# CHAPTER II
LITERATURE REVIEW

2.1 Biorefineries Overview

There are two major platforms to produce biofuel from biomass: the gasification platform and the carbohydrate platform. Carbohydrate platform would break biomass down into different types of component sugars, starch, or cellulose for fermentation or other biological processing into various fuels and chemicals. While, gasification platform would convert biomass into synthesis gas (hydrogen and carbon monoxide) which is then fermented into ethanol and butanol by microorganisms.
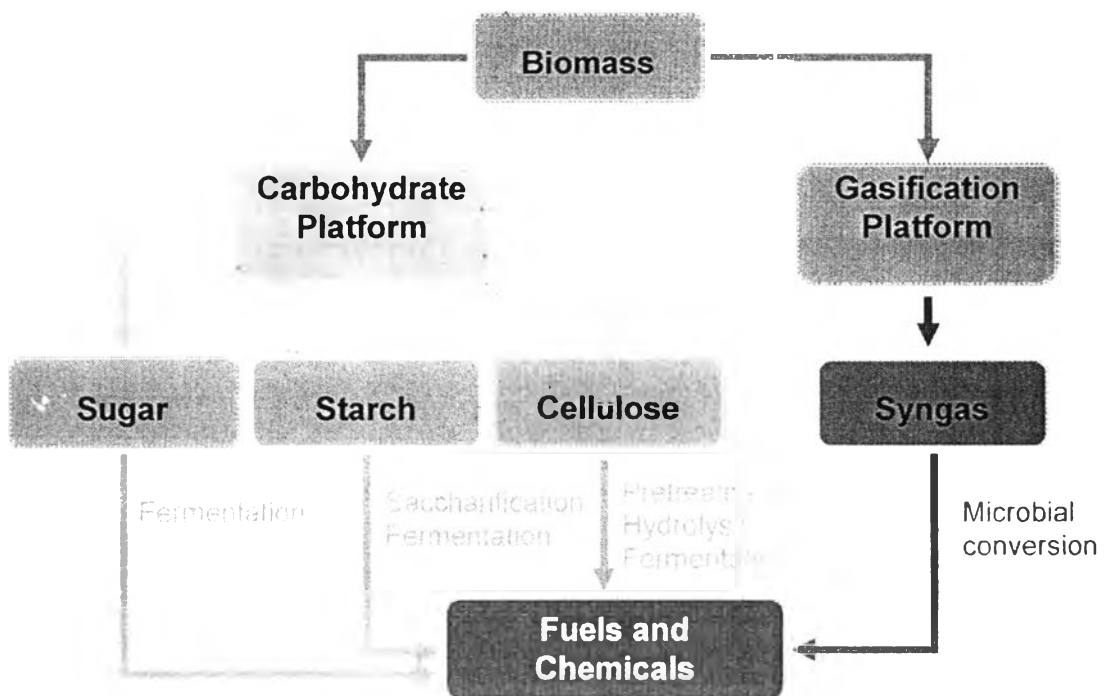

Figure 2.1 Schematic of pathways to produce fuels and chemical from biomass.

2.2 Biobutanol Production

Biobutanol can be produced by acetone-butanol-ethanol fermentation process or ABE fermentation process, as shown in Figure2.2. This process has been

improved by using various strains of the bacterium either *Clostridium acetobutylicum* or *Clostridium Beijerinckii* and different substatrates such as corn and molasses for many years. However, these subtstrates have high cost resulting in high price of butanol. Therefore, to produce butanol by using biomass as a feedstock is another choice to reduce butanol price.
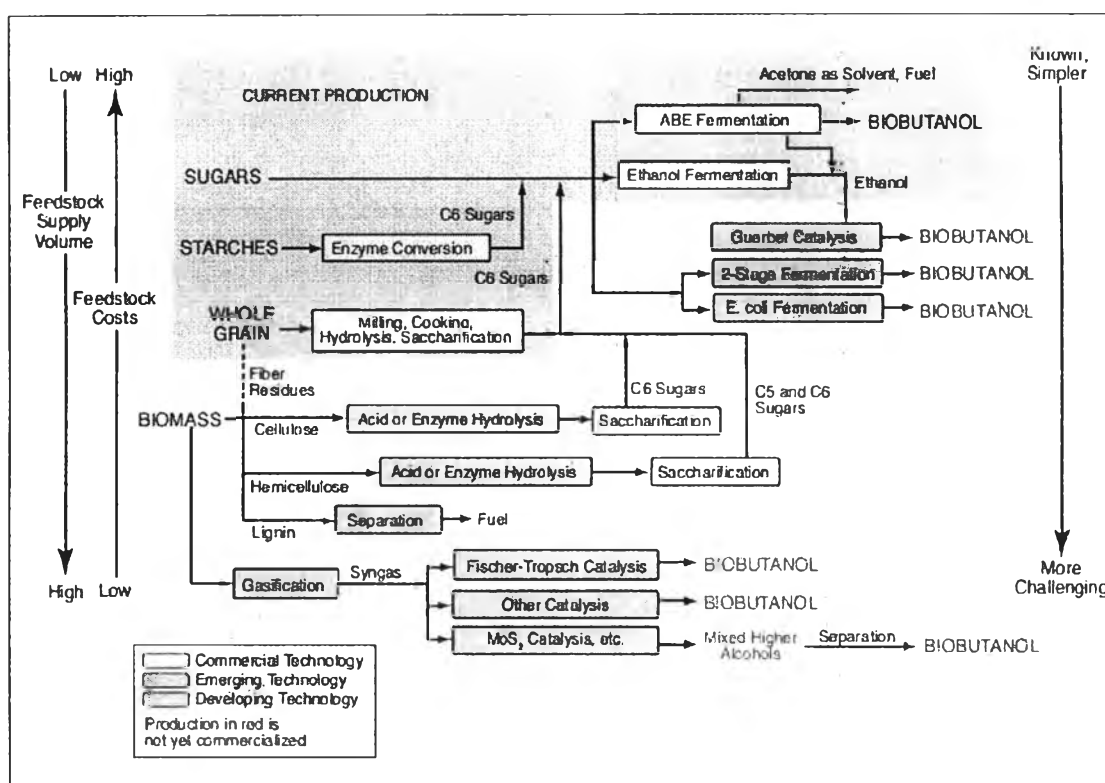
Figure 2.2 Schematic of variety of fermentation process to produce butanol (Cascone, 2008).

