CHAPTER 2

Literature Reviews

This study emphasizes on an integrated process for value adding of corncob, a low cost-agricultural waste, to high valuable products of xylooligosaccharides (XOs) and bioethanol using a chemo-enzymatic method. The literature reviews relating to this study are described in this chapter.

2.1 Lignocellulosic materials (LCMs)

Plant cell walls are divided into two parts of primary cell wall and secondary cell wall based on chemical component and structure. Generally, secondary cell walls are the source of fermentable sugar and more abundant than primary cell walls (Bayer et al., 2010) (Figure 2.1A).

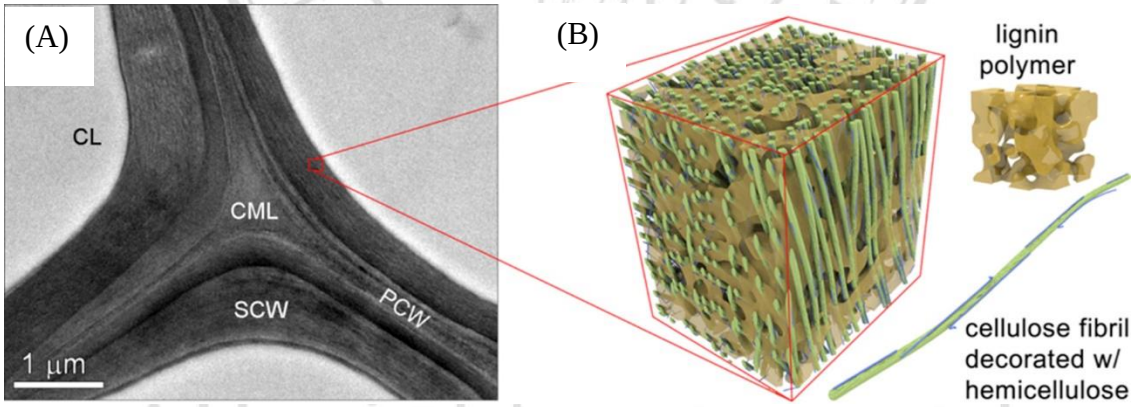

Figure 2.1 (A) The transmission electron micrograph of maize cell wall and (B) the structure of the lignocellulose framework in secondary cell walls (CL, cell lumen; CML, compound middle lamella; SCW, secondary cell wall; and PCW, primary cell wall).

Source: Bomble et al. (2017)

LCMs or lignocellulosic materials are an organic compound occurring in secondary cell walls of plant (Bayer et al., 2010). It composes of three main components of cellulose, hemicellulose and lignin (Figure 2.1B). The structural polymer of plant cell wall is cellulose with an association of hemicellulose, while, lignin plays a significant role on cell wall protection (Mousdale, 2008). LCMs are more attractive for high value chemical production because of the availability in a massive quantity with an inexpensive price (de Jong and Gosselink, 2014). Among the source of LCMs, agricultural residues such as rice straw, sugarcane bagasse, corncob and corn stovers etc., are an important source of LCMs according to the biorefinery strategy. The different contents of celluloses, hemicelluloses and lignins in various agricultural residues are listed in Table 2.1.

Table 2.1 Approximate contents of celluloses, hemicelluloses and lignins in various agricultural residue.

Type of agricultural residues	**Composition (%)**			**References**
	Celluloses	**Hemicelluloses**	**lignins**	
Barley straw	37	21	22	Yang et al. (2015)
Camellia oleifera shell	14	29	44	Zhu et al. (2013)
Cashew apple bagasse	21	16	34	de Barros et al. (2017)
Coffee pulp	15	10	10	Pleissner et al. (2016)
Corncob	46	37	15	Brar et al. (2016)
Corn stover	37	21	14	Qing et al. (2017)
Empty fruit bunch of oil palm	35	18	23	Tan et al. (2016)
Rice husk	34	15	19	Dagnino et al. (2018)
Rice straw	43	25	9	Akhtar et al. (2017)
Sugarcane bagasse	39	24	21	Liu et al. (2015)
Wheat straw	39	27	13	Narra et al. (2015)

2.1.1 Celluloses

Celluloses are one of the most important organic compounds in the world. They are synthesized by numerous living organism such as marine animal, plant, bacteria and fungi. In 1839, the powder form cellulose had been firstly isolated from plant tissue. After that, the structure of cellulose had been determined in 1920 (Trache et al., 2016). Celluloses the most abundant amphiphilic polysaccharides are a linear homopolymer made up from glucose linked by β-(1→4)-glycosidic bond (Coseri, 2017; Menon and Rao, 2012; Nagarajan et al., 2017). The amphiphilic property of cellulose is resulted from an equatorial orientation of 3 molecules of anhydrous glucose (hydrophilic property) and axial orientation of the hydrogen atoms from its C-H bonds (hydrophobic property) (Nagarajan et al., 2017). Asymmetric celluloses chain contains two different end-unit of a reducing and a non-reducing end (Figure 2.2). The carbonyl group poses at the reducing end, while supplementary OH group in position C-4 poses at the non-reducing end of celluloses chain (Trache et al., 2016). The degree of polymerization of celluloses is commonly a number of 800–1,000 units.

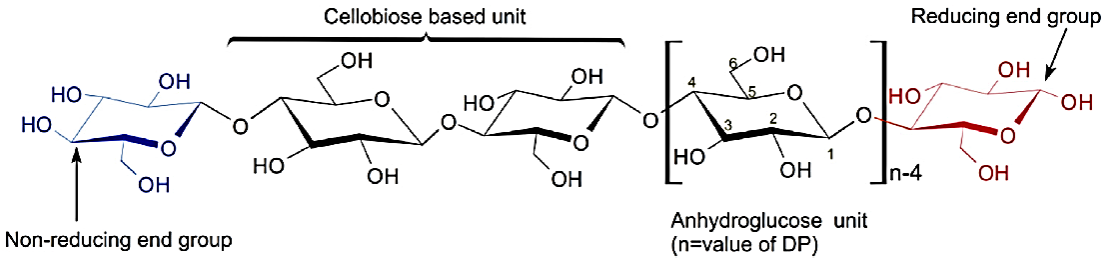

Figure 2.2 Molecular structures of celluloses.
Source: Trache et al. (2016)

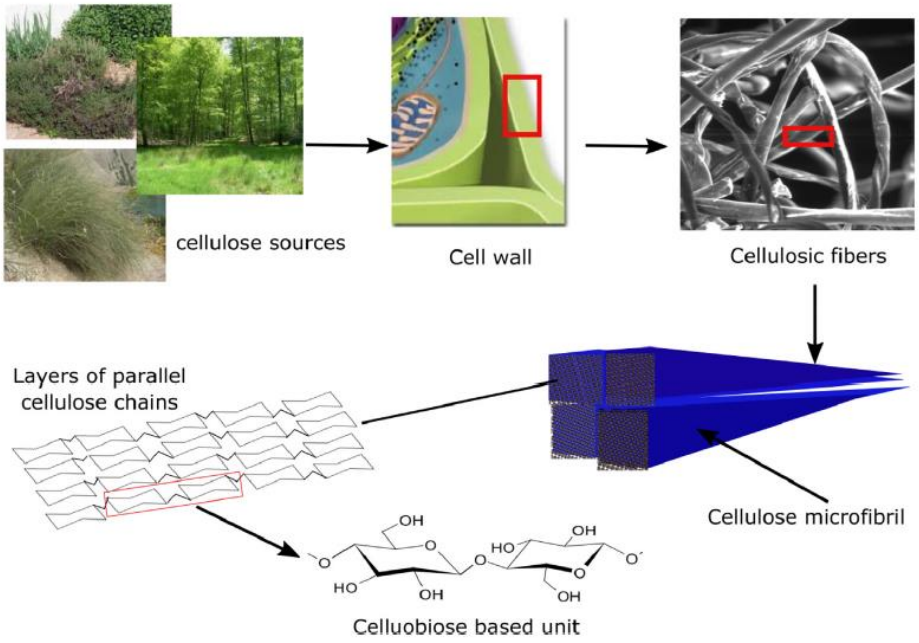

Figure 2.3 Celluloses organization in structural level from natural celluloses sources.
Source: Trache et al. (2016)

Celluloses can absorb 8–14% water and soluble in dilute acid and insoluble in water. In alkaline solution, celluloses become swell and low molecular weight celluloses are dissolved (Harmsen, 2010). Celluloses normally occur in nature in crystalline form, while amorphous region is found as a minor structure. The straight chains of cellulose assemble together into the bundle, which called cellulose microfibril (Figure 2.3) (de Jong and Gosselink, 2014; Xu, 2010). Linear celluloses chains are bound by hydrogen bonds and hydrophobic interactions or van der Waals interactions. Accordingly, these hydrogen bonds and hydrophobic interactions make celluloses resist chemical and biological hydrolysis (Nagarajan et al., 2017). The strong hydrogen bonds between the celluloses chains can be only broken down by aprotic solvent, strong acid and strong alkali solution. After strong alkali treatment, the neutralized mixture of celluloses is separated into 3 parts of α-cellulose (insoluble fraction), β-cellulose (precipitate fraction) and γ-cellulose (soluble fraction) (Xu, 2010). The hydrolysis of celluloses is divided into 2 methods of hydrolysis by enzyme (cellulase), and acid hydrolysis by mineral acids (H_2SO_4 and HCl) or solid-acid catalysts (Deng et al., 2014).

2.1.2 Hemicelluloses

In LCMs, hemicelluloses are the second most abundant polysaccharides after cellulose. They are non-crystalline heteropolysaccharides, which are the most complex part of the cell wall. They represent in the inner and outer part of plant secondary cell wall. The linkages between celluloses and hemicelluloses are resulted by the hydrogen bonds. Whereas, hemicelluloses are bonded to lignin by covalent bonds of α-benzyl ether linkage and bonded to acetyl unit by ester linkage (Xu, 2010). Hemicelluloses are amorphous polysaccharides with degree of polymerization of 80–200 (Ren and Sun, 2010). They compose of various types of pentose sugar, hexose sugar and uronic acid. The most commonly sugar that found in hemicelluloses are D-xylose, L-arabinose, D-galactose and D-mannose. While, L-rhamnose and L-fucose are found as minor component. D-glucuronic, 4-*O*-methyl-D-glucuronic acid and D-galacturonic acid are a group of uronic acids presented in hemicelluloses (de Jong and Gosselink, 2014; Ren and Sun, 2010; Xu, 2010). The factors affecting the hemicelluloses composition are plant source, tissue type, plant maturation and cultivation place and season (de Jong and Gosselink, 2014; Sedlmeyer, 2011). Hemicelluloses can be categorized into 3 groups namely; xyloglycan (xylan), mannoglycan (mannan) and mixed-linkage β-glucan (Naidu et al., 2018; Ren and Sun, 2010) as shown in Figure 2.4. Among these, xylan-type hemicelluloses are considered as the most abundant hemicellulose in secondary cell wall of hardwood (Angiosperms), grass and herbaceous plant. In softwood (Gymnosperms), glucomannan and galactoglucomannan are the main hemicelluloses components (de Jong and Gosselink, 2014). The types of hemicelluloses, their occurrence in nature and chemical components are described in Table 2.2.

