CHAPTER 2 LITERATURE REVIEWS

2.1 Ethanol

Ethanol is currently being important product that will be changed recruitment fossil fuel in the fuel market. Worldwide requirement of ethanol has been increasing since 2006, 39 billion liters and producted to reach 100 billion liters in 2015 (Licht, 2006). At least 4% production of ethanol is produced synthetically from oil and other is produced by fermentation from bioresources. At present, ethanol is still produced from two major groups of bioresources such as sugar substances and starchy materials. There is competition between these two feedstocks for fuel ethanol production. Sugar substances were being the feedstock more than 60% of production ethanol in 2000s and decreased to 47% by 2006, when grains accounted for 53% of the production (Licht, 2006). Production of sugar substances and grain are limited in the world. They are relatively expensive feedstock for ethanol production, and ethanol competes with human food for these raw materials. This competition between human food an fuel influence to the price of grains and sugar in the future.

Utilization of sugar substances and grain can be replaced by lignocellulosic material which is renewable, largely unused, and abundantly available sources of raw materials (biomass) for the production of the fuel ethanol. Lignocellulosic materials have a price which can be obtained from a variety of resources, e.g. forest residues, municipal solid waste, waste paper and crop residue resources (Wyman, 1996). This biomass were composed polymerized sugars in the form of cellulose and hemicellulose, which can be liberated by hydrolysis and subsequently fermented to ethanol by microorganisms (Millati et al., 2002 and Palmqvist et al., 2000).

2.2 Biomass

Biomass defined as the total mass of living matter within a given unit of environmental area, but is now used also to describe plant derived materials, a full range of plant and vegetation. Biomass are also known as a renewable fuel resources which cover almost any biologically degradable fuel from farmyard manure through industrial liquid effluents and solid waste, agro-industrial and forestry waste. Generally, biomass of the plant consisted of cellulose, hemicellulose, and lignin. Nowadays, biomass of plant are the main resources which are renewable and sustainable available resources in the production of ethanol and other chemicals.

2.2.1 Biomass potential in Thailand

Thailand is one of the agricultural base country in Asia, there are a lot of agricultural crops, such as paddy rice, sugarcane, cassava and palm oil. During the harvesting and processing of these agricultural crops, some residues including left over, such as rice straw and rice husk from paddy rice, bagasse and sugarcane leave from sugarcane, cassava rhizome from cassava as well as palm oil shell, palm oil fiber and palm oil empty fruit bunch from the palm oil fruit are the waste from palm oil. These residues can further be used as the substitute for fossil fuel for energy production and consequently, can solve the problem of high energy price as well as global warming. Oil Palm Empty Fruit Bunch (OPEFB) rank sixth agricultural of all biomass in Thailand

despite the energetic quality is high due to its high heating value but it has not yet been prioritized to use as alternative fuel due to its high moisture and volatile matter with low ash melting temperature, therefore the scope of this study focuses on renewable energy utilization from OPEFB (Kerdsuwan et al., 2011).

2.2.2 Oil palm plantation

There are two families of the oil palm tree, *Elaeis guineensis* which is native oil palm tree in western Africa and *Elaeis oleifera* whose origin is in tropical Central America and South America. The palm family widely cultivated in Thailand is Elaeis guineensis. It was first introduced to Thailand in 1968 (Prasertsan et al., 2006). Nowadays, the plantation of the palm oil in Thailand is continuously increased because of the Thai government announced the policy of producing palm oil based on biodiesel as renewable energy. The Office of Agricultural Economics (OAE, 2010) reported the oil palm plantation area in 2009 was accounted 3,165,000 Rai (1 Rai = 1,600 m^2) which they increased by 56 % from the last five years ago and the target of 10 million Rai should be achieved in 2029 (Yangdee, 2011). The oil palm production is increased by 64 % from 5,003,000 tons in 2005 to 8,223,000 tons in 2009. More than 90 % of palm oil plantation area in Thailand are located in Southern part of Thailand, especially in Chumporn, Surat Thani and Krabi (Kerdsuwan et al., 2011).

2.2.3. Oil palm empty fruit bunch (OPEFB)

Oil Palm Empty Fruit Bunch (OPEFB) is waste residue generated from palm oil industries. After harvesting fresh fruit bunches from oil palm tree, these bunches are sterilized in a horizontal steam sterilizer to inactivate enzymes which present in pericarp and loosen fruits from the bunches. The sterilized bunches are fed into a rotary drum thresher in order to remove the sterilized fruit from the bunches. These bunches without fruit are called as empty fruit bunch (EFB) which are conveyed to the damping ground, whereas the sterilized fruits are further used as feedstock for palm oil production in palm oil extraction process by the screw type press. The effluents from the screw type press are nuts and fibers which are separated from each other by the cyclone. After this separation, nuts are cracked into shells and kernels. The former are solid waste and left unused, the latter is sent to the kernel oil mill (Mahlia et al., 2001 and Prasertsan et al., 2006). Apparently, 100 tons of fresh fruit bunch creates 20-22 tons of empty fruit bunch, 14 ton oil-rich fiber and 5 tons of shell which they become solid waste from the oil extraction process (Perez, 1997; and Katamanee, 2006).

