CHAPTER 2

LITERATURE REVIEW

2.1 Longan

Longan, known in many scientific names *i.e. Euphoria longan* Lam., *Dimocarpus longan* Lour. and *Nephelium longan* Camb., is a subtropical fruit of the Family *Sapindaceae* closely related to lychee (Jeerasantikul, 2005; The Plant Variety Protection Office, 2002; Yang *et al*., 2008). The longan is native to southern China. It is commonly grown in Thailand, Vietnam, Myanmar, Sri Lanka and India. In Thailand, it is widely distributed in some northern provinces of Thailand including Chiang Mai, Chiang Rai, Lamphun, Lampang, Phayao, Phrae and Nan because of their suitable topography and climate (Kamwong, 2008).

Longan is an economically valuable fruit of Thailand. It is sold as fresh longan domestically about 25-30% of total production and exported to other countries about 30-35% and the other is transformed to other product (Kamwong, 2008). The amount of longan in the market and its price varies every year depending on the capacity of longan production each year (Table 2.1). If the production was over 480,000 ton/year, the price would be low because it glut in the market (Chaimongkol, 2003). Therefore, fresh longan must be transformed into other products such as dried longan, canned longan, longan wine and longan juice in order to solve this problem and, simultaneously, to increase product's value (Chaimongkol, 2003; Panyatep, 2005; Teechapoonyong, 2003; Thailand's Agricultural Information Center, 2007).With this strategy, longan can be exported in many forms such as dried longan, canned longan and deep-freeze longan to other countries, for instances, China, Indonesia, Hongkong and Singapore and provides higher income to Thailand (Table 2.2) (Office of Agricultural Economics, 2009; Thailand's Agricultural Information Center, 2007).

Table 2.1: Production of longan in 2005 – 2007.

Production			
List	**2005**	**2006**	**2007**
Number of household (household)	235,447	238,633	238,921
Field for product (rai)	820,985	870,125	939,029
Production (ton)	712,178	471,892	495,457
Production/rai (kg)	867	542	528
Cost (baht/ton)			
- Total cost	12,928	10,799	12,059
- Variable cost	10,081	7,895	9,056
Price			
- Type A (baht/ton)	12,560	17,140	16,062
- Type AA (baht/ton)	17,890	20,680	20,182
Total profit (baht/rai)	-1,410	217	-1,045
(baht/ton)	-1,626	401	-1,979

Source: Thailand's Agricultural Information Center (2007)

Table 2.2: Longan trading.

Trade			
List	**2005**	**2006**	**2007**
World trading (millonton)	0.569	0.256	0.267
World market sharing (%)	80.00	80.00	80.00
Sold in country (ton)	119,994	66,512	52,570
Export (ton)	592,184	405,380	490,200
- Fresh longan			
Quantity (ton)	133,651	119,430	160,352
Value (millonbaht)	2,165	2,116	2,269
- Dried longan			
Quantity (ton)	133,646	78,390	112,784
Value (millonbaht)	2,351	1,606	2,018
- Canned longan			
Quantity (ton)	12,669	11,206	11,284
Value (millonbaht)	443	405	408
- Deep-freeze longan			
Quantity (ton)	787	354	377
Value (millonbaht)	33	21	22
Price for export (baht/ton)			
- Fresh longan	16,440	19,363	16,018
- Dried longan	29,106	23,312	19,501
- Canned longan	35,182	36,108	36,326
- Deep-freeze longan	45,992	57,254	58,316

Source: Thailand's Agricultural Information Center (2007)

The lower grade and over production fresh longan are commonly transformed into dried longan to increase value of longan fruit. The black and smoky-flavoured dried pulp is mainly used to prepare drinks (Yunchalad *et al*., 2008). The moisture of dried longan is as low as 17.80% (Angkasit *et al*., 1999). Rezaei and VanderGheynst (2010) studied about critical moisture content for microbial growth in dried food-processing residues and found that the critical moisture content of sugar beet pulp, tomato pomace, fermented grape pomace and grape pomace were 24-31,16-21, 23-33 and 43-46% (dry basis), respectively. Hence, the microorganism can grow on dried longan when it was kept in unsuitable condition. Therefore, if dried longan is stored for a long time, it will expire and become useless. In addition, exporting of expired dried longan would make a severe problem to Thailand's reputation on export and economic (Panyatep, 2005). Therefore, the expired dried longan should be destroyed by burying or burning but the more benefited way is to used them in the production of fertilizer or biofuel (Krobthong, 2001; Ruamchai, *et al*., 2012).

Moreover, there are many studies mentioned about the utilization of unsalable dried longan. Palakul *et al*. (2006) used dried longan to produce alcohol by fermenting with *Saccharomyces cerevisiae*. The result showed that dried longan with 20^{o}brix was able to produce the highest alcohol up to 7.70% (v/v).

Kumtip *et al*. (2008) produced organic compounds from expired dried longan mixed with molasses using 15 microbial strains in static condition. The results illustrated that *S. cerevisiae* TISTR 5606 can produce highest ethanol and glycerol at 38.4 ± 1.3 and 4.51 ± 0.16 g/l, *Zymomonas mobilis* TISTR 405 can produce 3.93 ± 0.15 g/l of lactic acid, *Escherichia coli* TISTR 1261 can produce 29.1 ± 1.7 and 2.92 ± 0.99 g/l of citric and formic acid and *Candida utilis* TISTR 5001 can produce propanoic acid about 7.71 ± 0.39 g/l.

Agustina (2009) used overproduction of dried longan as carbon source for ethanol and whole cell pyruvate decarboxylase (PDC) production by using 15 strains microorganisms. The results showed that, in the case of using 6 years old dried longan extract as substrate, the highest level of ethanol and PDC activity were produced by *C. utilis* TISTR 5001 (1.26 ± 0.82 g/l) and *S. cerevisiae* TISTR 5606 (0.01 ± 0.00 U/ml), respectively. For 10 months old dried longan extract, the results showed that the highest level of ethanol was 24.71 ± 3.34 g/l which fermented by *S. cerevisiae*

TISTR 5606 and PDC activity was 0.05 ± 0.00 U/ml which fermented by *C. utilis* TISTR 5198.

Sumphanwanich (2006) used many agricultural wastes i.e. corn cob, bagasse, dried longan pomace and rice straw as substrates for producing ethanol by *S. cerevisiae*. The results showed that dried longan pomace which has cellulose about 78.36% (w/w) yielded the maximum glucose at 159.38 mg/g after pretreatment with hydrolysis of sulfuric acid, xylose at 3.50 mg/g and it can produce maximum amount of ethanol after fermented by *S. cerevisiae* at 24.43% (w/w).

Arbsuwan (2009) used pectinase, cellulase and diluted sulfuric acid to hydrolyze dried longan pomace which contained less sugar content for maximum reducing sugar yield at various conditions. It was shown that the hydrolysis of dried longan pomace with the mixture of 1.0% (v/w) pectinase and 1.0% (v/w) cellulase and incubation time of 12 h produced the highest reducing sugar at 43.15 mg/g longan pomace compared to other conditions.

2.2 Compositions of longan

Longan is a non-climacteric fruit which has sweet taste and fragrant (Panyatep, 2005). Components and nutrients in longan are shown in Table 2.3. The main components in fresh and dried longan are carbohydrate which composed of three sugars including glucose, fructose and sucrose. Besides, longan also has many important organic acids such as glucinic acid, malic acid and citric acid (Angkasit, 1999; Panyatep, 2005). Furthermore, Sumphanwanich (2006) reported that the main component in longan pomace is cellulose at 78.36 % (w/w) approximately.

Table 2.3: Chemical components and nutrients of fresh and dried longan.

Component	Fresh longan	Dried longan
Moisture (%)	81.10	17.80
Fat (%)	0.11	0.40
Fiber (%)	0.28	1.60
Protein (%)	0.97	4.60
Ash (%)	0.56	2.86
Carbohydrate (%)	16.98	72.70
Heating Value (kg/100 g)	72.79	311.80
Calcium (mg/100 g)	5.70	27.70
Iron (mg/ 100g)	0.35	2.39
Phosphorus (mg/ 100g)	35.30	159.50
Vitamin C (mg/ 100g)	69.20	137.80
Sodium (mg/ 100g)	-	4.50
Potassium (mg/ 100g)	-	2,012.00
Niacin (mg/ 100g)	-	3.03
Pantonic acid (mg/ 100g)	-	0.57
Vitamin B2 (mg/ 100g)	-	0.375

Source: Angkasit *et al*. (1999)

The main components of the other fruit's cell wall are similar to longan, composing of cellulose, hemicelluloses, pectin and lignin (Table 2.4). El Kossori *et al*. (1998) reported that cactus plup has pectin around 14.4% (w/w). Sreenath *et al*. (1995) reported that cellulase and pectinase can hydrolyze mango plup, therefore, mango plup has cellulose and pectin as substrates.