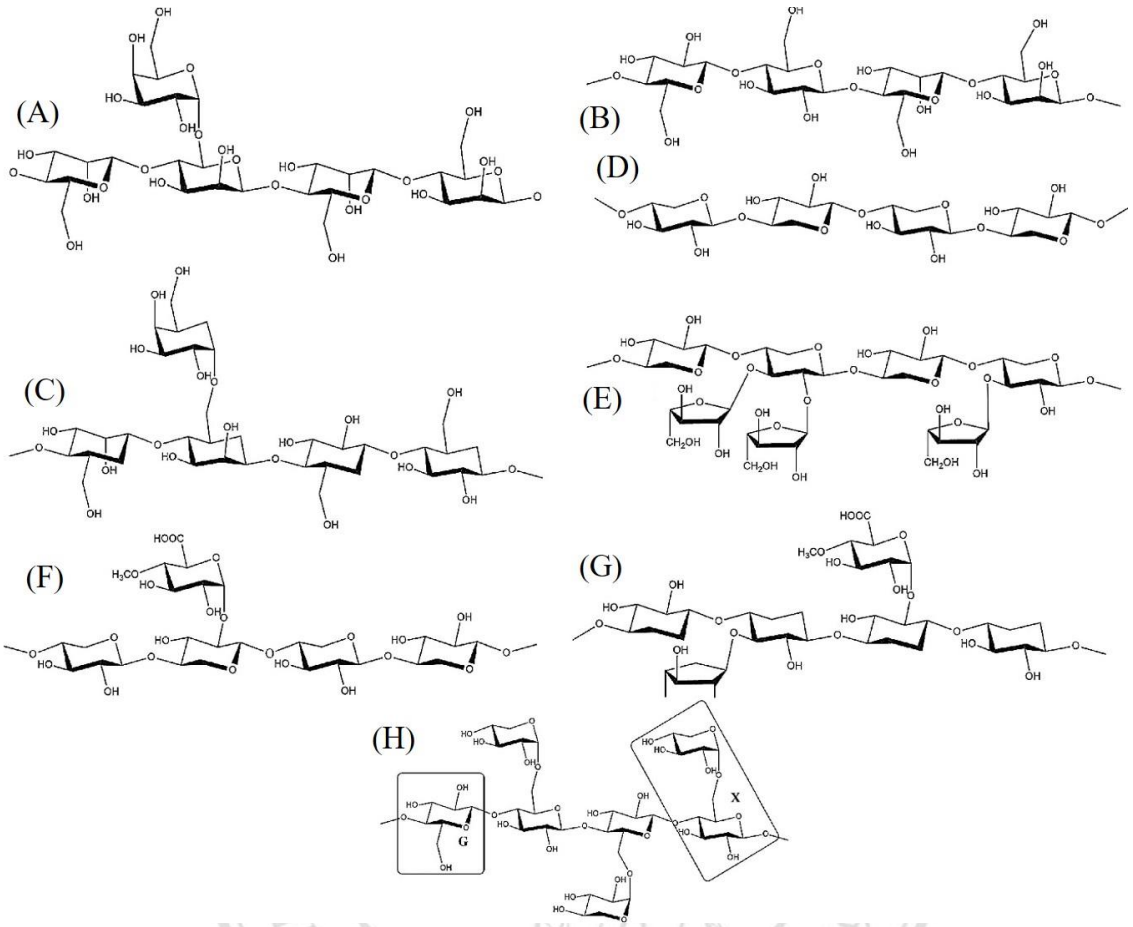

Figure 2.4 Various categories of hemicelluloses: (A) galactomannan, (B) glucomannan, (C) galactoglucumannan, (D) homoxylan, (E) arabinoxylan, (F) glucuronoxylan, (G) arabinoglucuronoxylan and (H) xyloglucan.

Source: Naidu et al. (2018)

Table 2.2 Types of hemicelluloses, their occurrences in nature and chemical components.

Types	Occurrences	Backbone and linkages	Substitutions
Xylans (Xyloglycans)			
Homoxylans (X)	Green algae (*Caulerpa* sp.)	β-(1→3)-D-xylopyranosyl or	-
	Red seaweed (*Palmariales* and *Nemaliales*)	β-(1→3)-D-xylopyranosyl and β-(1→4)-D-xylopyranosyl	-
Arabinoxylans (AX)	Cereal grain (wheat, rye, barley, oat, rice and corn), endospermic and pericarp tissue	β-(1→4)-D-xylopyranosyl	α-L-arabinofuranosyl, ferulic acid and coumaric acid
Glucuronoarabinoxylans (GAX)	Nonendospermic tissue of cereal grain	Arabinoxylans	α-D-glucopyranosyl uronic acid, 4-*O*-methyl-D-glucuronic acid and α-L-arabinofuranosyl
Glucuronoxylans (GX)	Hardwood, fruit, storage tissue, fruit fiber and sugar beet pulp	β-(1→4)-D-xylopyranosyl	4-*O*-metylglucoronic acid, acetyl group, α-D-glucuronic acid and galacturonic acid

Table 2.2 Types of hemicelluloses, their occurrences in nature and chemical components. (continued)

Types	Occurrences	Backbone and linkages	Substitutions
Arabinoglucuronoxylans (AGX)	Coniferous species and cell wall of lignified supporting tissue	Glucuronoxylans	2-*O*-α-D-glucopyranosyl uronic acid, 4-*O*-methyl-D-glucuronic acid and α-L-arabinofuranosyl unit
Complex heteroxylans (CHX)	Cereal, seed, gum exudate, mucilage, and leaves and bark of dicotyl plant	β-(1→4)-D-xylopyranosyl	α-(1→2) and α-(1→3) arabinose
Mannans (Mannoglycans)			
Glucomannans (GM)	Coffee bean and softwood	β-(1→4)-D-mannopyranosyl and D-glucopyronosyl	Mannose and acetyl group
Galactoglucomannans (GGM)	Softwood	β-(1→4)-D-mannopyranosyl and D-glucopyronosyl	α-D-galactopyranosyl and acetyl group
Mixed-linkage β-glucan			
β-glucans (β-(1→3, 1→4)-glucan; linear (1,3;1,4)-β-glucans)	Subaleurone and endospermic of cereal grain cell wall and nonendospermic tissue of gramineous and monocotyl plant	β-(1→4)-D-glucopyranosyl with mix β-(1→3) and (1→4)-linkage	-

Table 2.2 Types of hemicelluloses, their occurrences in nature and chemical components. (continued)

Types	Occurrences	Backbone and linkages	Substitutions
Xyloglucan	Dicotyl plant, grass, onion and fir trees	β-(1→4)-D-glucopyranosyl	D-xylopyranosyl, D-galactopyranosyl, D-glucopyranosyl and α-L-arabinofuranosyl

Source: Modified from de Jong and Gosselink (2014); Ebringerová and Heinze (2000); Ren and Sun (2010)

2.1.3 Lignins

Lignins are a non-carbohydrate polymer composed of a highly branch and amorphous molecules with variable compositions. Lignins are the plant phenolic polymer constructed by phenylpropane monomers with the broad composition and a variety of linkage between building units. The complexity of lignin structure is the main reason for ambiguous definition of lignins (Lu and Ralph, 2010). In higher plants, lignins are considered as the major structural components protecting the plants from environmental stress and microbial decomposition (de Jong and Gosselink, 2014).

Three essential hydroxycinnamyl alcohols or monoolignols in lignins are *p*-coumaryl alcohol, coniferyl alcohol and sinaphyl alcohol. The phenylpropane monomers which named *p*-hydroxyphenyl-, guaiacyl- and syringyl- unit are derived from these monoolignols as shown in Figure 2.5 (Lu and Ralph, 2010; Norgren and Edlund, 2014; Xu, 2010). The molecular composition and linkage type of phenylpropane monomers influence on the heterogenicity of lignin (de Jong and Gosselink, 2014). In plants, the proportion of each monomers depended on plant species, type of plant tissue and inter-connecting patterns between a monomer (Norgren and Edlund, 2014). However, recent report has been showed that lignins contain more monomers than those original ones such as coniferaldehyde, 5-OH coniferyl alcohol and acetylated monolignols (Lu and Ralph, 2010).

Generally, lignins have been categorized into three major groups of softwood lignins, hardwood lignins, and cereal straw and grass lignins based on type of building unit and variation of monomer ratio. Softwood lignins contain large amount of coniferyl alcohol. In hardwood and dicotyl fiber crop, lignins represent the variable ratio of coniferyl and sinaphyl alcohol. While, *p*-hydroxyphenylpropane structure is normally found in cereal straw and grass lignins (de Jong and Gosselink, 2014; Xu, 2010). Lignins are insoluble in water (Roopan, 2017). They are easily oxidized, dissolved in hot alkaline and bisulfite, condensed with phenol or thiol but they resist to acid hydrolysis. The reaction of lignin and nitrobenzene in hot alkaline solution generates vanillin, syringaldehyde and *p*-hydroxybenzene as final products (Lu and Ralph, 2010).

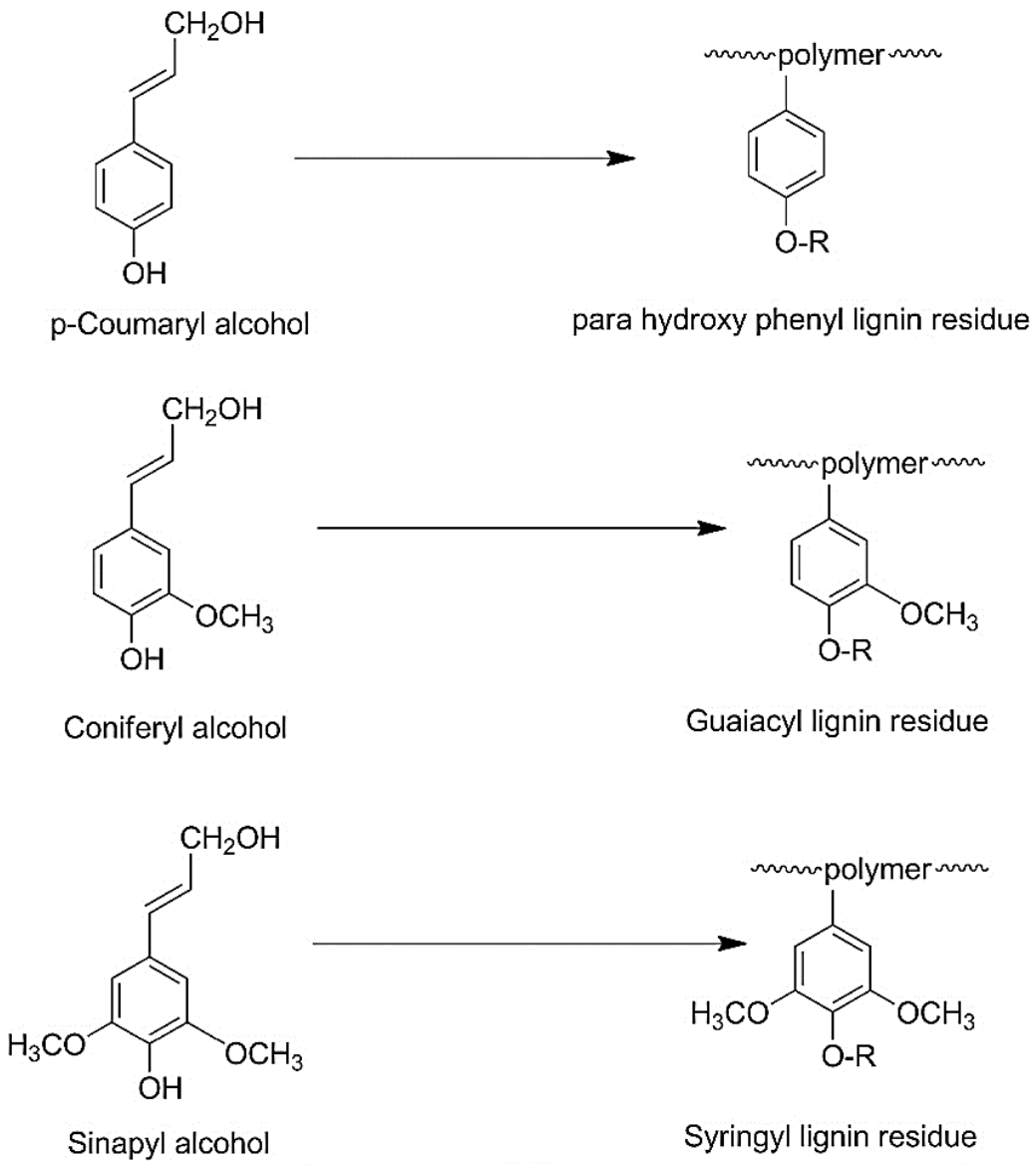

Figure 2.5 Structure of three monoolignols and three phenylpropane monomers of lignin.