2.3 Properties of Butanol

Butanol's properties are more attractive than ethanol with respect to gasoline. Because the fuel properties of butanol are more similar to gasoline than ethanol. There is a research that can present we can use 100 % butanol as a fuel or use it blend with gasoline like ethanol. And it will not dissolve in water so it can be transport in pipeline, tanks, and other equipment.

Table 2.1 Properties of n-butanol, ethanol, and gasoline (Kiplinger, 2007)

FUEL PROPERTIES			
	Butanol	**Ethanol**	**Gasoline**
Energy content Btu/gal.	110,000	84,000	115,000
Motor octane rating	94	92	96
Flash Point* (°F)	95	55	-45
Reid vapor pressure** (100°F)	0.3	2.3	8-15
Water solubility at 70°F	9%	100%	Negligible
Transportation Infrastructure	Existing	Segregated	Existing
Feedstock	Same as ethanol	Sugar or starch crops	Crude oil
Production costs	Not expected to differ from ethanol	About $1.30/gal.	Variable
Availability	Available in commercial quantities post-2010	Available now	Available now

* Fuels with higher flash points are less flammable than fuels with lower flash points.
** Vapor pressure is an indication of a liquid's evaporation rate.
Sources: ButylFuels, LLC, Department of Energy, BP, DuPont, Kiplinger

2.4 Composition of Lignocellulosic Biomass
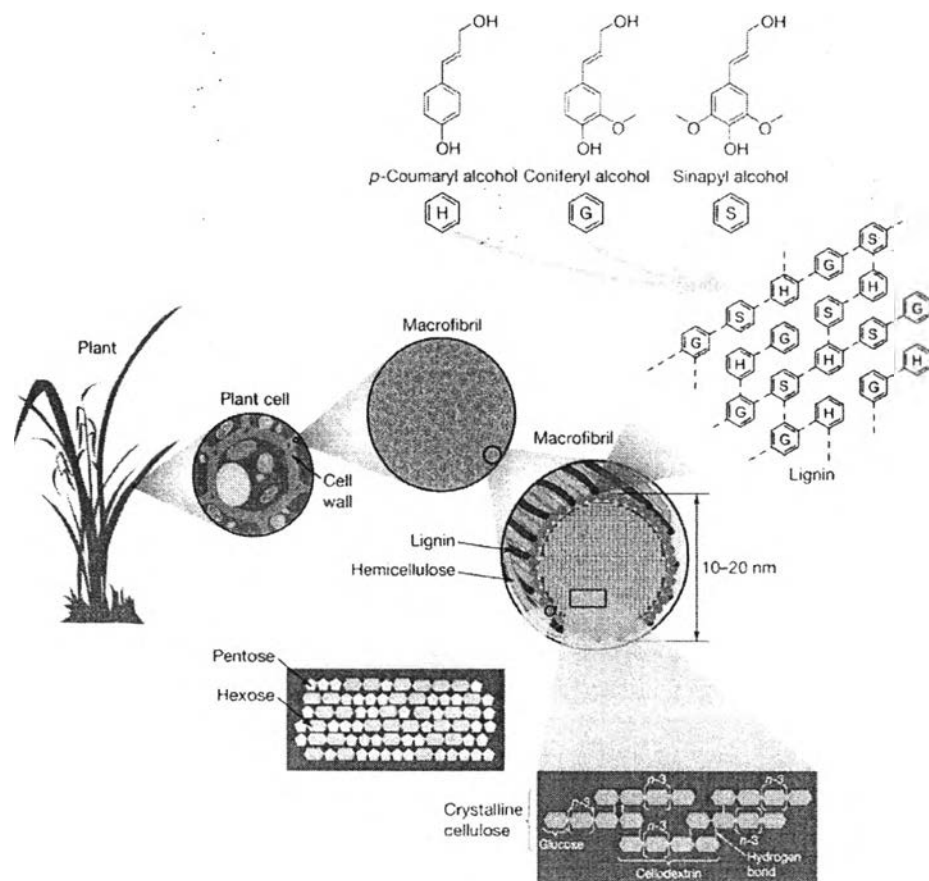
Figure 2.3 Schematic of lignocellulosic biomass (Rubin, 2008).

Lignocellulosic biomass consists of cellulose, hemicellulose, and lignin, which form complex structure result in enzymatic hydrolysis accessibility limited. The content of lignocellulose depends on type of lignocellulosic biomass. Table 2.2 shows the typical compositions of the three components in various lignocellulosic materials.