Figure 2.1 Palm oil plantation.
Source : http://gadflyketch.wordpress.com/2011/08/05/a-problem-with-palm-oil/

Figure 2.2 Oil palm empty fruit bunch.
Source : http://www.alibaba.com/product-free/112800708/EFB_empty_fruit_bunch_palm_oil/showimage.html

Figure 2.3 Solid waste palm oil (oil palm empty fruit bunch).
Source : http://www.etawau.com/OilPalm/EFB.htm

2.2.4 Composition of oil palm empty fruit bunch

Oil palm empty fruit bunch consists of 45-50% cellulose and hemicellulose and lignin equal amounts (25-35%) (Deraman, 1993). Due to oil palm empty fruit bunch is available in large quantities and has fairly high cellulose contents, so, empty fruit bunch fiber is considered to be a potential available substrate for cellulase production and glucose production (Deraman, 1993).

Table 2.1 Chemical composition of oil palm empty fruit bunch

Reference	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Hill and Abdul Khalik, (2000)	22	48	25
Rozman et al., (2005)	17.1	47.9	24.9
Sabutek, (2008)	68.3	41.9	13.2
Abdul Khalik et al.,(2006)	83.5	49.8	20.5
Sreekala and Thomas, (1997)	-	65	19

Source : Hasan et al., (2010).

2.3 Cellulose

Cellulose is most abundant a polymer compound in plants which its structure is associated with lignin. Dominant cellulose is the main component in woody materials, such as straw, weeds, leaves, stems and twigs of plants (Sutedja et al., 1991; Javis, 2003; and Zhang et al., 2004). According to Schlegel (1992), cellulose chains are built from ß-D-glucose units consisting of 14,000 glucose monomers. Cellulose is not soluble in water, mostly in the form of natural cellulose, which consists of carbohydrate binding 100-14000 glucose, or microfibril-shaped beam, parallel and lead molecules bound by hydrogen bonds (Beguin et al., 1992). Hydrogen bonding between cellulose molecules reinforces the structure microfibrile (Haygreen et al., 1989). According to Schwarz (2001), cellulose is difficultly degraded because of the hard crystalline structure and

insoluble property of cellulose in water. In addition, cellulose in nature is rarely found in pure form but it links to with lignin and hemicellulose.

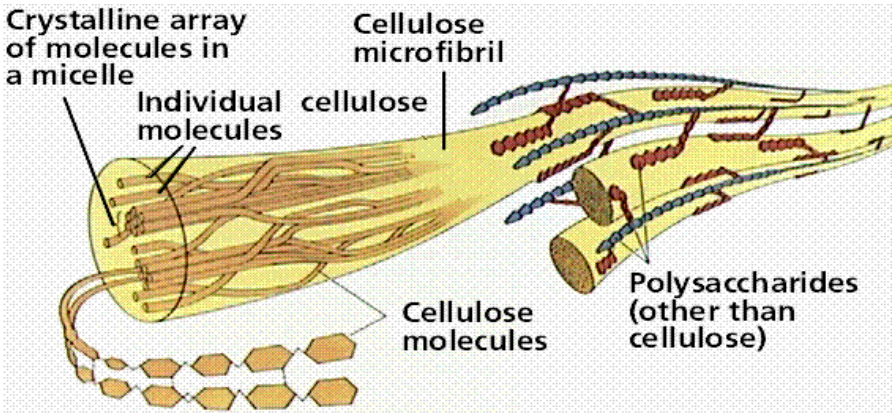

Figure 2.4 Cellulose in plant structure.
Source : Bailey, 1986.

2.3.1 Structural unit of cellulose

Glycosidic linkage in cellulose occurs between the first and second carbons of successive glucose units, the glucose subunits are bonded differently in amylase (α-1,4-glycosidic linkage). Various microorganisms, plants and animals have successive enzymes to break down (hydrolyze) the α-1,4-glycosidic bonds which are found in starch and glycogen, few living creatures can hydrolyze the β-14-bonds of cellulose. One of the common products of enzymatic cellulose hydrolysis is cellobiose, a dimmer of two glucose units is joined by the β-1,4-glycosidic linkage.

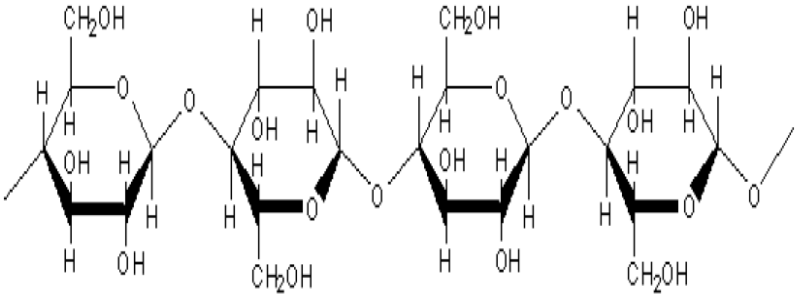

Figure 2.5 Molecule of cellulose.
Source : Bailey, 1986.

Figure 2.5 shows the molecule of cellulose in plant cell. Cellulose is a polymer, or more specifically a polysaccharide, which is made of more than 3,000 glucose units.