Source: Roopan (2017)

2.2 Pretreatment technology for lignocellulosic materials

Pretreatment is an essentially step in the biorefinery process of LCMs because the structure of crystalline cellulose microfibrils in nature is highly resistant to enzymatic hydrolysis. Generally, the aims of pretreatment are including size reduction of materials, improving the enzymatic accessibility and hydrolysis yields, and minimizing the overall process cost and energy consumption (Balat et al., 2008; Zabed et al., 2017). The

pretreatment is one of the most expensive steps in the biorefinery process. Therefore, numerous studies have studied on the different pretreatment methods in order to find out the suitable method for each LCMs. The pretreatment can be grouped in to four categories of 1) physical pretreatment, 2) chemical pretreatment, 3) physico-chemical pretreatment and 4) biological pretreatment (Zabed et al., 2017). Process conditions, effects on biomass, advantages and disadvantages of each pretreatment method are listed in Table 2.3.

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods.

Methods	Process conditions	Effect on biomass	Advantages	Disadvantages
Physical pretreatment				
Mechanical and milling	Physically reduced biomass size by cutting, chopping or material breaking methods	- Size reduction - Increasing surface area - Decreasing crystallinity - Disruption cell structure	- Effective way to reduce the size, increase surface area and decrease crystallinity of biomass - Can be combined with other pretreatment processes	- Vast power consumption - Non-economical process - Time-consuming process
Microwave irradiation	Internal heating a biomass with electromagnetic field	- Disruption the biomass structure at the polar bonds - Vibration of internal structure - Segregation and extension of biomass structure	- Simple operation process - Effective energy consumption - Short operation time - Can be applied with chemical pretreatment	- Need to study the dielectric properties of biomass before pretreatment - High capital cost

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods. (continued)

Methods	Process conditions	Effects on biomass	Advantages	Disadvantages
Chemical pretreatment				
Concentrated acid	Using high concentration acid above >30% with low temperature at atmosphere pressure	- Separation and removal of lignin - Conversion of biomass into celluloses dextrin	- Relatively high sugar conversion rate - Generation of amorphous cellulose	- Requirement of subsequently dilute acid hydrolysis and acid removal - High toxicity and corrosion - High operational and maintenance costs - Requirement of acid recycling
Diluted acid	Using low acid concentration under high temperature with a high pressure	- Decreasing the crystallinity of cellulose - Improving cellulose hydrolysis	- Less equipment corrosion than concentrate acid pretreatment	- High energy consumption - pH adjustment is necessary - Generation of fermentation inhibitors - Poor lignin removal

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods. (continued)

Methods	Process conditions	Effects on biomass	Advantages	Disadvantages
Chemical pretreatment (continued)				
Alkali	Application of alkali solution to pretreat biomass with a heating or operation at ambient temperature in atmosphere pressure	- Saponification reaction of ester bonds between hemicellulose and, lignin or other substitutions - Increasing porous structure and internal area in biomass - Solubilization of lignin - Removing acetyl group from hemicellulose	- Less sugar degradation - Effective method for agricultural residues - Specific degradation of lignin	- pH adjustment is necessary
Ozonolysis	Using ozone gas at room temperature	- Disruption of hemicellulose and lignin structure - Increasing enzymatic hydrolysis	- Effective removal of lignin - No toxic byproduct	- The toxic properties of ozone - Expensive process - Requirement of cooling system

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods. (continued)

Methods	Process conditions	Effects on biomass	Advantages	Disadvantages
Chemical pretreatment (continued)				
Organic solvent	Mixing an organic solvent with the catalyst (inorganic acid)	- Decomposition of internal chemical bond in biomass - Rearrangement of cellulose, hemicellulose and lignin structure	- Separation of cellulose, hemicellulose and lignin in the high purity form	- Formation of fermentation inhibitor - Expensive solvent and acid - Requirement of solvent recycling
Physico-chemical pretreatment				
Steam explosion	High pressure (0.69–4.83 MPa) and high temperature (190–270°C) in short duration (1–10 min)	- Breaking down and opening the 3-D structure - Partial solubilization of hemicellulose and lignin - Lignin transformation	-Short operation time - Low environmental impact - High glucose yields - Cost effective -The most effective method for hardwood and agricultural residue	- Less effective method for softwood - Need of acid catalyst when using softwood - Generation of toxic compounds - Incomplete disruption of lignin

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods. (continued)

Methods	Process conditions	Effects on biomass	Advantages	Disadvantages
Physico-chemical pretreatment (continued)				
Ammonia fiber explosion (AFEX)	Liquid ammonia treatment at high temperature and pressure	- Reduction of cellulose crystallization - Lignin breakage - Hemicellulose degradation	- Doesn't liberate any sugar - Inactivation the reaction between lignin and enzyme - Effective method for herbaceous plants and low lignin content substrate - Low inhibitor formation	- Not suitable for lignin-rich feedstock - High energy consumption -High cost of ammonia
Ammonia recycling percolation (ARP)	Using the flowed aqueous ammonia though the biomass reactor	- Solubilization of hemicellulose - Removal of lignin and modification of lignin structure	- Strong lignin removal - Generation of high quality pretreatment product -Suitable for hardwoods and lignin-rich materials	- High energy consumption - Consideration of environmental problem - Using high content of liquid ammonia

Table 2.3 Process conditions, effects on biomass, advantages and disadvantages of different pretreatment methods. (continued)

Methods	Process conditions	Effects on biomass	Advantages	Disadvantages
Physico-chemical pretreatment (continued)				
Wet oxidation	Pretreatment of biomass at high pressure and high temperature	- Solubilization of hemicellulose and lignin	- Low inhibitor formation	- High energy consumption and capital cost - Requirement of special equipment
Liquid hot water	The water acts as dilute acid at high temperature	- Hydrolysis of hemicellulose and releasing oligomer and acetic acid	- High xylose recovery yields - Avoidance of the inhibitory formation - Environmentally friendly process	- Requirement of large amount of water - High energy demand
Biological pretreatment				
Wood-rod fungi	Activity of lignolytic enzymes from fungi	- Enzymatic degradation of lignin and hemicellulose	- Low energy consumption and inhibitor formation - Environmentally friendly process	- Low reaction rate - Difficulty in large-scale industrial application

Source: Modified from Aditiya et al. (2016); Aguilar-Reynosa et al. (2017); Arora et al. (2015); Balat et al. (2008); Chen et al (2017); de Jong and Gooselink (2014)

2.3 The enzymatic hydrolysis of lignocellulosic materials

The utilization of LCMs feedstock for production of bioethanol and value-added compounds requires multi-enzyme complex and supplemental enzyme for hydrolysis of LCMs into fermentable sugar prior to bioconvert this sugar to those chemicals. Main types of these mentioned enzymes are cellulases and hemicellulases. The microorganisms are an essential source of those lignocellulolytic enzymes due to their advantage on higher feasibility to manipulate in an industrial scale, and higher stability and catalytic activity than plant and animal sources. Many studies have reported that bacteria, fungi, yeast and actinomycetes are a good lignocellulolytic enzyme producers (Behera et al., 2017).

2.3.1 Cellulases

Cellulases are defined as a group of enzymes, which catalyze the hydrolysis of β-1,4-glucosic bond in the cellulose polymer chain (Xu et al., 2007). Cellulases are composed of three major types of enzyme namely; endo-glucanase, exo-glucanase and β-glucosidase.

2.3.1.1 Endo-glucanases

Endo-glucanases or endo-1,4-β-glucanase or 1,4-β-D-glucan 4-glucanohydrolases (E.C. 3.2.1.4), which randomly hydrolyze 1,4-β-D-glucosidic linkages in cellulose. The hydrolysis products from endo-glucanase are long chain oligomers. These oligomers are further hydrolyzed in to short chain oligosaccharides by exo-glucanase or cellobiohydrolase (Juturu and Wu, 2014; Xu et al., 2007).

2.3.1.2 Exo-glucanases

Exo-glucanases or exo-1,4-β-D-glucanases, can be divided in to two groups of exo-glucanase (E.C. 3.2.1.176), acting from the reducing ends of oligomers, and exo-glucanase (E.C. 3.2.1.91), acting from the non-reducing ends of oligomers. However, two types of those exo-glucanases generate the cellobiose as the final product (Juturu and Wu, 2014; Xu et al., 2007).

2.3.1.3 β-glucosidases

β-glucosidases or β-D-glucoside glucohydrolases (E.C. 3.2.1.21), which hydrolyze the cellobiose into two glucose molecules. The cellulose hydrolysis rate is depended on this reaction step (Parisutham et al., 2017).

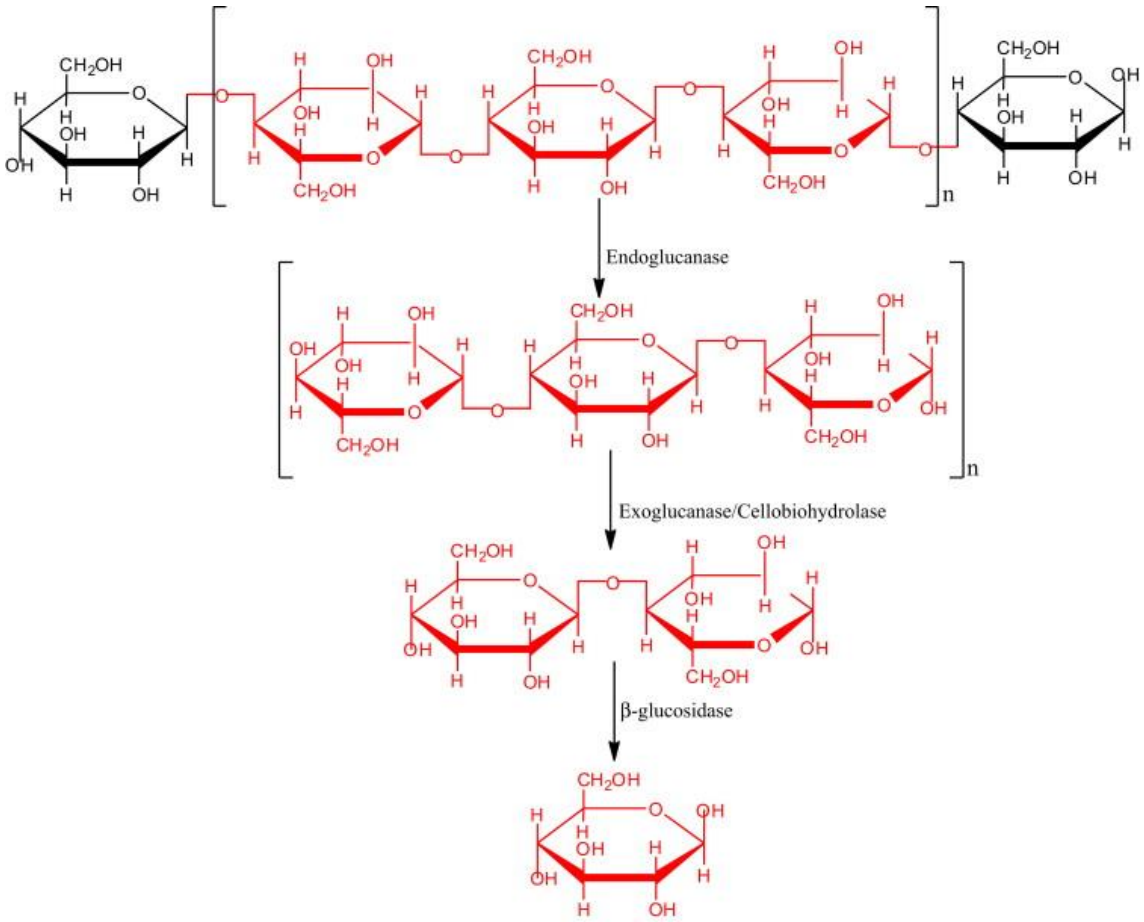

Figure 2.6 Action of different cellulases on cellulose polymer.
Source: Juturu and Wu (2014)

Figure 2.6 shows the randomly attack of endo-glucanase on amorphous cellulose chain to release small oligomers with free reducing and non-reducing ends. Then, exo-glucanase acts on free ends to release cellobiose, which is finally hydrolyzed to glucose as the end final product by β-glucosidase (Singhania, 2009). Nowadays, cellulases play a significant role on numerous industries, for example, food, animal feed, textile and laundry, biofuel, pharmaceutical, pulp and paper industries as well as waste management system (Behera et al., 2017).