Table 2.2 Lignocellulose contents of some agricultural wastes (Howard *et al.*, 2003)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Nut shells	25-30	25-30	30-40
Corn cobs	45	35	15
Wheat straw	30	50	15
Rice straw	32.1	24	18
Fresh bagasse	33.4	30	18.9
Switch grass	45	31.4	12
Coastal Bermuda grass	25	35.7	6.4

2.4.1 Cellulose

Cellulose is a homopolymer of β-glucose units that are linear linked via β-1-4 glycosidic bonds. To identify the β-glucopyranose, hydroxyl groups on C1 and C6 positions should be on the same plane. The structure of cellulose is illustrated in Figure 2.4. This linearity causes packing of cellulose chains called elementary and microfibrils (Figure 2.3) attached to each other by hemicelluloses, amorphous polymers of different sugars, and covered by lignin. Therefore, cellulose exists as crystalline fibers which are a major of enzymatic hydrolysis accessibility (Keshwani *et al.*, 2007). Since there are several hydroxyl groups in each monomer unit, their secondary inter-chain hydrogen bonding extensively occurs (Wang *et al.*, 2007). Hydrogen bonds are formed between the manifold hydroxyl groups on the glucose units within a chain or on adjacent chains, retaining the firmly network of hydrogen bonds and generating rigid microfibril structure. Furthermore, cellulose has both crystalline and amorphous zones in its structure according to the microstructure

periodically or randomly distributed along the orientation of cellulose fibrils (Shen and Gu, 2009). Each region has different activity which adds more complexities.
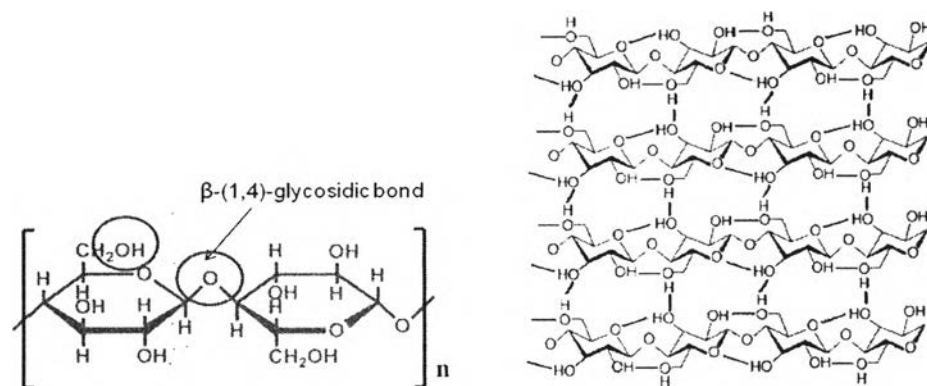
Figure 2.4 Cellulose structure and hydrogen bond network in cellulose molecules.

2.4.2 Hemicellulose

Hemicelluloses are heteropolymers, which consist of five-carbon sugars (xylose and arabinose) and six-carbon sugars (galactose and mannose). In contrast to cellulose, which is the same for all lignocellulosic biomass result in high crystallinity and strong, hemicelluloses have random (Figure 2.5), amorphous, and branched structure with little resistance to hydrolysis, and they are more easily hydrolyzed to their monomer components (Taherzadeh and Karimi, 2008).
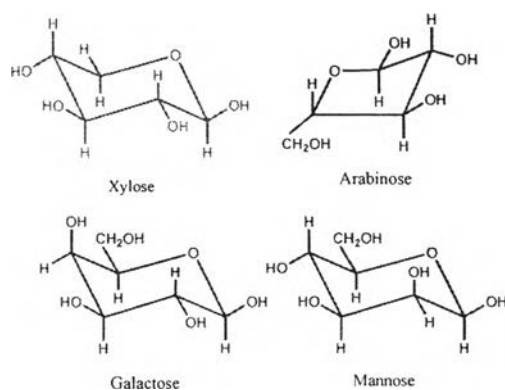
Figure 2.5 Monosaccharide represent in hemicellulose.

2.4.3 Lignin

Lignin is a very complex molecule made up of three types of phenolic acids (p-coumaryl alcohol, coniferyl alcohol and synapyl alcohol) linked in a three dimensional structure which is particularly difficult to biodegrade (Figure 2.6). Lignin is the most recalcitrant component of the plant cell wall, and the higher the proportion of lignin, the higher the resistance to chemical and enzymatic degradation (Taherzadeh and Karimi, 2008).

p-Coumaryl alcohol   Coniferyl alcohol   Sinapyl alcohol
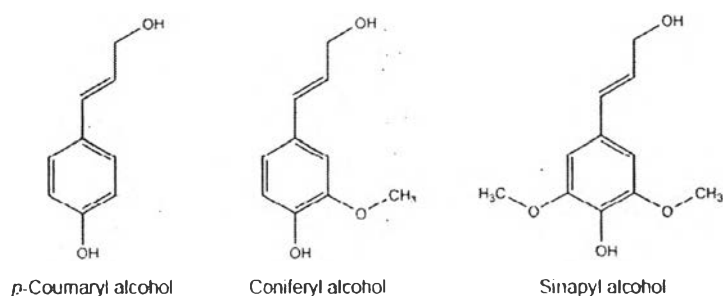
Figure 2.6 Structures of monolignols (Taherzadeh and Karimi, 2008).

2.5 An Emerging Technology: Microwave Technology

The development of recent and emerging technologies has been driven by the need to find alternatives to those traditional and conventional technologies due to their poor energy efficient and their generated environmental pollutions. In the process and manufacturing industries, heating both for drying and promoting chemical/physical changes can be considered as one of the most common employed processes (Appleton *et al.*, 2005). Microwave irradiation is an attractive emerging technique that shows the potential use as an improved energy efficient alternative as compared with current heating technologies. Furthermore, it is also regarded as one of green and sustainable energy with environmental compatibility.