Table 2.4: Fruit cell wall composition in g/kg fresh matter.

Friut	EIR*	Pectin	Hemicellulose	Cellulose	Lignin	Protein	Total
Apple	20	272	169	349	2	76	868
Pear	15	281	148	267	69	82	847
Mango	25	408	91	236	27	127	889
Pineapple	13	163	267	210	85	94	819
Strawberry	12	411	66	232	11	255	975
Raspberry	20	168	89	177	73	277	784
Cherry	13	396	49	130	169	244	988
Papaya	26	364	165	124	4	127	784

*Ethanol-insoluble residue.

Source: Aehle, (2007)

Yang *et al*. (2008) studied about characterization of polysaccharides from longan plup by DEAE-cellulose anion-exchange chromatography, fourier transform infrared spectroscopy (FTIR) and high performance liquid chromatography (HPLC). It was found that 3 polysaccharides in longan plup, LPS-N, LPS-A1 and LPS-A2 were composed of monosaccharides such as xylose, glucose, rhamnose, arabinose, galactose and uronic acid.

For longan seed, it is a by-product material from canned longan pulp and dried longan pulp manufacturing, there are thousands of tons of waste longan seed (Chanrittisen and Chomsri, 2010). The seeds contain starch as high as 40-60% of weight, which can be prepared for active carbon and starch gum (Lai *et al*., 2000; Viet *et al*., 2005).

2.3 Cellulose, hemicellulose, pectin and starch

2.3.1 Cellulose

Cellulose is the most abundant organic compound on Earth. Cellulose polymer is a linear chain of thousands of glucose units which joined by β-1,4glycosidic bonds (Figure 2.1). The basic recurring unit is cellobiose (Glazer and Nikaido, 1995). The linear polymers are held together by hydrogen bonding to from crystalline fibrils (Branen *et al*., 2002). Cellulose has a high tensile strength. It is water-insoluble and much more resistant to degradation than other glucose polymers such as starch (Glazer and Nikaido, 1995).

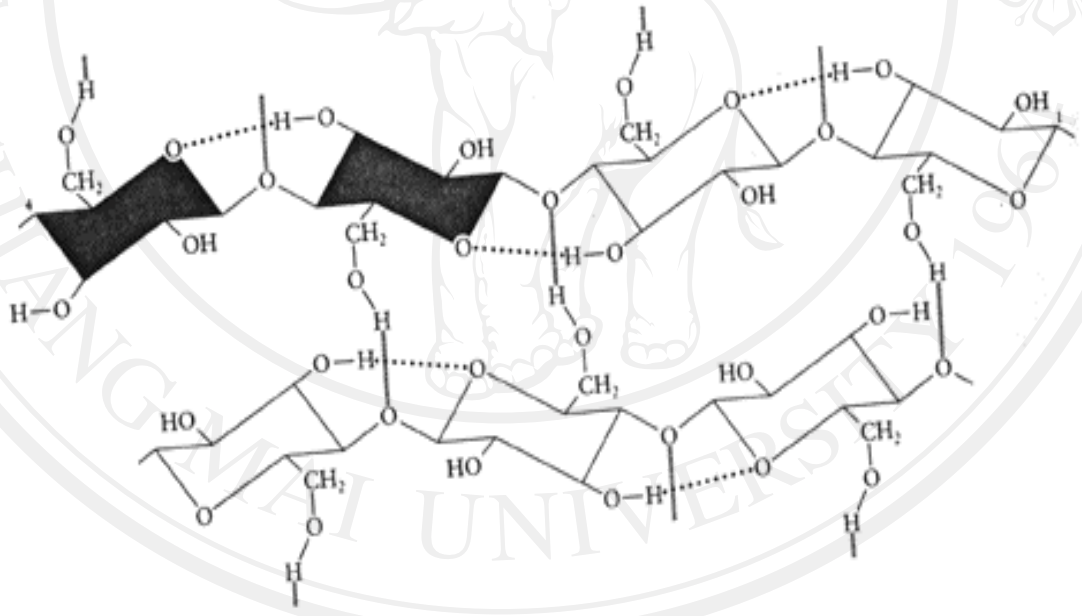

Figure 2.1: Cellulose chains showing the β-1,4-linked glucose residues rotated through 180^{o} with respect to their neighbors in the chain. The conformation shown is stabilized by intramolecular hydrogen bonds (dots) within each chain. Intermolecular hydrogen bonds contribute to the interaction of adjacent chains within microfibril. A cellobiose unit is indicated by shading within its pyranose rings (Glazer and Nikaido, 1995).

2.3.2 Hemicellulose

Hemicellulose is non-crystalline heteropolysaccharides in the cell walls, beside cellulose and pectin. Many sugars are represented in hemicellulose *i.e.* pentoses (β-D-xylose, α-L-arabinose), hexoses (β-D-glucose, β-D-mannose, α-D-galactose) and/or uronic acids (α-D-glucuronic, α-D-4-*O*-methylgalacturoni and α-D-galacturonic acids) as well as other sugars such as α-L-rhamnose and α-L-fucose with variously modified by acetylation or methylation. The main residues in the backbone are xylan, mannan and glucan, with xylan and mannan found as the most prevalent (Dumitriu, 2005; Girio *et al*., 2010; Glazer and Nikaido, 1995).

Xylan is a catchall for polysaccharides that has xylose in blackbone joined by β-1,4-xylopyranosidic linkage. Xylan has a variety of side chains with gluconicacid, arabinose and methyl esters of gluconic acid (Dumitriu, 2005). It is the main constituent of secondary cell walls constituting about 20-30% of the biomass of hardwoods and herbaceous plants (Girio *et al*., 2010).

Mannan is a polysaccharides that has mannose more than 6 molecules joined by β-1,4-mannopyranosidic linkage. Normally, it is rare to find mannan with pure mannose but mannan with mannose linked with other sugars such as galactomannan and glucomannan is mostly found in plant (Dumitriu, 2005).

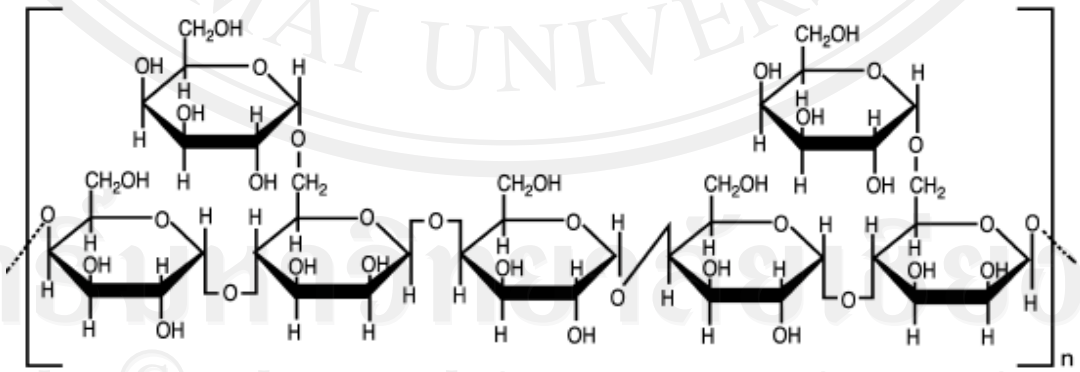

Figure 2.2: Tentative structure of galactoglucomannan (Dumitriu, 2005).

2.3.3 Pectin

Pectin, a complex colloidal carbohydrate, is the major structural polysaccharide component of fruit lamellas and cell walls. Pectin is made of anhydro galacturonic acid units with α-1,4-glycosidic linkages (Figure 2.3). The carboxyl groups of galacturonic acid in pectin can be partly esterified by methyl groups or partly or totally neutralized by one or more bases. There are three pectic polysaccharides found in all primary cell walls: homogalacturonan and rhamnogalacturonans I and II. (Albersheim *et al*., 1996; Branen *et al*., 2002; Shatty *et al*., 2006).