2.3.2 Hemicellulases

The complete breakdown of hemicelluloses requires the action of several types of hemicellulases enzymes (Figure 2.7). These enzymes are the group of endo-xylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase and acetylxylan esterase (Juturu and Wu, 2012). Endo-xylanase, the key enzyme of hemicellulose hydrolysis, randomly acts on the xylan backbone to produce xylooligomers or xylooligosaccharides (XOs). Then, β-xylosidases release xylose units from XOs. While, the removal of xylan side chains is catalyzed by α-L-arabinofuranosidases, α-D-glucuronidases, acetyl xylan esterases, ferulic acid esterases and *p*-coumaric acid esterases (Javier et al., 2007).

2.3.2.1 Endo-xylanases

Endo-xylanases (endo-1,4-β-xylanase; E.C. 3.2.1.8) are classified in to families 5, 7, 8, 10, 11, 26 and 43. Among these families, family 10 (GH10, formerly known as F) and family 11 (GH11, formerly known as G) endo-xylanase had been widely studied (Juturu and Wu, 2012). GH10 family is mostly found in bacteria and GH11 family is mostly found in fungi (Chakdar et al., 2016).

The family 10 (GH10) endo-xylanases are one of the well characterized and intensively studied. The TIM-barrel fold with catalytic domain of 250–450 amino acids of family 10 xylanase contains a carbohydrate-binding module connected to the catalytic domain. While the active site is a conserved region (Javier et al., 2007). The 3D structure of TIM-barrel fold, which likes a bowl, comprises of the arrangement of eight β-sheets. Disulfide bonds and salt bridges influence on the thermostabillity of family 10 xylanase (Juturu and Wu, 2012).

The family 11 (GH11) endo-xylanases can be divided into two sub-groups of alkali and acidic based on their isoelectric points (pIs). The small β-jelly roll structure of these xylanase gives their ability to pass though the pore in hemicellulose structure (Juturu and Wu, 2012). This β-jelly roll structure, the catalytic domain with 180–200 amino acid residues, contains two antiparallel β-sheets namely, β-sheet A and β-sheet B. The stabilization of these xylanases depends on hydrogen bonds between the β-strands

(Javier et al., 2007; Paës et al., 2012). Whereas, the disulfide bonds and uniform distribution of charged amino acids affect the thermostability of this enzyme (Juturu and Wu, 2012).

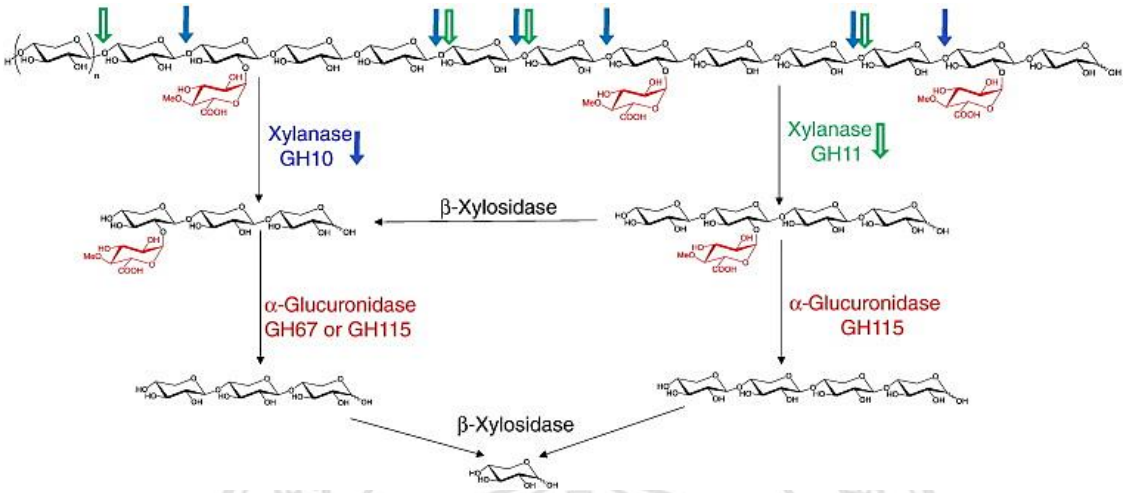

Figure 2.7 Schematic degradation of glucuronoxylan by xylanase GH10, xylanase GH11, α-glucuroninase and β-xylosidase.

Source: Biely et al. (2016)

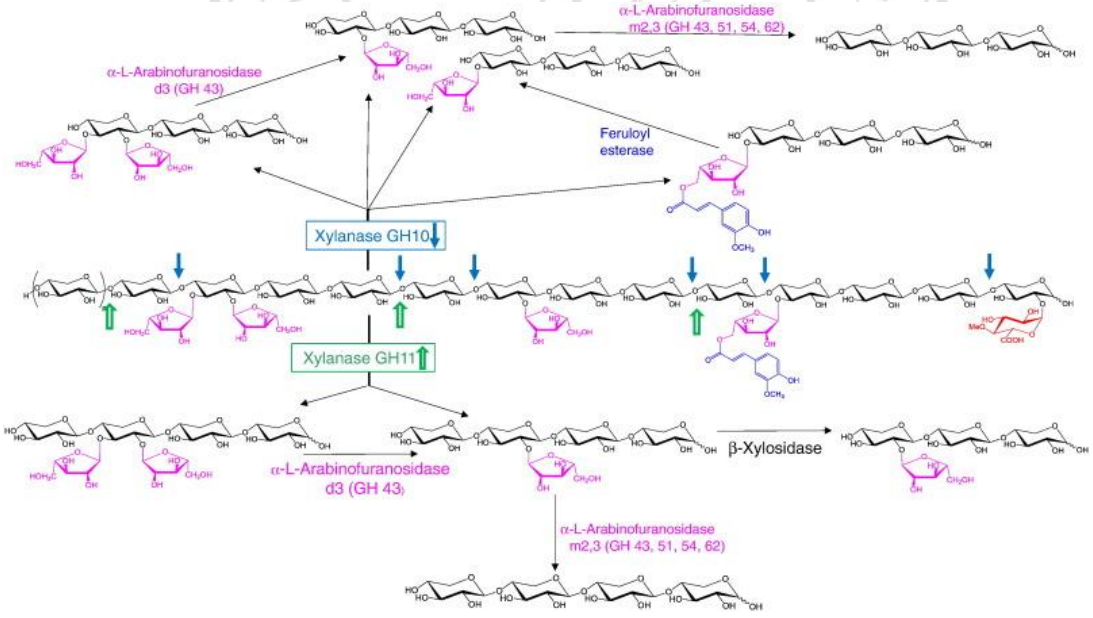

Figure 2.8 Schematic degradation of arabinoglucuronoxylan by xylanase GH10, xylanase GH11, α-L-arabinofuranosidase, ferulic acid esterase (feruloyl esterase) and β-xylosidase.

Source: Biely et al. (2016)

The different catalytic property between GH10 and GH11 had been described.

1) **Substrate specificity:** Family 10 endo-xylanase shows the lower substrate specificity than family 11 (Javier et al., 2007).
2) **Binding side:** Family 10 endo-xylanase has a smaller binding side than family 11, which active on short chains xylooligosaccharides (Javier et al., 2007).
3) **Substituents specificity:** Family 10 endo-xylanase can hydrolyze xylan with the substitutions, while, family 11 can hydrolyze only unsubstituted regions of xylan (Javier et al., 2007).
4) **Molecular weight:** Family 10 endo-xylanase is a high molecular weight enzyme with a cellulose-binding domain and a catalytic domain, whereas, family 11 is a low molecular weight enzyme (Juturu and Wu, 2012).
5) **Structure:** Family 10 endo-xylanase has a $(\beta/\alpha)_8$ fold TIM-barrel fold structure and family 11 has a β-jelly roll structure (Juturu and Wu, 2012).

Recently, several types of microbial xylanase have been discovered and purified from numerous microorganism such as *Bacillus* spp., *Aspergillus* spp., *Chaetomium* spp., *Streptomyces* spp. and *Neocallimastix* spp. (Juturu and Wu, 2012). Among these, *Streptomyces* is the good candidate for xylanase production because their enzymes are an extracellular enzyme, with high level of activity, thermal stability and pH stability across a broad pH range (Boonchuay et al., 2016). Table 2.4 shows the list of xylanases from *Streptomyces* spp. and their characteristics.

Table 2.4 The list of xylanases from *Streptomyces* spp. and their characteristics.

Strains	**MW*** **(kDa)**	**pH_{opt}** **	**T_{opt}** *** **(°C)**	**K_m (mg/mL)**	**V_{max} (U/mg)**	**Substrates**	**Methods**	**Specific activity (U/mg)**	**Purification fold**	**Yields (%)**	**References**
S. thermocarboxydus subsp. MW8	52	7.0	50	1.71	357	Birch wood xylan	$(NH_4)_2SO_4$ precipitation, DEAE Sepharose and Resource-Q	84	8.4	5	Chi et al. (2013)
Streptomyces sp. CS624	40	6.0	60	5.61	75	Beech wood xylan	$(NH_4)_2SO_4$ precipitation, CM Tris acryl and Sephadex G- 75	61,415	3.7	24	Mander et al. (2014)
Streptomyces sp. TN119	35.9	7.0	50	15.10	441	Oat spelt xylan	Ni- chromatography	91	ND****	ND****	Zhou et al. (2011)
S. matensis DW67	21.2	7.0	65	ND****	ND****	Birch wood xylan	$(NH_4)_2SO_4$ precipitation, DEAE-52 and Sephadex G-50	638	14.5	14	Yan et al. (2009)
S. olivaceus MSU3	42	8.0	40	8.16	250	Birch wood xylan	$(NH_4)_2SO_4$ precipitation, DEAE–cellulose and Sephadex G-75	153	4.3	16	Sanjivkumar et al. (2017)

Table 2.4 The list of xylanases from *Streptomyces* spp. and their characteristics. (continued)

Strains	**MW** [*] **(kDa)**	**pH_{opt}** [**]	**T_{opt}** [***] **(°C)**	**K_m (mg/mL)**	**V_{max} (U/mg)**	**Substrates**	**Methods**	**Specific activity (U/mg)**	**Purification fold**	**Yields (%)**	**References**
S. megasporus DSM 41476	47.6	5.5	70	1.68	437	Oat spelt xylan	$(NH_4)_2SO_4$ precipitation and HiTrap Q Sepharose XL	242	ND[****]	13.5	Qiu et al. (2010)
Streptomyces sp. CS428	37	7.0	80	102.3	3,225	Beech wood xylan	$(NH_4)_2SO_4$ precipitation and CM Trisacryl	926,103	26	55	G.C. et al. (2013)

[*]MW: molecular weight; [**]pH_{opt}: optimal pH; [***]T_{opt}: optimal temperature; [****]ND: not determined

2.3.2.2 β-xylosidases

β-xylosidases (β-1,4-xylosidases; E.C. 3.2.1.37) are type of hydrolase enzyme that have the responsibility to hydrolyze xylobiose (X2) and xylotriose (X3) at β-1,4-xylosidic linkages (Figure 2.7 and 2.8). This enzyme liberates the xylose as products from the non-reducing end of X2 and X3 (Biely et al., 2016). β-xylosidase are grouped into GH3, 30, 39, 43, 52, 54, 116 and 120 (Lagaert et al., 2014). Previous study reported that β-xylosidase was not only hydrolyzed XOs at non-reducing end but also hydrolyzed *p*-nitrophenyl-β-D-xylopyranoside which is artificial substrates. Moreover, many β-xylosidase also exhibited the transxylosidase activity (Javier et al., 2007).

