lignocellulosic biomass to fuel.

Pretreatment step is mainly required for ethanol production process, because this step enhances the rate of production and the total yield of monomeric sugar in the hydrolysis step. This process can significantly improve the hydrolysis process by removal of lignin and hemicelluloses, reduction of cellulose crystallinity, and increase of porousity (Mcmillan *et al.*, 1994) . It alters the microscopic and macroscopic size, chemical composition and structure of lignocellulosic material. A number of pretreatment processes such as alkaline treatment (Carrillo *et al.*, 2005), sodium chlorite treatment (Sun *et al.*, 2004), and organic solvent treatment (Xu *et al.*, 2006), are invented to improve the hydrolysis process.

The hydrolysis process is important to generate fermentable reducing sugar which is converted to ethanol by microbial activity. This process is generally catalyzed by acid or enzymatic approaches with various efficiencies, depending on treatment conditions, type of biomass, and hydrolytic agent properties. Cellulose can be broken into glucose via cellulose enzyme and sulfuric or other acid. Hemicelluloses are hydrolyzed by hemicellulases or acid hydrolysis to release its sugar component. The dilute-acid process is considered as harsh process because the formation of toxic degradation products from this process can interfere with the fermentation process. The factors affecting the hydrolysis of cellulose include porosity (accessible surface area) of the biomass materials, cellulose fiber crystallinity, and content of both lignin and hemicelluloses (Mcmillan *et al.*, 1994).

Fermentation process is a conversion process of the reducing sugar to ethanol by the action of microorganism. The six-carbon sugars, or hexoses, glucose, galactose, andmannose, are readily fermented to ethanol by many naturally occurring organisms (Mosier *et al.*, 2005), while the fermentation of pentose (five carbon sugars) is only done by a few strains and usually results in relatively low yields. Xylose, one kind of pentose sugar, is converted to ethanol by *S. pombe*, *S. cerevisiae*, *S. amucae*, and *Kluveromyces lactis* (Gong, 1983). Because the carbohydrate in lignocellulosic material contains both pentose and hexose sugar, the ability of microorganism to ferment the whole range of sugars is important for economical process for lignocellulosic bioethanol conversion. Genetic modificationof bacteria (Ingram *et al.*, 1998, 1999) and yeast (Ho *et al.*, 1998, 1999) has produced strains capable ofco-fermenting both pentoses and hexoses to ethanoland other value-added products at high yields.

The problem in this fermentation process is the ethanol product, formed being an inhibitor for the activity of the yeasts/bacteria. This causes a limit to the concentration of fermentable sugars (Hendriks and Zeeman, 2009). Furfural and other inhibitors like soluble lignin compounds formed are also a problem for the fermentation step because such compounds can inhibit or even stop the fermentation (Laser *et al.*, 2002).

Because of the different characteristics of hydrolysis and fermentation processes, the following terms are used to call various operation units of bioethanol conversion process (Fig 2.10).

- Separate hydrolysis and fermentation (SHF): Enzymatic hydrolysis is separated from fermentation step.
- Simultaneous saccharification and fermentation (SSF): Cellulose hydrolysis is performed in the presence of the fermentative microorganism.
- Simultaneous saccharification and co-fermentation (SSCF): Both cellulose and hemicelluloses are hydrolyzed, and the genetic engineering microorganism will ferment both xylose and glucose in the same broth.

SSF and SSCF are preferred since both unit operations can be done in the same tank, resulting in lower costs (Wright *et al.*, 1988).

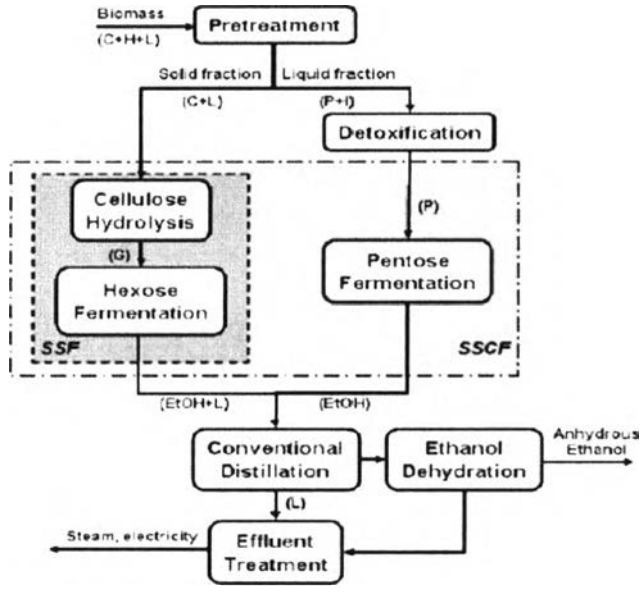


Figure 2.10 Generic block diagram of bioethanol production from lignocelluloses biomass. Possibilities for reaction–reaction integration are shown in side the shadedboxes: SSF – simultaneous saccharification and fer mentation; SSFC – simultaneous saccharification and co-fermentation. Main stream components are: C – cellulose; H– hemicellulose; L – lignin; G – glucose; P – pentose; I – inhibitors; EtOH – ethanol (Car dona Alzate *et al*, 2006).

In separation and purification processes, ethanol is recovered from the fermentation broth by distillation or distillation combined with adsorption (Gulati *et al.*, 1996; Ladisch and Dyck, 1979; Ladisch *et al.*, 1984).The residual lignin, unreacted cellulose and hemicellulose, ash, enzyme, organisms, and other components end up at the bottom of the distillation column. These materials may be concentrated, and burned as fuel to power the process, or converted to various coproducts (Wyman, 1995a; Hinman *et al.*, 1992; Wooley *et al.*, 1999).

From the process overview, the lignocellulosic bioethanol conversion is not easy due to following reasons.

i) The resistant nature of lignocellulosic biomass structure to prevent hydrolysis

ii) The various types of reducing sugars from the hydrolysis need an effective microorganism with effective fermentation activities.

iii) Cost for collection and storage of low lignocellulosic material.

Many researchers try to develop the bioethanol conversion process of lignocellulosic biomass to compete with petroleum fuel, and capable to utilize in large scale fuel.

2.5 Pretreatment of lignocellulosic biomass.

Pretreatment step is very crucial step in bioethanol production. It utilizes pretreatment additive and/or energy to form solids that are more reactive than native material and/or generate soluble oligo- and monosaccharide (Fig 2.11). The goal of this process is to decrease crystallinity of cellulose, increase biomass surface area, remove hemicelluloses, and break lignin in lignocellulosic structure (Fig 2.12).The beneficial effects of pretreatment of lignocellulosic materials have been recognized for some time (Mcmillan *et al.*, 1994). In this pretreatment process, if the pretreatment is not efficient enough, the resultant residue is not easily hydrolyzed and if it is more severe, the production of toxic compounds will be resulted which inhibitthe microbial metabolism (Kodali and Pogaku, 2006).

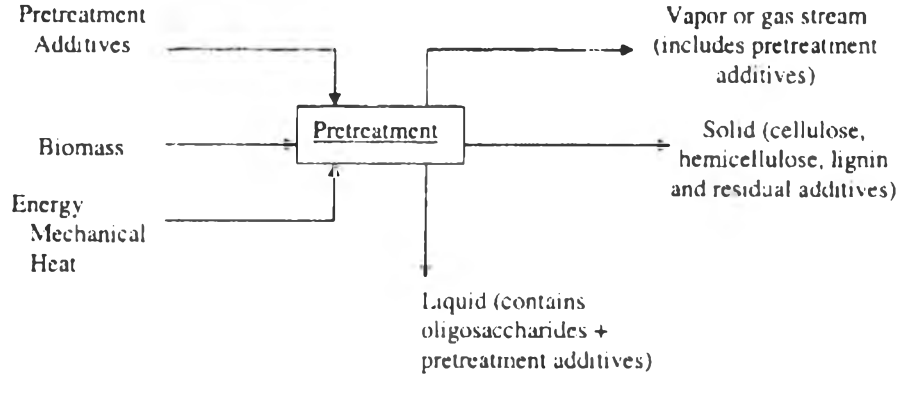


Figure 2.11 Schematic of pretreatment process (N. Mosier *et al.*, 2005)

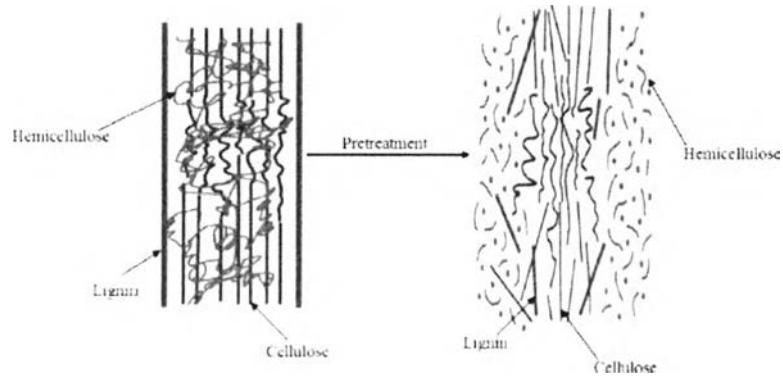


Figure 2.12 Schematic of the role of pretreatment in the conversion of biomass to fuel (Hsu *et al.*, 1980).

Although this step is very important, the cost of this step is the most expensive processing steps within the conversion of biomass to fermentable sugar (Zhang *et al.*, 2009). Therefore, the pretreatment of lignocellulosic material with cost effective manner becomes a major challenge in bioethanol technology research and development.

Taherzadeh and Karimi (2008) summarized the prerequisites for an ideal lignocellulose pretreatment, as follows;

i) Production of reactive cellulosic fiber for enzymatic attack
ii) Avoiding destruction of hemicelluloses and cellulose
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

2.5.1 Physical pretreatment

i) **Mechanical comminutions:** Size reduction of lignocellulosic material through the combination of chipping, grinding, and/or milling to reduce

cellulose crystallinity. The reduction in particle size leads to an increase of specific surface and a reduction of the degree of polymerization (DP).

ii) **Thermal treatment (pyrolysis):** Lignocellulosic material is heated. It can be used as substrate for a fast pyrolysis for thermal conversion of cellulose and hemicelluloses into fermentable sugars with good yields (Tomas-Pejo, 2008). When the temperature increases above 150°–180 °C, parts of the lignocellulosic biomass, firstly the hemicelluloses, followed by lignin, will start to solubilize (Bobleter, 1994; Garrote *et al.*, 1999). When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char (Kilzer and Broido, 1965; Shafizadeh and Bradbury, 1979).

2.5.2Physicochemical pretreatment

i) **Steam explosion (autohydrolysis):** Stream explosion is the most commonly used method for lignocellulosic biomass pretreatment (Mcmillan *et al.,* 1994) with lower capital investment and environmental impact. In this process, lignocellulosic material is treated with high pressure saturated stream, and then the pressure is suddenly reduced, leading to an explosive decomposition of materials.