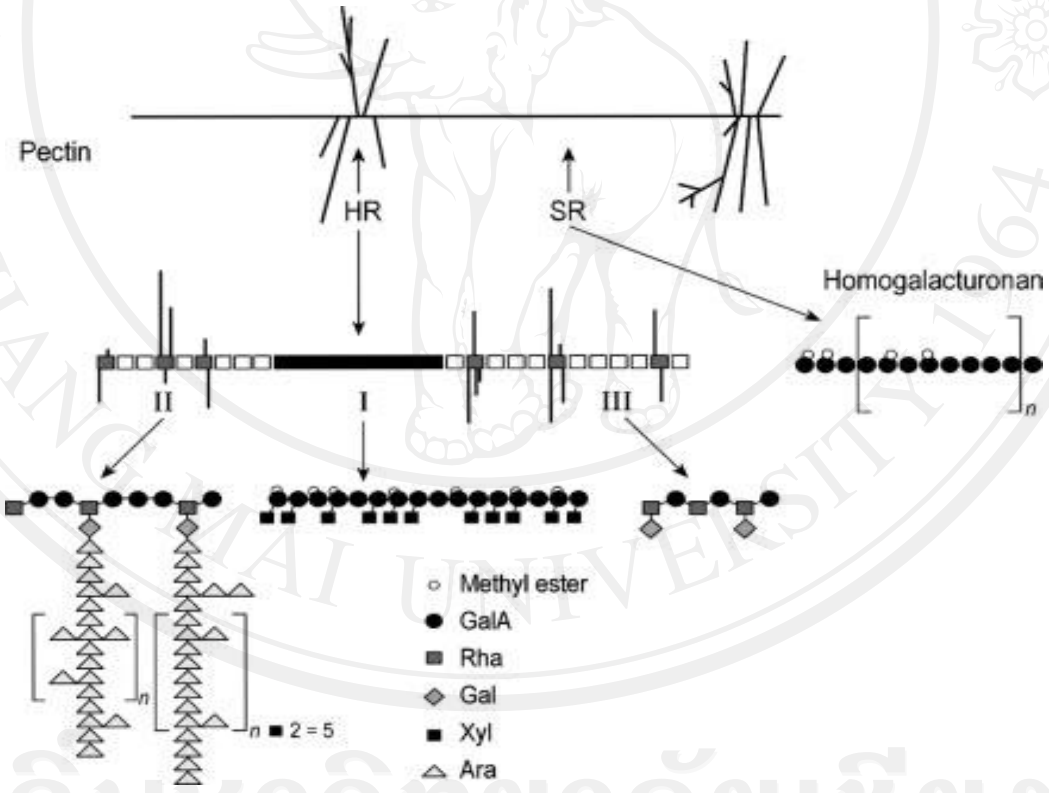

Figure 2.3: Model of pectin smooth regions (SR) and hairy regions (HR) model; Ara = arabinose, Gal = galactose, GalA = galacturonic acid, Rha = rhamnose and Xyl = xylose (Schols and Voragen, 1996).

2.3.4 Starch

Starch is the main storage carbohydrate of plant which accumulated as insoluble, semi-crystalline granules in amyloplasts which are specialized plastids present in plant storage organ such as, seeds, tuber and root (Andersson, 2001; Copeland *et al*., 2009). The amount of starch in some plant seeds is demonstrated in Table 2.5.

Table 2.5: Total starch content in some seed fruit.

Plant seed	% (dry weight basis)	References
Longan seeds	50	Lai *et al*. (2000)
Jackfruits	77.76 ± 0.96	Tulyathan *et al*. (2002)
Beans	44.64	Chiou *et al*., (2011)
Sesbania seeds	17.5-20.4	Hossein and Becker (2001)
Peas	53.61-57.23	Dostalova *et al*. (2009)
Raw seeds	30.7	Capriles *et al*. (2008)

Starchs' stability, transformations and physical properties are largely dependent on the nature of the amorphous and crystalline domains present in native granules or processed starch materials (Biliaderis, 2009). Starch is composed of two polymers of D-glucose: amylose, a main linear molecule of α-1,4-linked glucan with a small fraction of α-1,6-linkages, and amylopectin which has chains of α-1,4-linked glucoses arranged in a highly branched structure with α-1,6 branching links (Andersson, 2001; Copeland *et al*., 2009). Jane *et al*. (1999) reported that the physical properties of starch are depended on amylase and amylopectin ratio and chain length distributions of amylopectin.

Amylose is a polyglucan which has a molecular weight range of approximately 10^5-10^6, corresponding to a degree of polymerization (DP) of 1000-10,000 glucose units (Copeland *et al*., 2009; Taniguchi and Honda, 2009). Less than 0.5% of the glucoses that found in amylose are in α-1,6-linkages resulting in a low degree of branching and a structure with 3-11 chains of approximately 200-700

glucose residues per molecule (Copeland *et al*., 2009). Amylose is generally found in starch granule about 20-30% of total starch (Andersson, 2001).

2.4 Cellulase, hemicellulase, pectinase and amylase

2.4.1 Cellulase

Cellulases are complex enzyme system that cleave β-1,4-glycosidic bonds in cellulose (Aehle, 2007; Pandey *et al*., 1999). Complete and efficient degradation of crystalline cellulose to glucose requires the synergistic action of three cellulolytic enzymes; endoglucanase, exoglucanase and β-glucosidase (Schauer and Borriss, 2004). Endoglucanase which hydrolyzes β-1,4glycosidic bond in a random fashion was commonly measured by detecting the decrease in viscosity or reducing groups released from carboxymethycellulose (CMC) (Branen *et al*., 2002; Dumitriu, 2005). Exoglucanase includes both the 1,4-β-glucanglucohydrolases, which liberates glucose from 1,4-β-glucans and slowly hydrolyzes cellobiose, and 1,4-β-glucancellobiohydrolases, which cleaves off cellobiose unit from the nonreducing ends of cellulose polymers (Branen *et al*., 2002; Dumitriu, 2005). For β-glucosidase, its function is to release glucose units from cellobiose and soluble cellodextrins derived from cellulose (Branen *et al*., 2002; Dumitriu, 2005).

Cellulases are mostly produced by fungi such as *Trichoderma reesei* (Ahamed and Vermette, 2009; Martins *et al*., 2008; Sukumaran *et al*., 2009), *Aspergilus niger* (Farinas *et al*., 2010; Ja'afaru and Fagade, 2007; Sohail *et al*., 2009; Sukumaran *et al*., 2009) and *Penicillium echinulatum* (Martins *et al*., 2008; Camassola and Dillon, 2009) and some bacteria such as *Cellulomonas flavigena* (Rojas-Rejon *et al*., 2010), *Bacillus amyoliquefaciens* (Lee *et al*., 2008).

Cellulases are used in textile and laundry biotechnology. Cellulases are able to modify cellulosic fibers in a controlled and desired manner; bio-polishing and bio-stoning. These enzymes constitute the best known illustrations. In laundry, cellulases are also increasingly used in household washing powders because they enhance detergent performance, allow the removal of small, fuzzy fibrils from fabric surfaces, and improve the appearance and color brightness (Baht, 2000).

Cellulases also used in pulp and paper processing to increase paper machine runnability, and save energy during two-stage refining resulting in increased tensile strength and high fiber qualities. Moreover, the enzymes are used in animal feeds to improve weight gain and feed efficiency in poultry and swine (Aehle, 2007; Pere *et al*., 1995).

2.4.2 Hemicellulase

Hemicellulases are a group of enzymes that are able to degrade gel-forming polysaccharides like xylan, xyloglucan, arabinan, arabinogalactan, and galactomannan. The most common hemicellulases are xylananse and mannanase (Aehle, 2007).

Xylanases are glycan hydrolases that catalyze the endo-hydrolysis of β-1,4-D-xylosidic linkages in xylans. Xylanases are divided into three types *i.e.* endoxylanase which catalyzes the random hydrolysis of β -1,4-xylosidic linkages in xylans, exoxylanase which catalyzes β -1,4-xylosidic linkages from non-reducing ends to xylose and β–xylosidase which catalyzes xylobiose and xylooligosacharide (Aehle, 2007; Dumitriu, 2005). Xylanase are produced by bacteria mainly from *Bacillus* spp., cyanobacteria, actinomyces, and some fungi found in protozoa, snail, insect and seed plant (Aehle, 2007). The most commercial xylanse is produced by *Aspergillus* spp., and *Trichoderma* spp. (Dumitriu, 2005). Xylanase is used commercially in the pulp and paper making, baking, textile for fiber treatment, animal feed and food industry. For animal feed, xylanases are used to increase the digestibility of cereals. Furthermore, xylanase was used to degrade xylan in plant to five-carbon sugar D-xylose which can be converted into a wide variety of useful products (Aehle, 2007; Pandey *et al*., 1999; Whitaker *et al*., 2003).

There are two types of mananase *i.e.* endo-1,4-β-mannanase and exo-1,2-1,3-α-mannoxidase. Endo-1,4-β-mannanase called β-mannanase can catalyze the random hydrolysis of β-D-1,4 mannopyranosyl linkages within the main chain of mannan and various polysaccharides consisting mainly of mannose, such as glucomannan, galactomannan, and galactoglucomannan (Aehle, 2007; Dumitriu, 2005). β-Mannanase is produced by some species of bacteria such as *Bacillus subtilis*,

Aerobacter mannanolyticus and *Streptococcus* sp., some fungi such as *Aspergillus* spp. and *Trichoderma* spp., and also some plants. The most commonly used carbon sources for microbial β-mannanase production are galactomannan and other mannans (Aehle, 2007; Dumitriu, 2005; Whitaker *et al*., 2003).