2.3.2.3 Hemicellulose debranching enzymes

To completely hydrolyze the hemicellulose, the synergism action between accessory enzymes and xylanase is necessary. These accessory enzymes include α-L-arabinofuranosidases, α-glucuronidases, acetylxylan esterases and hydroxycinnamic acid esterases (Javier et al., 2007).

α-L-arabinofuranosidases (E.C. 3.2.1.55) release arabinose residue from the side chain of xylan and other arabinose-containing polysaccharide by exo-action (Figure 2.8) (Biely et al., 2016; Javier et al., 2007). They can be devided into the major group and minor group of m2,3 α-L-arabinofuranosidases and d3 α-L-arabinofuranosidases, respectively. The major group of m2,3 α-L-arabinofuranosidases, particularly active on xylopyranosyl residues that substituted by 1 unit of L-arabinofuranosyl residues at either position 2 or 3. While, d3 α-L-arabinofuranosidases selectively hydrolyze 2 unit of α-1,3-linked arabinofuranosyl residues. However, they are unable to release the substituted arabinofuranosyl residues (Biely et al., 2016).

α-glucuronidases (E.C. 3.2.1.131) catalyze the hydrolysis of α-1,2-linkage between 4-*O*-methylglucuronic acid or glucuronic acid and xylopyranosyl residue, which is one of the most acid stable linkages (Figure 2.7). Till date, the mode of action and example of these enzymes are complicate. The well-known is α-glucuronidases family GH67. This mentioned family can hydrolyze the linkage between uronic acid

and oligosaccharides at the non-reducing end to liberate 4-*O*-methyl-D-glucuronic acid (Biely et al., 2016; Javier et al., 2007).

Acetylxylan esterases (E.C. 3.1.1.72) are one type of carbohydrate esterase, which remove the acetyl group from acetylated xylan. The released acetic acids are liberated from position 2 and 3 on mono- and di-*O*-acetylated xylopyranosyl residue (Biely et al., 2016; Javier et al., 2007).

Ferulic acid and *p*-coumaric acid esterases are classified as hydroxycinnamic acid esterases (Figure 2.8). The structure-function relationship of these enzymes is still complicate. They cleave the ester link at *O*-5 position between ferulic acid or *p*-coumaric acid and the arabinofuranosyl side chain (Biely et al., 2016; Javier et al., 2007).

2.4 Xylooligosaccharides (XOs)

XOs are potential prebiotics that can be produced commercially from xylan-containing LCMs by various methods such as chemical hydrolysis, enzymatic hydrolysis and chemical pretreatment combined with enzymatic hydrolysis (Aachary and Prapulla, 2011). The term ‘‘oligosaccharide’’ is usually used for DP in the range 3–6 but xylobiose or X2 (DP2) has also been considered as an oligosaccharide in food application (Samanta et al., 2015). XOs show biological effects related to other oligosaccharide, such as fructooligosaccharides, galactooligosaccharides, soybean oligosaccharides and isomaltooligosaccharides (Moure et al., 2006). Recent studies have reported on the manufacture of XOs from various alkali-pretreated LCMs catalyzed by endo-xylanases, e.g. corncob (Aachary and Prapulla, 2009; Ai et al., 2005; Li et al., 2012), wheat bran (Manisseri and Gudipati, 2010), natural grass (Samanta et al., 2012a), oil palm frond fiber (Sabiha-Hanim et al., 2011) and sugarcane bagasse (Jayapal et al., 2013). A comparison of XO yields with different production methods is provided in Table 2.5.

Table 2.5 A comparison of xylooligosaccharide yields with different production methods.

Substrate	Source of xylanase	Conditions	XO yields (mg/g_{xylan})*	References
NaOH-treated corncob	Immobilized xylanase (*Streptomyces olivaceoviridis* E-86)	55°C, pH 6.3, 24 h	387.5 [387.50]	Ai et al. (2005)
NaOH-treated corncob powder	Xylanase from *Aspergillus oryzae* MTCC 5154	50°C, pH 5.4, 14 h	340.00 [347.33]	Aachary and Prapulla (2009)
Wheat bran soluble polysaccharides	Purified Ragi malt xylanase	50°C, pH 5.0, 2.5 h	75.00 [300.00]	Manisseri and Gudipati (2010)
Steam explosion treated-corncob	Xylanase from *Paecilomyces themophila* J18	70°C, pH 7.0, 2.5 h	286.00 [**]	Teng et al. (2010)
Autoclaved-oil palm frond fibers	Commercial xylanase (*Trichoderma viridae,* Sigma, USA)	40°C, pH 5.0, 24 h	175.00 [403.00]	Sabiha-Hanim et al. (2011)
NaOH-extracted corncob xylan	Purified xylanase from *Streptomyces rameus* L2001	50°C, 12 h	150.00 [150.00]	Li et al. (2012)
NaOH and stream-treated *Sehima nervosum* grass	Commercial xylanase (*Trichoderma viridae*, Sigma, USA)	45.19°C, pH 5.03, 10.11 h	180.60 [180.60]	Samanta et al. (2012a)

Table 2.5 A comparison of xylooligosaccharide yields with different production methods. (continued)

Substrate	Source of xylanase	Conditions	XO yields (mg/g xylan)*	References
KOH-treated sugarcane bagasse	Xylanase from *Pichia stipitis*	50°C, pH 5.4, 12 h	318.00 [318.00]	Bian et al. (2013)
NaOH-treated sugarcane bagasse	Commercial xylanase (*Trichoderma viridae*, Sigma, USA)	40°C, pH 4.0, 8 h	367.79 [58.90]	Jayapal et al. (2013)
KOH combined with $NaBH_4$-extracted corncob xylan	Commercial xylanase (Shearzyme 500L and Veron 191)	40°C, pH 5.0, 120 h	143.00 [143.00]	Uçkun Kiran et al. (2013)
Microwave treatment-wheat bran powder	Commercial xylanase (Bakezyme BXP 5001 BG)	55°C, 24 h	** [64.00]	Wang and Lu (2013)

*XO yields (mg/g substrate) are shown in brackets [ ] beneath XOs yields (mg/g xylan or hemicellulose); ** not provided

2.4.1 Prebiotics properties and health benefits of xylooligosaccharides

Nowadays, numerous studies found that XOs have several prebiotics properties and health benefits (Table 2.6 and Table 2.7). Various studied have reported that XOs are having the highest properties to increase number of bifidobacberia, whereas few lactobacilli have also been reported (Boonchuay et al., 2014).

Table 2.6 The ability of xylooligosaccharides from different origins to promote the growth of probiotic lactobacilli and bifidobacteria.

Microorganisms	Types of XOs	References
Lactobacillus	Commercial XOs (Xylo-oligo 70)	Crittenden et al. (2002)
brevis	Corncob XOs	Moura et al. (2007)
	Wheat bran XOs	Manisseri and Gudipati (2010)
L. fermentum	Corncob XOs	Moura et al. (2007)
L. maltromicus	Corncob XOs	Samanta et al. (2012b)
L. plantarum	Wheat bran XOs	Manisseri and Gudipati (2010)
L. viridiscens	Corncob XOs	Samanta et al. (2012b)
Bifidobacterium	Commercial XOs (Xylo-oligo 70)	Crittenden et al. (2002)
aldolescentis	Corncob XOs	Moura et al. (2007)
	Rice husk XOs	Gullón et al. (2008)
	Wheat bran XOs	Manisseri and Gudipati (2010)
	Corncob XOs	Chapla et al. (2012)
B. bifidum	Corncob XOs	Chapla et al. (2012)
	Wheat bran XOs	Manisseri and Gudipati (2010)
B. breve	Rice husk XOs	Gullón et al. (2008)
B. infantis	Rice husk XOs	Gullón et al. (2008)
B. lactis	Commercial XOs (Xylo-oligo 70)	Crittenden et al. (2002)
B. longum	Corncob XOs	Moura et al. (2007)
	Rice husk XOs	Gullón et al. (2008)
B. pseudolongum	Commercial XOs (Xylo-oligo 70)	Crittenden et al. (2002)

Table 2.7 Potential health benefits of xylooligosaccharides.

Health benefits	**Types of study**	**References**
Increasing short-chain fatty acids	*In vivo* (rats) *In vitro*	Campbell et al. (1997) Rycroft et al. (2001)
Antimicrobial (*Helicobacter pylori*) activity	*In vitro*	Christakopoulos et al. (2003)
Increasing the population of bifidobacteria and lactobacilli	*In vitro* *In vivo* (elderly treatment group) *In vivo* (diabetic rats) *In vivo* (healthy adults)	Rycroft et al. (2001) Chung et al. (2007) Gobinath et al. (2010) Childs et al. (2014)
Increasing fecal moisture content and decreasing the fecal pH value	*In vivo* (elderly treatment group)	Chung et al. (2007)
Improving body weight	*In vivo* (diabetic rats)	Gobinath et al. (2010)
Reducing hyperglycemia	*In vivo* (diabetic rats)	Gobinath et al. (2010)
Reducing cholesterol	*In vivo* (diabetic rats)	Gobinath et al. (2010)
Antioxidant activity	*In vitro* *In vitro*	Veenashri and Muralikrishna (2011) Bian et al. (2013)
Inhibiting the adhesion of *Listeria* to the intestinal epithelium	*In vitro* (gut model)	Ebersbach et al. (2012)
Improving aspects of the plasma lipid profile	*In vivo* (healthy adults)	Childs et al. (2014)
Modulating the markers of immune function	*In vivo* (healthy adults)	Childs et al. (2014)

2.5 Bioethanol

Bioethanol is a liquid biofuel produced from several biomass feedstock and conversion technology (Balat et al., 2008). Recent studies have reported that various types of LCMs can be used as the feedstock for bioethanol production, such as corncob (Chang et al., 2012; Chen et al., 2010; Cheng et al., 2010; Fan et al., 2013; Gu et al., 2014; Kahar et al., 2010; Zhang et al., 2010b), rice straw (Diep et al., 2012; Singh and Bishnoi, 2012), bagasse (Wanderley et al., 2013) and wheat straw (Zhang et al., 2013). Bioethanol is appropriate for mixing with gasoline because of its high octane number (Balat et al., 2008). Ethanol contains 35% oxygen, which results in a complete combustion of fuel and lowers emission of harmful gases (Bhatia et al., 2012). Nowadays, most of the global fuel ethanol is produced from sugar-based feedstock or sucrose from molasses and cane juice. The ethanol fermentation from sugar is normally defined as the first generation bioethanol. While the second generation ethanol from lignocellulosic biomass, a low-cost feedstock, is a remarkable process for the long-term bioethanol production. The released sugar from enzymatic hydrolysis of both feedstock is mainly glucose that can be convert into ethanol by microorganism via a glycolysis pathway (Baeyens et al., 2015; Singhania, 2009).