This process increases crystallinity of cellulose by promoting crystallization of amorphous portions, enhances hemicelluloses hydrolysis, and promotes delignification.

ii) **Ammonia fiber explosion(AFEX):** The concept of AFEX is similar to the steam explosion. Lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. It can be used for the pretreatment of many lignocellulosic materials including alfalfa, wheat straw, wheat chaff (Mes-Hartree *et al.,* 1988), barley straw, corn stover, rice straw (Vlasenko *et al.,* 1997).

iii) **Carbon dioxide explosion:** Similar to stream and ammonia explosion pretreatment, super critical CO_2 explosion is used for pretreatment of lignocellulosic materials with a lower temperature than stream explosion, and a reduced expense is compared to the ammonia explosion. It was hypothesized that CO_2 would form carbonic acid and increase the hydrolysis rate (Dale and Moreira, 1982).

iv) **Liquid hot-water pretreatment (LHW):** Liquid hot water is used instead of stream to solubilize mainly hemicelluloses to make cellulose better accessible and to avoid inhibitor formation. LHW subjects biomass to hot water in liquid state at high pressure during a fixed period and it presents elevated recovery rates for pentoses and generates low amount of inhibitors (Tomas-Pejo *et al.*, 2008). A difference between the LHW and the steam pretreatment is the amount and concentration of solubilized products. In a LHW pretreatment, the amount of solubilized products is higher, while the concentration of these products is lower, as compared to steam pretreatment (Bobleter, 1994) because of the amount of water input.

2.5.3 Chemical pretreatment

i) Ozonolysis

Ozone gas is used to break down the lignin and hemicelluloses and increase the biodegradability of the cellulose. This process effectively removes lignin and does not produce toxic residue for the down stream process, and the reaction is carried out at room temperature and pressure (Vidal and Molinier, 1988). However, a large amount of ozone is required, making the process expensive.

ii) Alkaline pretreatment

Lignocellulosic material can be treated with alkaline solution to remove lignin and various uronic acid substitutions on hemicelluloses that lower the accessibility of enzyme to the hemicelluloses and cellulose (Silverstein *et al.*, 2008 and Han *et al.*, 2009). This pretreatment process can be achieved at ambient condition and utilize lower temperature and pressure than other pretreatment technologies, but pretreatment time is measured in term of hours or days rather than minutes or seconds

(Mosier *et al.*, 2005). Sodium, potassium, calcium, and ammonium hydroxide are the appropriate chemical in this pretreatment process. Among these chemicals, NaOH has been studied the most (Kumar *et al.*, 2009) while calcium hydroxide (slake lime) shows the effective pretreatment agent with the least expensive per kilogram of hydroxide. In addition, alkaline peroxide is the effective pretreatment method too. During the alkaline pretreatment, the solvation and saphonication occur and lead to a swollen state of the biomass that increases the internal surface area, decreases crystallinity, separates structural linkages between lignin and carbohydrates, and disrupts the lignin structure (Fang *et al.*, 1987). Compared with acid processes, alkaline processes cause less sugar degradation, and many of the caustic salts can be recovered and/or regenerated.

iii) Acid pretreatment

Lignocellulosic biomass is treated with acid solution for high yield of sugar. The main goal of this process is to solubilize the hemicelluloses, and to make the cellulose better accessible. Acid pretreatment involves the use of sulfuric, nitric, or hydrochloric acids. It can operate either under a high temperature and low acid concentration (dilute acid pretreatment) or under a low temperature and high acid concentration(concentrated acid pretreatment) (Taherzadeh *et al.*, 2008). The main reaction in the acid pretreatment process is the hydrolysis of hemicelluloses and precipitation of solubilized lignin. Solubilized hemicelluloses (oligomers) can be subjected to hydrolytic reactions producing monomers, furfural, HMF, and other (volatile) products in acidic environments (Pereira Ramos, 2003). During the acid pretreatment, solubilized lignin will quickly condensate and precipitate in acidic environments (Liu and Wyman, 2003; Shevchenko *et al.*, 1999).

There are primarily two types of dilute acid pretreatmentprocesses:

1) high temperature (T greater than 160 °C), continuous-flow process for low solid loading (5–10% [weight of substrate/weight of reaction mixture]) (Brennan *et al.*, 1986; Converse *et al.*, 1989)
2) lowtemperature (T less than 160 °C), batch process for high solid loading (10–40%) (Cahela *et al.*, 1983; Esteghlalian *et al.*, 1997).

In recent years, acid pretreatment by sulfuric acid at concentration below 4% has been the most interestingly effective pretreatment system that can achieve high reaction rate and improve hydrolysis (Esteghlalian *et al.*, 1997).Dilute sulfuric acid pretreatment (0.2–2.0% sulfuric acid, 394–493 K) of lignocellulose serves three important functions in the conversion process(Silverstein *et al.*, 2004).

1) Hemicellulose hydrolysis to produce a monomeric sugar.
2) Exposure of cellulose for enzymatic digestion by removal of hemicelluloses and part of the lignin.
3) Solubilization of heavy metals whichmay be contaminating the feedstock.

Although this method shows many benefits in the pretreatment process, dilute sulfuric acid also has some important disadvantages (Zheng *et al.*, 2009).

1) Corrosion that mandates expensive materials of construction
2) Acidic prehydrolyzates must be neutralized before the sugars proceed to fermentation
3) Gypsum has problematic reverse solubility characteristics when neutralized with inexpensive calcium hydroxide
4) Formation of degradation products and release of natural biomass fermentation inhibitors are other characteristics of the acid pretreatment
5) Disposal of neutralization salts is needed
6) Biomass particle size reduction is necessary

From these disadvantages, the cost of the dilute acid pretreatment is relatively high, as compared to physicochemical pretreatments (Viola *et al.*, 2008). Recently, the acid pretreatment has been used on a wide range of feedstocks ranging from hardwoods to grasses and agricultural residues.

Martin *et al.* (2007) studied the potential of dilute-acid prehydrolysis as a pretreatment method for sugarcane bagasse, rice hulls, peanut shells, and cassava stalks. The prehydrolysis was performed at 122 °C for 20, 40, or 60 min using 2% H_2SO_4 at a solid-to-liquid ratio of 1: 10. Under the conditions tested, prehydrolysis using dilute sulfuric acid was efficient for obtaining sugars from sugarcane bagasse and rice hulls

hemicelluloses, and for improving the enzymatic convertibility of bagasse cellulose, but it was not efficient for the other materials. This work demonstrates the potential in using dilute sulfuric acid for the pretreatment before enzymatic hydrolysis of bagasse.

iv) Oxidative pretreatment

Lignocellulosic biomass is treated by the addition of an oxidizing compound, such as hydrogen peroxide or peracetic acid which is suspended in water to remove the hemicelluloses and lignin, so the accessibility of the cellulose increases. During oxidative pretreatment, several reactions can take place, like electrophilic substitution, displacement of side chains, cleavage of alkyl aryl ether linkages or the oxidative cleavage of aromatic nuclei (Hendriks and Zeeman, 2009). The used oxidant is not selective in many cases, so losses of hemicelluloses and cellulose can occur. Moreover, a high risk on the formation of inhibitors exists, such as aromatic compounds, formed by lignin oxidization.

v) Organosolv process

Organosolv process is used to treat the lignocellulosic material. Organosolv is the mixture of an organic or aqueous organic solvent with inorganic acid catatlysts (HCl or H_2SO_4). It 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). Solvents may be inhibitory to microorganism growth and enzymatic hydrolysis, thus, the solvent removal is necessary in this process.

2.5.4 Biological pretreatment

In biological pretreatment processes, a microorganism, such as brown-, white-, and soft-rot fungi, is used to degrade lignin and hemicellulose in waste materials (Schurz, 1978). Brown rots mainly attack cellulose, while white and soft rots attack both cellulose and lignin. White-rot fungi are the most effective basidiomycetes for biological pretreatment of lignocellulosic materials (Fan *et al.*, 1987). This pretreatment process is considered as a safe and environmentally friendly method. It

is increasingly promoted as a process that does not require high energy for lignin removal from a lignocellulosic biomass (like most pretreatment technologies),despite extensive lignin degradation (Okano *et al.*, 2005).

2.5.5 Pulsed-electric-field pretreatment

Pulsed-electricfield(PEF) pretreatment involves application of a short burst of high voltage to a sample placed between two electrodes. Lignocellullosic material is treated by PEF using high field strengths in the range of 5-20 kV/cm to rapture plant cells and create permanent pores in the cell membrane. This pore facilitates the entry of acids or enzymes used to break down the cellulose into its constituent sugars. PEF pretreatment can be carried out at ambient conditions and energy use is low because of short pulse times.

2.5.6 Microwave pretreatment

Microwave is an alternative method for conventional heating in lignocellulosic biomass pretreatment while the other pretreatment methods, such as stream explosion, liquid hot water, and ammonia fiber explosion, are usually achieved through a convection or conduction based heating, which is based on superficial heat transfer (Liu and Wyman., 2005; Mosier *et al.*, 2005).

Microwave heating is based on the ability of a particular substance, such as a solvent or substrate, to absorb microwave energy and effectively converts the electromagnetic energy to heat (kinetic energy). Molecules with a dipole moment (permanent or induced) try to align themselves with the oscillating electric field of the microwave irradiation, leading to rotation. Under microwave irradiation, a large number of molecules are rotationally excited and, as they strike other molecules, rotational energy is converted into translational energy (i.e., kinetic energy) and, as a consequence, heating is observed (Fig 2.13)(Jason R. Schmink and Nicholas E. Leadbeater., 2011). Therefore, the heating in this process is volumetric and rapid (A. de la Hoz *et al.*, 2005). The more polar solvents, such as dimethyl sulfoxide, dimethyl formamide, ethanol, and water, better convert microwave irradiation into heat as compared to nonpolar ones, such as toluene or hexane, because microwave heating depends on the dipole moment of a molecule.

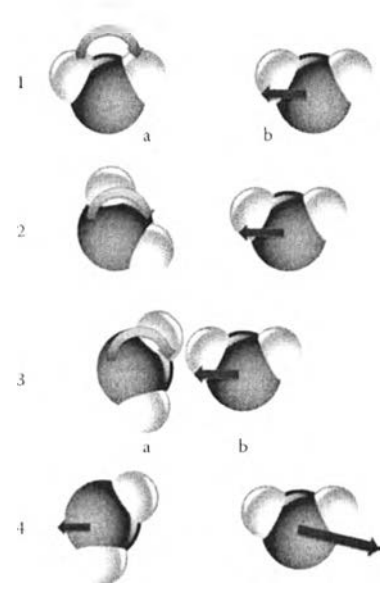


Figure 2.13 Microwave heating. Panels 1–3 show a molecule that has been rotationally excited by microwave irradiation being approached by a second molecule b. Upon impact (panel 3), the rotational energy of molecule is converted to the translational movement of molecule b. In panel 4, note the increase in translational vector magnitude, the consequence of which leads to an increase in molecular collisions (kinetic energy).

The one of microwave effect is the inversion of temperature gradients when using microwave irradiation. In contrast to heating by conventional means, microwave irradiation raises the temperature in the whole reaction volume simultaneously, without intervention through the vessel wall (Fig 2.14). This means that the synthesis can proceed uniformly throughout the reaction vessel.