Mannanases have several potential and existing applications in particular in pulp and paper making, laundry detergent, textile and animal (Aehle, 2007; Whitaker *et al*., 2003). β-Mannanase is used to solubilize softwood pulp mannan and used as an aid in pulp bleaching (Clark *et al*., 1990). Moreover, in coffee production, β-mannanase is used to reduce the viscosity of the extract during production of instant coffee (Wong and Shaddler, 1993).

2.4.3 Pectinase

Pectinases, a group of hydrolytic enzymes, usually referred as pectolytic enzymes. Pectolytic enzymes degrade pectic material and reduce the viscosity of a solution (Shatty *et al*., 2006). There are three different types of pectic enzymes; pectinesterase, lyase, and polygalacturonase (Figure 2.4) (Branen *et al*., 2002).

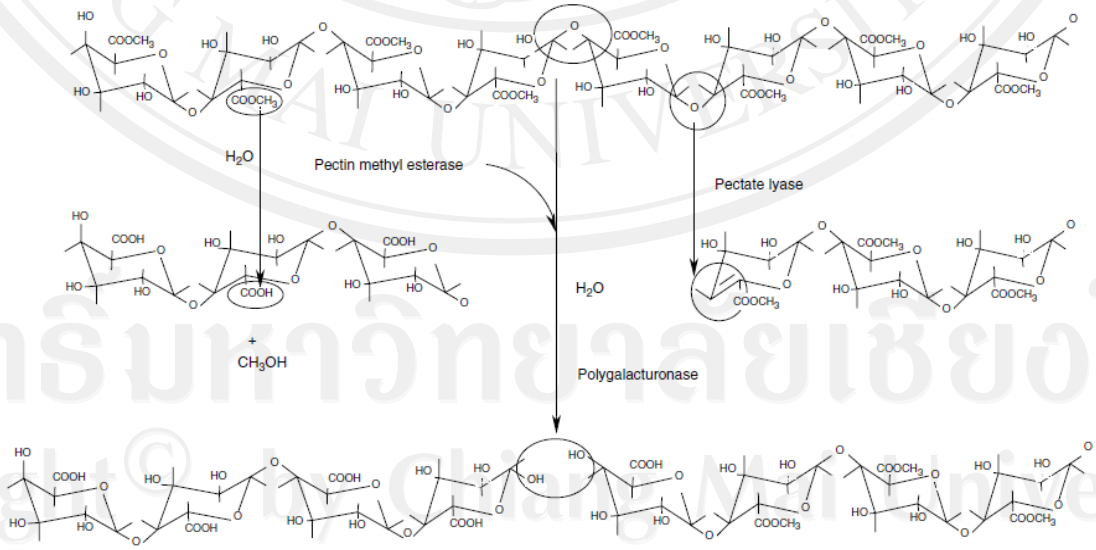

Figure 2.4: Pectin main chain structure and enzymes involved in degradation (Shatty *et al*., 2006)

Pectinesterase de-esterify pectin to produce pectic acid and methanol. The enzymes are specific for the methyl ester of pectic acid and do not attack the methyl ester of polymannuronic acid (alginic acid) or gum tragacanth (Branen *et al*., 2002). Lyase directly attacks highly esterified polymethylgalacturonic acid. There are two types of pectin lyase; endopectatelyase which randomly splits partially or completely de-esterified pectin chains and exopectatelyase which liberates unsaturated dimers from the reducing end of pectic acid (Branen *et al*., 2002). Polygalacturonase hydrolyzes internal bonds in pectic acid resulting in rapid reduction in viscosity (Kelly and Fogarty, 1978; Rombouts and Pilnik, 1980). There are two types of polygalacturonase; endopolygalacturonase which hydrolyzespectic acid into monomers, dimers and trimers of galacturonic acid and exopolygalacturonases which hydrolyzes the terminal α-1,4 bonds in pectates at the nonreducing end to release galacturonic acid monomers (Branen *et al*., 2002; Mount *et al*., 1970). Pectinase are produced by bacteria, actinomyces, mold, and yeasts, most of the commercial enzymes are produced from the mold *Aspergillus ssp*. (Branen *et al*., 2002; De Vries and Visser, 2001; Shatty *et al*., 2006)

Pectic enzymes are used in food industry to facilitate pressing and to help in the separation of the flocculent precipitated by sedimentation, filtration, or centrifugation in the extraction of the clarified juice. Pectinases are used in wine making to reduce haze of grape juice and to promote faster aging of wine (Arguelles *et al*., 1994; Branen *et al*., 2002). Pectinases are also used in coffee and tea fermentation (Jones and Jones, 1984).

2.4.4 Amylase

Amylases are a family of enzymes that catalyzes the breakdown of starch and glycogen which cleave on α-1,4- and α-1,6-glucosidic bonds into sugars (Taniguchi and Honda, 2009). Amylases can be divided into two types; endoamylases and exoamylases. Endoamylases catalyze hydrolysis in a random manner in the interior of the starch molecule producing linear and branched oligosaccharides of various chain lengths. Exoamylases can hydrolyze non-reducing end into short end products (Gupta *et al*., 2003). Microbial enzymes that are classified as amylases are

shown in Table 2.6. α-Amylase (endo-1,4-α-D-glucanglucohydrolase) randomly cleaves the α-1,4-D-glucosidic bonds between the adjacent glucose unit in linear amylose chain (Pandey *et al*., 1999). Glucoamylase (exo-1,4-α-D-glucanglucohydrolase) hydrolyzes single glucose unit from the nonreducing ends of amylose and amylopectin in a stepwise manner and it also hydrolyzes the α-1,6-linkages at the branching point of amylopectin, although at a slower rate than α-1,4-linkages (Pandey *et al*., 1999). The most widely used enzyme for glucose production from starch is the glucoamylase and α-amylase from strains of the fungal genus *Aspergillus* (Smith, 1994). Furthermore, in the industry, α-amylase and glucoamylase are popularly used to hydrolyze starch form maltooligosaccharides into glucose (Aehle, 2007; Reilly, 2007). Amylase and glucoamylase are produced by various microorganisms *i.e. Aspergillus niger* (Dojnovand Vujcic ,2012; Pascoal *et al*., 2011), *Aspergillus oryzae* (Sahnoun *et al*., 2012), *Aspergillus awamori* (Negi and Banerjee, 2009), *Bacillus* sp. (Hashemi *et al*., 2011).

Table 2.6: Microbial enzymes that are classified as amylases.

Enzyme name	EC number	Mechanism	Mode
α-Amylase	3.2.1.1	Retaining	Endo
β-Amylase	3.2.1.2	Inversion	Exo
Glucoamylase	3.2.1.3	Inversion	Exo
Oligo-1,6-glucosidase	3.2.1.10	Retaining	Endo
α-Glucosidase	3.2.1.20	Retaining	Exo
Amylo-1,6-glucosidase	3.2.1.33	Retaining	Exo?
Pullulanase	3.2.1.41	Retaining	Endo
Cyclomaltodextrinase	3.2.1.54	Retaining	Endo
Glucan-1,4-α-maltotetraohydrolase	3.2.1.60	Retaining	Exo?
Isoamylase	3.2.1.68	Retaining	Endo
Glucan-1,4-α-maltohexaohydrolase	3.2.1.98	Retaining	Exo?
Glucan-1,4-α-maltotriohydrolase	3.2.1.116	Retaining	Endo
Glucan-1,4-α-maltohydrolase	3.2.1.133	Retaining	Endo
Neopullulanase	3.2.1.135	Retaining	Endo
4-α-D-Glucanotrehalosetrehalohydrolase	3.2.1.141	Retaining	Exo?
Branching enzyme	2.4.1.18	Retaining	Endo
Cyclomaltodextringlucanotransferase	2.4.1.19	Retaining	Endo
4-α-glucanotransferase	2.4.1.25	Retaining	Endo
4-α-glucan 1-α-D-glucosylmutase	5.4.99.15	Retaining	Exo?

Source: Taniguchi and Honda (2009).

2.5 Ethanol

2.5.1 Ethanol definition, utilization and production

Ethanol (CH_3CH_2OH; also known as ethyl alcohol, grain alcohol, and EtOH) is a kind of clear and colorless liquid with a characteristic and agreeable odor. Its molecule contains a hydroxyl group (-OH) bonded to a carbon atom (Melendez *et al*., 2009). It has a higher octane number (108), broader flammability limits and higher flame speeds than gasoline (Balat and Balat; 2009*).*

Since oil crises in 1970s, ethanol has been used as a renewable liquid biofuel for motor vehicles because fossil fuels are limited sources and increased levels of greenhouse pollution from their combustion (Balat and Balat; 2009; Demirbas, 2005). Ethanol is more environmentally friendly because it contains 35% oxygen which helps complete combustion of fuel and thus reduces particulate emission that causes health hazard to living beings (Ali, 2010; Balat and Balat; 2009). Moreover, ethanol fuel is lower cost than fossil fuel (Yamaji *et al*., 2003). Therefore, it is considered to replace fossil fuel in vehicle by ethanol. Ethanol is used as fuel in transportation by mean of directly used or blended with gasoline. It has been used in Germany and France as early as 1894 by the incipient industry of internal combustion (Demirbas and Karslioglu, 2007). Since 1980s, ethanol has been widely focused as an alternative fuel for transportation (Balat and Balat; 2009). Nowadays, the various blending of gasoline and ethanol is found in the market. E10 or gasohol, a blend of 10% ethanol with 90% gasoline, is the most commonly blended and sold. This blend is acceptable for operation in conventional gasoline vehicles produced since the mid-1980s (Melendez *et al*., 2009). Moreover, the ethanol fuel also sold as E20, 20% ethanol and 80% gasoline, E85, 85% ethanol and 15% gasoline, and 100% ethanol with or without a fuel additive (Demirbas, 2005; Gallagher, 2009).