2.5.1 Bioethanol fermentation through the Embden-Meyerhof-Parnas (EMP) pathway

Glycolysis pathway is the cellular breakdown of the glucose into energy usually represents in the cytosol of prokaryotic and eukaryotic. The glycolysis pathway in Eukaryotic such as yeast and human is the Embden-Meyerhof-Parnas (EMP) pathway. Whereas, certain prokaryotes e.g. *Zymomonas* and *Pseudomonas* convert glucose into energy though Entner-Doudoroff pathway (Godbey, 2014).

2.5.1.1 The entry of glucose into cell and the fructose 1,6-bisphosphate formation

The first step consists of three minor steps: a phosphorylation, an isomerization and a second phosphorylation reaction.

i) **The first phosphorylation:** The phosphoryl group from ATP is transferred to glucose by hexokinase. This reaction requires the Mg^{2+} because the true substrate of hexokinase is the $MgATP^{2-}$ complex (Figure 2.9) (Berg et al., 2002; Nelson and Cox, 2004).

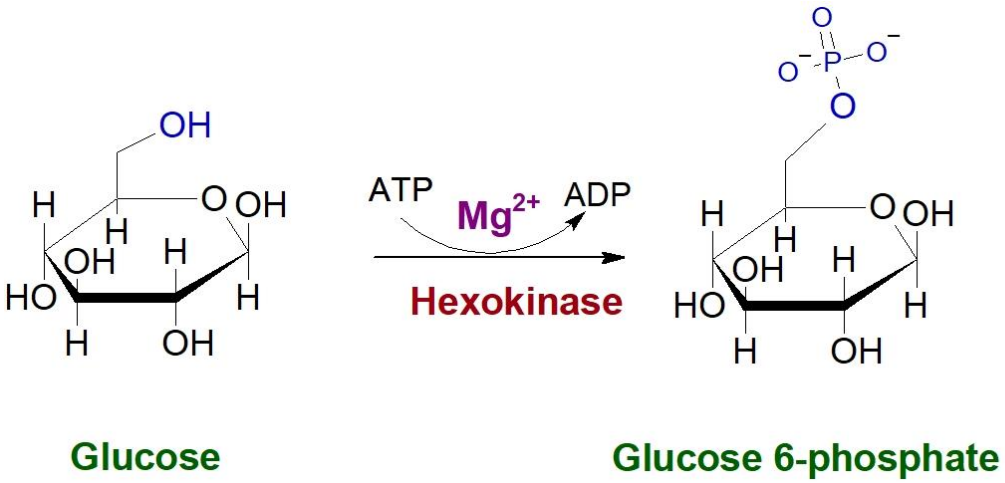

Figure 2.9 The first phosphorylation of glucose.

ii) **Isomerization:** The glucose of glucose 6-phosphate is isomerized from a 6-carbon sugar (an aldose) into the 6-carbon sugar fructose as fructose 6-phosphate (a ketose) by phosphohexose isomerase (Figure 2.10). This is a significant step in glycolysis, as the carbonyl and hydroxyl groups rearrangement (Berg et al., 2002; Godbey, 2014; Nelson and Cox, 2004).

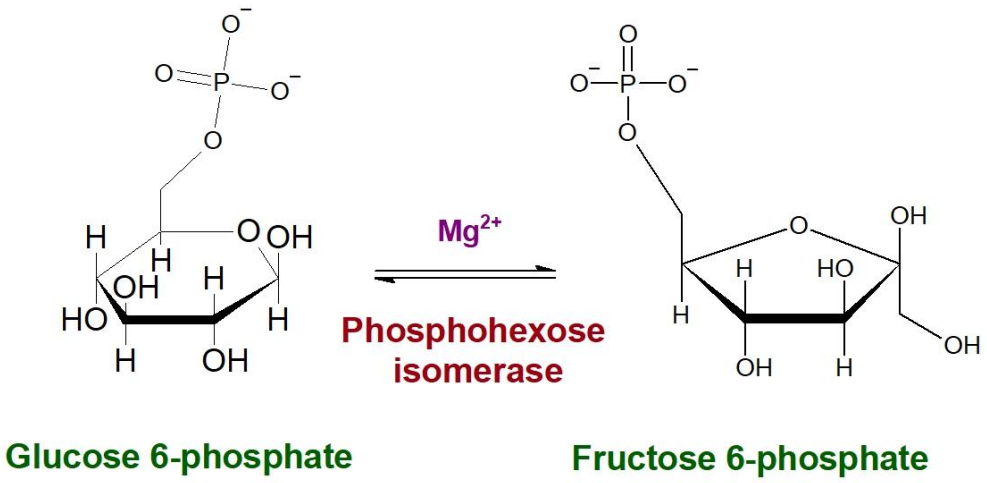

Figure 2.10 An isomerization to convert glucose 6-phosphate into fructose 6-phosphate.

iii) **The second phosphorylation:** Phosphofructokinase catalyzes the transfer of a phosphoryl group from ATP to fructose 6-phosphate at carbon 6 to yield fructose 1,6-bisphosphate, ADP and H^+ (Figure 2.11) (Nelson and Cox, 2004; Godbey, 2014).

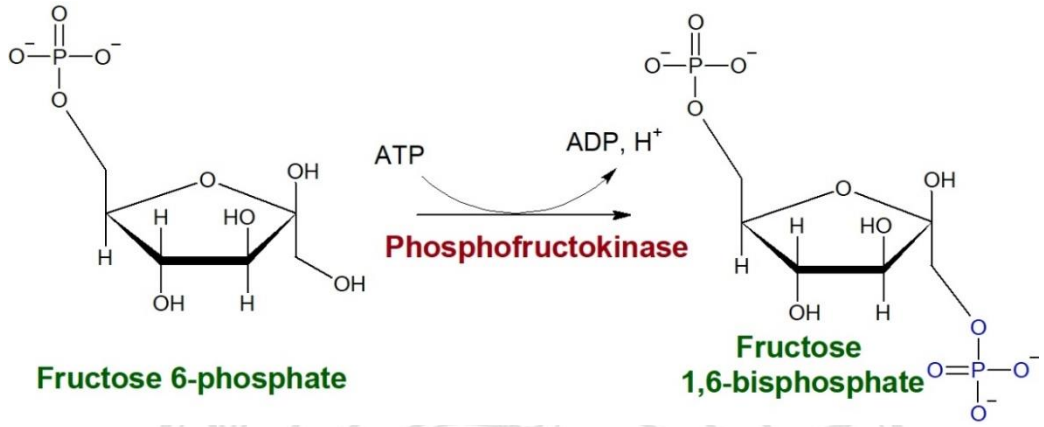

Figure 2.11 The second phosphorylation of fructose 6-phosphate to fructose 1,6-bisphosphate.

2.5.1.1 The cleavage of six carbon sugar

Fructose 1,6-bisphosphate is cleaved to yield two different isomers of triose phosphates, glyceraldehyde 3-phosphate (an aldose) and dihydroxyacetone phosphate (a ketose) by fructose 1,6-bisphosphate aldolase (aldolase) as shown in Figure 2.12 (Berg et al., 2002; Godbey, 2014; Nelson and Cox, 2004).

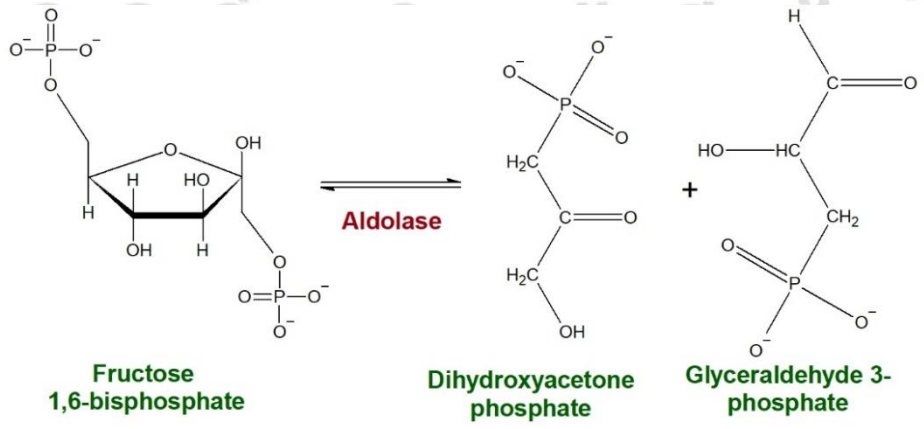

Figure 2.12 Cleavage of fructose 1,6-bisphosphate into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate.

2.5.1.2 The recovery of tree-carbon fragment

Glyceraldehyde 3-phosphate is directly degraded in the step of glycolysis, whereas dihydroxyacetone phosphate is further converted to glyceraldehyde 3-phosphate by triose phosphate isomerase (Figure 2.13) (Berg et al., 2002; Nelson and Cox, 2004).

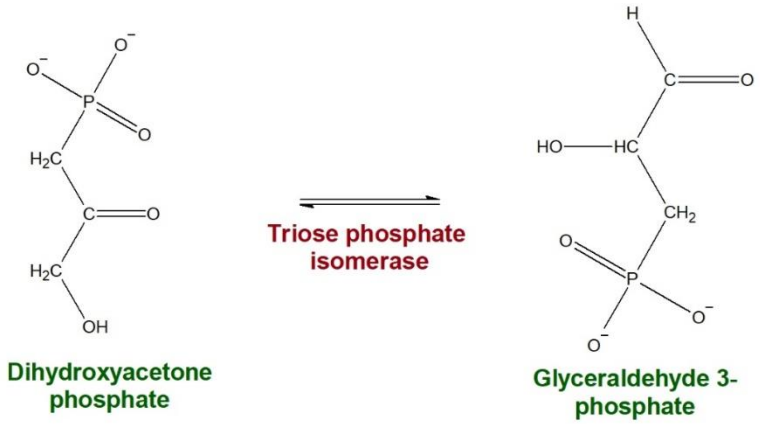

Figure 2.13 The conversion of dihydroxyacetone phosphate into glyceraldehyde 3-phosphate.

2.5.1.3 The phosphorylation and oxidation of glyceraldehyde 3-phosphate

Till this step, the reaction gives 2 molecules of glyceraldehyde 3-phosphate, but ATP is not formed. Glyceraldehyde 3-phosphate dehydrogenase oxidizes aldehyde group on glyceraldehyde 3-phosphate molecule into a 1,3-bisphosphoglycerate (Figure 2.14) (Berg et al., 2002; Nelson and Cox, 2004).