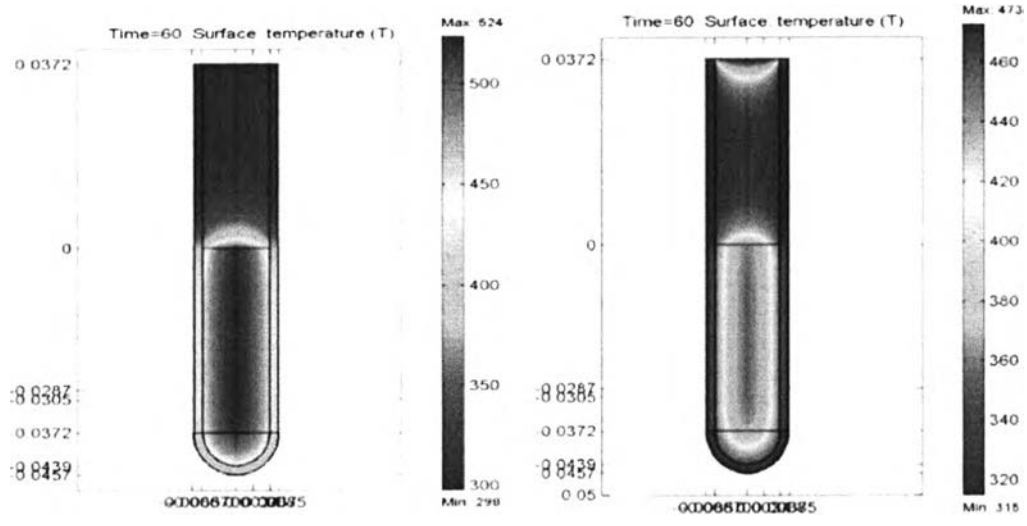


Figure 2.14 The temperature profile after 60 sec of microwave irradiation (left) compared to treatment in an oil-bath (right). Microwave irradiation raises the temperature of the whole reaction volume simultaneously, whereas in the oil heated tube, the reaction mixture in contact with the vessel wall is heated first. Temperature scale in Kelvin. '0' on the ver tical scale indicates the position of the meniscus (Schanche, J.-S., *Mol. Diversity* 2003, *7*, 293–300)

Microwave pretreatment of lignocelluloses was initially reported by Ooshima *et al.* (1984) and Azuma *et al.* (1984). This pretreatment process selectively heats the more polar (lossy) part and creates a "hot spot" within the inhomogeneous materials. It is hypothesized that this unique heating feature results in an "explosion"effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulose. In addition, the electromagnetic field used in microwave might create non-thermal effects that also accelerate the destruction of the crystal structures (A. de la Hoz *et al.*, 2005).

For initial study, Ooshima *et al.* (1984) investigated the microwave treatment for wet rice straw and bagasse with water content 84 or 94%. This treatment enhanced the accessibility of the cellulosic materials for the enzymatic hydrolysis: for example, 1.6 times in the rice straw by the microwave treatment at 170 °C for 5

min and 3.2 times in the bagasse by the treatment at 200 °C for 5 min, compared with the untreated ones.

Recently, microwave-assisted alkali/acid/H_2O_2 pretreatment of rice and wheat straw was investigated by Zhu *et al.*, (2006). They also investigate the simultaneous saccharification and fermentation (SSF) of the microwave-assisted and conventional alkali pretreated wheat straw to ethanol. The SSF optima for conventional alkali pretreated wheat straw were 100 g/l substrate, 40 °C, 20 mg [cellulase] g^{-1} [substrate], initial pH 5.3, and 96 h. Under this optimum conditions the ethanol concentration and its yield were 31.1 g /l and 64.8%, respectively, whereas the SSF optima for microwave-assisted alkali pretreated wheat straw were 100 g/l substrate, 40 ^{o} C, 15 mg [cellulase]g-1[substrate], initial pH 5.3, and 72 h. Under the optimum conditions the ethanol concentration reached 34.3 g/l and the ethanol yield was 69.3%. It shows that production of ethanol from microwave-assisted pretreated wheat straw had lower enzyme loading, shorter reaction time and could achieve higher ethanol concentration and yield than that from the conventional alkali pretreatment.

The sugar yield based on dry weight of switch grass by microwave-assisted alkali pretreatment was reported by Hu *et al.* (2008). In this study, microwave-based heating was used to pretreat switchgrass, which was then hydrolyzed by cellulase enzymes. When switchgrass was soaked in water and treated by microwave, total sugar (xylose + glucose) yield from the combined treatment and hydrolysis was 34.5 g/100 g biomass, equivalent to 58.5% of the maximum potential sugars released. This yield was 53% higher than that obtained from conventional heating of switchgrass. To improve the sugar yield, switchgrass was presoaked in different concentrations of alkali solutions and then treated by microwave or conventional heating. Moreover, the effects of temperature, solid content, and treatment time on microwave pretreatment of switchgrass were investigated. At optimal conditions of 190 °C, 50 g/L solid content, 0.1 g/g of alkali loading, and 30 min treatment time; the sugar yield from the combined pretreatment and hydrolysis was 58.7 g/100 g biomass, equivalent to 99% of potential maximum sugars. The results demonstrate that the microwave-assisted alkali treatment is an efficient way to improve the enzymatic digestibility of switchgrass.

2.5.7 Summary of pretreatment method

Many pretreatment processes have been developed to improve bioethanol conversion process. The choice of the pretreatment technology used for a particular biomass depends on its composition and the byproducts produced as a result of pretreatment. There are 2 things to emphasize.

1) One technology that is efficient for a particular type of biomass material might not work for another material.
2) It is not always possible to transfer the results of pretreatment from one type of material to another material

Advantages and disadvantages of the various pretreatment processes are summarized in (Tables 2.5, 2.6),and the effect of various pretreatment methods on the chemical composition and physical structure of lignocellulosic biomass is shown in (Table 2.7). There have been some reports comparing various pretreatment methods for biomass. Among these reports about the pretreatment process, the combination of the pretreatments usually performed the effective pretreatment process in ethanol conversion. For examples, Rosgaard *et al.* (2007) evaluated the efficacy of three different pretreatment procedures, i.e., acid or water impregnation followed by steam explosion versus hot water extraction, on barley and wheat straw. The pretreatments were compared after enzyme treatment using acellulase enzyme system. The acid or water impregnation followed by the steam explosion of barley straw was the best pretreatment in terms of the resulting glucose concentration in the liquid hydrolysate after enzymatic hydrolysis.

Table 2.5 Advantages and disadvantages of various pretreatment processes for lignocellulosic materials (Kumar *et al.*, 2009)

Pretreatment process	**advantages**	**Limitations and disadvantages**
Mechanical comminution	Reduces cellulose crystallinity	Power consumption usually higher than inherent biomass energy
Steam explosion	Causes hemicellulose 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
AFEX	Increases accessible surface area, removes lignin and hemicellulose to an extent;does not produce inhibitors for down-stream processes	Not efficient for biomass with high lignin content
CO2 explosion	Increases accessible surface area; cost-effective; does not cause formation of inhibitory compounds	Does not modify lignin or hemicelluloses
Ozonolysis	Reduces lignin content; does not produce toxic residues	Large amount of ozone required; expensive
Acid hydrolysis	Hydrolyzes hemicellulose 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
Organosolv	Hydrolyzes lignin and hemicelluloses	Solvents need to be drained from the reactor, evaporated, condensed, and recycled; high cost

Pretreatment process	**advantages**	**Limitations and disadvantages**
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

Table 2.6 Comparison of advantages and disadvantages of different pretreatment options for lignocellulosic materials (Girio *et al.*, 2010).

Desirable features	Concentrated acid	Dilute acid	Steam explosion	Autohydrolysis	Organosolv	Solid Superacids	Alkaline	Ionic liquids	Supercritical fluids
High hemicellulose solubilisation	++	++	++	++	+	+	+	++	+
High hemicellulosic monosaccharides production	++	++	0		0	+	0	0/+	
Low hemicellulosic oligosaccharides 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 inhibitors formation			0	0	+	0	+		0
Low corrosion problems			0	0		0		0	0
Low need for chemicals			0	++		0/+	0	+	++
Low neutralisation 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.

Table 2.7 Effect of various pretreatment methods on the chemical composition and chemical/physical structure of lignocellulosic biomass (Mosier *et al.*, 2005)

	Increases accessible surface area	Decrystalizes cellulose	Removes hemicellulose	Removes lignin	Alters lignin structure
Uncatalyzed steam explosion	■		■		▩
Liquid hot water	■	ND	■		▩
pH controlled hot water	■	ND	■		ND
Flow-through liquid hot water	■	ND	■	▩	▩
Dilute acid	■		■		■
Flow-through acid	■		■	▩	■
AFEX	■	■	▩	■	■
ARP	■	■	▩	■	■
Lime	■	ND	▩	■	■

■ Major effect
▩ Minor effect
ND: Not determined

2.6 Hydrolysis (Saccharification) process

Hydrolysis process is a very important process to convert the carbohydrate polymers in lignocellulosic material to fermentable sugar. Several products can be resulted from hydrolysis of lignocellulosic materials (Fig 2.15).

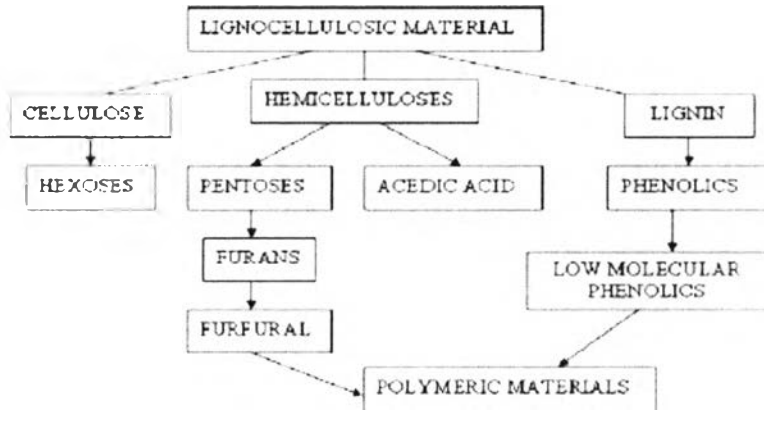


Figure 2.15 Main degradation products occurring during hydrolysis of lignocellu losicmaterial (Demirbas A., 2008).

Hemicelluloses can be hydrolyzed to xylose,mannose, acetic acid, galactose, and glucose. It can be generalized as

Hemicellulose ⟶ Xylan ⟶ Xylose ⟶ Furfural

Acetic group ⟶ Acetic acid

Among these products, xylose has the main application to form xylotol, a functional sweetener, via bioconversion process.

The degradation of xylan yields eight main products: water, methanol, formic, acetic, and propionic acids, hydroxy-1-propanone, hydroxy-1-butanone and 2-furfuraldeyde (Gullu DE., 2003). Xylose is further degraded to furfural under high temperature and pressure (Dunlop AP., 1948). 5-hydroxymethyl furfural (HMF) is also formed from hexose degradation (Ulbricht *et al.*, 1984).

For cellulose hydrolysis, the following reaction is proposed.

Cellulose ⟶ Glucan ⟶ Glucose

⟶ Decomposition products

The common hydrolysis methods can be divided into 2 types: chemical hydrolysis (dilute and concentrated acid hydrolysis) and enzymatic hydrolysis. The other techniques, such as gamma-ray, electron-beam irradiation, or microwave irradiation, are used to hydrolyze the lignocellulosic material too, but commercially unimportant.

2.6.1 Chemical hydrolysis

In the chemical hydrolysis, the pretreatment and the hydrolysis may be carried out in a single step. Acids are predominantly applied in chemical hydrolysis (Taherzadeh *et al.*, 2007). This acid hydrolysis can be classified into 2 types.

i) Dilute acid hydrolysis

The dilute acid process is one of the oldest, simplest, and most efficient methods of producing ethanol from biomass. This process is involved in H_2SO_4 solution of about 1% concentration under high temperature and pressure, and has a reaction time in the range of seconds or minutes, which facilitates continuous processing. The reactor in this method is very expensive because of the utilization of acid and high temperature and pressure. Most dilute acid processes are limited to a sugar recovery efficiency of around 50% (Badger PC., 2002). At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis (McMillan, 1994).

Dilute acid hydrolysis is considered as the most employed technique for the hemicellulose breakdown. In this process, the use of diluted acids (1–4%), under moderate temperatures (120° to 160 °C), has proven to be adequate for hemicelluloses hydrolysis, promoting little sugar decomposition (McMillan *et al.*, 1994; Mussatto and Roberto., 2006). The mechanism in acid hydrolysis of hemicellulose is the

breakdown of long hemicellulose chains by acid to form shorter chain oligomers and then to sugar monomers. However, because hemicellulose is amorphous, less severe conditions are required to release hemicellulose sugars.