Ethanol can be produced from hydration of alkanes obtained from the cracking of petroleumor produced from fermentation process using sugar, starch or agro-residues (Demirbas, 2005). At present, the interesting source for ethanol production is agricultural wastes *i.e.* rice straw, wheat straw and cornstalks, etc. because they are the most abundant biomass that can be converted into sugar and used

a substrate in ethanol production. The production processes can be performed by acid hydrolysis, enzymatic hydrolysis and microbial fermentation (Abedinifar *et al*., 2009; Demirbas, 2005; Dias *et al*., 2010; Karimi *et al*., 2007; Lu *et al*., 2009).The ethanol which was produced by biological process are called bioethanol. Recently, bioethanol is attractively growing interest around the world (Balat and Balat; 2009). Figure 2.5 shows World tendencies of ethanol production from 2005 to 2012.

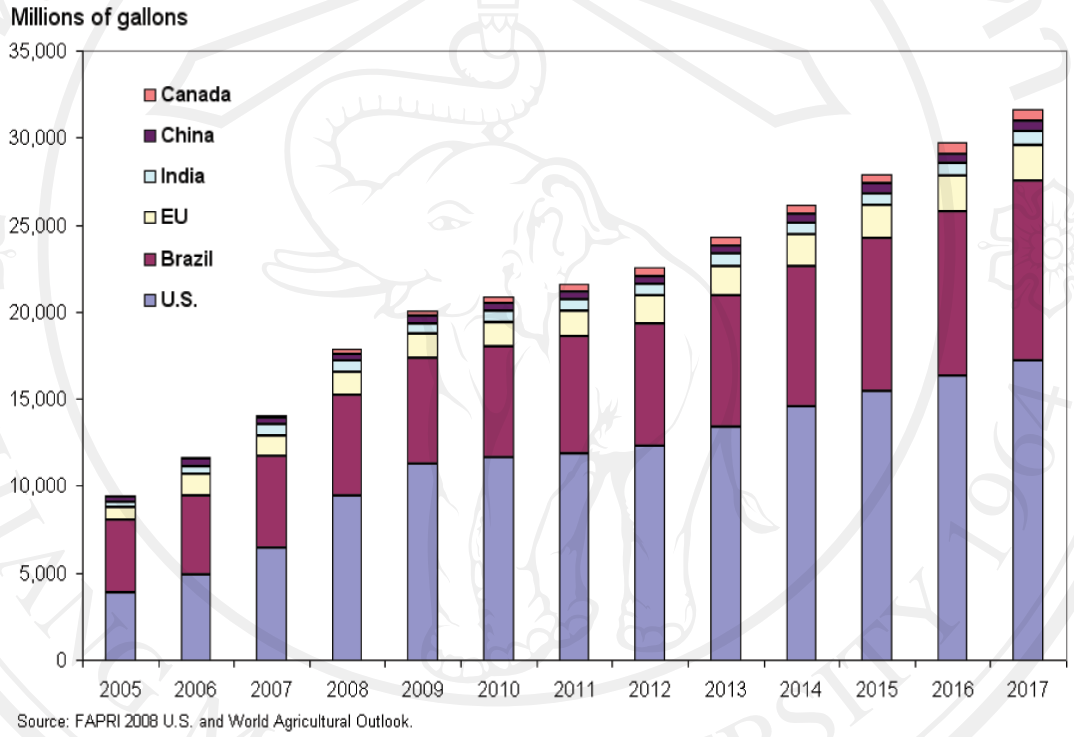

Figure 2.5: World tendencies of ethanol production from 2005 to 2012 (Food and Agricultural Policy Research Institute, 2008).

2.5.2 Ethanol Fermentation

Ethanol fermentation is a biological process which fermentable compounds such as sugars are converted into ethanol by microorganisms. There are three steps of fermentation: the formation of solution of fermentable sugars, the fermentation of these sugars to bioethanol and the separation and purification of the ethanol, usually by distillation (Demirbas, 2005). Some sugars can be converted directly into bioethanol, but starch and cellulose must be hydrolyzed to sugar before

fermentation. Glucose is a kind of sugar which can be fermented to ethanol by wide-type *S. cerevisiae* and several other microbial strains such as *Zymomonas mobilis*, *Candida utilis* (Agustina, 2009; Lu and Mosier, 2008). However, *S. cerevisiae* cannot convert pentose i.e. xylose into ethanol (Toivari *et al*., 2004). The using of some microorganism such as *Pichia stipitis*, *Candida shehatae* and *Pashysolen tannophilus* can be solved this problem (Hahn-Hagerdal and Karhumaa, 2007 and Chandel *et al*., 2008). Moreover, the genetic engineering can be used to add the genes of pentose converting pathway in to *S. cerevisiae* (Du, 2012; Sanchez *et al*., 2010). There are several reports stated about the uses of microorganisms to produce ethanol by fermentable sugars (Table 2.7).

Table 2.7: Yeast species which produce ethanol as the main fermentation product.

Substrate	**Microorganism**	**References**
Dried longan juice	*Saccharomyces cerevisiae* *Candida utilis*	Agustina (2009)
Mahula (*Madhuca latifolia* L.) flower	*S. cerevisiae* CTCRI *Zymomonas mobilis* MTCC	Behera *et al*. (2010)
Sweet sorghum juice	*S. cerevisiae* NP01	Laopaiboon *et al*. (2009)
Glucose	*S. cerevisiae* GT4608	Liu *et al*. (2009)
Olive prunings hydrolyzed	*Candida tropicalis* NBRC 0618	Martin *et al*.(2010)
Sugar beet thick juice	*Kluyveromyces marxianus* KD-15	Oda *et al*. (2010)
Glucose and xylose mixtures	*Pachysolen tannophilus* 1771	Zhao *et al*. (2008)
Xylose	*Candida shehatae* UFMG HM52.2 *Pichia stipitis* NRRL Y-7124	Dussan *et al*. (2011)

One of the most common pathways used by many microorganisms which metabolize glucose into ethanol is Embden-Meyerhof (EM) pathway or glycolysis (Lin and Tanaka ,2006). It is a pathway of glucose catabolism. It is widely found in microorganisms as well as plants and animals (Chauhan, 2008). Nevertheless, some bacteria such as *Zymomonas mobilis* has another pathway so-called Entner-Doudoroff (ED) which is the opposite of EM or glycolytic pathway of glucose consumption in anaerobic condition (Lin and Tanaka, 2006).

S. cerevisiae is the most common microbe used for ethanol production by using hexoses such as glucose under anaerobic condition (van Maris *et al*., 2006*;* Demirbas, 2005). The anaerobic ethanol production rate in defined media is as high as 30 mmol g biomass^{-1}h^{-1}at 30ºC (Bakker *et al*., 2001). *S. cerevisiae* contains complex system for hexose transport (van Maris *et al*., 2006). Glucose dissimilation was processed by the Embden-Meyerhof pathway (Figure 2.6). This pathway oxidizes glucose into 2 pyruvate resulting in the net formation of 2 ATP per glucose. Under anaerobic condition, the NADH formed by glyceraldehyde-3-phosphate dehydrogenase isreoxidizedby alcoholic fermentation and pyruvate is further reduced to ethanol with the release of CO_2 (van Maris *et al*., 2006; Bai *et al*., 2008). Theoretically, the yield is 0.511 for ethanol and 0.489 for CO_2 on a mass basis of glucose metabolized (Bai *et al*., 2008; Demirbas, 2005). However, in practice, the microorganisms use some of glucose for growth and the yield is less than 100% (Demirbas, 2005).