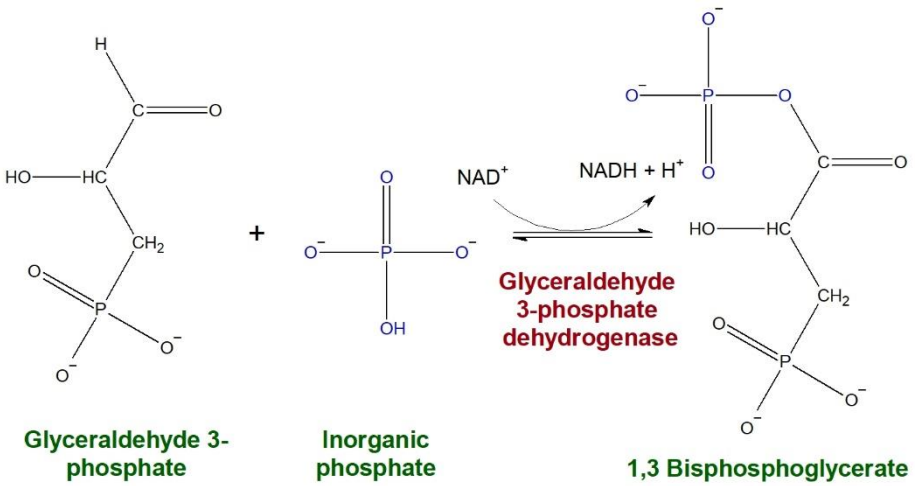

Figure 2.14 Oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate.

2.5.1.4 The generation of ATP from 1,3-bisphosphoglycerate

The enzyme phosphoglycerate kinase catalyzes a transfer of high-energy phosphoryl group on 1,3-bisphosphoglycerate to ADP. This reaction generates ATP and 3-phosphoglycerate as products (Figure 2.15) (Berg et al., 2002; Nelson and Cox, 2004).

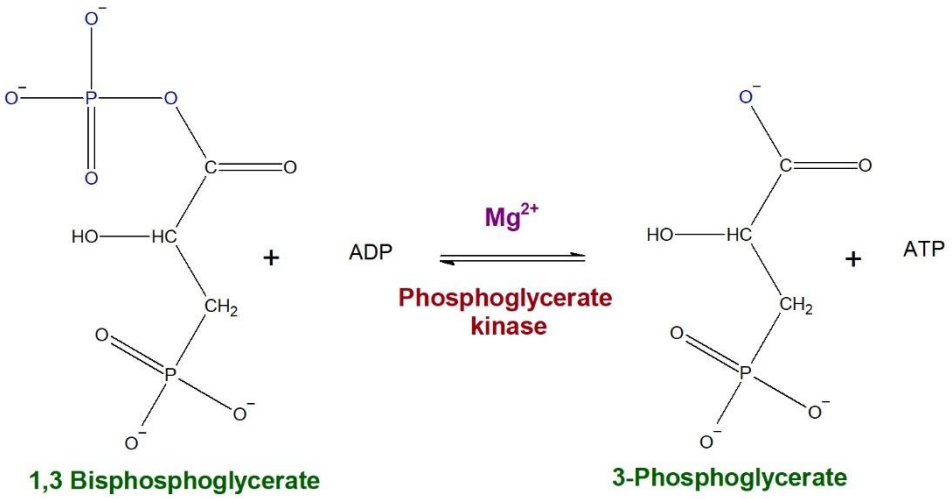

Figure 2.15 Phosphoryl transfer from 1,3-bisphosphoglycerate to ADP.

2.5.1.5 The generation of ATP and the formation of pyruvate

The first minor reaction is an intramolecular shifted of the phosphoryl group between carbon 2 and carbon 3 of glycerate by phosphoglycerate mutase. The second minor reaction requires the enolase to remove a molecule of water from 2-phosphoglycerate for the generation of phosphoenolpyruvate (PEP). The last minor reaction, pyruvate kinase transfers a phosphoryl group from phosphoenolpyruvate to ADP, which requires K^+ and Mg^{2+} or Mn^{2+} (Figure 2.16). The final products are pyruvate and ATP (Berg et al., 2002; Nelson and Cox, 2004).

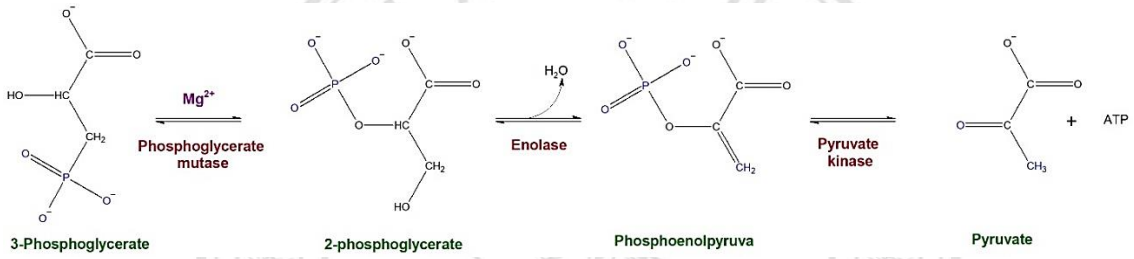

Figure 2.16 The generation of ATP and the formation of pyruvate.

2.5.1.6 The ethanol fermentation

The first scientific study of alcoholic fermentation was researched by Antoine Lavoisier. He not only described that sugar can be converted into alcohol and carbon dioxide (CO_2), but also determined the composition of both fermentable substances and fermentation products (Ishizaki and Hasumi, 2014). Till date, there are numerous microorganisms which can ferment the glucose into ethanol. In yeast and other microorganisms, the pyruvate is converted to ethanol and CO_2. Pyruvate which is the product from glycolysis is decarboxylated by pyruvate decarboxylase. This enzyme requires Mg^{2+} and the cofactor, namely, thiamine pyrophosphate (TPP) that derives from thiamine (vitamin B1). After this reaction, acetaldehyde is generated. Then, alcohol dehydrogenase reduces acetaldehyde into ethanol and CO_2 (Figure 2.17) (Berg et al., 2002; Nelson and Cox, 2004).

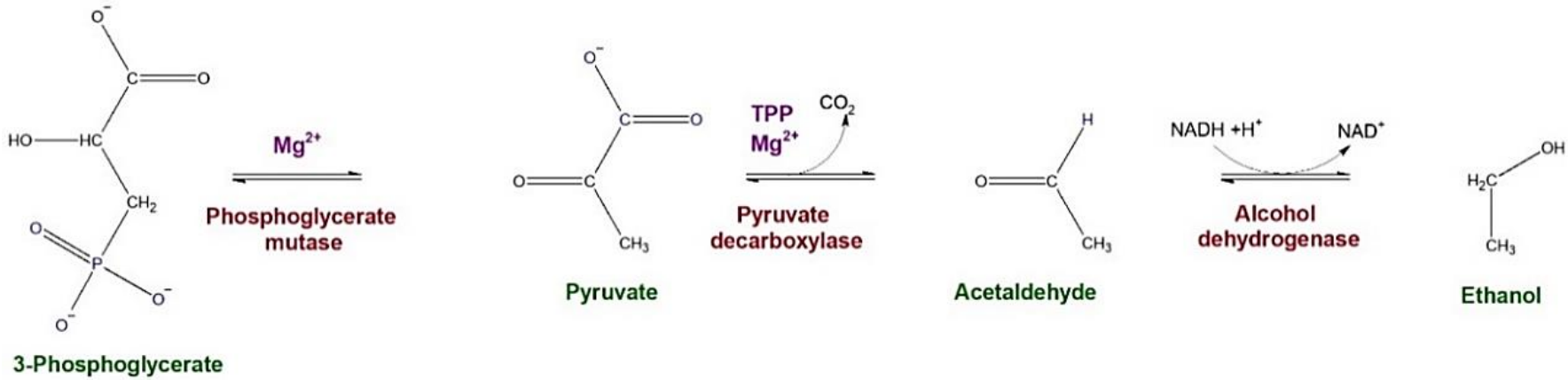

Figure 2.17 Ethanol fermentation.

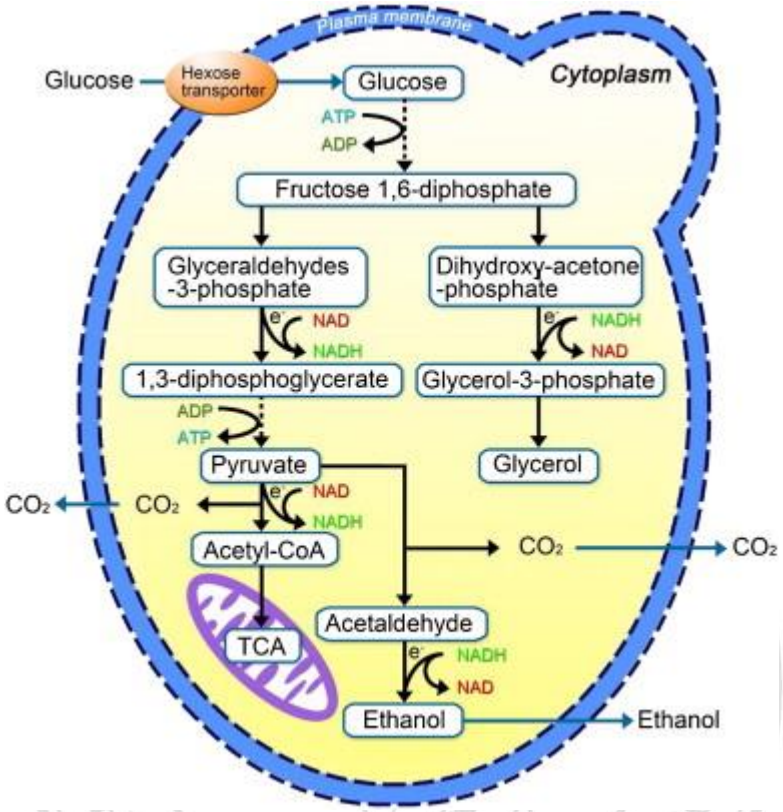

Figure 2.18 Glucose metabolism through glycolysis pathway and the ethanol fermentation by *Saccharomyces cerevisiae.*

Source: Zabed et al. (2017)

The ordinary microorganism that has been wildly used in ethanol fermentation is *S. cerevisiae.* In cytoplasm of *S. cerevisiae*, the ethanol fermentation from glucose is converted through the glycolysis pathway. In anaerobic condition, the two moles of pyruvate from 1 mole of glucose are converted into 2 moles of ethanol and 2 moles of CO_2 (Figure 2.18) (Zabed et al., 2017). The simplest term of sugars fermentation into ethanol by yeast resulted from the sequence actions of enzymes can be described by this reaction (Häggström et al., 2014):

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

From this mentioned reaction stoichiometry, the maximum theoretical yield for ethanol production from glucose ($Y_{EtOH/Glucose}$) is 2.0 mol/mol or 0.511 g/g, nevertheless

the actual yields is only in the range of 90–95% of the maximum theoretical yield because of the generation of glycerol, other minor metabolites and cell biomass (Häggström et al., 2014). While, theoretical yields of ethanol production from glucose has been compared with other sugars as shown in Table 2.8.

Table 2.8 Theoretical yields of ethanol production from different type of sugars.

Sugar types	**Theoretical yields ($g_{ethanol}/g_{sugar}$)**	**References**
Fructose	0.511	Thammasittirong et al. (2013)
Glucose	0.511	Häggström et al. (2014)
Maltose	0.538	Thammasittirong et al. (2013)
Sucrose	0.538	Silva et al. (2005)
Xylose	0.511	Mousdale (2010)

Recent studies have report on production of bioethanol fermentation by various microorganisms such as *S. cerevisiae* (Chang et al., 2012; Chen et al., 2010; Kahar et al., 2010; Sindhu et al., 2012; Singh and Bishnoi, 2012; Zhang et al., 2010b), *Kluyveromyces marxianus* (Zhang et al., 2010a), *Zymomonas mobilis* (Su et al., 2013), *Candida shehatae* (Chen et al., 2010), *Scheffersomyces stipites* (Singh and Bishnoi, 2012), *Hohenbuehelia* sp. (Liang et al., 2013) and *Paecillomyces variotii* (Zerva et al., 2014). The advantages and limitations of using each ethanolic microorganism are summarized in Table 2.9.

Table 2.9 Ethanolic microorganisms and their characteristics.