2.6 Fundamentals of Microwave Technology

Microwaves are electromagnetic energy with wavelengths ranging from 1 cm to 1 m and frequencies of 30 GHz to 300 MHz, which consist in the electromagnetic radiation spectrum between infrared radiation and radio frequencies (Strauss, 2002). However, not all frequencies can be used for microwave chemistry. According to international agreement, a frequency of 2.45 GHz or 2450 MHz corresponding to 12.2 cm wavelength (λ) and energy of $1.02 \times 10\text{-}5$ eV is preferred for scientific microwave heating and drying in laboratory reactions to avoid intervention with other designated bands for other applications such as radar and telecommunications.
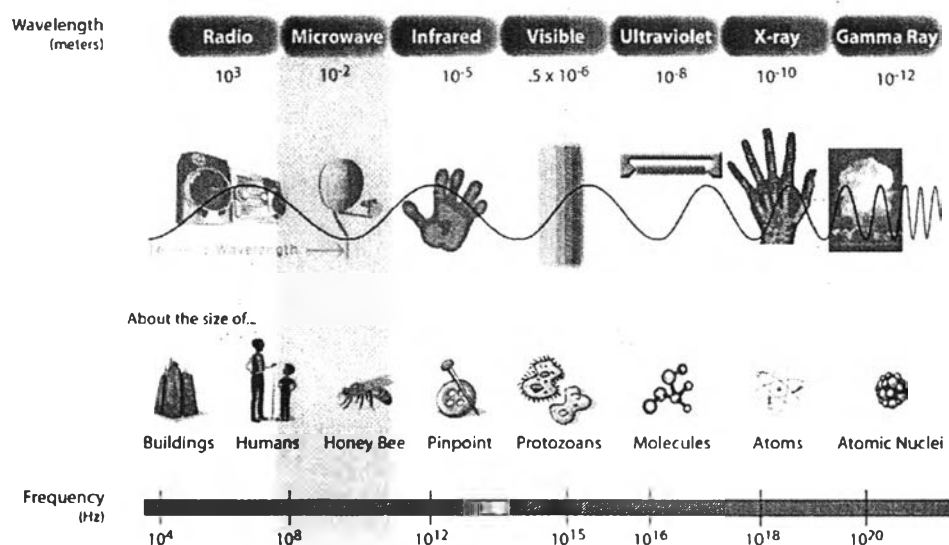
Figure 2.7 The electromagnetic spectrum with their applications at various frequencies (http://mc2.gulf-pixels.com/?p=281).

One of the main limitations of microwave energy for heating is an ability of each material to absorb microwaves. Due to differences in the response of target samples to microwave radiation, materials can be classified into three main categories as follows:

- Reflecting materials that reflect microwaves out of the surface of materials, e.g., metals

- Insulating materials that transmit microwave energy through without storing the energy in the form of heat, e.g., quartz

- Absorbing materials that can couple with microwave energy generating heat, e.g., water
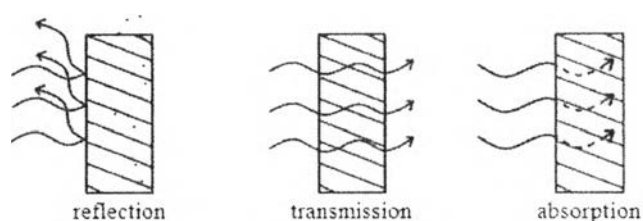
Figure 2.8 Interactions of microwave radiation with materials.

When a material is subjected to microwave energy, there are two basic mechanisms of microwave heating which arise from the displacement of charged particles in the material:

- Polarization or dipole rotation is an alignment of dipole, either induced or permanent, molecules tend to agitate and reorient themselves under the influence of an oscillating electric field. Energy dissipation is induced in the form of heat due to dielectric relaxation time of the sample.

- Ionic conduction generates heat through resistance within the structure of materials to an electric current or oscillation of ions which is generated by the oscillating electromagnetic field.

However, the microwave technology has been shown possibilities to be an energy efficient technique for chemical processing. The advantages and challenges of microwave processing are summarized in Table 2.3.

Table 2.3 Benefits and challenges of microwave processing (Clark and Sutton, 1996)

Benefits	Challenges
Rapid heating	Heating low-loss poorly absorbing materials
Precise and instantaneous controlled heating	Efficient tranfer of microwave energy to workpiece
Selective heating	Controlling accelerated heating
Volumetric and uniform heating	Economics
Short processing times	
Increased product yields	
Reduction of hazardous emission	
Time, energy and cost saving	
Clean power	