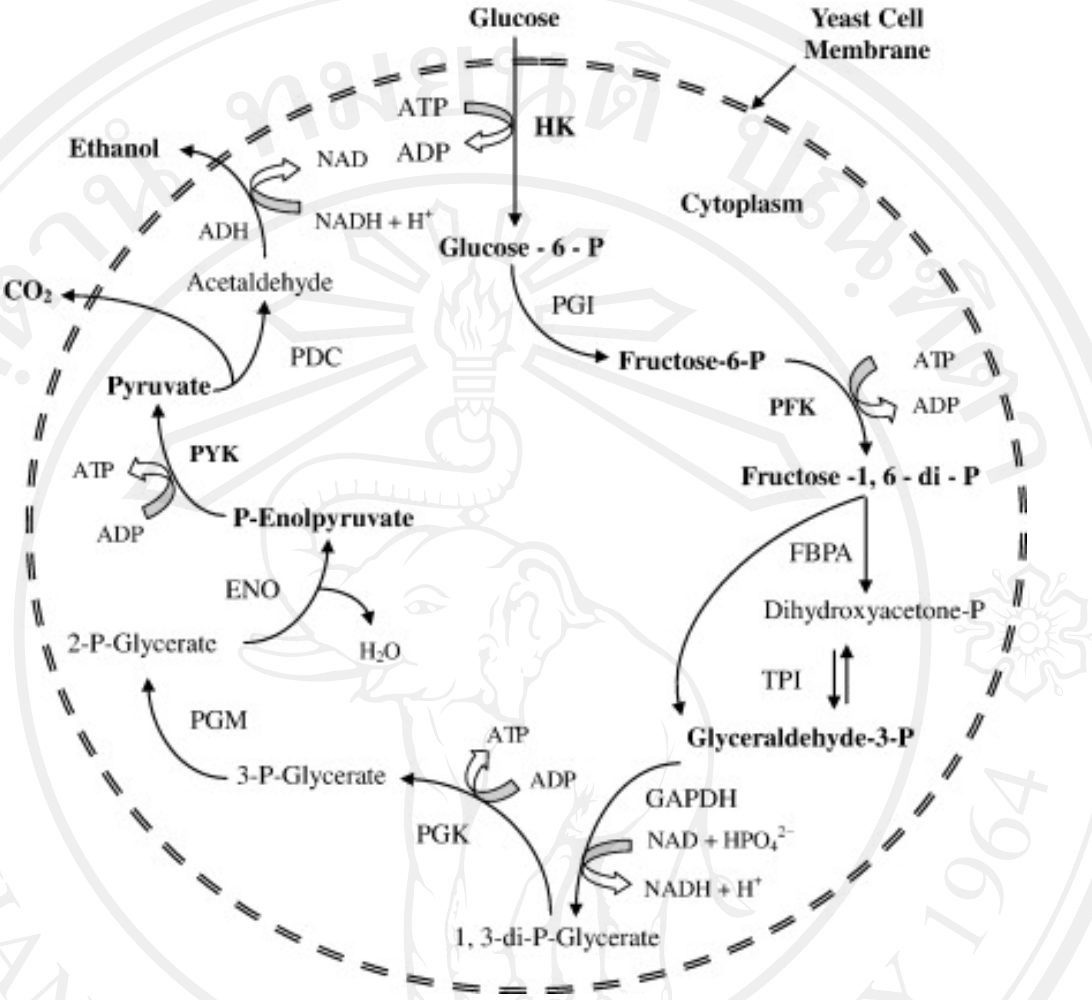

Figure 2.6: The Embden-Meyerhof pathway of alcohol fermentation in yeast (*Saccharomyces* spp.) (Bai *et al*., 2008).

The performance parameters of fermentation are: temperature range, pH range, alcohol tolerance, growth rate, productivity, osmotic tolerance, specificity, yield, genetic stability, and inhibitor tolerance (Demirbas, 2005). One of the obstacles in ethanol production from lignocellulosic material is the inhibitor which can decrease ethanol productivity or ethanol production rate. Fermentation inhibitors have been divided into five groups; substances released during pretreatment (acetic acid and extractives such as terpenes, alcohols and tannins), sugar degradation products (furfural, hydroxymethyl furfural, laevulinic acid, formic acid and humic substances), lignin degradation products (wide range of aromatic and polyaromatic compounds), fermentation products (ethanol, acetic acid, glycerol, lactic acid) (Gredd and Saddler, 1995).

2.6 Ethanol Production from Agricultural Wastes

There is an increasing interest worldwide to find out new and cheap carbohydrate sources for bioethanol production (Mohanty *et al*., 2009). However, biomass sources should be highly considered in several issues (Gnansounou *et al*., 2005) such as chemical composition, cultivation practices, availability of land and land use practices, use of resources, energy balance, emission of greenhouse gases, acidifying gases and ozone depletion gases, absorption of minerals to water and soil, use of pesticides, soil erosion, contribution to biodiversity and landscape value losses, farm-gate price of the biomass, logistic cost (transport and storage of the biomass), direct economic value of the feedstocks taking into account in the co-products, creation or maintaining of employment, and water requirements and water availability. Agricultural residues are the interesting sources for ethanol production because they are the most abundant and cheap feedstocks (Balat and Balat, 2009). The substrate price is estimated at about 60 – 70% of ethanol price (Balat and Balat, 2009). Therefore, the ethanol cost is reduced if the cheaper substrate is used. Environmentally, this energy source generates very low net greenhouse gas emission so it can reduce environmental impacts (Galbe and Zacchi, 2002). Most of agro-residues are lignocellulosic materials with low sugar content (Table 2.9). Hence, these materials are potential candidates that should be hydrolyzed into sugar for ethanol production. However, bioconversion of lignocellulosic materials into ethanol is difficult, the factors that should be considered are the resistant nature of biomass in breaking down, the various types of sugar released and the costs of collecting and storing of low density lignocellulosic materials (Balat and Balat, 2009).

There are many processes in degrading agricultural wastes which can utilize less sugar to increase high amount of reducing sugar for ethanol production such as acid hydrolysis, enzyme hydrolysis and using microbes in degradation. There are three main stages of ethanol production from agricultural wastes *i.e.* pre-treatment, hydrolysis and fermentation (Figure 2.8) (Balat and Balat, 2009; Margeot *et al*., 2009).

Table 2.8: Sugar content in some lignocellulosic materials.

Material	Total sugar (mg/l)	Reducing sugar (mg/l)
Wheat straw	0.4	0.0175
Rice straw	0.7	0.0175
Rice husk	2.6	0.0325
Bagasse	1.3	0.175

Source: Patel *et al*. (2007)

Nowadays, there are many studies about ethanol production from lower cost substrates especially agricultural wastes such as corn cob, rice hull, bagasse, wheat straw, rice straw and also fruit wastes (Abedinifar *et al*., 2009; Kuhar *et al*., 2008; Lu *et al*., 2009; Mohanty *et al*., 2009; Patel *et al*., 2007; Shrestha *et al*., 2008; Sumphanwanich, 2006; Yoswathana *et al*., 2010; Prasad *et al*., 2007).

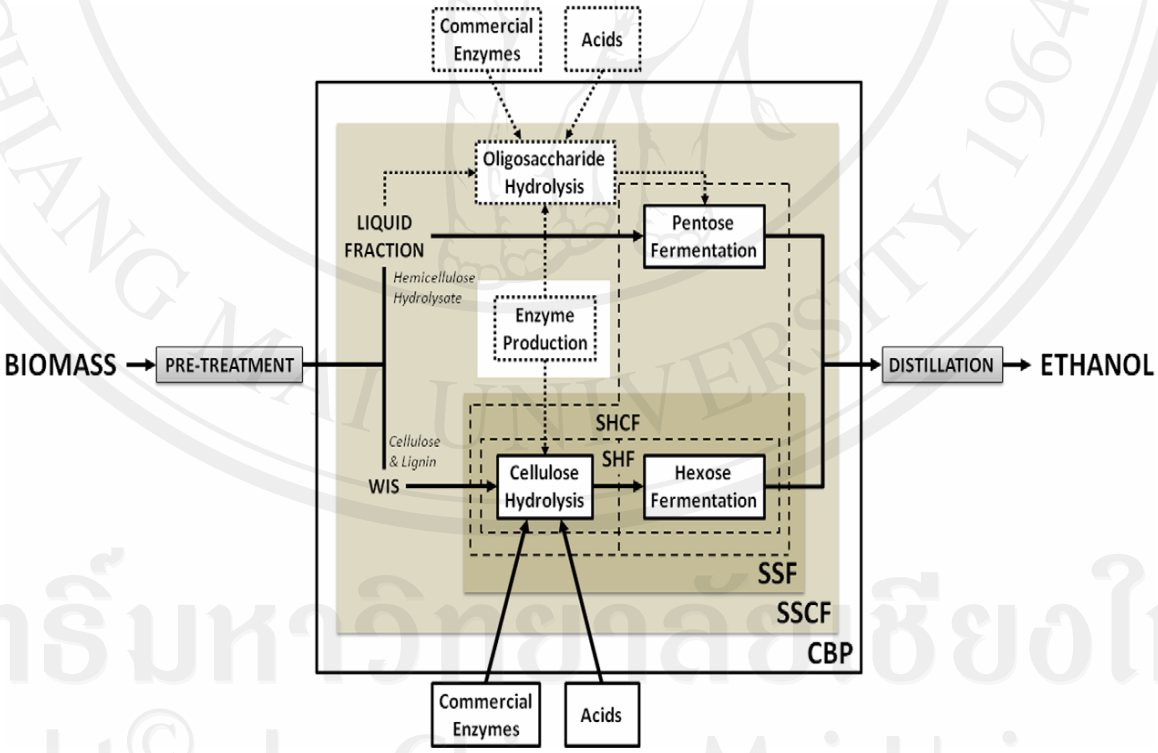

Figure 2.7: Lignocellulosic ethanol bioproceses; SSF = simultaneous saccharification and fermentation, SSCF = simultaneous saccharification and cofermentation, CBP = consolidated bioprocessing (Girio *et al*. , 2010).