A cellulose polymer is simply a larger molecule consisting of many smaller, subunit repeated subunits; in this case, subunit contains chain of glucose.

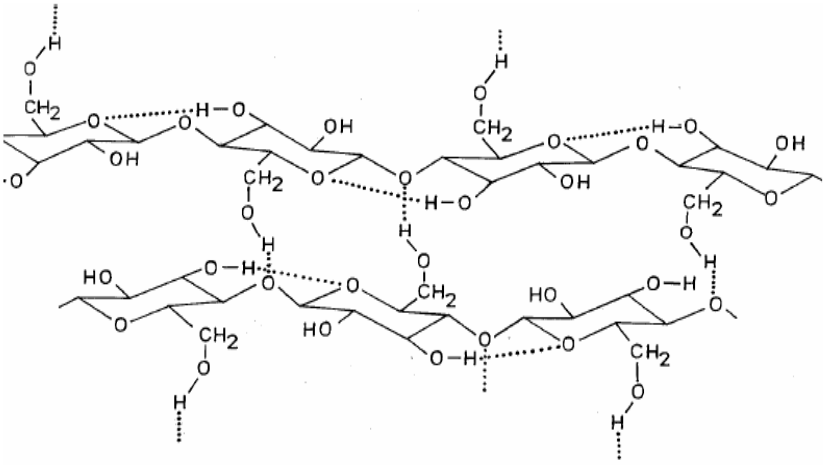

Figure 2.6 Schematic view the hydrogen bonding between glucose residues.
Source : Bailey, 1986.

Figure 2.6 shows the schematic view the hydrogen bonding between glucose residues. ß-1,4-Connected glucose residues are trapped in a plane of the crystal by hydrogen bonds. Each glucose residue is turned by 180° towards with its neighbors, making cellobiose the smallest subunit. This picture explains that why cellulases which active in crystalline cellulose usually releases cellobiose (not glucose).

2.3.2 Functionality

Cellulose is often used as an antique agent, emulsifier, stabilizer, dispersing agent, thickener, and gelling agent but these are generally subsidiary to its most important uses of holding on to water. Another important derivative of cellulose is carboxymethylcellulose (Chaplin, 2004).

2.3.3 Cellulase enzyme

Cellulases (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4) are multienzyme complexes, which containing three main components including endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.9.1) and β-glucosidase (EC3.2.1.21), which have been shown to act synergistically in the hydrolysis of cellulose (Emert et al., 1974; and Ryu et al., 1980). Cellulases are being studied increasingly due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which as raw materials in the microbial production for a wide variety of chemicals, food and fuel (Ekperigin, 2007).

2.3.4 The mechanism of cellulase enzyme in producing glucose

Three general types of cellulases based on the type of reaction catalyzed (Cao et al., 2000):

- β-1-4-endoglucanase, which attack low crystalline region in the cellulose fiber creating free chain ends
- β-1-4-exoglucanase or cellobiohydolase, which degrades the molecule futher by removing cellobiose unit from the free chain ends, resulting in the tetrasaccharides or disaccharide such as cellobiose
- β-glucosidase or cellobiose, which hydrolyzes cellobiose to produce glucose

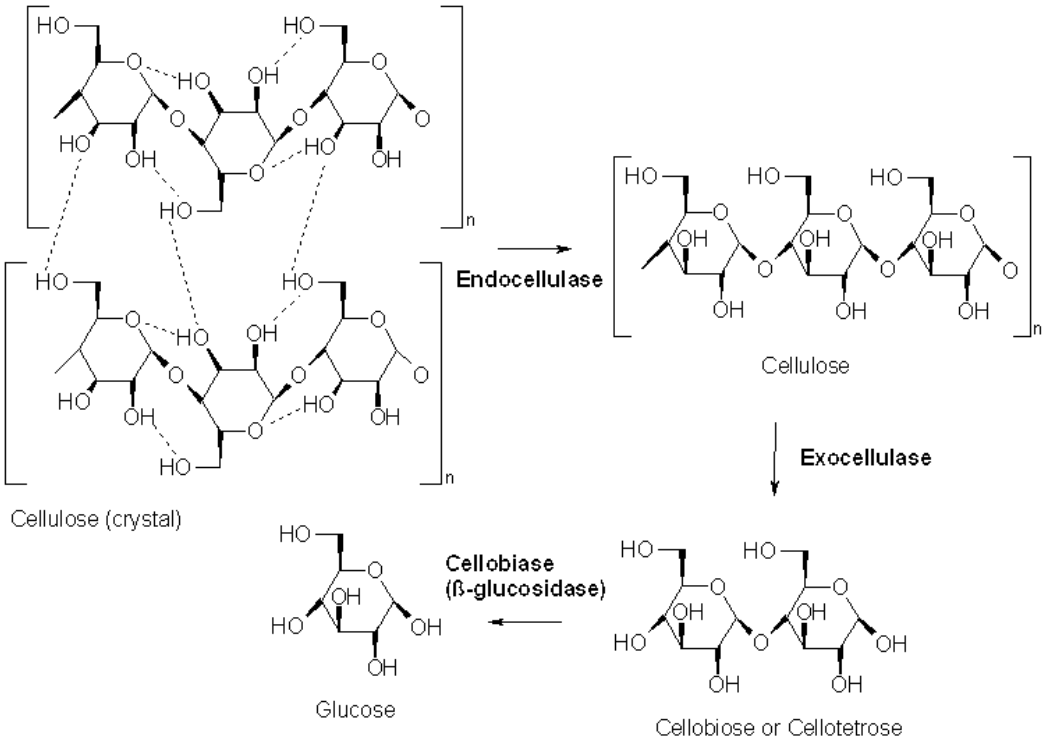

Figure 2.7 The mechanisms of cellulase in producing cellulose.