2.7 Effective Parameters in Pretreatment of Lignocelluloses
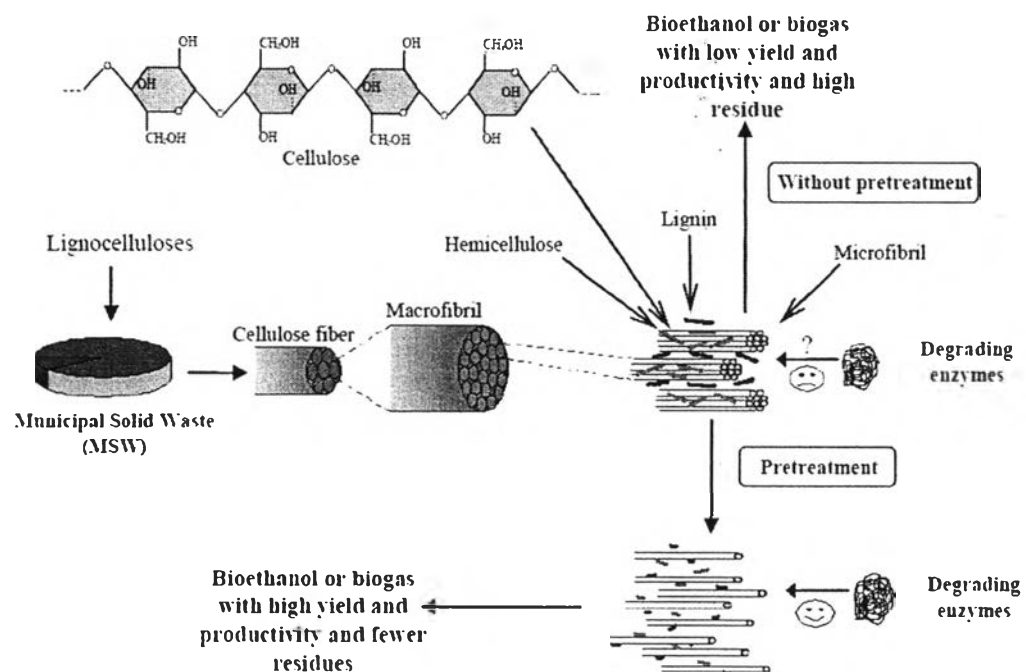
Figure 2.9 Schematic of effect of pretreatment on enzymatic hydrolysis (Taherzadeh and Karimi, 2008).

2.7.1 Cellulose Crystallinity

The crystallinity is given by the relative amounts of crystalline (around 2/3 of total cellulose) and amorphous form. Since amorphous region is more accessible by enzymatic hydrolysis compared with crystalline region. As a result, high cellulose crystallinity will be hard to hydrolysis by enzyme, while low cellulose crystallinity will be easy to hydrolysis by enzyme (Taherzadeh and Karimi, 2008).

2.7.2 Substrates Available Surface Area

The main objective of pretreatment is to remove lignin and hemicellulose to increase the available surface area for improvement in enzymatic hydrolysis (Alvira *et al.*, 2009).

2.7.3 Lignin Barrier

Lignin acts as a physical barrier to prevent the digestible parts of substrate (Figure 2.9), which result in limiting the enzyme accessibility (Alvira *et al.*, 2009).

2.7.4 Hemicellulose Content

Hemicellulose is a physical barrier like lignin which surrounds the cellulose fiber and can protect cellulose from enzymatic attack. Thus, removal of hemicellulose and lignin will increase the enzymatic accessibility (Taherzadeh and Karimi, 2008).

2.8 Pretreatment of Lignocellulosic Biomass

Owing to complex structure of lignocellulose, pretreatment is a necessary process to increase enzymatic hydrolysis accessibility by removing hemicellulose and/or lignin (Figure 2.10). However, an effective and economical pretreatment should meet the following requirements: (a) production of reactive cellulose fiber for enzymatic attack, (b) avoiding the degradation or loss of carbohydrate, (c) avoiding formation possible inhibitors for hydrolytic enzymes and fermenting microoraganisms, (d) producing less residues, (e) minimizing energy demand, (f)

consumption of little chemical and using cheap chemical (Sun and Cheng, 2002; Taherzadeh and Karimi, 2008).
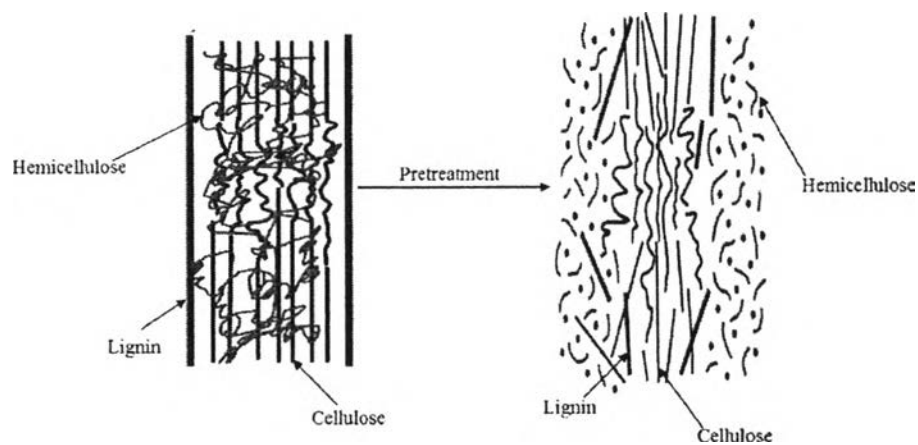
Figure 2.10 Schematic of the role of pretreatment (Kumar *et al.*, 2009).

There are several pretreatment methods, which can be classified into biological, physical, chemical, and physico-chemical pretreatment. Because each pretreatment has its native advantages and disadvantages (Table 2.4). Therefore, none of pretreatment method can be expressed as a best method.