To take advantage of the differences between hemicelluloses and celluloses, dilute acid hydrolysis is performed in 2 stages. The first stage is performed at low temperature to maximize the yield from the hemicelluloses, and the second, higher temperature stage isoptimized for hydrolysis of the cellulose portion of the feedstock (Farooqi R and Sam AG., 2004) (Fig 2.16). The first stage uses 0.7% sulfuric acid at 190 °C to hydrolyze the hemicelluloses present inthe biomass. The second stage is optimized to yield the more resistant cellulose fraction. This is achieved by using 0.4% sulfuric acid at 215 °C. The liquid hydrolyzates are then neutralized and toxic compounds are removed before fermentation of sugar solution (Brennan *et al.*, 1986). In dilute acid hydrolysis, feedstock must be reduced in size in the range of a few millimeters to allow sufficient acid penetration (Badger PC., 2002).

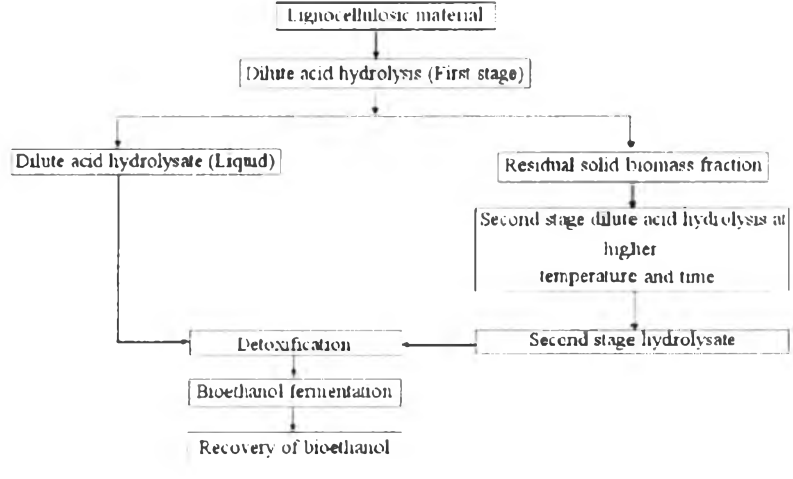


Figure 2.16 Dilute acid hydrolysis (first-stage and two-stages) and separated fer mentation of pentose and hexose sugars (Chandel *et al.*, 2007).

ii) Concentrated acid hydrolysis

Concentrated acids, such as H_2SO_4 and HCl, have been used to treat lignocellulosic materials. This process provides a complete and rapid conversion of cellu-

lose to glucose and hemicelluloses to five-carbon sugars with little degradation. The acid concentration used in concentrated acid hydrolysis process is in the range of 10–30% at temperatures of about 160 °C and pressures of about 10 atm (Sun *et al.*, 2002; Kumar *et al.*, 2009). These severe conditions are required to release glucose tightly associated chains because of crystalline in cellulose. Acid concentration, temperature, and time are crucial factors in this process, so it has to control well to avoid the sugar and lignin degradation to by-product (McMillan *et al.*, 1994). Reaction time of this process is normally longer than dilute acid process.

This concentrated acid hydrolysis is normally involved in 2 steps: a decrystallization step to break down the crystal structure of fiber using sulfuric acid of more than 60% concentration, and a hydrolysis step with acid of around 20–30% concentration to liberate sugars from the decrystallized fiber (Bayat-makooi *et al.*, 1985). Iranmahboob *et al.*, (2002) studied a concentrated acid hydrolysis of mixed wood chip, and achieved a high glucose yield of 72–82%.

In comparison to dilute acid hydrolysis, concentrated acid hydrolysis leads to little sugar degradation and gives sugar yields approaching 100% (Yu *et al.*, 2008), but it could cause serious environmental concerns (Sun and Cheng, 2002). Environment and corrosion problems, the high cost of acid consumption, and recovery present major barriers to economic success in a concentrated acid hydrolysis process.

2.6.2 Enzymatic hydrolysis

Enzymatic hydrolysis is a hydrolysis process catalyzed by the action of several enzymes. The most important of which are cellulases, producing from both bacteria and fungi. Three major types of cellulose activities are recognized (Lynd, 1996):

1) Endoglucanases (1,4-b-D-glucanohydrolases): Cut at random the internal amorphous sites in the cellulose polysaccharide chain generating oligosaccharides of various lengths, and consequently shorter chains appear.
2) Exoglucanases: act in a progressive manner on the reducing and non-reducing ends of the cellulose chains liberating either glucose (glucano hydrolases) or

cellobiose, and act on microcrystalline cellulose peeling the chains from the microcrystalline structure (Sheehan and Himmel, 1999).

3) β-Glucosidases (β-glucoside glucohydrolases): hydrolyze soluble cellodextrins and cellobiose to glucose.

The schematic representations of the cellulases enzyme activities are shown in Fig 2.17 and 2.18. In addition to three major groups of cellulase enzymes, there are also a number of ancillary enzymes that attack hemicellulose, such as glucuronidase, acetyl esterase, xylanase, b-xylosidase, galacto mannanase, and glucomannanase (Duff and Murray, 1996).

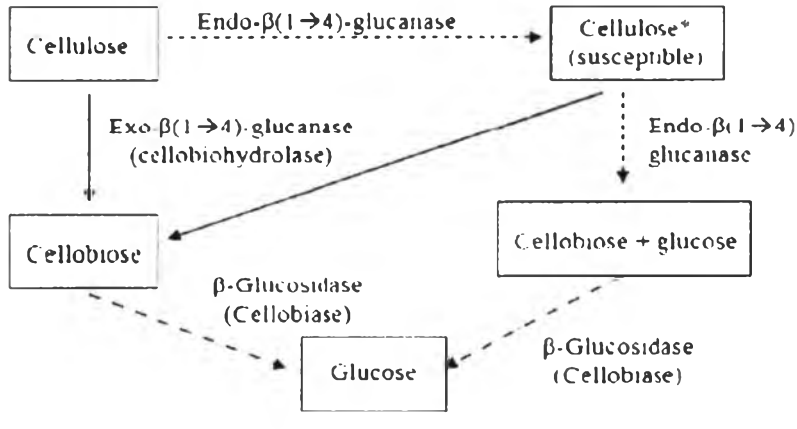


Figure 2.17 Mode of action of cellulolytic enzymes (Kim SH., 2004)

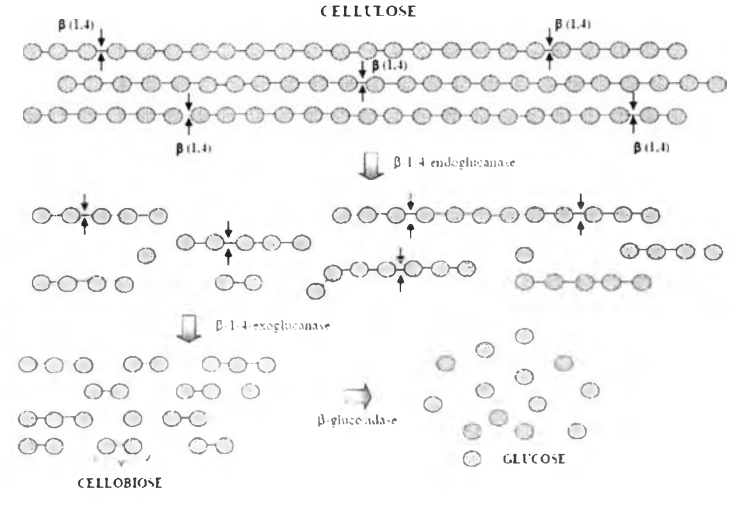


Figure 2.18 Schematic representation of the cellulase enzymes over the cellulose

structure (Mussatto *et al.*, 2010).

Cellulase enzyme is highly specific to enzymatic hydrolysis. The products of the hydrolysis are usually reducing sugars, including glucose. Utility cost of enzymatic hydrolysis is lower than acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50 °C), and does not have a corrosion problem (Duff and Murray, 1996). Moreover, enzymatic hydrolysis is an environmentally friendly process (Keshwani and Cheng, 2009). However, enzymatic hydrolysis of natural lignocellulosic materials is a very slow process. It is limited by several factors: crystallinity of cellulose, degree of polymerization (DP), moisture, available surface area, and lignin content (Chang and Holtzapple, 2000; Koullas *et al.*, 1992; Laureano-Perez *et al.*, 2005; Puri, 1984). This comparison is summarized in Table 2.8 (Hamelinck *et al.*, 2005).

Table 2.8 Comparison of process conditions and performance of three hydroly sis processes (Hamelinck *et al.*, 2005)

	Consumable	Temperature (K)	Time	Glucose yield (%)	Available
Dilute acid	<1% H_2SO_4	488	3 min	50-70	Now
Concentrated acid	30-70%	313	2-6 h	90	Now
Enzymatic	Cellulase	323	1.5 days	75-95	Now-2020

2.6.3 Characterization of the pretreated solid residues before hydrolysis

Hsu *et al.* (2010) investigated the operation condition for the dilute acid pretreatment followed by enzymatic hydrolysis of rice straw. A maximal sugar yield of 83% was achieved when the rice straw was pretreated with 1% (w/w) sulfuric acid with a reaction time of 1–5 min at 160 ° or 180 °C, followed by enzymatic hydrolysis. The pretreated rice straw is characterized by FTIR spectrum analysis (Fig 2.19).

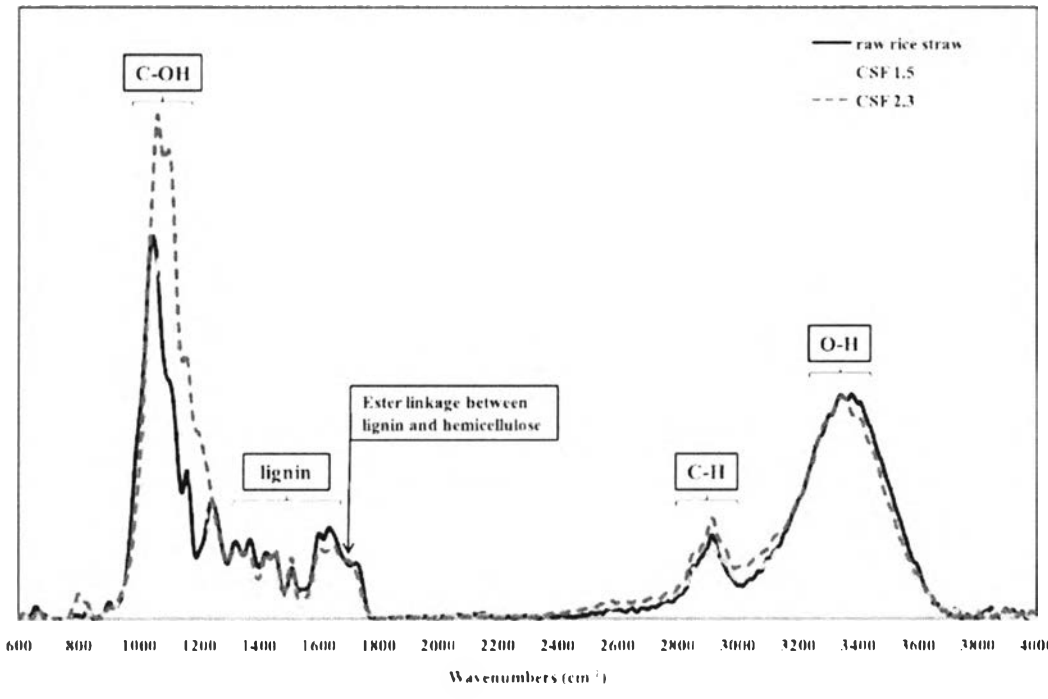


Figure 2.19 FTIR spectra of raw rice straw and pretreated solid residues under CSF of 1.5 (180 °C/0.7% H_2SO_4/1 min) and 2.3 (180 °C/1.0% H_2SO_4/4 min).