2.4 Pretreatment process

Lignocellulosic biomass contains cellulose, hemicellulose, lignin, and ash making strong structure. Pretreatment process is required to digest the structure of cellulosic biomass into soft structure which the enzymes can convert the carbohydrate polymer into fermentable sugars. In addition, pretreatment may increase the surface area of the cellulose thereby enhancing its reactivity with the enzyme. Pretreatment refers to solubilization and separation of one or more of the major components of biomass – hemicellulose, cellulose, lignin and extractives. Hydrolysis can break down the hydrogen bond the hemicellulose and cellulose or sugar components such as pentoses and hexoses (Eggeman et al., 2005).

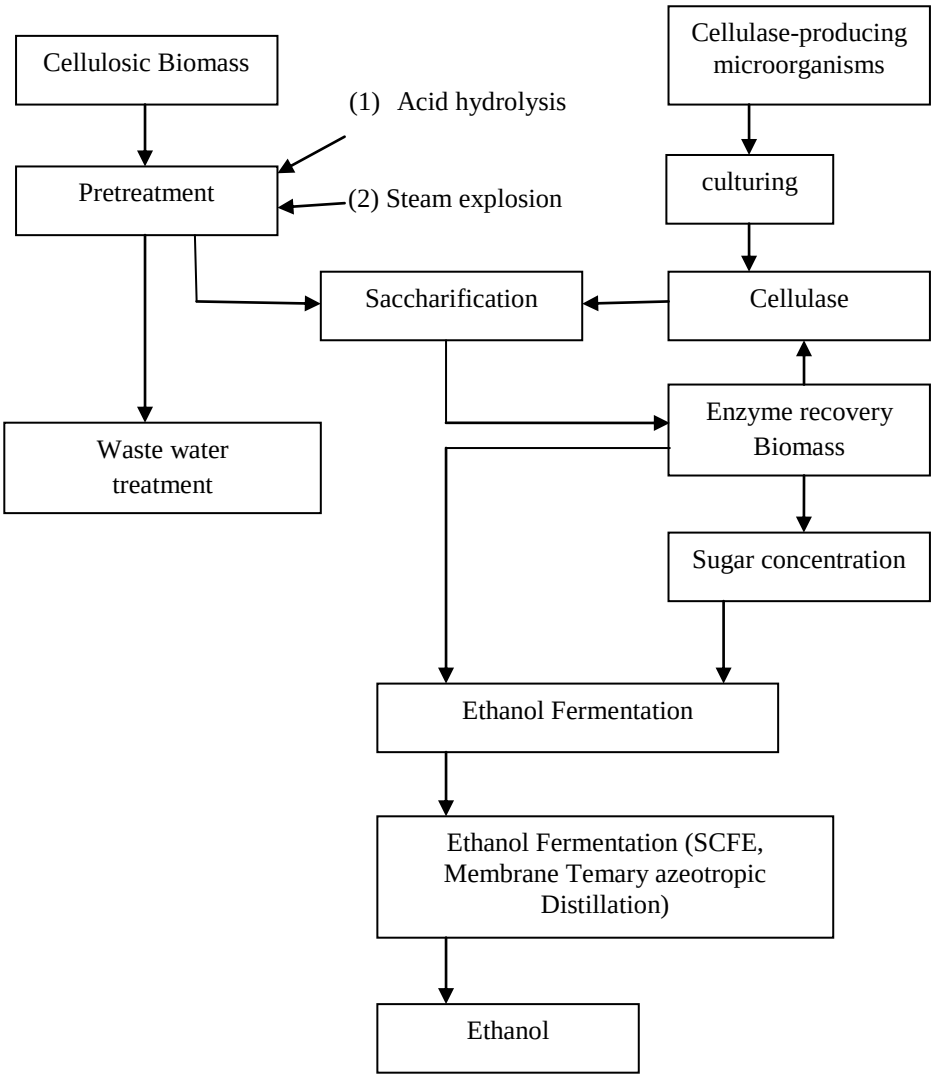

Figure 2.8 Flow diagram outlining processes in an integrated bench plant.