Table 2.4 Summary of advantages and disadvantages with different methods for lignocellulosic biomass pretreatment (Alvira *et al.*, 2009; Zheng *et al.*, 2009)

Pretreatment method	Advantages	Disadvantages
Biological	- Degrades lignin and hemicellulose - Low energy consumption	- Low rate of hydrolysis
Milling	- Reduces cellulose crystalinity	- High power and energy consumption
Steam explosion	- Lignin transformation - Hemicellulose solubilization - Cost-effective - Higher yield of glucose and hemicellulose in the two-step method	- Partial hemicellulose degradation
AFEX	- Increases accessible surface area - Low formation of inhibitors	- Not efficient for raw materials with high lignin content - High cost of large amount of ammonia
CO_2 explosion	- Increases accessible surface area - Cost-effective - Do not imply generation of toxic compounds	- Does not affct lignin and hemicelluloses - Very high pressure requirements
Wet oxidation	- Efficient removal of lignin - Low formation of inhibitors - Minimizes the energy demand	- High cost of oxygen and alkaline catalyst

Table 2.4 (Cont.) Summary of advantages and disadvantages with different methods for lignocellulosic biomass pretreatment

Pretreatment method	Advantages	Disadvantages
Ozonolysis	- Reduces lignin content - Dost not imply generation of toxic compound	- High cost of oxygen
Organosolv	- Causes lignin and hemicellulose hydrolysis	- High cost - Solvents need to be drained and recycled
Concentrated acid	- High glucose yield - Ambient temperature	- High cost of acidand need to be recovered - Formation of inhibitors
Diluted acid	- Less corrosion problems than concentrated acid - Less formation of inhibitors	- Generation of degradation products - Low sugar concentration in exit stream
Alkali	- Ambient temperature - Low pressure	- Long pretreatment time - Formation of salts

Although there are several pretreatment methods, chemical and physico-chemical are currently the most effective method. Combination of different methods might be interesting and gain very high yields (Alvira *et al.*, 2009). Table 2.5 shows the effect of different pretreatment technologies. However, these effects do not depend only on the method but also depend on type of lignocellulosic biomass and operating conditions.

Table 2.5 Effects of different pretreatments on physical/chemical composition or structure of lignocellulose (Hendriks and Zeeman, 2009)

Pretreatment method	Mechanical	Acid	Alkali	Thermal + acid	Thermal + alkali (lime)	AFEX
Increase accessible surface area	+	+	+	+	+	+
Decrystallization cellulose	+			N/A	N/A	+
Solubilization hemicellulose	+	-		+	-	+
Solubilization lignin	-	+/-	+/-	+/-		+
Formation furfural	+	-		+	-	-
Alteration lignin structure	+	+	+	+	+

+ = major effect, - = minor effect and N/A = not avialable.

This project is focuses on a combined of microwave and alkali pretreatment method. Sodium, potassium, calcium, and ammonium hydroxides are suitable alkali, which is more effective for lignin solubilization and minor hemicellulose solubilization than acid. Chen et al. (2009) used four different chemicals (dilute sulfuric acid, calcium hydroxide, aqueous ammonia acid, and sodium hydroxide) to pretreat corn stover. The authors found that sodium hydroxide (2 % sodium hydroxide solution at 120 °C for 30 min) was the best chemical to remove 73.9 % of lignin and sodium hydroxide-pretreated sample gave the hydrolysis yield reach 81.2 % by 48 h. Alkali treated rice straw was studied by Zhang and Cai (2008), they showed the SEM micrographs before and after pretreatment (Figure 2.11) and they

found that after pretreatment, the basic tissue in transverse section shrank and the fiber of pretreated samples were distorted in longitudinal section.
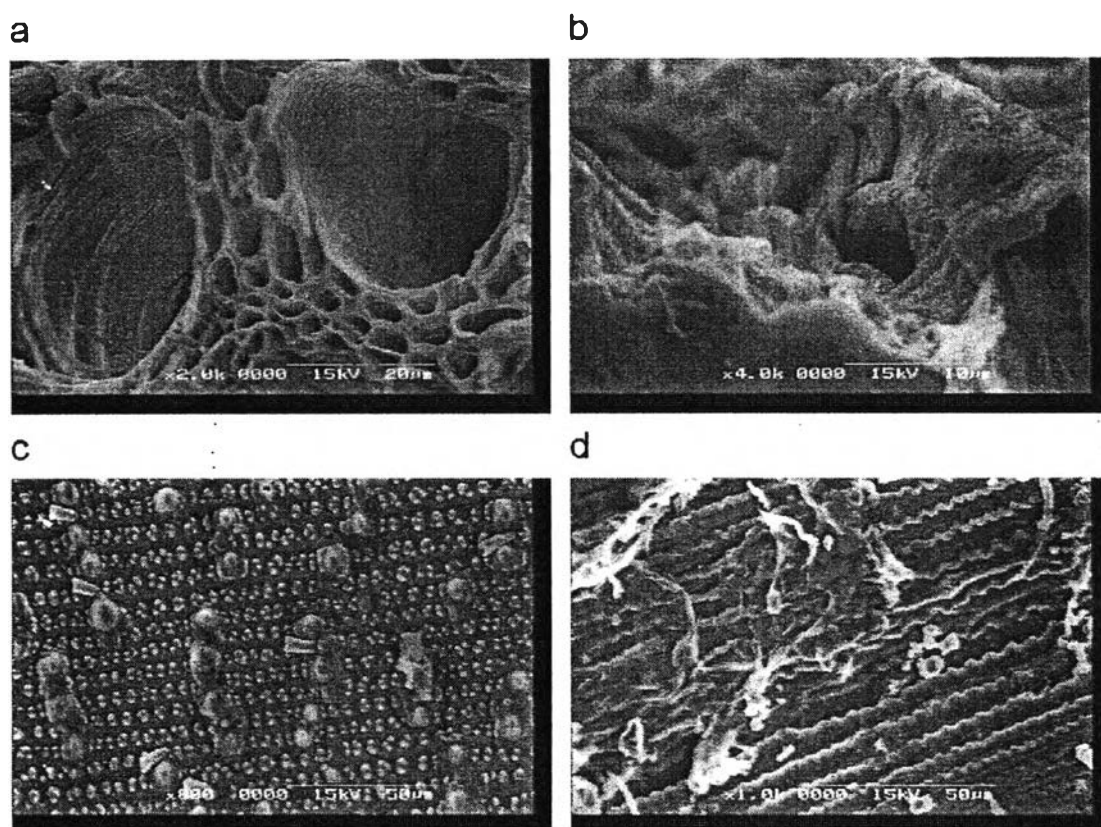
Figure 2.11 SEM micrographs of rice straw before and after pretreatment in the following forms: (a) Transverse section of rice straw before pretreatment. (b) Transverse section of rice straw after pretreatment. (c) Longitudinal section of rice straw before pretreatment. (d) Longitudinal section of rice straw after pretreatment (Zhang and Cai, 2008).