- The broad band at 3350 cm^{-1}: O–H stretching of the hydrogen bonds of cellulose(The absorption peak in the untreated rice straw was similar to that in pretreated solid residues, implying that most of the crystalline cellulose in the rice straw was not disrupted by the acid-catalyzed reaction at current condition.)
- The band at 2900 cm^{-1}: C–H stretching within the methylene of cellulose (This peak was slightly enhanced after the pretreatment (Kumar *et al.*, 2009)).
- The prominent bands at 1200–1000 cm^{-1}: The overlapped bands relate to the structural cellulose and hemicelluloses:
- The C–O–H stretching of primary and secondary alcohols at 1064 cm^{-1}
- The C–O–C glycosidic bond stretching at 1160 cm^{-1}
- The C–O–C ring skeletal vibration at 1100 cm^{-1}

The band at 910 cm^{-1} was dominated by the β-(1, 4)-glycosidic bond (C–O–C), which was not easily cleaved by the acid catalyzed reaction (Xiao *et al.*, 2001).

After the dilute acid pretreatment, the adsorption peaks of these bands were enhanced because the content of cellulose increased in the pretreated solid residue from hemicelluloses releasing in the acid hydrolysis reaction.

- The bands at 1740 and 1245 cm-1: related to the alkyl ester of the acetyl group in hemicelluloses (Both of these bands were absent in the pretreated solid residue, as a result of the removal of the hemicelluloses from the rice straw).
- The peak at 1720 cm^{-1}: the acetyl group in hemicelluloses structure and/or the linkage between hemicelluloses and lignin

The distribution of lignin can be observed by these FTIR bands that were diminished after the dilute acid pretreatment. It is suggested that the release of acid-soluble lignin in the pretreated solid residue has occurred.

- The bands at 1610 and 1516 cm^{-1}: aromatic skeletal stretching
- The bands at 2860, 1460, and 1425 cm-1: C-H deformation within the methoxyl groups of lignin
- The peaks at 1375 and 1330 cm^{-1}: aromatic hydroxyl groups (may be generated by cleavage of ether bonds within the lignin)
- The peak at 1640 cm^{-1}: the C=O groups in the alkyl groups of the lignin side chain conjugating with the aromatic structure

They found that the absorption peak for C=O groups was reduced in the pretreated solid residues since the acid hydrolysis reaction may cause partial lignin structure to release from raw rice straw.

The degradation of the ester and ether linkages within the lignin by the acid-catalyzed reaction may also destroy the matrix structure and generate small lignin fragments. This redistribution of the lignin has been suggested to generate a trap effect that may hinder cellulase as it begins to attack the surface of the cellulose. However, these smaller lignin fragments easily congregate into drops due to hydrophobic

interactions within the solid residue and this congregation increases the hydrophobic areas within the matrix, which then allows more adsorption of cellulase. This naturally reduces the amount of cellulase available for the hydrolytic reaction (Selig *et al.*, 2007).

2.7 The potential of lignocellulosic materials for second generation bioethanol production

The lignocellulosic materials are considered to be a potential feedstock for bioethanol production with sustainability while the biofuels from crops are lack of sustainability and disturb the food chain. Many countries around the world try to research and develop the utilization of lignocellulosic materials to be biofuels. In Europe, the C_4 perential grass *Miscanthus x giganteus* has proved a promising biomass crop while switchgrass (*Panicum virgatum*) has been tested at several locations in North America.

In Australia, Y.J. Jeon *et al.*, (2010) studied the potential of different lignocellulosic materials for bioethanol production. They evaluated sugar recoveries and fermentabilities of eight lignocellulosic materials: Sorghum straw (*Sorghum bicolor*), wheat straw (*Triticumaestivum*), sugarcane bagasse, and sugarcane tops (SCT) (*Saccharum officinarum*), Hardwood (*Eucalyptus dunnii*),softwood chips (*Pinus elliotii*), *Eucalyptus loxophleba ssp. lissophloia* (oil mallee), and *Arundo donax*. These biomass samples were analyzed for cellulose, hemicelluloses and lignin using the detergent fibre method (Van Soest *et al.*, 1991) and the results are shown in Table 2.9.

Table 2.9 The list of plant species used in this study and their sugar composi tions (Y.J. Jeon *et al.*, 2010)

Raw materials	Component (%)*		
(common name)	Cellulose	Hemicellulose	References
Wheat straw	36	26	This study
	33.7	25.0	Ali et al. (1991)
Sugarcane bagasse	39.6	26.5	This study
	35.0	35.8	Sasaki et al. (2003)
Sorghum straw	32.4	27	This study
	35.1	24	Tellez-Lus et al. (2002)
Arundo donax	42.5	31.2	This study
	36	30	Pascoal Neto et al. (1997)
Sugarcane tops	35	32	This study
Oil mallee	53	33	This study
Pine	49.8	13.1	This study
	45.3	22.2	Araque et al. (2008)
Eucalyptus	51.9	18.1	This study
	46.8	16.6	Garrote et al. (2003)

*Composition percentages are on dry-weight basis.

All biomass material was milled following dilute acid pretreatment (2% H_2SO_4 (v/v) with 10% (w/v) raw material at 134°C for 60 min) and enzymatic hydrolysis. The acid/enzyme-treated hydrolysates were fermented by a recombinant strain of *Zymomonas mobilis*. The analysis of Acid/enzyme hydrolysate samples for glucose, xylose and arabinose as well as the degradation products including acetate, furfural, hydroxymethyl furaldehyde (HMF), levulinic acid and formate were achieved by HPLC using an Aminex column HPX-87H equipped with a refractive index detector and a computer interfaced electronic integrator. Separations were performed at 50 °C and eluted at 0.6 ml/min using 5 mmol/l H_2SO_4. The analyzed results are summarized in Table 2.10.

Table 2.10 Sugar production for fermentationof raw materials (10% w/v) from acid /enzyme pretreatment(Y.J. Jeon *et al.*, 2010)

Raw material	Glucose (g/l)	Xylose (g/l)	Arabinose (g/l)	Total sugar (g/l)	Sugar recovery yield (%)*
Wheat straw	23.3	19	3.0	45.3	74
Sugarcane bagasse	26.9	18.2	2.8	47.9	72
Sorghum straw	22.0	16.9	3.7	42.6	72
Arundo donax	17.7	23.3	2.2	43.2	59
Sugarcane tops	22.3	18.7	3.1	44.1	66
Oil mallee (Bark and hard wood)	10.6	3.4	5.0	19.0	25
Pine (soft wood)	10.6	19.7	1.5	31.8	51
Eucalyptus (hard wood)	8.9	14.6	0.3	23.8	34

Acid hydrolysis carried out using 2% H_2SO_4 (v/v) at 134°C for 1 h.
Enzyme hydrolysis carried out using 2% cellulase and 4% b-glucosidase at 60_C for 22 h.
*Sugar recovery yields were calculated based on composition (on dry-weight basis) of cellulose and hemicellulose (see Table 2.9).

The results show that pretreatment and enzyme hydrolysis of the herbaceous raw materials resulted in higher sugar recoveries when compared to those of woody raw materials. Among the herbaceous materials, wheat straw, sugarcane bagasse, and sorghum straw(72–74%) showed the highest sugar recoveries. The hydrolysate from A. donax (Adx) showed the lowest sugar recovery (65%), while the sugar recoveries of woody raw materials showed poorer recoveries (<55%). From evaluation of lignocellulosic materials in this study, the herbaceous raw materials have a high potential for bioethanol production and may be used as feedstock in large scale production.

Besides this study, the other research groups have also tried to estimate the ethanol production ability from various lignocellulosic materials that suit for their home country, such as water hyacinth, giant reed, banana, wild sugarcane, miscanthus grass, and so on.

2.7.1 Water Hyacinth [*Eichhornia crassipes (Mart.) Solms*]

Figure2.20 *Eichhornia crassipes (Mart.) Solms*
(http://www.mpbd.info/plants/eichhornia-crassipes.php)

Water Hyacinth (Fig 2.20) was studied as cheap feedstock for the bioethanol production by Mukhopadhyay, S., and Chandra Chatterjee, N. (2010). Water hyacinth is a noxious aquatic weed found in many tropical and sub-tropical freshwater habitats with faster growth rate. The utilization of water hyacinth as a feedstock for ethanol production is a environmentally friendly method to take it away. In this research, water hyacinth [*Eichhornia crassipes (Mart.) Solms*] was pretreated with 0.1 N H_2SO_4 and 1% (w/v) NaOH, followed by enzymatic hydrolysis using the enzyme blend from *Trichoderma reesei* ATCC 26921 and *Aspergillus phoenicis* ATCC 52007. After the pretreated water hyacinth was hydrolyzed by the enzyme blends from two fungal cultures for 72 hours, the total sugar of 18.28 g/l was obtained.

The ethanol production from the pretreated water hyacinth was also studied in four different modes of enzymatic hydrolysis (saccharification) and fermentation process with or without prefermentation hydrolysis for better ethanol production. The results of the ethanol production by different methods are shown in Table 2.11.

Table 2.11 Concentration (g/l) and yield g/g pretreated water hyacinth of ethanol produced under different modes of enzymatic hydrolysis and fermen tation (Mukhopadhyay, S., and Chandra Chatterjee, N. (2010)).

Mode of fermentation	**Concentrate (g/l)***	**Yield (g/g dry substrate)***
Separate hydrolysis and fermentation (SHF)	4.5±0.3	0.11±0.007
Simultaneous saccharification and fermentation (SSF)	5.2±0.2	0.14±0.007
Single batch bioconversion (SBB)	5.0±0.2	0.13±0.006
Prefermentation hydrolysis- Simultaneous saccharification and fermentation (PH-SSF)	8.3±0.5	0.21±0.011
CD at 5% level	1.72	0.045
*Results are mean of three replication ± SD Different letter in the superscript denotes significant difference at 5% level.		

From these results, water hyacinth, which is being used to absorb heavy metal pollutants in waste water, could be utilized as abundant cheap feedstock for the production of fuel ethanol.

2.7.2 Banana pseudostem

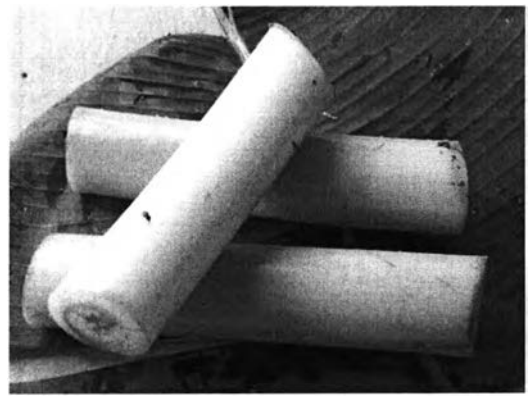


Figure 2.21 Banana pseudostem
(http://s3.amazonaws.com/readers/2010/04/15/posola_1.jpg)

Banana pseudostem (Fig 2.21) was used as raw material for the bioethanol production by Chittibabu *et al.*,(2011). They are interested in banana pseudostem because India is the largest producer of banana. India contributes to 27% of world's banana production. 60–70% t/ha of banana pseudostem is generated in the field after harvesting that is dumped on roadside orburnt or left *in situ,* causing impact on environment. The potentials of banana pseudostem (BPS) for ethanol production are consist of three factors: High concentration of holocellulose (72%)with low lignin content (10%), and its easy availability.