Figure 2.8 shows the processes in ethanol production. Cellulase production is the most important step in the economical production of ethanol. In order to enhance cellulase production, the substrate should be performed with pretreatment and cellulose surface area in also increased. The goal of a pretreatment is to alter or remove structural and compositional impediments to hydrolysis in order to improve the rate of enzyme hydrolysis and increase yields of sugars from cellulose or hemicellulose. Many current pretreatment methods (i.e., dilute acid, ammonia fiber explosion, ammonia recycles percolation, hot water, and lime pretreatment) have limitations such as capital-intensiveness, the tendency to form inhibitors, as well as low yields (Han et al., 1974). There are available of various pretreatment process. One of them is physical pretreatment, the function of these pretreatment is to break down the feedstock size by milling or steam processing. By reducing the substrate size, the reaction on the waste becomes more effective and efficient. Pretreatment also has a great potential for

improvement of efficiency and lowering of cost through research and development (Mielenz, 2001).

Effective pretreatment is characterized by several criteria. Methods used for cellulosic material required much more intense physical pretreatment such as steam explosion. The other common methods used in pretreatment are dilute acid, alkaline, organic solvent, ammonia, sulfur dioxide, carbon dioxide or other chemicals to make the biomass more digestible by the enzyme (Fan et al., 1982).

Table 2.2 Pretreatment processes of lignocellulosic material

Pretreatment method	Processes	Studied application	Possible change in biomass
Physical pretreatments	Milling: - Hammer milling - Two-roll milling, etc.	Ethanol	- Increase in accessible surface area and pore size - Decrease in cellulose crystallinity - Decrease in degree of polymerization
	Irradiation: - Gamma-ray - Microwave, etc.	Ethanol and biogas	
	Other : - Hydrothermal - High pressure steaming, etc.	Ethanol and biogas	
Chemical and physicochemical pretreatments	Explosion: - Steam explosion - CO_2 explosion, etc	Ethanol and biogas	- Increase in accessible surface area - Partial or nearly complete delignification - Decrease in cellulose crystallinity - Decrease in degree of polymerization - Partial or complete hydrolysis of hemicelluloses
	Alkali: - Sodium hydroxide - Ammonium sulfite, etc.	Ethanol and biogas	
	Acid : - Sulfuric acid - Hydrocloric acid, etc.	Ethanol and biogas	
	Gas : - Chlorine dioxide - Nitrogen dioxide, etc.	Ethanol and biogas	
	Oxidizing agent: - Hydrogen peroxide - Ozone, etc.	Ethanol and biogas	
	Solvent extraction of lignin: - Ethanol-water extraction - Benzene-water extraction, etc.	Ethanol	
Biological pretreatments	Fungi and Actinomycetes	Ethanol and biogas	- Delignification - Reduction in degree of polymerization of cellulose - Partial hydrolysis of hemicellulose

Source : Taherzadeh et al., (2008).

2.4.1 Hydrolysis

Hydrolysis is a chemical decomposition process that uses water to split chemical bonds of substances. There are three types of hydrolysis: acid, enzymatic and thermochemical. Feed stocks that may be appropriate for acid or enzymatic hydrolysis typically are plant-based materials containing cellulose. These include forest material and sawmill residue, agricultural residue, urban waste, and waste paper.

Lignocellulosic materials predominantly contain a mixture of carbohydrate polymers (cellulose and hemicellulose), lignin, extractives, and ashes. The term “holocellulose” is often used to describe the total carbohydrate contained in a plant or microbial cell. Holocellulose is therefore comprised of cellulose and hemicellulose in lignocellulosic materials (Taherzadeh et al., 2007). Cellulose and hemicellulose are chains of sugar molecules that can be broken down by chemical or biological reaction into the simple sugars. The sugars are then fermented using yeast or bacteria to produce ethanol, which is then distilled to make high concentration for final use (Fan et al., 1987).

2.4.2 Enzymatic hydrolysis

Almost of the biomass can be decomposed by enzymes obtained from various microorganisms. The polysaccharide hydrolysis thus are one of the most important enzymatic processes on earth, and cellulose synthesis and hydrolysis are a great part of the carbon cycle. Enzymatic cellulose hydrolysis are generally a slow and incomplete process. However, in a relatively short time (up to 48 hrs) the microbial consortium in the bovine rumen hydrolyzes cellulose to 60 - 65 %, and the lower termites were even reported to assimilate wood cellulose to an extent greater than 90 % (Breznak et al., 1994).

The crystalline material is hydrolyzed by a number of simultaneously active side interacting enzymes, or alternatively by a multienzyme complex. The investigation of the hydrolysis mechanisms of cellulases has been reported: the dualism between mechanical and structural "preparation" of the insoluble (crystalline) substrate followed by the hydrolytic activity on a released molecule. Recently increased research in cellulases aims at the enzymatic mechanisms at the surface of the insoluble substrate. It also tries to solve the problems with direct conversion of biomass into valuable products by using isolated enzymes or cellulolytic microorganisms (Sheehan et al., 1999).

2.4.3 Acid hydrolysis

Acid hydrolysis is a direct hydrolysis method used for biomass conversion and single stage acid hydrolysis is considered as the simplest and single reaction vessel. Typically, a single stage with diluted acid (less than 5 % acid concentration) was used rather than concentrated acid. A high temperature is needed to achieve a maximum conversion within a short residence time. However, implementation of high temperature resulted in product contamination with the presence of soluble derivatives, furfural and hydroxy-methyl furfural, which is generated in the presence of acids from sugars; C5 and C6, reduce the formation of decomposed products (Yan et al., 1996).