Microorganisms	Characteristics	Advantages	Limitations	References
Saccharomyces cerevisiae	Facultative anaerobic yeast	- The ability to tolerate a broad pH range and osmotic pressure - Higher ethanol yields - Superior performance to growth under restricted condition e.g. anaerobic condition and low nutrients	- Unable to utilize xylose	Aditiya et al. (2016); Mohd Azhar et al. (2017); and Zabed et al. (2017)
Zymomonas mobilis	Gram-negative bacteria	- Rapid fermentation - High ethanol yields - Low biomass yields	- Narrow substrate utilization range	Aditiya et al. (2016); Mohd Azhar et al. (2017); and Zabed et al. (2017)
Candida shehatae	Xylose fermenting yeast	- The ability to ferment arabinose and xylose	- Xylose fermentation only occur under oxygen-limited conditions	Gírio et al. (2010)

Table 2.9 Ethanolic microorganisms and their characteristics. (continued)

Microorganisms	Characteristics	Advantages	Limitations	References
Candida glabrata	Facultative anaerobic yeast	- Thermotolerant yeast (≤42°C) - High acid concentration tolerance - High ethanol production rate and specific growth rate	- Unable to utilize xylose	Choudhary et al. (2016); and Merico et al. (2007)
Kluyveromyces marxianus	Thermophilic yeast	- A wide variety sugars utilization - Thermophilic yeast (≤45°C)	- Cannot grow under strictly anaerobic conditions - Generation of unwanted-product such as xylitol - Low capacity to tolerate high ethanol concentration	Arora et al. (2015); and Zabed et al. (2016)
Scheffersomyces (*Pichia*) *stipitis*	Xylose fermenting yeast	- Thermotolerant yeast (≤42°C) - Capable to ferment xylose - Low byproduct formation	- Cannot tolerate high ethanol concentration - Low ethanol production rate - Requirement of microaerophilic condition	Arora et al. (2015); and Zabed et al. (2016)

2.5.2 The second generation bioethanol production

The second generation bioethanol is defined as the process that use cellulose and hemicellulose-released sugar, including glucose, xylose, arabinose and others as the substrate for ethanol fermentation (Mekala et al., 2014; Ortíz and Quintero, 2014). Ethanol production from lignocellulosic biomass composes of 4 main steps: pretreatment, hydrolysis, fermentation and distillation. The pretreatment and hydrolysis were already described in section 2.2 and 2.3. Moreover, the cellular bioethanol fermentation through the EMP pathway was also explained in section 2.5.1. Here, the fermentation and distillation technologies to produce bioethanol from LCMs are discussed as following.

The ethanol fermentation process can be proceeded through two main different approaches of separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF). The SHF is conventional process to produce ethanol via the consecutive step of hydrolysis and fermentation. While, SSF, the hydrolysis and fermentation are performed together in same time and same reactor (Cha et al., 2015; Paulova et al., 2015). The operation of these processes is simplified in Figure 2.19 and 2.20. Among both fermentation process, SSF provides several advantages such as improving the enzymatic hydrolysis rate, reducing the cooling cost, reducing the chance of contamination, reducing end product inhibition, reducing the overall process time, and reduce the bioreactor using and investment cost. Unfortunately, a different temperature between the hydrolysis and fermentation are the limitation of SSF process. Therefore, a potential thermotolerant ethanolic microorganisms has been applied to overcome this problem (Antil et al., 2015; Cha et al., 2015; Hasunuma and Kondo, 2012). The examples of ethanol production by various substrates via SHF or SSF processes are shown in Table 2.10.

This fermentation broth of ethanol still remains other unwanted product such as water. Therefore, the distillation process of fermentation broth is required to obtain a high purity ethanol. The downstream production of ethanol fermentation includes: adsorption distillation, azeotropic distillation, chemical dehydration, diffusion distillation, extractive distillation and membrane distillation (Aditiya et al., 2016).

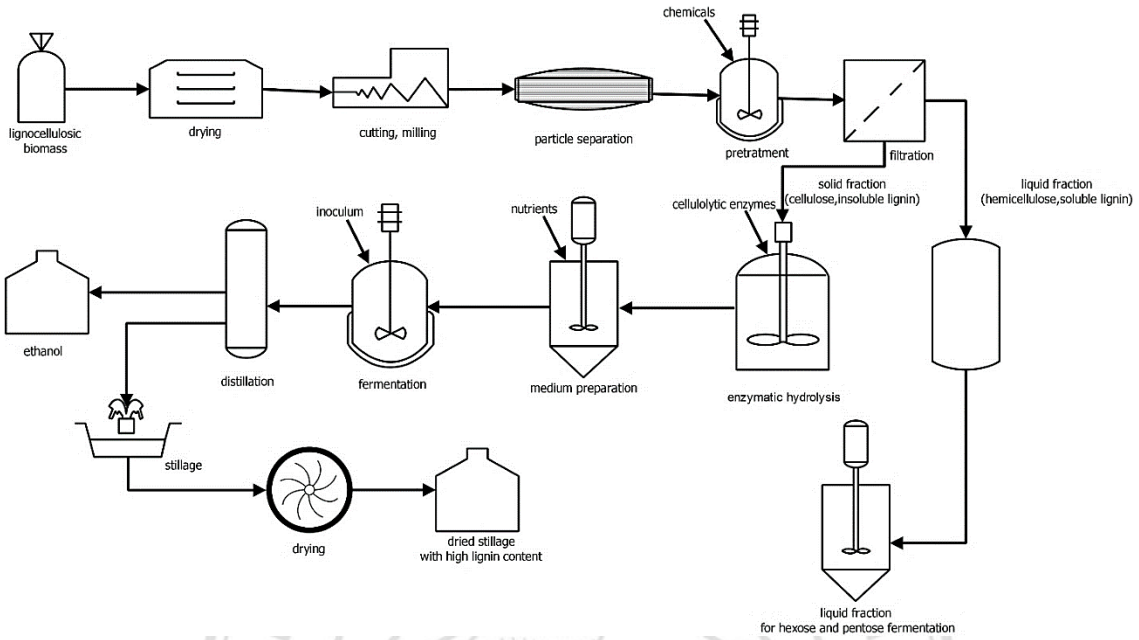

Figure 2.19 The unit operation of separate hydrolysis and fermentation (SHF) process.

Source: Paulova et al. (2015)

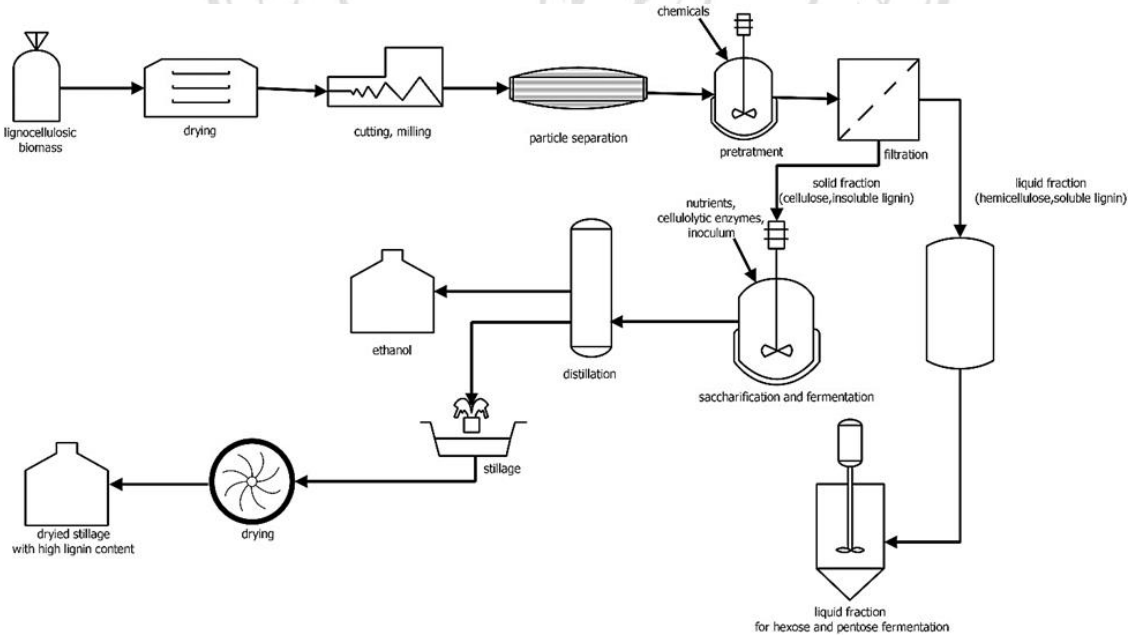

Figure 2.20 The unit operation of simultaneous saccharification and fermentation (SSF) process.

Source: Paulova et al. (2015)

Table 2.10 Bioethanol production from various lignocellulosic materials.

Substrates	Type of pretreatment	Enzyme sources	Fermentation methods	Vessel types	Temperature (°C)	Microorganisms	T* (h)	Yields (g/L)	Yields (g/g LCMs)	References
Corncob	Acid pretreatment	A mixture of commercial cellulase	SSF (10% (w/v))	2-L jar bioreactor	30	*Saccharomyces cerevisiae* NBRC2114	36	12.3	0.12	Kahar et al. (2010)
Corncob	ND**	Commercial cellulase (*Trichoder-ma reesei* ATCC 26921, Sigma)	SSF (12.5% (w/v))	250-mL flasks	37	*Kluyveromyces marxianus* 6556	72	5.7	0.05	Zhang et al. (2010a)
Corncob	The combination of acid and alkali pretreatment	Commercial cellulase (GC220)	Fed-batch SSF	6-L bioreactor	37	*S. cerevisiae*	96	84.7	0.34	Zhang et al. (2010b)
Corncob	Acid pretreatment	A mixture of commercial cellulase	Fed-batch	Conical flask	25	*S. cerevisiae* BCRC 21812	48	32.3	ND**	Chang et al. (2012)
Wheat straw combined with wheat starch hydrolysate	Steam pretreatment	Commercial cellulase (Cellic CTec2)	SHCF (18.5% (w/v))	2-L bioreactor	32	Recombinant *S. cerevisiae* TMB3400	120	60.5	0.53	Erdei et al. (2012)

Table 2.10 Bioethanol production from various lignocellulosic materials. (continued)

Substrates	Type of pretreatment	Enzyme sources	Fermentation methods	Vessel types	Temperature (°C)	Microorganisms	T* (h)	Yields (g/L)	Yields (g/g LCMs)	References
Wheat bran	Acid pretreatment	Purified endoglucanase from *Aspergillus flavus*	SSF (5% (w/v))	250-mL flasks	28	*S. cerevisiae*	96	5.8	0.12	Gomathi et al. (2012)
Rice straw	Microwave alkali pretreatment	A mixture of cellulase from *A. heteromorphus* and *T. Reesei*	SHF	Stopper-ed flasks	28	Co-culture of *S. cerevisiae* and *Scheffersomyces stipites*	36	21.7	0.48	Singh and Bishnoi (2012)
Corncob	Alkali pretreatment	Cellulase (GC220)	SSCF with fed-batch mode	3-L bioreactor	30	Recombinant *Zymomonas mobilis* CP4	72	60.5	0.24	Su et al. (2013)
Wheat straw	Acid pretreatment	Cellulase from *Penicillium janthinellum*	SSF (10% (w/v))	2-L bioreactor	40	*K. marxianus* MTCC 4136	48	21.6	0.22	Singhania et al. (2014)

*T: time; **ND: not determined

SSF: simultaneous saccharification and fermentation; SHCF: separate hydrolysis and co-fermentation; SHF: separate hydrolysis and fermentation; SSCF: simultaneous saccharification and co-fermentation.