Optimization of the microwave assisted alkali pretreatment and enzymatic hydrolysis of banana pseudostem (BPS) for the production of bioethanol were studied by Chittibabu *et al.*, (2011). Pretreatment of BPS was performed at different alkali concentrations, liquid-solid ratios, temperatures and microwave exposure times, followed by enzymatic hydrolysis. The experimental results showed that when BPS was pretreated using microwave heating with 10% NaOH, 4:1 liquid-solid ratio at temperature of 90°C for 8 min pretreatment time, the yield of reducing sugars(YRS%) reached its maximum 84% after 110 h of enzymatic hydrolysis at 30 FPU/g of solid enzyme loading. The reducing sugars produced by enzymatic hydrolysis was determined by DNS (3,5- dinitro salicylic acid) method.

The yield of reducing sugars (YRS) in % is defined as:

$$YRS\ \% = (W_{Rs} \times 0.9 \times 100) / (W_{Is})$$

W_{Rs} – weight of reducing sugars produced by enzymatic hydrolysis
W_{Is}– weight of initial solids. (the initial solid concentration was 2% w/v)

Experiments were also performed under optimum pretreatment conditions for both microwave and convectional heat pretreated techniques with different enzyme loadings (5–30 FPU/g of solid) (Fig 2.22).The residual cellulase activity of microwave pretreated BPS samples was higher than that of the conventional heat treated BPS. In brief, the results of this study can serve for further optimization of bioethanol production process from lignocellulosic biomass.

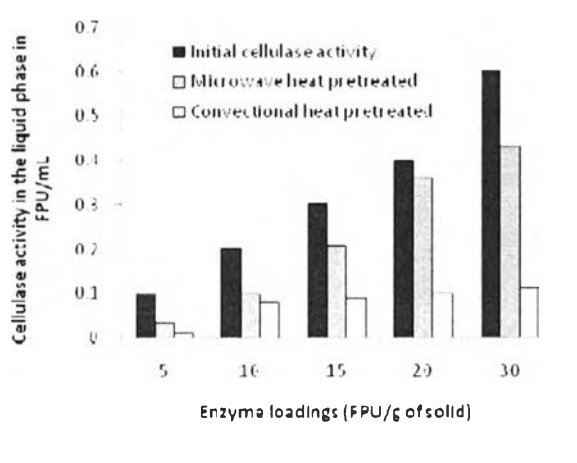


Figure 2.22 Residual cellulase activity in the liquid phase after 110 h of enzymatic hydrolysis

2.7.3 Giant reed (*Arundo donax*)

Figure 2.23 Giant reed (*Arundo donax*) (http://luirig.altervista.org/cpm/albums/bot-units03/arundo-donax8606.jpg)

Giant reed (Arundo donax L.) (Fig 2.23), a perennial, herbaceous rhizomatous crop of Poaceace family, which occurs over a wide range of climatic habitats, was used as substrate to test its suitability for second generation ethanol production. It is native in Asia and countries surrounding Mediterranean Sea; it has a C_3 photo-

synthetic cycle, but its high rates of photosynthesis and productivity are similar to those of C_4 species (Lewandowski *et al.*, 2003). Normally, it uses for pulp production in some country. It is able to grow well on marginal and non-agricultural lands, and its raw material has carbohydrate content similar to those of agricultural residues, such as corn stover and wheat straw. Moreover, it has been recognized as very robust species with the potential to avoid competition with food crops for lands. The chemical and energetic characterization of *Arundo donax* is shown in Table 2.12, and lignocelluloses and lignin from Arundo donax was analyzed in data following these experiments: ash determination and characterization by dispersive X-ray analysis, solubility, UV, FTIR, molecular weight determination (Oskar *et al.*, 1989).

Table 2.12 Chemical and energetic characterization of *Arundo donax* (Franscisco *et al.*, 2010)

	Glucan (%)	Klason lignin (%)	Holocellulose (%)	Xylan (%)	Araban (%)	Acetyl group (%)	Higher heating value (constant volume) cal/g over dry basis	Higher heating value (constant volume) cal/g (% moisture)
Arundo donax	34.8	23.0	64.5	19.4	1.5	3.4	4573 (9.1)	4166 (8.2) (8.9%)

- Percentages relative to the raw material (100 kg dry mass). Average value of four replicated variation coefficient less than 2%.

Scordia *et al.*, (2011) studied the effect of temperature, reaction time, and dilute oxalic acid (OA) concentration during the steam-pretreatment of giant reed. The giant reed biomass was collected along the Tellaro torrent in Southern Italy. It was pretreated with stream and oxalic acid. The oxalic acid, dicarboxylic organic acids, can hydrolyze β-(1,4)-bonds more selectively than sulfuric acid. The basis for this is not really known, however, hemicellulolytic and cellulolytic enzymes catalyze hydrolysis through a general acid-base

mechanism mediated by two carboxylic acids, and oxalic acid with its two pKa's might mimic this reaction through similar ion-pair mechanisms. In pretreatment step, the effects of temperature (170–190 °C), acid loading (2–10% w/w) and reaction time (15–40 min) were handled asa single parameter, combined severity. Then, the pretreated giant reed is followed by enzymatic hydrolysis.

Combined severity parameter describes the effect of pretreatment conditions, hereinafter referred as CS, was described by Chum *et al.*, (1990).

$$Log\left[t \times \exp\left(\frac{Tp - Tref}{14.75}\right)\right] - pH$$

t is the time (min), T_p is the pretreatment temperature (°C)

T_{ref} is the reference temperature

pH was measured from the amount of oxalic acid added before reaction.

The combined severity (CS) was plotted against the residual hemicellulosic and cellulosic polymers. At high combined severities dilute oxalic acid pretreatment can remove hemicelluloses and prepare the solid residue for enzymatic hydrolysis and SSF. After the pretreatment step, the simultaneous saccharification and fermentation (SSF), of this biomass was tested by two different yeast strains; *Scheffersomyces (Pichia) stipitis* CBS 6054, which is a native xylose and cellobiose fermenter, and *Saccharomyces carlsbergensis* FPL-450, which does not ferment xylose or cellobiose, along with commercial cellulolytic enzymes. *S. carlsbergensis* attained a maximum ethanol concentration of 15.9 g/l after 48 h at pH 5.0,while *S. stipitis*, at the same condition, took 96 h to reach a similar ethanol value; increasing the pH to 6.0 reduced the S. stipitis lag phase and attained 18.0 g/l of ethanol within 72 h.

Carbohydrate compositions of the original and pretreated giant reed samples were measured by using an improved high-performance anion exchange chromatography (ICS-3000, Dionex, Sunnyvale, California) with pulsed amperometric detection (HPAEC-PAD), according to the method of Davis MW.,(1998), and monomeric sugar concentrations in the hydrolyzate fraction were determined by HPLC equipped with a refraction index detector.

Moreover, Scordia *et al.* (2012) also evaluated the capacity of *S. stipitis* CBS6054, a native xylose fermenting yeast, to produce ethanol from sugars con-

tained in the giant reed (*Arundo donax*) hemicellulosic hydrolysate. Severity factor and oxalic acid concentration ranging from 2.87 to 4.05 and from 2 to 8 (% w oxalic acid/w solid dry matter), respectively, were employed to minimize degradation products and maximize sugar release. However, at the optimum condition for sugar release (43.8 g/l), levels of toxic degradation products (acetic acid, furfural, HMF and phenolic compounds) were considered too high for yeast fermentation. The condition to minimize degradation products and maximize sugar yields was judged to be 2.87 severity factor and 5.0% oxalic acid concentration. At this condition 26.0 g/l xylose, 5.0 g/l glucose and 2.4 g/l arabinose were recovered in giant reed hydrolysate fraction. In fermentation at pH 6.0 after 48 h, 8.2 g/l of ethanol was obtained with ethanol yield of 0.33 (g_e/g_s) and a productivity of 0.17 g/l*h.

2.7.4 Wild sugarcane or Kans grass (*Saccharum spontaneum*)

Figure 2.24 Wild sugarcane or Kans grass (*Saccharum spontaneum*) (http://endigitalkinmen.kmnp.gov.tw/ezfiles/1/1001/plugin/o_kmnp/pictures/75/2875/bigkmnp-slide-plant_%20Po-0016-0001.jpg)

Wild sugarcane or Kans grass (*Saccharum spontaneum*) (Fig 2.24) is a perennial grass with a deep roots and rhizomes up to 6 m in height. It is believed to be a predecessor of the important species S. officinarum L. (cultivated sugarcane). It is wild in Mediterranean areas and it has adapted to live over a wide range of grassland climatic habitats:from oriental Asia to the southern region, in the warm-temperateare as of Africa and in Mediterranean regions (Australian Government,2004) while mis-

canthus, perennial energy crop which is native to subtropical areas, encounters difficulty in the Mediterranean semi-arid environment because of the dry summer period (Cosentino *et al.*, 2007). In addition, it is the one of the most distributed weeds worldwide infesting millions of acres lands, often causing abandonment of agricultural field (Chandel *et al.*, 2009).

Saccharum genus is similar to the Miscanthus genus. It differs from the latter by the different dispositions of the spikelets in the bloom and the rachis fragility (Scally *et al.*, 1997). Besides, the crop shows high aboveground biomass yield, starting after the second year of establishment, whereas this occurs only after the third year in the Miscanthus genus (Cosentino *et al.*, 2006). Surprisingly, it rarely has a pretreatment studies for wild species of saccharum genus that can grow in more arid habitat while sugarcane (saccharum hybrids) is studied in various aspects.

Scordia *et al.* (2010) evaluated oxalic acid as a pretreatment for bioconversion. Overall sugar yields, sugar degradation products, enzymaticglucan hydrolysis and ethanol production were studied as effects of temperature (150°–190 °C), reaction time (10–40 min), and oxalic acid (OA) concentration 2–8% (w/w). Time and temperature were combined into a single parameter, called Severity Factor (SF) [Log (R_0)] and related to oxalic acid using a response surface methodology.

Severity factor(SF): $\log(R_0) = Log\left[t \times \exp\left(\frac{Tp-Tref}{14.75}\right)\right]$

t is the time (min), T is the pretreatment temperature (^oC)

T_{ref} is the reference temperature, usually set to 100 ^oC

Carbohydrate compositions of Saccharum raw materials and dilute OA pretreated solid samples were measured by using an improved high performance anion exchange chromatography. The composition is shown in Table 2.13.

Table 2.13 Raw material composition of *Saccharum spontaneum* L. ssp. Aegyp tiacum (Willd.) Hack (Scordia *et al.*, 2010).

Composition	Dry matter[a] (%)
Glucan	36.81±0.13
Xylan	21.53±0.04
Galactan	0.72±0.01
Arabinan	2.16±0.01
Mannan	0.16±0.04
Rhamnan	0.14±0.01
Acetyl groups	3.68±0.00
Protiens	2.35±0.18
K. lignin	20.03±0.12
Ash	5.40±0.21
AL ash	1.21±0.09

[a]Mean values and standard deviation of two determinations.

From composition analysis, carbohydrate in Kans grass cell wall accounts for 61.5% of total dry weight (tdw) while cellulose constitutes the main glucan fraction (36.8%). The main sugars in the hemicelluloses fraction are arabinan and xylan with 2.1 and 21.5% (tdw).