Treatment of lignocellulosic materials with acid and a high temperature can efficiently improve the enzymatic hydrolysis. Sulfuric acid is often applied invarious report, while

other acids such as HCl and nitric acid were also reported (Taherzadeh et al., 2007). The acid pretreatment 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). The use of low temperature and high concentrated-acid pretreatment (e.g. 40 °C) is a clear advantage compared to dilute-acid processes. However, high acid concentration (e.g. 30-70%) causes corrosive and dangerous for machine and human. Therefore, this process requires specialized non-metallic constructions or expensive alloys.

Acid hydrolysis can be divided into two groups are dilute acid and concentrated acid. Dilute acid processes are conducted under high temperature and pressure. The reaction times in the range of seconds or minutes. Most dilute acid processes are limited to a sugar recovery efficiency around of 50%. The reason for this is that at least two reactions are part of this process. The first reaction converts the cellulosic material to sugar and the second reaction converts the sugar to other chemicals. The condition that causes in the first reaction to occur also the right condition for the second reaction. Once the cellulosic molecules are broken apart, the reaction proceeds to break down sugar into other products.

In the high concentration acid process, mild temperature is used and the high pressure is usually applied. For example, in total volatile acid (TVA) concentrated acid process, corn stover is mixed with dilute (10%) sulfuric acid, and heated to 100°C for 2 to 6 hours in the first hydrolysis reactor. The low temperature and pressure minimize the degradation of sugars. To recover the sugar, the hydrolyzed material in the first reactor is soaked in water and drained several times. The waste from first stage then dewatered and soaked 30%nd 40% concentration of sulfuric acid for 1 to 4 hours as a pre-cellulose hydrolysis step. This material is then dewatered and dried with the effect that acid concentration in the material increased to 70%. After reacting in another vessel for 1 to 4 hours at 100°C, the reactor contents are filtered to remove solids and recover the sugar and acid. The sugar from the second vessel is recovered in the liquid from the first stage hydrolysis.

2.4.4 Dilute acid

Dilute acid hydrolysis which is one of the common method is used for pretreatment of the lignocellulosic material. Generally, the condition of the process set at a temperature between 140°C and 170°C for one stage and 120°C in two stages process. (Torget et al., 1994). The hydrolyzation of lignocellulosic occurs in an auto-hydrolysis process which involves heating in water at 180°C (Lee et al., 1997). The water pretreatment can be applied to remove hemicellulose from lignocellulose.

2.4.5 Steam explosion

Steam explosion is an effective pretreatment which is able to separate the lignocellulosic feedstock. In this pretreatment, heat is carried out by direct steam injection. Initially, the pressure of the process is high (220-270°C) and short residence time (40-90s), whereas recent investigator use lower temperature (190-200°C) and longer residence time (10 minutes) (Weil et al., 1994).

2.5 Saccharification

Saccharification or enzymatic hydrolysis of cellulose is conversion process to change cellulose to cellobiose, and then futher convert to simple sugar, such as glucose. Saccharification is carried out by acid solution or cellulase enzymes (Beguin et al., 1994). Utility cost of enzymatic hydrolysis is low compared to acid or alkaline hydrolysis because enzyme hydrolysis is usually conducted at mild conditions (pH 4.8 and temperature 45–50^{o}C) and does not have a corrosion problem (Duff et al., 1996).

2.6 Fermentation process

The fermentation step is central in the overall ethanol production process since it represents the actual transformation of conditioned and pretreatment feedstock into the main product using bio-agents such as yeast or other ethanol-producing microorganism. In traditional process ethanol is produced by fermentation of microbial namely yeast, *Saccharomyces cereviseae*. The yeast is valid for practically everyone of the main types of the feedstock employed for the ethanol production (Power, 2003).

Many years ago, fermentation process used to convert grape sugar into alcohol and carbon dioxide by the action of yeast. For dry wines, the process is allowed to continue until all the sugar has been converted into alcohol. For wines such as port, fermentation is stopped by the addition of high level alcohol which kills the yeast and allows some sugars to remain in the juice, unfermented (Bailey, 1986).

In addition, fermentation is also uses to produce cellulase by *Aspergillus fumigatus* using wheat straw as a carbon source. ß-glucosidase and CM-cellulase activities were assayed by using Salicin and CM cellulose as substrate. The biodegradation of cellulose to be soluble sugar is a process which is only possible after the action of the multienzyme system of cellulases produced by cellulolytic microorganism (Steinkraus, 1995).

For a successive fermentation, chemist has determined and defined nutritional conditions. Factors such as carbon sources, nitrogen sources, trace metals, vitamins, carbon loading, and carbon-and-nitrogen ratios were considered to develop the new growth media because of they have been shown to influence biomass accumulation, propagule formation, and biocontrol efficacy. Several nitrogen sources found as yield excellent of fungal growth were identified, and by varying the carbon-and-nitrogen ratios, biomass production were increased (Bailey, 1986).

Now a day, application of fungi in ethanaol fermentation are able to utilize in the fermentation process. The nutrient required by a fungus in the greatest amount is the carbon source (Bailey, 1986).