2.7 Pretreatment of Agricultural Wastes

There are several pretreatments used in pretreating agricultural wastes (Table 2.9). However, the purpose of pretreatment process is to degrade lignin and hemicellulose, break down cellulose crystallinity and increase the porosity of materials for enzyme reaction in order to increase reducing sugar content (Sun and Cheng, 2002). The lignocellulosic material which was pretreated can increase production up to more than 90% (Hamelinck *et al*., 2005).

Table 2.9: Processing of pretreatment from different raw materials.

Raw material	Processing	Reference
Wood	Acid hydrolysis or Enzymatic hydrolysis	Balat and Balat, 2009
Straw	Acid hydrolysis or Enzymatic hydrolysis	
Corn stalk	Acid hydrolysis	
Rape Seed	Acid hydrothermal hydrolysis and enzymatic hydrolysis	Lu *et al*., 2009
Rice straw	Acid hydrolysis	Karimi *et al*., 2006
Sugarcane bagasse, paddy straw and wheat straw	Alkali hydrolysis, acid hydrolysis, enzymatic hydrolysis and microbial pretreatment	Prasad, 2008

2.7.1 Physical pretreatment

This process pretreats materials by using chipping, grinding and/or milling to increase accessible surface area and pore size of materials and breaks down the cellulose crystallinity to improve enzymatic hydrolysis and biodegradability of agro-residues (Taherzadeh and Karimi, 2008). The material size after chipping is about 10–30 mm and after grinding or milling is about 0.2–2 mm (Sun and Cheng, 2002). Moreover, irradiation, hydrothermal, high pressure steaming and pyrolysis are used in the physical pretreatment as well. Another defect of this pretreatment process is its high energy requirement and it is not suitable to use for industry applications (Taherzadeh and Karimi, 2008).

2.7.2 Acid hydrolysis

Acid hydrolysis has been used to pretreat lignocellulosic material since 1819 and there are many applied technologies used in converting the materials into sugars (Balat and Balat, 2009; Harris and Beglinger, 1946). This process is divided into 2 types: using concentrated acid at mild temperature condition and using diluted acid at high temperature and high pressure condition (Badger, 2002; Girio *et al*., 2010).

2.7.2.1 Concentrated acid

Highly concentrated sulfuric or hydrochloric acids are used in this process. The popularly type of acid are 72% sulfuric acid or 41% hydrochloric acid. Also, some other acids are used in this process such as 100% trifluoroacetic acid (Demirbas, 2005; Fengal and Wegener, 1983). In the first stage hydrolysis, the material that is pretreated by physical pretreatment is mixed with 10% sulfuric acid and heated to 100ºC for 2 to 6 hr. The second stage hydrolysis, the solid residue from the first state is dewatered and soaked in a 30 to 40% concentration of sulfuric acid for 1 to 4 hr. After that this solution is dewatered and dried in order to increase acid concentration up to approximately about 70%. After incubated at 100ºC for 1 to 4 hr,

the solids are removed and sugar and acid solution are recovered. The sugar/acid solution from the second stage hydrolysis can be recycled and used as solution in the first stage hydrolysis (Badger, 2002; Demirbas, 2005).The advantages of concentrated acid hydrolysis are high sugar recovery production (Table 2.10). By using low temperature, the operation cost and the cost of material such as tanks or piping are reduced. Moreover, it can also reduce the needless product occurred from high temperature condition and by using recycled acid solution, the process cost can be further reduced (Badger, 2002; Demirbas, 2005; Girio *et al*., 2010). The disadvantages of this process are that the process was slower than diluted acid and higher cost occurs from acid recovery and hydrolysate neutralization before fermentation (Demirbas, 2005).

Table 2.10: Yields of bioethanol by concentrated sulfuric acid hydrolysis from cornstalk.

Amount of corn stalk (kg)	1000
Cellulose content (kg)	430
Cellulose conversion and recovery efficiency	0.76
Ethanol stoichiometric yield	0.51
Glucose fermentation efficiency	0.75
Ethanol yoeld from glucose (kg)	130
Amount of corn stalk (kg)	1000
Hemicelluloses content (kg)	290
Hemicelluloses conversion and recovery efficiency	0.90
Ethanol stoichiometric yield	0,51
Xylose fermentation efficiency	0.50
Ethanol yoeld from xylose (kg)	66
Total ethanol yield from 1000 kg of cornstalks	196 kg (225.7 l = 59 gallons)

Source: Demirbas (2005)

2.7.2.2 Diluted acid

Diluted acid hydrolysis is carried out under high temperature and high pressure condition and less reaction time (Badger, 2002; Demirbas, 2005). As an example, using 1% sulfuric acid as diluted acid in pretreatment process and incubated at 236.85ºC in continuous flow reactor can produce sugar around 50% of sugar recovery efficiency (Badger, 2002; Demirbas, 2005). There are two reaction steps in this process. In the first reaction, substrate is hydrolyzed by acid into sugar and the sugar is further converted into other products in the second reaction (Badger, 2002).

Lu *et al*. (2009) used diluted acid hydrolysis in their study and found that the most optimal condition in rapeseed straw pretreating is using 1% sulfuric acid and 20% solid content at 100ºC for 10 min.

The main advantage of diluted acid hydrolysis is the fast rate of reaction (Badger, 2002). Furthermore, less acid is needed (Galbe and Zacchi, 2002). The biggest disadvantage of this process is low sugar yield production (Badger, 2002). Moreover, high temperature used to achieve conversion of lignocellulose into glucose in this process is another disadvantage because it causes sugar degradation and equipment corrosion (Galbe and Zacchi, 2002). Other degradation products can be poisonous to microorganisms they can also inhibit ethanol fermentation process (Badger, 2002; Larsson *et al*., 1999).

2.7.3 Enzymatic Hydrolysis

Enzymatic hydrolysis is a basic method of lignocellulosic material hydrolysis that has been used since 1960s (Keller, 1996). This method is usually employed after pretreating by some kind of pretreatments such as physical pretreatment and chemical hydrolysis in order to increase the effectiveness of enzymatic processes because the crystalline structure of lignocellulose is broken and lignin is removed causing the exposure of cellulose and hemicellulose (Badger, 2002; Galbe and Zacchi, 2002; Margeot *et al*., 2009). Abedinifar *et al*, (2009) reported that the pretreatment of rice straw by diluted-acid hydrolysis and using enzymatic hydrolysis had higher sugar yield than untreated rice straw.

2.7.4 Microbial Pretreatment

Microorganisms can be used also to treat the agro-residue. Various types of fungi are used to treat many agricultural wastes (Table 2.11). Hemicelluloses and lignin are usually degraded but cellulose is more resistance to microbial degradation (Taherzadeh and Karimi, 2008). Furthermore, the agricultural wastes have only carbon sources for microbial growth, therefore, adding nitrogen sources into substrate can promote microbial growth and improve efficiency of degradation for increasing sugar production (Hammel, 1996).

Table 2.11: Microbial pretreatment of agricultural wastes**.**

Substrates	Microbes	References
Rice straw	*Phanerocheate chrysosporium*	Bak *et al*., 2009
Corn fiber	*P. chrysosporium*	Shrestha *et al*., 2008
Wheat straw	*Pycnoporus cinnabarinus* *P. chrysosporium*	Kuhar *et al*., 2008
Wheat straw and rice straw	*Pleurotus sajor-caju* *Aspergillus niger* *Aspergillus awamori* *P. chrysosporium*	Patel *et al*., 2007
Bamboo residues	*Coriolus versicular*	Zhang *et al*., 2007
Pinusradiata wood chips	*Gloeophyllum trabeum*	Monrroy *et al*., 2010
Paddy straw, wheat straw and sugarcane bagasse	*A. niger* *A. awamori* *P. chrysosporium* *Trichoderma reesei* *T. viride*	Havannavar and Geeta, 2007
Corn stover	*Cyathus stercoreus* *P. chrysosporium*	Keller *et al*., 2003
Wheat straw	*Poria subvermispora* *Irpex lacteus*	Salvachua *et al*., 2011
Cassava waste	*A. niger* *T. reesei*	Fungsin *et al*., 2007

Havannavar and Geeta (2007) used 6 fungal cultures to degrade agricultural wastes in order to release reducing sugars (Table 2.12). The results showed that the fungal cultures *T. reesei* yielded maximum reducing sugar of 22.30 mg/g in paddy straw, 25.56 mg/g in wheat straw and 28.56 mg/g in sugarcane bagasse.