The composition of *Saccharum spontaneum* was also determined by Chendel. (2009) using the Technical Association of Pulp and Paper Industries method (TAPPI, Atlanta, Georgia, USA). It contains (on a dry weight basis, mg/g): 45.10cellulose, 22.75hemicelluloses, 24.56lignin, 4.85 moisture and 2.64ash. The total carbohydrate content (TCC) present in the *S. spontaneum*cell wall was 67.85 mg/g of dry weight consistant with Scordia *et al.* (2010) report.The level of TCC is comparable to those of other substrates used for bioethanol production, viz. sugar cane bagasse (63), wheat straw (54), birch (73), spruce (63.2), corn stover (59.9),and poplar (58.2 mg/g) (Chandel *et al.*, 2007a).

Monomeric sugar concentrations in the hydrolysate fraction (HF) were determined by HPLC equipped with a refraction index detector. A multiple variant response surface analysis for total sugars was carried out to optimize the dilute-OA-pretreatment process for HF fermentation (Fig 2.25).Maximum total sugar yield was

attained at a severity factor (SF) of 2.93 and 6.79% (w/w) oxalic acid, while maximum formation of sugar degradation products was observed at the highest SF (4.05) and 5% (w/w) oxalic acid.

In simultaneous saccharification and fermentation (SSF) process, commercial cellulases and *Saccharomyces cerevisiae* attained 89.9% glucan conversion and 17.8 g/l ethanol while *Pichia stipitis* CBS 6054 fermented hemicellulosic hydrolysates from less severe conditions to ethanol with a yield of 0.35 (g_e/g_s).

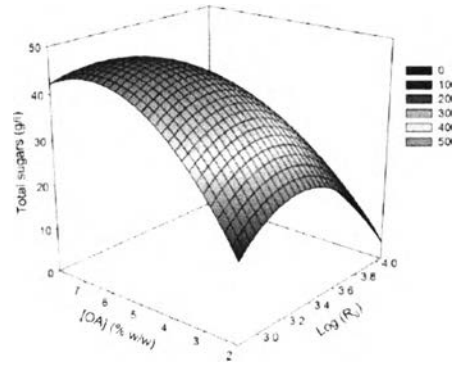


Figure 2.25 Perspective plot of the fitted total sugars (g/l) response surface of severityfactor [Log (R0)] versus oxalic acid concentration after dilute-OA-pretreatment of Saccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack (Scordia *et al.*, 2010).

Except oxalic acid pretreatment followed by emzymatic hydrolysis, acid hydrolysis of *Saccharum spontaneum* was studied by Chandel *et al.,* (2011). In this study, *S. spontaneum* was collected from Sathedi, Muzaffarnagar Dist., India. Dry stem pieces including leaf sheath were processed and thermochemically saccharified with varying concentrations of dilute sulfuric acid [0.75%, 1.50% (v/v)], autoclaved under pressure (15, 20 and 25 psi)for varying time period ranges from 15 to 60 min using initial solid to liquid ratio of 1:10.A maximum(35.86±2.33 g/L) total sugar was released with 1.5%H_2SO_4 in 15 min at 25 psi (Table 2.14).

Table 2.14 Total sugars and fermentation inhibitors profile from acid hydroly sates of *S. spontaneum* at different parameters (Chandel *et al.*,2011).

Autoclave pressure (psi)	H_2SO_4 (% v/v)	Time (min)	Total sugars (g/L)	Furfurals (g/L)	Phenolics (g/L)
15	0.75	15	9.44 ± 0.22	0.23 ± 0.02	0.33 ± 0.03
		30	12.82 ± 0.714	0.41 ± 0.03	0.56 ± 0.04
		45	15.02 ± 0.75	0.53 ± 0.02	0.85 ± 0.05
		60	17.52 ± 0.85	0.65 ± 0.04	0.98 ± 0.05
	1.50	15	10.11 ± 0.45	0.27 ± 0.01	0.85 ± 0.04
		30	13.95 ± 0.62	0.45 ± 0.02	0.95 ± 0.04
		45	16.02 ± 0.0.75	0.60 ± 0.03	1.08 ± 0.06
		60	18.25 ± 0.94	0.72 ± 0.04	1.22 ± 0.06
20	0.75	15	12.25 ± 0.65	0.64 ± 0.02	1.09 ± 0.05
		30	16.36 ± 0.0.84	0.74 ± 0.03	1.21 ± 0.07
		45	19.76 ± 0.88	0.92 ± 0.05	1.35 ± 0.08
		60	21.75 ± 1.10	1.21 ± 0.07	1.46 ± 0.07
	1.50	15	15.10 ± 0.88	0.84 ± 0.05	1.21 ± 0.06
		30	18.25 ± .96	1.02 ± 0.06	1.51 ± .08
		45	22.35 ± 1.11	1.11 ± 0.07	1.88 ± 0.07
		60	20.91 ± 1.16	1.31 ± 0.06	2.25 ± 0.10
25	0.75	15	20.62 ± 1.12	1.11 ± 0.04	1.33 ± 0.08
		30	28.32 ± 1.22	1.63 ± 0.07	1.98 ± 0.09
		45	25.52 ± 1.410	1.81 ± 0.08	2.21 ± 0.12
		60	22.85 ± 1.26	2.05 ± 0.09	2.85 ± 0.15
	1.50	15	35.86 ± 2.33	1.54 ± 0.0610	2.01 ± 0.120
		30	30.73 ± 2.26	1.89 ± 0.06	2.65 ± 0.13
		45	27.92 ± 1.85	2.06 ± 0.09	3.01 ± 0.21
		60	24.90 ± 1.85	2.15 ± 0.08	3.45 ± 0.22

The insight structure deviation of *Saccharum spontaneum* in the pretreatment process was revealed using scanning electron microscopic (SEM). It shows cell wall disruption, ultra structure and surface properties of native and acidic saccharified *S. spontaneum* (Fig. 2.26a and 2.26b). The anatomy of chopped and untreated S. spontaneum was easily recognizable, with sheath leaves surrounding the straw itself as thick-walled fiber cells.

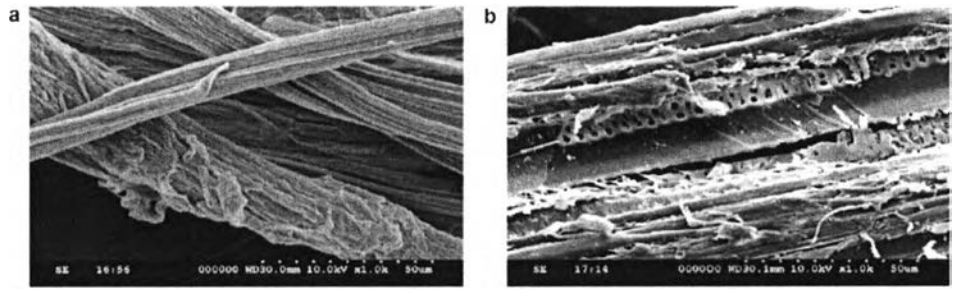


Figure 2.26 Scanning electron microscopic (SEM) observations of substrate *S. spontaneum*a) Native view of S. spontaneum (control/untreated), re vealing compact anatomy and b) Acidic pretreated *S. spontaneum* at 25 psi pressure for 15 min with 1.50% (v/v) sulfuric acid, showing de lignification caused by treatment.

Chandel *et al.,* (2010) also assessed SSF of aqueous ammonia pretreated *S. spontaneum* with thermotolerant *S. cerevisiae* VS_3 and naturally xylose fermenting *P. stipitis* NCIM 3498. The cellulase enzyme used for saccharification was preparedfrom the culture supernatants of *Aspergillus oryzae* MTCC1846. During SSF, *P.stipitis*, VS_3 and mixed culture showed ethanol production of 15.73±0.44 g/l (productivity, 0.218±0.04 g/l/h), 14.22±0.15 g/l (productivity, 0.197±0.02 g/l/h), and 17.73±0.25 g/l(productivity, 0.246±0.02 g/l/h), respectively. In addition, the enzyme mixture from *Trichoderma reesei* was studied by Kataria and Ghosh (2011) to hydrolyze Kans grass. Maximum total sugar was found to be 69.08 mg/g dry biomass with 20 FPU/g dry biomass of enzyme dosage. These researches show the possibilities for using *Saccharum spontaneum* as a feedstock for bioethanol production.

2.8 Biomass energy potential and research in Thailand

Thailand is a developing country in Southeast Asia, with a population of approximately 67 million in 2007.The economic growth and the inefficient energy utilization in Thailand are responsible for the rising energy elasticity. As a country depending on more than 50% of imported energy, development of a renewable energy program such as biomass energy in Thailand, is needed to reduce energy supply risk and to contribute to global GHG emission reduction. Renewable energy has continu-

ously contributed to Thailand's energy supply at a portion of around 17%, butal-mostall renewable energy use is from traditionally burning of firewood, charcoal, and agricultural wastes. Less than 1% has been converted to modern forms of energy, such as electricity, methane, and liquid biofuels. Energy supply and demand are shown in Table 2.15.

Table 2.15 Thailand's energy balance in 2008 (in ktoe) (Department of Alterna tive Energy Development and Efficiency (DEDE))

Energy supply	Components of energy demand							
	Coal and its products	Crude oil	Natural gas	Condensate and NLG	Petroleum products	Electricity	Renewable energy[b]	Total
Domestic production	4743	7318	24,969	3900		1577[a]	20,188	62,695
Imports	10,026	40,516	8261		884	237	44	59,967
Exports	471	2388		109	9009	101	57	11,711
Stock changes	225	281		446	39		25	1046
Total primary energy supply	14,947	48,727	33,230	3345	9204	1713	20,199	112,957
Supply from secondary sources, less own uses and losses[c]	7203	48,727	30,077	177	41,254	9828	7954	42,702
Total final energy output	7744		3153	3522	32,050	11,541	12,245	70,255
Non energy uses				3522	843			4365
Final energy consumption	7744		3153		31,207	11,541	12,245	65,890

[a] From hydro and other sources.
[b] Excludes electricity generation.
[c] Includes supply from power and processing plants.

Accordingly given the high and rising price of oil since 2004, the government has become more aware of the need to promote domestic renewable energy, particularly biomass fuel, in order to reduce reliance on energy imports and improve fuel security. In January 2009, the National Energy Policy Council (NEPC) approved a 15-year renewable energy development plan (2008-2022) which is categorized into three stages: short, medium, and long runs, as shown in Table 2.16. The plan focuses on increasing domestic alternative energy use to replace fossil fuel imports and a roadmap was developed to promote raw material/feedstock for ethanol production, to increase production efficiency of ethanol feedstocks and to increase planting areas for ethanol feedstocks.

Table 2.16 Targets for Thailand's 15-year renewable energy development plan (Department of Alternative Energy Development and Efficiency (DEDE))

Forms of energy	Sources	2008	2011	2016	2022	Unit
		Existing	Short run	Medium run	Long run	
1. Electricity	Total	1750	3273	4191	5680	MW
	Biomass[a]	1610	2800	3220	3700	MW
	Other	140	473	971	1980	MW
2. Heat	Total	3007	4150	5582	7433	ktoe
	Biomass	2781	3660	5000	6760	ktoe
	Other	226	490	582	673	ktoe
3. Bio-liquid fuel	Total	725	2190	3591	4927	Million liters
	Ethanol	328	1095	2263	3285	Million liters
	Biodiesel	397	1095	1328	1642	Million liters
4. Compressed natural gas		28,236	144,540	217,540	251,850	MMscf
Targets: fossil fuel reduction			10,960	15,580	19,800	ktoe

[a] In 2008 electricity generated from biomass was 1610 MW, with half of this amount sold to the national grid.