2.6.1 Separate hydrolysis and fermentation (SHF)

SHF is a separation of the hydrolysis and fermentation by running the reactions in separation units. Separation of hydrolysis is pretreated lignocellulosic material is in a first unit degraded to monomeric sugars by cellulases and fermentation converted glucose to ethanol in a second, separate unit by microorganisms, *Saccharomyces cerevisiae.* The main advantage of this method is that the two processes (hydrolysis and

fermentation) that can be performed at their own individual optimal conditions. Cellulases have shown to be most efficient at temperature between 45-50°C, whereas commonly used fermenting organism has an optimum temperature of 30-37^{0}C (Taherzadeh et al., 2007).

Another advantage with SHF is possible possibility to run the fermentation process in a continuous mode with cell recycling. Lignin residue removal can occur before fermentation. This removal is much more problematic if lignin is mixed together with the yeast (Galbe et al., 2002). The major drawback of SHF is suppression of end products, i.e. glucose and cellobiose released in cellulose hydrolysis strongly inhibits the cellulase activity. Glucose inhibits β-glucosidase which results in an increase of cellobiose while β-glucosidase catalyze the hydrolysis of cellobiose to glucose. Cellobiose is inhibitor of cellulases that has directly effected on a cellulase activity (Alfani et al., 2000).

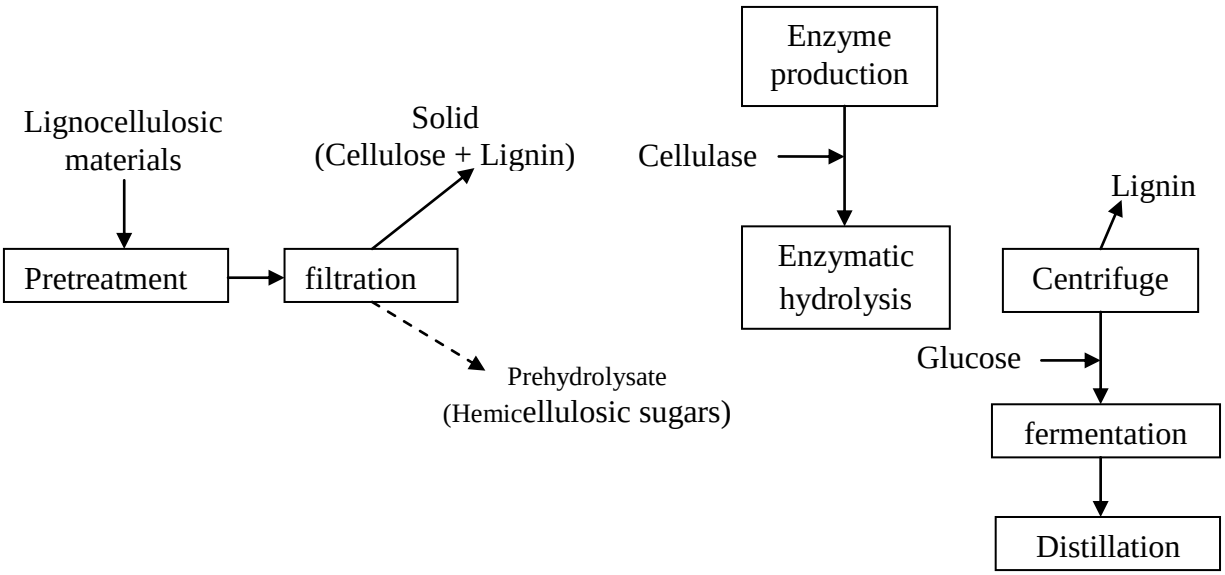

Figure 2.9 Flow chart a schematic picture of a possible bio-ethanol process using separate hydrolysis and fermentation (SHF)

2.7 Cellulolytic microorganisms

Biodegradation is the decomposition of a substance due to the action of biological agents, especially microorganisms. Generally, degradation is the process of renovation by microorganisms (De Long, 2004). According to Sutedja et al., (1991), cellulose can be decomposed easily and quickly by specific organisms that are found in various microbes such as bacteria, fungi, actinomycetes and lower animals. Microbiologist experts classify different cellulose degrading organisms are aerobic bacteria, mixobacteria, anaerobic bacteria, including thermophilic forms, actinomycetes, fungi, mushroom, protozoa, and insects.

A cellulose degrading organisms are a class of fungi, yeasts, actinomycetes, and bacteria. For fungi, many fungi involves in the cellulose degradation process in various environment such as *Acremonium spp*., *Trichoderma reesei*, *Trichoderma viride*, *Penicillium pinophilum*, *Phanerochaeta chrysosporium* (*Sporatrichum pulverulentum*), *Fusarium solani*, *Talaromyces emersonii*, *Trichoderma koningii*, *Fusarium oxysporium*, *Aspergillus niger and Rhyzophus oryzae* while the class of yeast is *Candida* (Bhat et al., 1997; Murashima et al, 2002; and Mach et al., 2003). *Actinomyces* group is *Micromonspora*, *Microbiospora, Nocordia, Streptomyces*, and *Streptosporangium*, and genus of bacteria is of major *Bacillus, Cellulomonas, Clostridium, Corynobacterium, Cytophaga, Pseudomonas, Vibrio* and *Sporohytophaga* (Anonymous, 2005).