Moreover, Alvira *et al.* (2009) reported that sodium hydroxide causes swelling, increasing the internal surface of cellulose, and decreasing the degree of polymerization and crystallinity, which provokes lignin structure disruption.

Chang *et al.* (1998) studied on the effect of lime pretreatment conditions on crops residues and found that short pretreatment times (1–3 h) with high temperatures (85–135 °C) were required to reach high sugar yields while long treatment times (e.g. 24 h) with lower temperatures (50–65 °C) were more effective.

However, the useful component in lignocellulosic biomass might be decomposed at high temperature (Zhu *et al.*, 2005). Thus, this project is interested in microwave radiation, which might help alkali pretreatment to operate at lower temperature and shorter time. The effect of microwave irradiation on rice straw was reported by Zhu *et al.* (2006). They indicated that rice straw treated by microwave alone (700 W) had almost the same reducing sugar yield as no pretreatment. In addition, the rice straw treated by microwave after alkali pretreatment (1 % sodium hydroxide solution for 1 h) almost had the same hydrolysis rate and yield as conventional alkali pretreatment while combination of microwave and alkali pretreatment had much higher initial hydrolysis rate, but the hydrolysis yield almost the same. Zhu *et al.* (2005) indicated different irradiation power result in the same final weight loss and composition.

2.9 Enzymatic Hydrolysis

The objective of this process is breaking the polymer of cellulose and hemicellulose by using enzyme. Glucose and xylose usually are the main products of enzymatic hydrolysis because cellulose contains glucans, while hemicellulose contains several sugars such as xylan, glucan, and arabinan. Cellulases can be produced by both becteria (such as *Clostridium cellulovorans*) and fungi (such as *Trichoderma reesei* and *A. niger*). Cellulases contain three major of enzymes, which are endo-glucanase, exo-glucanase and β-glucosidase (Figure 2.12). Endo-glucanase or 1,4-D-glucanohydrolas attacks regions of low crystallinity in the cellulose fiber and creats free chain-ends. Exo-glucanase or cellobiohydrolase degrades the molecule further by removing cellobiose units from the free chain-ends. β-glucosidase hydrolyzes cellobiose to produce glucose. Moreover, other enzymes such as glucuronidase, xylan, β-xylosidase and glucimannanase can break the polymer of hemicellulose (Sun and Cheng, 2002). Talebnia *et al.* (2009) reported that an optimum activity of the most cellulose enzymes is at temperatures and pH in the range of 45–55 °C and 4–5, respectively. In addition, cellulase dosage of 10–30 (FPU/g cellulose) is often used in laboratory studies because it results in an efficient hydrolysis with high glucose yield in a reasonable time (48–72 h) and enzyme cost.
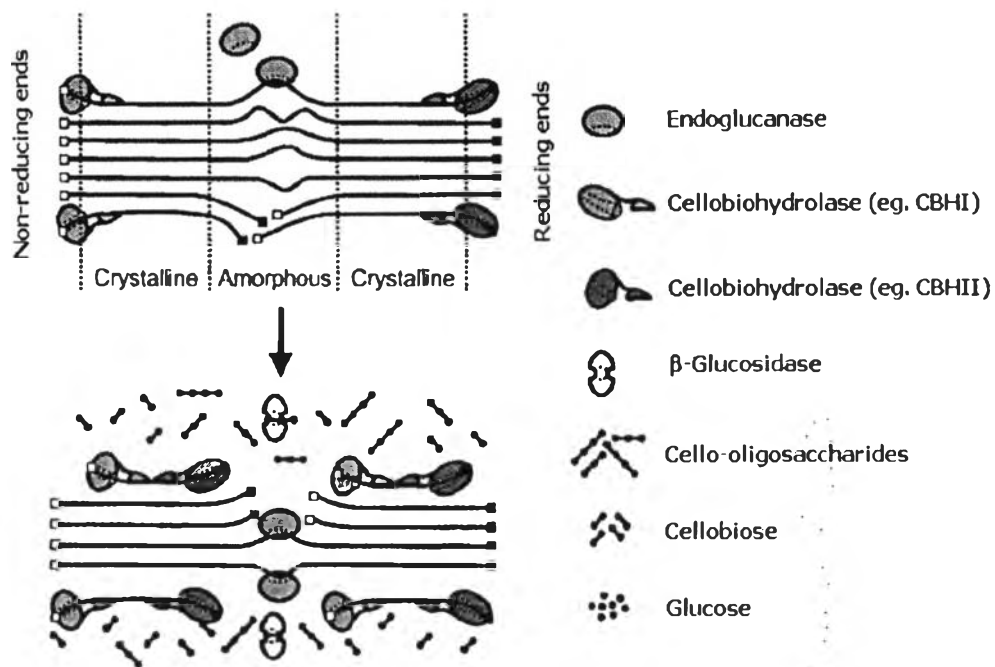
Figure 2.12 Mode of action of cellulase enzymes in the hydrolysis of cellulose (Lynd *et al.*, 2002).