Table 2.12: Fungal pretreatment on hydrolysis of paddy straw, wheat straw and sugarcane bagasse.

Cultures	**Paddy straw**	**Wheat straw**	**Sugarcane bagasse**
	Reducing sugar (mg/g)	Reducing sugar (mg/g)	Reducing sugar (mg/g)
Aspergillus niger	7.48	7.88	9.15
Aspergillus awamori	5.51	5.87	7.50
Pleurotus spp.	9.63	11.05	12.82
Phanerochaete chrysosporium	14.55	18.11	20.96
Trichoderma viridae	13.09	13.41	17.80
Trichoderma reesei	22.30	25.56	28.26
Control	1.43	1.53	1.81

Source: Havannavar and Geeta (2007)

Patel *et al*. (2007) conducted a study on ethanol production from microbial pretreated agricultural. Agricultural residues used in the study were wheat straw, rice straw, rice husk and bagasse. The authors found that wheat straw and rice straw, pretreatment with *A. niger* and *A. awamori* and fermented by *S. cerevisiae* had the highest amount of ethanol about 2.5 and 2.2 g/l respectively. For rice husk and bagasse, they were pretreated by *A. awamori* and *Pleurotus sajor-caju* and obtained ethanol yield about 8.5 and 9.8 g/l respectively

Fungsin *et al*. (2007) produced reducing sugar for ethanol production from cassava waste, which had starch as the main component, using *A. niger* TISTR 3352 in microbial pretreatment process. The results showed that the maximum yield of reducing sugar was 56.2 g/l.

Moonjai *et al*. (2006) studied the ethanol production from lignicellulosic rice by-products using *Trichoderma* sp. T-11 to hydrolyze the substrate in submerged and solid state fermentation (SSF). The fermented the hydrolyzates were fermented to ethanol production by *S. cerevisiae*. The results demonstrated that SSF gave higher ethanol yield than submerged fermentation (SmF). In the SSF, maximum ethanol yield were approximately 0.09 g/g when using rice bran or/and rice polish and SmF gave maximum ethanol yield around 0.03 g/g.

The main advantages of microbial pretreatment are low energy requirement, no corrosion of equipment and mild environmental impacts because this process requires low temperature and there is no chemical requirement such as acid (Keller *et al*., 2003). This pretreatment can remove lignin which is the inhibitor of enzyme reaction and degrade hemicellulose as well (Taherzadeh and Karimi, 2008). Furthermore, microbial pretreatment can produce less poisonous product and inhibitors for ethanol fermentation (Keller *et al*., 2003). However, the main problems of microbial pretreatment are the difficulty of growing microorganisms, low treatment rate and it is not suitable for commercial applications (Han and Challihan, 1974; Sun and Cheng, 2002; Taherzadeh and Karimi, 2008).

Several pretreatment methods are used to pretreat agricultural wastes and lignocellulosic material. However, each pretreatment method has different advantages and disadvantages (Table 2.13). Therefore, it is important to apply the appropriate methods to degrade the suitable material for suitable product.

Table 2.13: Comparison of Dilute-acid hydrolysis, concentrated-acid hydrolysis, enzymatic hydrolysis and microbial pretreatment.

Comparing variable	Dilute-acid hydrolysis	Concentrated-acid hydrolysis	Enzymatic hydrolysis	Microbial pretreatment
Mild hydrolysis conditions	No	Yes	Yes	Yes
High yields of hydrolysis	No	No	Yes	No
Product inhibition during hydrolysis	No	No	Yes	No
Formation of inhibitory by-products	Yes	Yes	No	No
Low cost of catalyst	Yes	No	No	Yes
Short time of hydrolysis	Yes	No	No	No

Source: Taherzadeh and Karimi (2008)

2.8 *Aspergillus niger*

A. niger is a filamentous ascomycete that is the most common species of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found (Samson *et al*., 2001; TSCA, 1997).The main component of *A. niger* cell wall is carbohydrate which is composed of about 73-83%, of four sugars including glucose, galactose, mannose, and arabinose, 9-13% of hexosamine which is glucosamine and galactosamine, 2-7% of fat and 0.5-2.5% of protein, etc. (Johnston, 1965).

A. niger can grow on a wide variety of substrates in solid state and submerge and surface fermentation but it has highest specific growth rate in solid state fermentation (Torres *et al*., 1998; TSCA, 1997). It is ubiquitous in the nature and commonly found as a saprophyte growing on dead leaves, stored grain, compost piles, and other decaying vegetation (TSCA, 1997).

Various strains of *A. niger* are used in the industrial preparation of citric acid, gluconic acid and enzymes such as α-amylase, amyloglucosidase, cellulase, lactase, invertase, pectinase, and acid protease which are important in the biotechnology industry (Baker, 2006; Bennett, 1985; TSCA, 1997; Ward,1989). Furthermore, the

strains of *A. niger* can also produce many enzymes, for instances, cellulase, β-glucosidase, α-galactosidase, xylanase, amylase, glucoamylase, polygalacturonase, CMCase, tannase, β-glucannase, lactase, pectinase, lipase and invertase. (Arulpandi *et al*, 2008; Glazer and Nikaido, 1995; Pandey *et al*, 1999; Schauer and Borriss, 2004).

2.9 *Aspergillus foetidus*

A. foetidus is a filamentous fungus. Eltem *et al*. (2004) reported that its colonies are 4.5-6.0 cm in diameter after culturing at 27ºC for 2 weeks, its texture is lanose, margin white, yellowish center. The basal mycelium sporulation is not dense. Sporulation is more at colony margin and center. Conidial heads are blackish brown and reverse bright yellow. The odor is indistinguishable.

A. foetidus can produce many enzymes such as xylanase (Chapla *et al*., 2010; Shah and Madamwar, 2005), amylase (Michelena and Castillo , 1984), lipase (Nair and Bone, 1987), pectinase (Cavalitto *et al*., 1996) and α-galactosidase (Liu *et al*., 2007). Moreover, it can also produce other products such as citric acid (Chen, 1993)

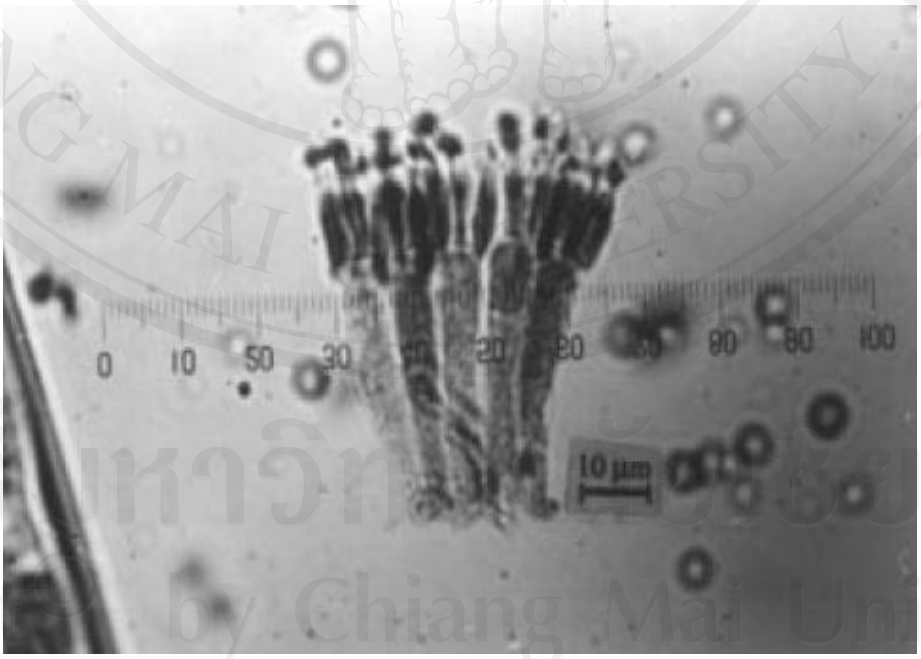

Figure 2.8: Phialide of *A. foetidus* (Eltem *et al*., 2004)

2.10 *Trichoderma reesei*

T. reesei (also known as *Hypocrea jecorina*) is a mesophilic and filamentous fungus which is one of the most efficient xylanase and cellulase producers (Xiong, 2004). It is a useful fungus for the large-scale production of enzymes for a variety of industrial applications because it has efficient secretory ability, the cheap and easy cultivation and can grow on a single carbon source, such as cellulose or xylan (Xiong, 2004). It is widely used in the production of many enzymes such as cellulase (Oinonen *et al*., 2005; Wen *et al*., 2005; Zhang *et al*., 2012), xylanase (Zhang *et al*., 2012) and amylase (Throndset *et al*., 2010).