Cellulose degradation can occur in aerobic or anaerobic conditions. In aerobic conditions, degradation by fungi, myxobacteria from the genus *Polyangium*, *Sorangium* and Eubacteria, while biodegradation under anaerobic conditions by *Clostridium* (Schlegel, 1992). Some cellulolytic fungi have a high efficacy to degrade cellulose, crystalline cellulose, such as *Tricoderma viride*, *Tricorderma koningii*, *Fusarium solani, Penicillium iriensis*, and species of *Basidiomycetes* (Ekawati, 2003). According to Chaplin (2005), microorganisms can decompose cellulose include fungi, yeast, and bacteria. A special group of bacteria includes the genus *Bacillus, Cellulomonas, Clostridium, Corynobacterium, Pseudomonas, Vibrio*, and *Cytophaga*.

Aerobic bacteria such as *Cytophaga* and *Sporocytophaga* have the ability to grow on a substrate cellulolytic (Schlegel, 1992). Cytophaga groups capable of growing at pH 6.1 to 9.1, optimum temperature 20-28^{o}C and the availability of sufficient oxygen can accelerate the decomposition of cellulose (Sutedja et al., 1991). Gram-negative bacteria such as *Xanthomonas* and *Pseudomonas c*an use cellulose (Fikrianda et al., 2000).

Anaerobic cellulose degradation in the environment is carried out by *Acetivibrio cellulolitic*us and various species of *Clostridium*, such as *Clostridium cellulolitic*um and *Clostridium celluvorans*, both are mesophilic or *Clostridium thermocellum* and *Clostridium stercorarium* that are thermophilic. Anaerobic microorganisms known to participate in the degradation of cellulose are *Clostridium thermocellum*. These bacteria can grow in simple synthetic media with cellulose or cellubiose (Schlegel, 1992).

Many microorganisms are able to degrade cellulose in anaerobic (without the presence of O_2) such as *Clostridium*. Some *Clostridium* species require cellulose as a source of energy. The end result of the decomposition of cellulose is CO_2, H_2O, ethanol and various organic acids. The final results will be used for methanogens by other bacteria (Marsiati, 2005).

2.7.1 Degradation of cellulose

According to Schwarz (2001), it is difficult to hydrolyzed cellulose because cellulose is insoluble in water and form crystals. Degradation by the enzyme only occurs on the surface. According to Walker et al., (1991), cellulose degradation can occur by enzymatic hydrolysis or acid hydrolysis. The amount of degradation of cellulose is determined by the amount of enzyme adsorbed on the surface of cellulose, cellulose degrading enzyme performance and the presence of other substances, such as lignin or hemicellulose. In acidic conditions the degradation of cellulose produces D-glucose and accumulation of solid waste such as calcium sulfate (Aguestin et al., 1995).

Enzymatically catalyzed degradation of cellulose by cellulase enzymes. This enzyme produced by many organisms such as bacteria and fungi (Begiun et al., 1992). Cellulolytic microbes have several cellulases that are bound to the outer surface of cells (Walker et al., 1991). Cellulases can be produced by fungi, bacteria, and ruminants. Commercial production of enzymes often uses fungi or bacteria. Fungi can produce cellulases include of *Tricoderma, Aspergillus,* and *Penicillium*. Types of fungi that are commonly used in the production of cellulase was *Aspergillus niger, Aspergullus nidulans, Aspergillus fumigates, Neuspora sitophila, Tricodema viride, Tricodema longibrachiatum,* and *Saccharomyces cerevisia*. While the bacteria that can produce cellulase are *Pseudomonas, Cellulomonas, Bacillus, Micrococcus, Cellovibrio* and *Sphorophytophaga* (Usama et al., 2008).

The process of degradation cellulose of microbial by using the help of enzyme extracellular, namely ß-1,4-endoglucanase and ß-1,4-exoglucanase. Endoglucanase hydrolyze cellulose polymer at random and produce smaller glucose molecules. While exoglucanase hydrolyze two glucose subunits in the tail to produce cellulose disaccharide. There is another enzyme that is known as the ß-glucosidase or cellubiase, which can hydrolyze cellubiose to glucose. Cellubiase can be included as an intracellular enzyme or extracellular (Maier et al., 2000).

Mechanism of cellulose degradation by microorganisms depends on the characteristics of microorganisms and the degrading conditions. Aerobic bacteria and fungi degrade cellulose produces byproducts such as furfural, acetic acid, CO_2, pigments, and a number of substances/ microbial cells (Sutedja et al., 1991). Cellulose degradation can occur based on the enzyme system, different types of bacteria can produce the same enzyme. It affects the ability of bacteria in degrading organic compounds (Atlas et al., 1981).

The factors that influence the degradation of cellulose are the environmental factors and properties of cellulose degrading microbes with conditions including moisture, aeration reaction, temperature and nitrogen availability of adequate and nutritional elements (Sutedja et al., 1991).