Because of aggressive policies of the Thai government in reducing foreign oil import and increasing domestic renewable energy utilization, production and consumption of biofuel in Thailand have continued to increase.

Biofuels nowadays are importantly alternative fuels for transport. From 2001 to 2007, the global annual production of bio-ethanol and biodiesel grew by 23 and 43%, respectively. In Thailand, the Royal Thai Government (RTG) has promoted biofuels for transport to reduce oil imports and spur rural development since 2004.Bio-ethanol derived from cane molasses, cassava and sugarcane has been strongly receiving attention by the RTG to partially substitute conventional gasoline. Although Thailand is an agro-industrial based country and has a variety of crops for first generation bioethanol, only cane molasses, cassava and sugarcane juice are the major feedstocks being promoted for the commercial ethanol plants due to their surplus availability and their economic and technical feasibility.

The actual production of ethanol (January 2009) in Thailand was at 1.33 million liters per day. The Energy Ministry has targeted the use of ethanol at 9 mil-

lion liters per day in 2023. The existence of ethanol plant in Thailand (2009) is shown in Table 2.17.

Table 2.17 Existing Ethanol Plants in Thailand (June 2009) (www.dede.go.th/dede/fileadmin/upload/pictures_eng/pdffile/Existing_Ethanol_Plant.xls)

Company	Installed Capacity (L/day)	Feedstock	Province	Commencing Date
1. Pornwilai International Group	25,000	Molasses	Ayuddhaya	Oct 03
2. Thai Alcohol	200,000	Molasses	Nakhon-Pathom	Aug 04
3. Thai Agro Energy	150,000	Molasses	Suphanburi	Jan 05
4. Thai Nguan Ethanol	130,000	Cassava	Khon Khan	Aug 05
5. Khon Khan Alcohol	150,000	Sugarcane Molasses	Khon Khan	Jan 06
6. PetroGreen	200,000	Sugarcane Molasses	Chaiyaphoom	Dec 06
7. Thai Sugar Ethanol	100,000	Sugarcane Molasses	Kanchanaburi	Apr 07
8. KI Ethanol	100,000	Sugarcane Molasses	Nakhon Ratchasima	Jun 07
9. PetroGreen	200,000	Sugarcane Molasses	Kalaseen	Jan 08
10. Ekarat Pattana[1]	200,000	Molasses	Nakhonsawan	Mar 08
11. Thai Rung Ruang Energy	120,000	Sugarcane Molasses	Saraburi	Mar 08
12. Ratchaburi Ethanol	150,000	Cassava Molasses	Ratchaburi	Jan 09
13. ES Power	150,000	Molasses Cassava	Sakaew	Jan 09
14. Maesawd Clean Energy	200,000	Sugarcane	Tak	May 09
15. SupThip	200,000	Cassava	Lopburi	May 09
Total	**2,275,000**			

Note: [1] Production for exporting, 95% purity

Although biofuel energy is promoted by Thai government, the current feedstock for bioethanol production in Thailand is still food crops. These raw materials compete with food crops demand, leading to higher or unstable global and regional prices (Office of Agricultural Economics, Ministry of Agriculture, Thailand, 2011). To utilize the non-food material for bioethanol production in Thailand, many lignocellulosic materials were researched for low cost, abundant and effective feedstock

that may lead to large scale ethanol production in the future. Among lignocellulosic materials, the perennial herbaceous energy crops are studied, such as Miscanthus grass, switchgrass, and Kans grass.

Perennial herbaceous energy crops, well adapted to the climatic and soil conditions of a specific area, could reduce the raw material cost for this technology. Once established, they do not require annual reseeding and require lower energy inputs of fertilizer and pesticide than annuals crops (Vecchiet and Jodice., 1996). They have a high production of biomass that most of them have not been cultivated for biomass production. Moreover, the knowledge about them for bioethanol conversion is not well known as traditional agricultural residues. In Thailand, many research groups also study in bioethanol conversion from herbaceous energy crops.

Purple guinea grass

Purple guinea grass (*Panicum maximum* cv. TD53) is one of the popular forage plants grown in Thailand. It gives a very high yield, is easy to harvest and self-generated after harvesting, resists to drough, and grows well on various types of soil. Bioethanol conversion of purple guinea grass was studied by Ratsamee *et al.*, (2012) from a Department of Microbiology, Faculty of Science, Chulalongkorn University.

Ratsamee *et al.* (2012) collected purple guinea grass from the Department of Livestock Development, Ministry of Agriculture, Pakchong distinct, Nakorn Ratchasima province, Thailand. This sample was determined chemical compositions by the Technical Association of Pulp and Paper Industry method (TAPPI 1988). The purple guinea grass used in this study was found to be composed of 41.7% (w/w,DS) cellulose, 27.1% (w/w,DS) hemicelluloses, and 10.4% (w/w, DS) lignin. After composition analysis, the purple guinea grass was treated with dilute sulfuric acid (H_2SO_4) or calcium hydroxide ($Ca(OH)_2$) followed by enzymatic hydrolysis.

Under the optimum condition for the acid pretreatment (6%(w/v) loading in 3% (w/v) H_2SO_4 at 121 °C, 103.4 kPa for 30 min), 173 mg/g DS reducing sugar was released while 110.5 mg/g DS was released after hydrolysis by cellulase GC220 (63 FPU/g, DS) under the optimum condition for alkali pretreatment (6%(w/v) loading in

4% (w/v) $Ca(OH)_2$ at 121 °C, 103.4 kPa for 5 min). Reducing sugars were quantified using the Somogyi-Nelson method (Somogyi 1952), and glucose, xylose and five pretreatment byproducts (furfural, hydroxymethyl furfural, 4-hydroxybenzaldehyde, syring aldehyde and vanillin) were analyzed by HPLC. Although the quantity of reducing sugar from H_2SO_4 pretreatment was higher than $Ca(OH)_2$ pretreatment, the level of glucose released from $Ca(OH)_2$ –pretreated purple guinea grass was slightly higher than that from dilute H_2SO_4 pretreatment. Fermentation of the $Ca(OH)_2$ pretreated purple guinea grass (100g containing 41.7 g of cellulose) by the SHF and SSF methods yielded 8.76 and 7.51 g of ethanol per 100 g pretreated purple guinea grass at a 0.05 L scale respectively. The high ethanol yield (96% theoretical yield) and high cellulose content show that the purple guinea grass is also a promising herbaceous energy crop in Thailand.

Miscanthus Sinesis

Miscanthus sinesis is widly found in South East Asia. It was studied to have a great potential as an energy crop for ethanol production. Release of monomeric sugar of Miscanthus sinesis was studied by Boonmanumsin *et al.* (2012) from the Petroleum and Petrochemical College, Chulalongkorn University. They collected the sample from Cha-Chueng-Sao province, Thailand. The *M. sinesis* sample was treated with microwave-assisted ammonium hydroxide (NH_4OH) followed by phosphoric acid (H_3PO_4). The two stage pretreatment with 1.0%(w/v) NH_4OH, 15:1 liquid-to-solid ratio (LSR) at 120 °C for 15 min, followed by 1.78%(v/v) H_3PO_4,15:1 LSR at 140 °C for 30 min provided the highest totoal monomeric sugar yield of 71.6g/100g dried biomass. The chemical composition of *M. Sinesis* sample was analyzed by using the method described by Lin *et al.*,(2010) and the monosaccharides were measured using a HPLC with pulsed refractive index detector.

Weeds

Thailand is a country that has plant diversity, including various types of weed disturbing the agriculture in the country. Many types of weed were screened the potential as the raw materials for production of biofuels by Supaporn *et al.* (2003)

from Department of Botany, Faculty of Science, Chulalongkorn University. The 10 weed samples were collected from 8 provinces of Thailand: Bangkok, Samutprakarn, Chonburi, Pathumthani, Lumpoon, Nakornpatom, Cha-Chueng-Sao, and Nakorn Ratchasima.

Ten screened weeds, the height more than 1 m, consist of *Coix aquatic*, *Imperata cylindrical*, *Panicum maximum*, *Pennisetum polystachyon*, *Pennisetum purpureum*, *Phragmites karka*, *Saccharum spontaneum*, *Sorghum propinquum*, *Thysanolaena maxima*, and *Typha angustifolia*. The chemical compositions of biomass samples were determined by Goering an Van Soest method in 1970 (Table 2.18).

Table 2.18 Chemical composition and source of 10 screened weeds in Thailand (Supaporn *et al.*, 2003).

Type	**Source**	**Composition (%)***			
		Cellulose	**Hemicellulose**	**Lignin**	**Ash**
*Coix aquatic*a	Nakornpatom	33.16	34.21	6.00	7.29
Imperata cylindrical	Nakornpatom	37.21	32.23	8.21	6.27
Panicum maximum	Chonburi	39.39	28.31	10.65	8.18
Pennisetum polystachyon	Pathumthani	38.69	27.46	10.56	8.46
Pennisetum purpureum	Nakornpatom	34.60	28.44	6.84	10.19
Phragmites karka	Samutprakarn	37.83	30.52	11.03	7.53
Saccharum spontaneum	Chonburi	42.23	31.91	8.30	4.95
Sorghum propinquum	Bangkok	33.81	30.80	8.15	8.79
Thysanolaena maxima	Lumpoon	39.81	26.72	14.44	5.47
Typha angustifolia	Nakornpatom	32.03	27.66	10.22	11.08

* relate to dried biomass weight

In pretreatment step, all biomass samples were cut and grinded. Then samples were soaked with 10% (w/v) of 2:1 NaOH solution:sample at 60 °C for 2 h. The change in composition of the pretreated biomass is shown in Table 2.19.

Table 2.19 Comparison of the chemical composition of the untreated and the NaOH-pretreated weeds.(Supaporn *et al.*, 2003)

Type	Cellulose content (%)		% difference	Hemicellulose content (%)		% difference	Lignin content (%)		% difference
	untreated	pretreated		untreated	pretreated		untreated	pretreated	
Coix aquatica	33.16	64.53	94.60	34.21	13.85	59.51	6.00	4.32	28.00
Imperata cylindrical	37.21	67.79	82.18	32.23	15.89	50.70	8.21	8.1	1.34
Panicum maximum	39.39	68.27	73.32	28.31	11.98	57.68	10.65	10.43	2.07
Pennisetum polystachyon	38.69	71.17	83.95	27.46	10.1	63.22	10.56	9.73	7.86
Pennisetum purpureum	34.60	67.71	95.69	28.44	1.84	44.30	6.84	8.09	-18.27
Phragmites karka	37.83	64.48	70.45	30.52	13.13	56.98	11.03	12.39	-12.33
Saccharum spontaneum	42.23	67.33	59.44	31.91	15.24	52.24	8.30	8.29	0.12
Sorghum propinquum	33.81	65.48	93.73	30.80	14.11	54.19	8.15	6.49	20.37
Thysanolaena maxima	39.81	60.48	51.92	26.72	17.38	34.96	14.44	12.43	13.92
Typha angustifolia	32.03	55.29	72.62	27.66	14.09	49.06	10.22	11.82	15.66

After alkaline pretreatment, the pretreated weeds were produced ethanol by simultaneous saccharification and fermentation (SSF) process using cellulase enzyme from *Acrophialophora* sp. UV10-2 and fermented by heat resistant yeast *Kluyveromyces marxianus* NRRL Y-1109 at 40 °C, pH 5.0. *Coix aquatica* gave the highest ethanol production of 4.9 g/l (0.16 g/ g substrate) among screened weeds. This research shows the possibility for weed in Thailand to be biofuel feedstock, however, the pretreatment and bioconversion method are needed to continue studying to get high total reducing sugar and ethanol production yield.