2.10 Improving Enzymatic Hydrolysis

In addition to temperature and pH, which affect enzymatic hydrolysis, other factors including substrates, cellulose and end-product inhibition of cellulose activity are the factors to improve yield and rate of enzymatic hydrolysis.

2.10.1 Substrates

Chen *et al.* (2009) indicates that high substrates concentration (8 % substrate) usually result in lower hydrolysis yield due to end product inhibition, while lower concentration (3 % substrate) gives higher hydrolysis yield. In addition to substrates concentration, the structure features of substrate including cellulose crystallinity, degree of cellulose polymerization, surface area, and content of lignin are also sensibility to cellulase.

2.10.2 Cellulase

Sun and Cheng (2002) reported that increasing of celluloses can increase the yield and rate of hydrolysis, but would increase the cost of the process. However, cellulase enzyme loading in hydrolysis depends on the type and concentration of substrates.

The enzymatic hydrolysis consists of three steps (Taherzadeh and Karimi, 2008): (1) adsorption of cellulase enzymes from liquid phase onto the surface of cellulose, (2) biodegradation of cellulose to simple sugars, mainly cellobiose and oli-gomers, and (3) desorption of cellulase to the liquid phase. Talebnia *et al.* (2009) reported that cellulase activity decreases during the hydrolysis and it is believed that the irreversible adsorption of enzyme on cellulose is partially responsible for this deactivation.

Cellulase from *T. reesei* is widely used but it is poor in cellubiase and limits the conversion of cellubiose to glucose. Thus, use of cellulase enzyme mixed with other enzymes has been suggested to reach higher enzymatic hydrolysis rate, because it is well known that conjugated action of cellulases and hemicellulase results in a higher ultimate sugar production (Talebnia *et al.*, 2009). Using the mixture of cellulose from *T. reesei* ZU-02 and cellobiase from *A. niger* ZU-07 to hydrolyze corn cobs was studied by Chen *et al.* (2009). First, the authors studied on the cellulase dosage from *T. reesei* ZU-02 and found that the optimum cellulose dosage is 20 FPU g^{-1} substrate at pH 4.8 and 50 °C. Second, they studied on the effect of hydrolysis time and found that hydrolysis yield and reducing sugar concentration for 48 h were 67.5 % and 49.4 g l^{-1}, respectively. And after 48 h hydrolysis yield was little increase. Last, they studied on the effect of the mixture of cellulase and cellubiase (20 FPU g^{-1} substrate; 6.5 CBU g^{-1} substrate) and found the cellobiose concentration was maintain at low level during the whole hydrolysis process since cellubiose was hydrolyzed to glucose which resulted in higher reducing sugar and hydrolysis yield. At 48 h, the hydrolysis yield and reducing sugar concentration were 83.9 % and 61.4 g l^{-1}, respectively.

2.10.3 End-product Inhibition of Cellulose Activity

Sun and Cheng (2002) reported that cellulase activity is inhibited by cellobiose and results in lesser glucose production. Several methods have been developed to reduce the inhibition such as the use of high concentrations of enzymes, the supplementation of β-glucosidases during hydrolysis, and the removal of sugars during hydrolysis by ultrafiltration or simultaneous saccharification and fermentation (SSF).

2.10 Literature Review

Lin *et al.* (2010) studied on the effect of cellulose, hemicellulose and lignin on the yield of reducing sugar and found out the yield of reducing sugar decreases as lignin content increases. Therefore, it can be conclude that lignin has a major effect on yield of reducing sugar while hemicellulose is considered to have a minor effect. In addition, Sun *et al.* (1995) indicated that sodium hydroxide is the most effective in removing lignin and hemicellulose. And the optimal condition is 1.5% NaOH for 144 h at 20 °C. As mention before, short pretreatment times (1–3 h) with high temperatures (85–135 °C) were required to reach high sugar yields while long treatment times (e.g. 24 h) with lower temperatures (50–65 °C) were more effective. Therefore, a combined of microwave and alkali pretreatment might be an alternative for operate in shorter pretreatment time and lower temperature. McIntosh and Vancov (2010) indicated that treated sorghum straw with 2% NaOH for 60 minutes at 121 °C followed by enzyme saccharification yielded gave the highest total sugar release of 798.8 mg/g pretreated material. Thus, the optimum condition of combined of microwave and alkali should not exceed 121°C and 60 minutes. This is reason why this work has study on the effect of the conditions of a combined pretreatment of corn cobs using microwave and sodium hydroxide (0.75 % to 3 % (w/v)) in the temperature range of 60 to 120°C for 5 minutes to 30 minutes. After removing of lignin and hemicellulose, hydrolysis is an essential step to convert cellulose and hemicellulose into five and six carbon sugars by either dilute acid or enzymatic hydrolysis. However, because of the better efficiency obtained from enzymatic hydrolysis, it has become more attractive method used for hydrolysis of cellulose and

hemicellulose in the present. Therefore, the residues from the pretreatment part were subjected to enzymatic hydrolysis to product sugar at pH 4.8 and 50 °C which is the appropriate conditions for cellulase enzyme. And the optimum conditions of a combined of microwave and sodium hydroxide pretreatment on corn cobs can be indicated by measuring the highest amount of sugar released from enzymatic hydrolysis of corn cobs by HPLC instrument.